Food and Agriculture Organization of the United Nations

ISTA Seed Quality Assurance

Guidelines for the establishment and management of seed testing laboratories

Joint FAO and ISTA Handbook

2023

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Guidelines for the establishment and management of seed testing laboratories

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Acronyms

AI	artificial intelligence
BIC	ISTA Blue International Certificate
BP	'between paper' germination method
FAO	Food and Agriculture Organization of the United Nations
FTE	full-time equivalent
GMO	genetically modified organisms
ISF	International Seed Federation
ISTA	International Seed Testing Association
ISTA Rules	*International Rules for Seed Testing*
LIMS	laboratory information management system
NIR	near infrared
NIT	near infrared transmittance
OECD	Organisation for Economic Co-operation and Development
OIC	ISTA Orange International Certificate
OSD	other seed determination
PCR	polymerase chain reaction
PP	'pleated paper' germination method
PPE	personal protective equipment
PT	ISTA Proficiency Test
QA	quality assurance
Q-documentation	quality documentation
Q-manual	quality manual
Q-objectives	quality objectives
Q-policy	quality policy
RH	relative humidity
SAP	sample administration programme
SI	(= Système International) International System of Units
SOP	standard operating procedure
TCOM	ISTA Technical Committee
TEZ	tetrazolium
TP	'top of paper' germination method
TSW	thousand-seed weight
UPOV	International Union for the Protection of New Varieties of Plants
WI	work instructions

Preface

Achieving sustainable food security and nutrition is a challenge for nations and international development organizations. The availability of quality seeds is a critical component of highly productive food systems. The International Seed Testing Association (ISTA) was formed in 1924 with the aim of facilitating the international trade of seeds of known quality. This led to the early development of the *International Rules for Seed Testing* (ISTA Rules), which provide internationally agreed methods for seed quality assessment.

Since its formation, ISTA has evolved into an organization that also accredits laboratories for the issuance of ISTA Certificates for seed analysis. The ISTA accreditation system, utilizing the ISTA Rules, ensures uniformity in seed testing worldwide and the provision of seed test results that are accurate and reproducible. This gives assurance to farmers who purchase the seeds and government seed control agencies that regulate the movement of seed.

With the aim of enabling the movement of seeds of known quality, it is with great pleasure that ISTA has collaborated with FAO on the revision of the 1983 publication *Project Seed Laboratory 2000–5000*. ISTA hopes that this publication will enable the development of new seed testing laboratories to build seed testing capacity worldwide, and assist both new and existing seed laboratories to develop their seed testing capabilities. ISTA also hopes that this joint publication will ultimately help seed testing laboratories achieve ISTA accreditation.

Keshavulu Kunosoth, PhD
ISTA President

November 2022

Foreword

The global prevalence of food insecurity and malnutrition, which has been rising steadily since 2014, has worsened sharply in the recent past as a result of the confluence of the impacts of climate variability, the COVID-19 pandemic and conflicts on agricultural and food production systems. While this worrisome trend suggests that the world is not on track to achieving the United Nations Sustainable Development Goal 2 on the eradication of hunger and malnutrition, any such forecast is not inevitable. The world can produce enough nutritious food, estimated at a 50 percent increase over the 2013 levels, to cater for everyone's needs. However, this feat will not be achieved through a business-as-usual approach.

The Food and Agriculture Organization of the United Nations (FAO) Strategic Framework 2022–2031 seeks to support countries to actualize the goals of the 2030 Agenda through the transformation to *more* efficient, inclusive, resilient and sustainable agrifood systems for better production, better nutrition, a better environment, and a better life, leaving no one behind. With about 80 percent of all foods being plant-based, a significant amount of the benefits from the transformation of agrifood systems will accrue from crops. The use of quality seeds is foundational to sustainable crop production systems, yet in many countries where hunger and malnutrition remain pervasive, less than 10 percent of crop acreage is planted with quality assured seeds. This is why FAO invests significant amounts of effort and resources to enhance the farmers' timely access to sufficient quantities of affordable quality seeds and planting materials of the improved and well-adapted crop varieties that satisfy end users' needs.

Well-equipped seed testing laboratories that are staffed by skilled personnel are critically important to the efforts to enhance the availability of quality seeds. FAO, therefore, welcomes yet another opportunity to collaborate with the International Seed Testing Association (ISTA) to generate this knowledge product that contributes to both the strengthening of institutional and human capacities for seed quality assurance and the harmonization of procedures across countries. The adoption and implementation of a uniform set of procedures for setting up and running seed testing laboratories facilitate the transferability of data across laboratories, including between countries, thereby enhancing the confidence of stakeholders in seed quality assurance mechanisms. Therefore, FAO takes pleasure in recommending these guidelines, which may be used as both a reference material and training resource, to everyone who is interested in seed quality assurance procedures.

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November 2022

Acknowledgements

Authors and contributors

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Florina Palada (Head of ISTA Accreditation and Technical Department, ISTA Secretariat, Switzerland), Christine Herzog (contracted ISTA Systems Auditor, Germany), Joël Léchappé (ISTA Technical Auditor; ISTA Honorary Life Member; ISTA Immediate Past President 2016–2019; ISTA President 2010–2016; formerly INRAe/GEVES–SNES, France), Eddie Goldschagg (ISTA Bulking and Sampling TCOM Chair until 2022; SANSOR, South Africa) and Steve Jones (ISTA Technical Auditor; ISTA Immediate Past President 2022–2025; ISTA President 2019–2022; formerly CFIA, Canada), who all drafted individual chapters and worked as a team to complete the handbook.

Revisions were provided by Eddie Goldschagg, Ruojing Wang (ISTA Purity TCOM Chair; CFIA, Canada), Andrea Jonitz (ISTA Purity TCOM Vice-Chair; Landwirtschaftliches Technologie Zentrum Augustenberg, Germany), Gillian Musgrove (ISTA Germination TCOM Chair; SASA, United Kingdom of Great Britain and Northern Ireland), David Johnston (ISTA Germination TCOM Vice-Chair; Louisiana Department of Agriculture and Forestry, United States of America), Axel Goeritz (ISTA Moisture TCOM Chair; LUFA Nord-West, Germany), Tanja Petrović (ISTA Moisture TCOM Vice-Chair; Maize Research Institute 'Zemun Polje', Serbia), Stefanie Krämer (ISTA Tetrazolium TCOM Chair until 2022; Landwirtschaftliches Technologie Zentrum Augustenberg, Germany), Jayanthi Nadarajan (ISTA Seed Storage TCOM Chair; Plant and Food Research, New Zealand) and G.V. Jagadish (ISTA Seed Storage TCOM member; Indo-American Hybrid Seeds, India). Feedback and input were also provided by Wilson Hugo (FAO), Chikelu Mba (Team Leader of the Seeds and Plant Genetic Resources Team at FAO), Dot Vittrup Pedersen (ISTA Germination and Purity TCOM member; DLF, Denmark), Didier Demilly (ISTA Proficiency Test TCOM Chair; GEVES, France) and Branislava Opra (ISTA Secretariat, Switzerland).

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Vanessa Sutcliffe of HeartWood Editorial prepared this handbook for publication through developmental editing and copyediting of the original and revised texts, drafting figures and establishing the design and layout in the three language versions. She was supported by Yoana Uzunova and Karen De La Rosa at the ISTA Secretariat who designed the front cover and helped with some of the figures. Fatma Rekik provided FAO editing.

Translation into French was provided by Joël Léchappé and into Spanish by Augusto Martinelli (member of ISTA Germination, Purity and Tetrazolium TCOMs; former ISTA Technical Auditor; Argentina).

The project coordination was done by Andreas Wais (ISTA Secretary General, ISTA Secretariat, Switzerland) and Wilson Hugo (FAO), with overall handbook coordination by Steve Jones (ISTA). Both ISTA and FAO provided financial support to develop and publish the current new handbook.

Other people and organizations

Thanks to the Government Seed Testing Station, Wageningen, Netherlands (Kindom of the), that published the first edition of *Project Seed Laboratory 2000–5000* in 1979. FAO helped distribute 200 copies of this edition before the revised second edition was published in *Seed Science and Technology* in 1983.

Copyright and acknowledgements for images and drawings

Many thanks go to all the ISTA member laboratories who have willingly shared photographs of their equipment and facilities. The original photographers and organizations retain their copyright and ability to use their own images. ISTA and FAO gratefully acknowledge being able to use the images in this handbook.

A list of figures and the laboratories that provided them is shown on page vi, and their full details are provided below. The staff of the Romanian laboratory RO05 have provided example worksheets used as the basis for Figures 12.3–12.7.

CA08 Saskatoon Laboratory, Seed Science and Technology Section, Canadian Food Inspection Agency (CFIA), Saskatoon, CANADA (image copyright CFIA, Her Majesty the Queen in Right of Canada, 2022)

CH01 Agroscope, Forschungsgruppe Saatgutqualität, Zürich, SWITZERLAND

DE19 Seed Testing Laboratory, Deutsche Saatveredelung (DSV) AG, Lippstadt, GERMANY

FR02 Station Nationale d'Essais de Semences, GEVES, Beaucouzé Cedex, FRANCE

GB04 Official Seed Testing Station, Science and Advice for Scottish Agriculture (SASA), Edinburgh, UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

IN39 Seed Testing Laboratory, Telangana State Seed and Organic Certification Authority (TSSOCA), Government of Telangana, Hyderabad, INDIA

IT01 Laboratorio di Ricerca e Analisi Sementi (LaRAS) DISTAL, Viale Fanin, 40, 40127 Bologna, ITALY

KE01 KEPHIS Seed Testing Laboratory, Nakuru, KENYA

RO05 Central Laboratory for Quality of Seeds and Planting Material (LCCMS), Bucharest, ROMANIA

SN01 National Seed Testing Laboratory, Division des Semences (DISEM, seysoo.com), Hann-Dakar, SENEGAL

UG02 Seed Testing Laboratory, Chemiphar (U) Ltd, Kampala, UGANDA

ZA01 Seed Testing Laboratory (OSTL, gov.za), Directorate of Plant Production, Department of Agriculture, Forestry and Fisheries, Pretoria, SOUTH AFRICA

Chapter 1: Introduction

1.1 Background

This revised joint publication from the International Seed Testing Association (ISTA) and the Food and Agriculture Organization of the United Nations (FAO) is based on the publication *Project Seed Laboratory 2000–5000* (second, revised edition; van der Burg *et al*., 1983). FAO helped fund and support the preparation and distribution of the 1983 publication. While the original publication covers how to build a new seed laboratory in the tropics or subtropics that could test 2000 to 5000 samples a year, FAO and ISTA agreed to produce an updated handbook that provides generic solutions for establishing and managing seed testing laboratories worldwide. In this new handbook, all sections have been updated and/or reorganized. New sections have been added on sampling, thousand-seed weight testing, quality assurance (QA), information technology (IT) and becoming an ISTA accredited laboratory. QA tips, Notes, and Safety tip boxes are used throughout the document to emphasize key points. Discussions covering special considerations for tropical or subtropical areas are also included in Chapter 15 (Region-specific considerations). The new Chapter 13 (Seed testing equipment and consumables) lists generic equipment and suggests essential criteria for sourcing equipment in different parts of the world. Information and considerations on expanding a laboratory to meet future testing needs are also discussed in different chapters. This handbook includes tables from the 2022 edition of the *International Rules for Seed Testing* (ISTA Rules). Whenever any technical procedure is mentioned in this handbook, the reader should consult the current edition of the ISTA Rules and current ISTA technical handbooks. The ISTA handbooks listed on the ISTA website[1] add extra information to the methods described in the ISTA Rules.

Currently, 237 seed testing laboratories are members of ISTA worldwide. Of these, 149 are ISTA accredited laboratories in 59 countries that facilitate seed export internationally. In addition, ISTA has personal members from 82 different countries, increasing the number of people involved in seed testing globally. Many countries have their own system for accrediting national or domestic seed testing laboratories, helping to provide information about the planting quality of seeds that supply grains to feed the world. In response to whether more seed testing laboratories are needed worldwide, FAO has identified a need to increase seed production with quality control in several areas of the world. Furthermore, ISTA annually receives at least two new applications for ISTA accreditation by seed laboratories.

Governments, national organizations and companies typically invest in seed testing laboratories to join one of the Seed Schemes of the Organisation for Economic Co-operation and Development (OECD), to produce their own seed for domestic use, to support the export of seed to other countries, or to support small-scale farmers by testing seed before planting to ensure good seed quality for proper germination and crop establishment. This publication aims to provide information to organizations, companies and people who want to establish a new seed testing laboratory. When building a new laboratory from the ground, the entire process – from planning to operating – could take 1–2 years. Whether a large national laboratory with 100 people or a three-person laboratory, the overview of what is needed to establish and manage a seed testing laboratory is similar. Established laboratories can also use this publication as a checklist to review what they already know or as a reference when needing to expand.

If seed testing is new to a country, the laboratory may not need to be ISTA accredited at establishment or at all. ISTA accreditation is a process that allows the issuance of internationally recognized seed testing reports, but this is not required for a country or company not planning to export seeds. However, if the laboratory wishes to operate in a traceable and sustainable way under a quality assurance system, this handbook serves as the starting point and provides an overview of five basic testing methods needed to ensure the quality of seed for planting. These methods include analytical purity, other seed determination, germination, moisture content and thousand-seed weight. Some information about tetrazolium (TEZ) testing is also included but not disease testing for seed health as it is a more specialized topic. Chapter 4 (Buildings and workflow) includes how to expand the laboratory to complete other more specialized tests. Once established and operational, a laboratory could take 3–5 years to become ISTA accredited. During this period, it could become an ISTA Member Laboratory to access ISTA membership benefits. The strategic needs essential to help plan what is required are provided in Chapter 2 (Deciding what is needed for a seed testing laboratory). Decision trees and flow charts are included to facilitate the planning process.

[1] www.seedtest.org

1.2 General Disclaimer

The examples, images and suggestions made in this handbook are provided to the best of the authors' and reviewers' knowledge, and every effort has been made to ensure the information included is accurate and current at the time of publication. ISTA and FAO take no responsibility or liability for how the information in this handbook is applied or for any errors or omissions. Any building or room plans are only illustrative and should not be used as building or construction blueprints. National building and safety regulations need to be considered and complied with for the region or country and take precedence over any information provided in this handbook.

Chapter 2: Deciding what is needed for a seed testing laboratory

Several questions need to be asked before starting to adapt or build a seed testing laboratory. This chapter provides a decision tree to help with those questions. It highlights why seed testing laboratories are essential in providing accurate information about the quality of seed lots and how this facilitates the national and international trade of seed and the efficiency of the agricultural production system. Answers to these questions allow planning for both the short and long term and result in a seed testing laboratory fit for purpose, one that can test the quality of seed for sale or replanting.

2.1 Step one: Why is a seed testing laboratory required?

Having a seed testing laboratory in a country or company means being autonomous in testing the quality of the seeds produced, used, processed, traded, imported or exported, or even those stored in gene banks. The decision to build and run a seed testing laboratory is usually part of a general policy or business strategy, such as developing agriculture schemes for government laboratories, or businesses for seed companies or private third parties involved in seed testing (see Appendix).

The defined needs will be influenced by the following:

- the crop species included and the equipment needed to analyse either unprocessed raw or cleaned seed lots ready for trade;
- the harvesting and planting season for the main crops to be tested, which will indicate peak times for the laboratory;
- the type of seed lot sampling, e.g. internal sampler belonging to the seed processing plant or external samplers from a network of samplers;
- the choice of standard methods (e.g. ISTA methods from the latest edition of the *International Rules for Seed Testing*, known as 'ISTA Rules') or the need to develop other in-house methods;
- whether ISTA membership and ISTA accreditation are necessary if using ISTA methods; and
- the issuance of test reports, e.g. in-house, national regulatory or international for export needs (ISTA Certificates).

Consequently, the laboratory first needs to define a strategy based on its role as a governmental laboratory, a company laboratory or a laboratory doing private third-party testing.

The types of laboratories are considered in the following sections.

2.1.1 Governmental seed testing laboratory

The objectives of a governmental laboratory are often described in seed laws, national seed regulations or breeding programmes established by the government or state regulatory organizations. For example, there is typically a need:

- To provide farmers with seed of known analytical purity (i.e. weed seed content) and level of germination produced by governmental organizations or delegates (subcontractors), to contribute to producing food and feed. This need is usually contained within the framework of a country's agriculture policy and programme. The laboratory focuses on the 'basic' or 'standard' tests such as analytical purity, other seed determination (OSD), germination, and often moisture and thousand-seed weight (TSW), but is limited to the species produced in the region. *In this case the testing methods should follow internationally agreed methods such as those described by ISTA*. Developing a quality assurance (QA) programme or seeking accreditation may be scheduled as a longer-term goal.

QA tips: The quality of seeds depends on the needs and requests of farmers or other end users. The basic criteria are the crop species, the other species content, the level of germination, the moisture content or the thousand-seed weight (TSW). Depending on local needs or regulations, information or results may also be required on variety, vigour, seed health, etc.

- To have an official 'tool' to be part of a national seed production scheme, possibly based on a seed certification scheme such as that recommended by the Organisation for Economic Co-operation and Development (OECD). The scope of activities of the laboratory should cover sampling and basic testing of all the species produced in the country. *In this case, the laboratory should implement the ISTA Rules (standard worldwide recognized methods)*, as recommended by OECD and probably by the national regulations. This implies purchasing equipment, having sufficient trained staff, and implementing a fully traceable system for sampling and testing, either paper-based or electronic. According to the national regulations, *the laboratory may be required to be certified or accredited following national, regional or specific international standards (ISTA accreditation and/or ISO 17025)*.

- To have an official 'tool' to guarantee the country's independence when importing/exporting seeds. International organizations such as the International Seed Federation (ISF) and OECD recommend that ISTA International Seed Certificates (Orange or Blue) are used. The laboratory will have to be *accredited by ISTA* to issue ISTA Certificates. This requires:
  - ✓ implementation of a QA programme;
  - ✓ calibration and regular verification of laboratory equipment;
  - ✓ training of staff and ensuring that trained deputies are assigned to key activities for secured continuity;
  - ✓ participation in ISTA Proficiency Test (PT) programmes to guarantee the uniformity of the laboratory's performance; and
  - ✓ full traceability from sampling to issuing test reports, by keeping records for at least 6 years.

Note: A seed testing laboratory is a tool to measure the quality of seeds. The seed standards that define whether the quality is good enough for its end use and purpose are usually set by a different body or organization, such as a national seed certification system or through import phytosanitary requirements. The separation of testing from standard compliance assessment helps to guarantee their independence and credibility. Decisions are usually made by certification agencies, officials, licensed (approved) persons on behalf of officials, customs authorities, seed company trade departments, customers or end users.

2.1.2 Seed company laboratory

The aim is to support the quality control of seed processing and trading of seed lots. International seed companies generally have their own network of seed testing laboratories. Some laboratories are accredited by ISTA to facilitate international trade, while others focus on testing the quality of freshly harvested seeds and those undergoing processing by the seed laboratories. Establishing a new laboratory will benefit from any existing network of company laboratories.

Regional, medium or small seed companies, or companies in developing areas, may plan to build a new laboratory. The laboratory can be designed to meet the company's strategic development that addresses the objectives of testing the quality of the seed produced. Four levels of need can be identified, and the laboratory can fulfil more than one of these needs:

1. *Testing the quality of freshly harvested seeds*. Sampling raw seed lots may require adapting the sampling methods and tools to conform with the raw material. Micro-cleaning samples to simulate the processing in the production plants may also be necessary before testing the analytical purity and germination level.

Safety tips: Sampling of unprocessed or raw seed lots and micro-cleaning are not part of this handbook. These activities require specially designed rooms with high ceilings and good ventilation since handling and processing raw seed lots releases dust that may decrease air quality.

2. *Testing the quality during seed processing* to identify problems, take early corrective measures, and plan further processing steps. *Quick* and *informative* are the key words; information must be given to the seed processing plant quickly to adjust the different processing stages if necessary. The main tests needed (analytical purity, germination and moisture) may not strictly follow standard ISTA methods regarding the size of working samples for analyses, duration of tests or accuracy. This may affect the equipment needs, but *the material and training of staff based on ISTA methods* must remain the same.

Note: With time, seed company laboratories may use in-house seed testing methods in the seed processing plant. Their establishment could fall under the research and/or method development units tasked to strategize the future development needs of the laboratory.

3. *Testing the quality of the seeds to be traded or distributed* to end users, farmers or seed growers. The production of seed can be part of a national seed certification scheme with field inspection and samples taken for official seed testing. Alternatively, seed can be produced by a seed company authorized to self-certify, or can be produced under a national legal framework where seed companies declare the quality. These conditions may vary in any given country, where some crops may have compulsory certification and others do not need certification. For all cases where seed is to be sold with the QA of the seed company, the laboratory will need to be equipped and the staff trained according to ISTA requirements. This may lead to the laboratory becoming *ISTA accredited,* thus *able to issue ISTA International Certificates used for export*.

Note: In the case of edible cereals and pulses where seeds may have a different end use, the seed laboratory may perform total quality tests before seed treatment. Once a seed lot is treated with chemicals, it cannot be sold or used for human consumption or animal feed, if it does not meet the minimum quality standards.

4. *Testing stocks of 'carried-over' seed* stored in the warehouse before the next sales season.

2.1.3 Private third-party testing

Note: This handbook does not cover farmers testing the quality of their produced seeds to store and re-sow the following year. See the FAO website for useful publications on this topic. In such cases, shelves in a room with natural light for plant growth coupled with basic methods, are sufficient for undertaking simple germination tests to ensure viable seeds are stored before they are re-sown. There is no need for a large laboratory or an extensive quality assurance (QA) system.

The aim is to provide all the services and testing described in sections 2.1.1 and 2.1.2, if allowed under national regulations. The premises, equipment and staff qualifications will depend upon the scope of activities. Private third-party laboratories could either receive samples directly from non-authorized samplers and report the quality of the sample received, or collect the sample through their own or an authorized sampler and report the quality of the entire seed lot. *The objectives or requirements of following ISTA methods and being ISTA accredited* guarantee the quality and reliability of the services provided.

Note: When the sample is received from a non-authorized sampler, the report must clearly state that it refers to the sample received. In contrast, when a sample is taken by an authorized sampler following ISTA sampling methods, the result will be an estimation of the quality of the entire seed lot (see Chapter 6: Sampling).

2.1.4 Defining the kind of laboratory needed

In the previous sections, we defined three laboratory types. Table 2.1 provides guidelines for choosing appropriate equipment, the qualifications needed for staff and the methods that fit closely to the operational and infrastructural needs of a laboratory. Each type of laboratory can also have different degrees of complexity and can be categorized accordingly. The laboratory categories described below and in Table 2.1 are for the purpose of this handbook only; they do not correspond to any standard.

A *basic seed testing laboratory* is sufficient to analyse freshly harvested seeds or seed lots during processing. This category of laboratory requires methods adapted to the laboratory's specific needs and does not necessarily follow ISTA methods. As such, in-house methods may be more appropriate. There may not be a need for complex equipment, and staff training can be adjusted to the in-house methods. This type of laboratory is the lowest cost option for a laboratory to start with and is strictly limited to the scope of activities defined.

A *standard seed testing laboratory* will be better prepared to analyse the quality of seeds for local needs and inform end users and farmers on the seed quality before sowing.

Note: Methods adapted to specific needs for basic seed testing laboratories can include:
- in-house methods for sampling (where no ISTA methods exist for this category of seed lots, i.e. freshly harvested seed lots or during seed processing);
- smaller working samples (fewer replicates) to determine analytical purity, germination or other characteristics of seed quality;
- shorter germination periods; and
- less strict control of germination temperatures.

This type of laboratory can also perform analyses on freshly harvested seeds or during processing. The methods should be based on standard methods (ISTA Rules). Consequently, the equipment and training of staff should follow as much as possible the requirements of the ISTA Rules. This category of laboratory can also be small (three people), with a limited scope of activities, but one that meets the local needs. Methods used can be based on the ISTA guidelines but do not necessarily strictly follow ISTA Rules. The investment and the operating costs are lower than the advanced and expert laboratories.

Note: Flexibility in methods applied by non-ISTA accredited laboratories is an option when the test gives an estimate of the characteristics of non-processed seed lots, such as germination and moisture. Examples of these flexible methods include:
- The size of submitted sample or number of primary samples taken may be smaller than what is mandatory in the ISTA Rules.
- Germination can be estimated on smaller working samples, with less precise temperature (variations larger than +/−2 °C), shorter or longer duration of tests and rapid evaluation of seedlings.
- Before seed processing, germination testing may be done in fewer days since the objective is to roughly estimate of germination potential.
- Moisture can be estimated with rapid measurement using non-calibrated moisture meters.
- Viability can be estimated on a smaller number of seeds.

An *advanced seed testing laboratory* performs tests in a way that guarantees the traceability from the seed lot to the issuance of the test results, which is important for national trade and/or being part of a national regulation system (e.g. seed certification). This laboratory may include a network of trained, evaluated and registered samplers. Standard methods (e.g. ISTA Rules) are used throughout the process, from sampling and sample preparation to testing. The equipment should be of certified precision, sufficient for all tests, checked for accuracy (calibrated/verified) and regularly maintained. The staff need to be well trained and qualified in the methodology. Training can be from experienced personnel in the seed laboratory as part of an official training system for seed analysts available in the country or from another recognized source. A quality system (see Chapter 5: Quality assurance) is recommended. This category of laboratory needs several years of experience to be run effectively and

efficiently. The investments for equipment, people and the operating budget are higher than basic and standard laboratories.

An *expert seed testing laboratory* tests seed quality for all requirements; it produces certificates of quality for national and international trade (import/export) and fulfils the needs of national regulatory bodies. This kind of laboratory guarantees full traceability from the seed lot to the test results and ensures uniformity of results worldwide, provided it is accredited by ISTA (see Chapter 5). The accreditation requires a fully implemented quality system, extra equipment (calibrated, verified, controlled and maintained), qualified staff, high performance in internal and external PTs, and verification of all activities during regular internal and external audits. An expert seed testing laboratory that can issue internationally valid certificates needs extensive experience and has additional operating costs linked to equipment, people, the QA system and the accreditation needs. This type of laboratory will cost the most to establish and maintain (see Chapter 16: How to become an ISTA accredited laboratory or sampling entity).

Table 2.1. Suggested categories of laboratories: equipment, level of qualification of staff, methods to fit needs

Category of laboratory	Laboratory objectives				
	Test quality of freshly harvested seeds	Assist quality control during seed processing	Inform end users and farmers on quality of seeds for sowing	Quality test for national or local trade according to national regulations and/or OECD certification[a]	Issue seed analysis certificates valid for import/export
Basic seed testing laboratory • limited costs for building and equipment • in-house methods (based on ISTA methods) used • basic training of staff • in-house traceability and uniformity of results • not inspected nor guaranteed by external accreditation or inspection body	Yes	Yes	No	No	No
Standard seed testing laboratory • standard costs for building, equipment and functioning • methods used based on standard methods (ISTA methods) • staff trained in using ISTA methods • standard traceability and uniformity of results • not inspected nor guaranteed by external accreditation or inspection body	Yes	Yes	Yes	No	No
Advanced seed testing laboratory • additional costs for calibration, verification and control of equipment • quality assurance (QA) system established • standard ISTA methods used • qualified staff trained in using ISTA methods • reliability, traceability and uniformity of test results guaranteed • sampling network exists • ISTA member laboratory (advised)	Yes	Yes	Yes	Yes	No
Expert seed testing laboratory • additional costs for extra equipment (calibrated, verified, controlled and maintained) • cost for QA system and accreditation • standard ISTA methods used and ISTA Certificates issued • reliability, traceability and uniformity of test results guaranteed • sampling network exists • ISTA accredited laboratory member	Yes	Yes	Yes	Yes	Yes

Note(s):
[a]OECD = Organisation for Economic Co-operation and Development.

2.2 Step two: Defining the scope of analyses needed in immediate, short and long term

The scope of laboratory analyses required in the immediate, short and long term need to be defined for the following reasons.

- The diversity of species to be analysed can significantly influence the laboratory equipment required and staff training needs. For example, grasses such as *Poa trivialis* and *Dactylis glomerata* need a seed blower for analytical purity tests. A grinder is needed to prepare the samples of cereals for a moisture test. Large seeds of agricultural species are germinated in larger germination rooms, whereas grasses can be germinated in small germination cabinets or on germination tables (Copenhagen tank type).
- Different species will have different peak times throughout the year. Variations will occur when testing 1000 samples each of two winter crops rather than the same numbers of a winter and a summer crop.
- Different species have a different time between harvesting and the next planting season, so the urgency in getting quality test results will vary.
- The number of samples expected in a year affects the network of approved samplers (if applicable), the size of the laboratory, the flow of samples and the number of staff. The laboratory supplies and staff needed may have to fit with a seasonal flow of samples.
- Different tests determine the space, equipment, supplies and staff training and qualifications. For example, natural light is essential for analytical purity and OSD tests. Other equipment such as balances (placed on vibration-free benches), analytical purity tools (spatulas, needles, magnifiers) and a reliable seed reference collection is also essential. Germination tests are supported by large quantities of consumables (clean water, substrates), space for planting and seedling evaluation, and growth in incubators, germination cabinets (germinators) or other temperature-controlled growth rooms.
- The laboratory may work only with national/export seed lot samples or with seed lot samples taken from imported seed lots from another region/hemisphere.

Note: The bigger the scope, the more complicated the analysis; or the more complex the seed identifications, the lower the number of samples an analyst can process in a day. For example, 1000 samples of cereals, vegetables, grasses or flowers require experienced and well-trained staff, diverse equipment, germinators set at different temperatures and more handling time. In comparison, 1000 samples of maize (*Zea mays*) require fewer staff, one germination temperature and therefore less handling time.

2.2.1 Which crop species will be analysed?

The species of seeds to be analysed is defined by the customers and stakeholders of the laboratory. A governmental laboratory must cover all regulated crop species. A seed company laboratory may only need to analyse the seed of the species produced and traded by that company. A private third-party laboratory must define its business to fit market needs.

Following a stepwise path to define the species to be tested is recommended (Figure 2.1).

1. Make a full inventory of species to be analysed, setting priorities according to the regulations, the strategy and the *80 percent vs. 20 percent* principle. This guiding principle focuses on the species that make up 80 percent of the analyses, the seed production or the profits. Keep in mind to include not only species produced in the region but also those that are imported.
2. Classify the species according to the ISTA crop groups (i.e. grasses; cereals; small legumes; pulses; other agricultural species; vegetables; spices, herbs and medicinal species; tree and shrub species; flower species). The crop groups are based on methods, equipment and competence needed. The laboratory can then define the needs for rooms, equipment, staff, material and supplies according to the main ISTA crop groups to be covered.
3. Select the two or three crop groups that cover your main needs. It is best to start with the crop groups that are easier to analyse (cereals, vegetables, pulses and other agricultural species). Limiting the number of species included in your scope is also recommended.
4. Start to operate the laboratory and accumulate experience in working with the essential species for your customers and establishing your system.
5. Plan for future extensions of the analyses to encompass other less frequently analysed species. Extensions to include other species are easier and quicker once a laboratory is established than starting with a very large scope at the onset.

Note: Remember to check the ISTA website for current versions of all ISTA documents (www.seedtest.org/en/services-header/documents.html) like the ISTA crop groups and free-to-download chapters of the ISTA Rules (www.seedtest.org/en/publications/international-rules-seed-testing-1168.html).

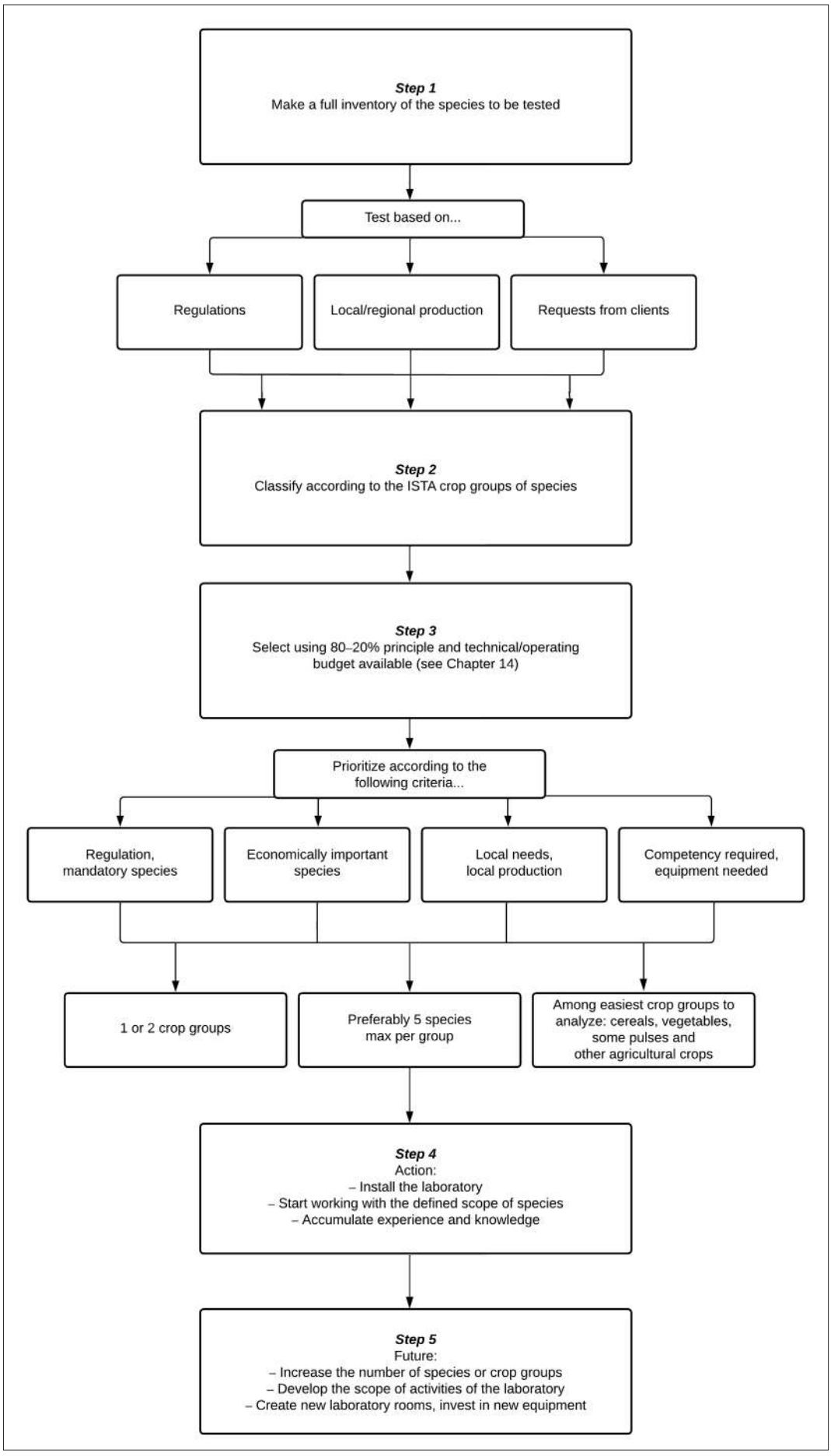


Figure 2.1. Decision tree to define the scope of species to be analysed by a seed testing laboratory

2.2.2 How many seed lots to sample and test per year?

The laboratory can estimate the number of seed lots to be sampled or analysed based on the principle that one seed lot equals one sample on which three different analyses/tests will be performed. For example, each sample needs analytical purity, OSD and germination tests and, based on experience, only one sample out of two needs a moisture test and one out of two needs a TSW determination. This average number of tests performed will be adjusted according to the needs, such as a requirement for systematic moisture tests, TSWs, seed health checks, tetrazolium tests, and so forth. Note that carryover seeds need a yearly germination test and should be added to the expected number of samples received.

Other parameters to consider include:

- The location of the seed lots to be sampled and the main seed production areas determine the laboratory's workload. Seed lots in warehouses near the laboratory are easy to sample and carry to the laboratory. When seed lots are in different locations, a network of samplers with their own equipment and transportation is needed.
- When seed tests are performed during seed processing, the number of tests can be five per lot or more. However, the size of the working samples is often smaller.
- The number of samples and the workload does not follow a linear model. Crop groups like cereals, some vegetables and pulses need less time to complete the analytical purity test and OSD test. Grasses, flowers, and tree and shrub species need more time (two to six times longer) for the analytical purity and OSD tests and may also need different equipment like a seed blower. Analysts conducting these tests will require greater competency and longer training (e.g. up to 5 years versus a few months for *Zea mays*).

The seasonal flow of samples (Figure 2.2) mainly depends on the harvest or the planting/sowing season. The peak season may affect space in the laboratory and the supplies available, but it mainly depends on the number of competent trained staff available for testing. A laboratory analysing all kinds of seeds (raw seeds, carryover seeds, seeds taken during processing and seed lots for trade) has a heavy workload throughout the year and may even have peaks where extra people are needed. The laboratory will need to organize the workflow and plan the staffing and ordering of supplies and materials to prepare for the seasonal nature of testing.

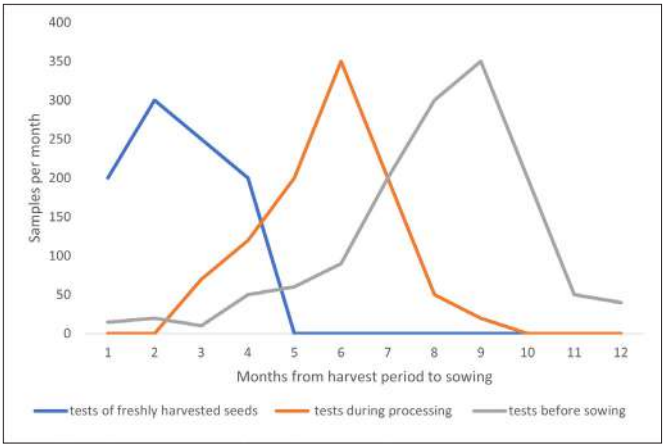


Figure 2.2. Estimated peak seasons for testing freshly harvested seeds, testing in the seed processing plant and testing before sowing (theoretical data for a medium- to large-sized laboratory)

Note: *Seed lot definition.*
The ISTA Rules define the maximum size of the seed lots to be tested. The Organisation for Economic Co-operation and Development (OECD) uses the same seed lot maximums and determines the specifications of OECD seed lot identification codes. The sizes of submitted and working samples are also given in Chapter 2 of the ISTA Rules, which is available for free download from the ISTA website (www.seedtest.org/en/publications/international-rules-seed-testing-1168.html). This information helps estimate the volumes for handling samples and space needed for sample storage during and after testing.

2.2.3 What tests are needed?

The ISTA Rules describe in detail the tests that can be performed on seed lots to determine their quality. Usually, as a minimum, seed lots need to be tested for analytical purity, OSD and germination. This guarantees that the seed lot quality has been scientifically verified using international standardized test methods. It confirms the crop species being sold and provides accurate information on the level of pure seeds of the species, the amount of inert matter present, the number of seeds of common or prohibited species in the sample and the capacity to germinate under good field conditions. The moisture content of the seed is often requested when seed lots are traded by weight as well as the weight of 1000 seeds (TSW). Nevertheless, the results of the tests have little meaning if not performed on a representative sample of the seed lot.

Any new laboratory should begin with the ability to conduct five 'basic' or 'standard' tests: analytical purity, OSD, germination, moisture and TSW. The laboratory should also have a sampling system to ensure the sampling of the seed lot links the laboratory test results to the seed lot. Otherwise, the laboratory result will refer only to the sample tested.

Additional tests may be requested to meet regional or national needs or according to the local regulations, for example:

- Seed health tests that estimate the level of infection or guarantee the absence of pests, insects or pathogens (disease) are increasingly requested.
- Viability tests such as the tetrazolium test are quicker than the germination test and can be done in a few hours. For example, they can replace a germination test for some tree species where the germination test lasts longer than 6 weeks, or the dormancy breakage is 18 months.
- Vigour tests are designed to predict the capacity to grow in some particular suboptimal conditions.
- Other tests may be required, such as varietal purity, adventitious presence of genetically modified organisms (GMO), seed mixture components or testing for dust-like seeds.

These tests require specific competencies, rooms, equipment, material and supplies. This handbook does not cover such detail, which would need input from staff with expertise in these areas and test methods. Readers may contact the Chairs of the relevant ISTA Technical Committees for guidance and to learn about the technical requirements of the specific test methods as described in the ISTA Rules chapters.

Safety tips: Is there a need to test treated seed? Based on health and safety regulations the laboratory may have to modify the room conditions when testing treated seeds. During a moisture test the chemicals used as seed treatment are heated above 100 °C and can be noxious. Therefore, separate rooms or adapted air extraction may be recommended, and the use of personal protective equipment (PPE) such as gloves, glasses, laboratory coats and/or masks may also be needed.

2.2.4 Developing the scope of analyses needed in short and long term

It is important to plan initially for a laboratory with a smaller scope of activities and species. The objective is to address the immediate needs, acquire experience, build stability and ensure continuity. The scope should be limited to sampling and analysing the selected species. The laboratory should focus on building experience and competency, starting with a combination of the most important and easiest species to analyse and limiting the number of species to a maximum of five from any of the main crop groups. Grasses, flowers, small legumes and some pulses are more difficult to analyse and should not be included in the initial scope unless necessary or mandatory.

The extension of the scope within 5 years could address expansion and different needs, such as:

- increasing the number of samples;
- extending the crop groups and species to be analysed; and
- including other tests to answer the needs of clients.

A seed testing laboratory, as described in this handbook, can easily deal with an increase in the number of samples and species to be analysed. This expansion mainly affects the number of trained staff, the supplies and the equipment needed (e.g. number of balances, size of incubators/germinators, etc.). Increasing the types of tests, including new tests or technologies, would influence the size of the premises and may require new or existing rooms to be adapted to the different needs. The staff (quantity and competencies) and the supplies of materials are also likely to be affected. As such, the laboratory expansion should be viewed as a second phase of the laboratory establishment, and new parts could be added to the original building through a modular approach.

The size and scope of activities and the steps for development can be based on a risk analysis. Examples are given on the kind of questions to consider and answer:

- *Starting with a small building* – This is less expensive to build, run and maintain. One future risk is whether funds to extend the building will be available in 5–10 years. Is this an acceptable risk?
- *Starting with a large building* – This gives possibilities to extend the activities. But is it too expensive to build and maintain?
- *Restructuring an existing building* – Can an old building be restructured to become a seed laboratory? This handbook gives guidance on what type, size and number of rooms may be necessary.
- *Starting with a limited number of important species* – It is better to gain experience in performing accurate analyses on a limited number of species. But is there a risk of not satisfying stakeholder needs, as some species would not be tested?
- *Only doing a few basic tests* – Again, it is better to gain experience performing accurate analyses. But is there a risk of not satisfying stakeholder needs as other but less frequent tests are needed?
- *Employing a small team of newly trained staff* – Is there a risk that competent staff get promoted or leave the laboratory? Retaining competent staff, or replacing them with new equally qualified staff with a period of overlap to ensure smooth work transition, is a challenge.
- *Availability of supplies* – Are there risks of running out of supplies such as sand for germination tests? Are there delivery issues, or is availability seasonal?

2.3 Step three: Deciding on the best location

Deciding on the best location for the seed testing laboratory is a combination of the assessment of many different criteria, for example:

- Should the laboratory be close to the main seed/ crop production area? The quality of the samples to be tested may be affected by factors such as climatic conditions, the distance between the collection site and laboratory, and the shipping transportation mode.
- Is there reliable sources of electric power, tap water and internet connections? A backup generator may be needed to maintain a reliable electrical supply.
- Are there climate-related risks such as seasonal heat or cold cycles, flooding, fires, earthquakes, tsunamis, hurricanes and tornados?
- Should the laboratory be close to other scientific institutes or universities to support the training and development of new staff?
- Access for staff employees to different facilities to encourage them to work at this location. Is it important to be close to other local infrastructures for staff and their families? Does the laboratory need to be close to a major city?
- Is the laboratory a new or existing building?
- What are the plans for the future extension of activities? Is it better to expand the laboratory or to build new laboratory units close to the production areas? An option is to build small new laboratories close to the production areas organized in a network to share experiences. This leads to better contact with the producers and end users, and greater efficiency can be attained as the peak season of the different species would be shared among the network of small laboratories. This model would mean less administration, less travel and reduced transport of samples.

All these considerations determine building size, staff needs, sample flow and budgets needed for investments and running the laboratory daily. Use a modular approach and think about an efficient workflow. Adding more specialized testing in the future is a critical part of the longer-term plan to establish a seed testing laboratory.

Chapter 3: Staffing

3.1 Job descriptions and qualifications required

Having people with good seed testing knowledge is essential for a seed testing laboratory to succeed. While seed analysts can cover many functions, additional competencies are necessary to start and run a laboratory, such as management, budgeting and communication. Hiring temporary staff during peak seasons may also be useful. All functions may be covered by just two or three people in small laboratories, or more people in large laboratories. Alsthough it is possible to have a two-person laboratory, the minimum number of staff is perhaps three to ensure continuity. If this is not possible, then the critical risk of not being able to test samples if the person is absent needs to be anticipated and managed. For example, if having three or more staff is not possible, arrange a way to subcontract work to another laboratory or bring in already trained people in the case of an emergency.

When planning to establish a laboratory that follows a quality assurance (QA) approach and aims to become ISTA accredited, just two people is not sufficient. All staff will need specific job descriptions. These can be as simple as explaining who does what and outlining the required qualifications. In some countries, seed science is still taught in universities and equips graduates with basic knowledge.

QA tips: As part of a quality assurance (QA) system, the staff members need a job description to understand their role and responsibilities in the laboratory. Even without a QA system, it is always good to know who is doing what, why and when.

People with an academic background may do well in seed testing, but it is not a requirement. Staff with a higher level of education may quickly move on from regular seed testing. As such, it is always necessary to continue training personnel that can perform seed testing. It can take just a few days to train someone to sample but a few weeks to ensure they do the job correctly. In a few weeks, people can learn to mix and divide samples to produce the working sample test weights. In 6 to 12 months, they can be trained in germination testing. In 18 months, they can be trained in simple analytical purity and seed identification (seed ID) training. It often takes 5 to 10 years to become highly skilled in analytical purity and seed ID testing in a wide range of species. In many situations, these training periods are like apprenticeships in seed testing: aptitude, skills and the ability to learn are essential prerequisites as well as good hand–eye coordination and vision. Having a university degree, MSc or PhD in seed technology, botany or agriculture might help in mastering these skills more quickly, but the skills still need to be acquired. Some countries have formal training courses for seed analysts that include managing a seed laboratory and issuing analysis reports. In others, there may be a requirement to have a seed analyst diploma or certification to work in a seed testing laboratory. Many countries do not have such training, and all the necessary knowledge is acquired as in-lab training delivered by experienced analysts.

Competent people are essential in any laboratory, and seed testing is no exception. Arguably, having experienced staff is more critical where automated testing machines are unavailable. Staff members need to be able to work reliably and proficiently, meet specific criteria and perform reliable testing. Their performance must be monitored to provide results on which the customer can rely. A good analyst is precise, meticulous, and works well both independently and as part of a team. Analytical purity and germination analysts may have slightly different skill sets, but in smaller laboratories, the same person needs to be able to perform all tests, including tests like thousand-seed weight and moisture. In larger laboratories, some analysts may specialize in certain tests or do tests on more difficult species. The experienced analysts will also need to train new and seasonal/temporary staff, supervise others or oversee the analytical purity or germination testing.

Note: Once trained, regular internal and external Proficiency Tests (PT) for staff will help maintain skill levels, e.g. using internal ring tests. External and internal PTs are a requirement for an accredited ISTA laboratory.

One might ask: Should the person running the laboratory be skilled in all seed testing and sampling techniques so they can help with testing and staff training daily? Or should they be a business manager with a qualification in administration? Or should they hold an academic qualification in seed science, technology or research? In-country regulations might dictate the answers to these questions, but it is generally a bad idea to rotate or transfer people out of their jobs into other governmental institutions or company departments for no good reason, without leaving experienced staff to allow for a transition or to train staff. A succession plan to bring new people into the laboratory, either as a replacement or an expansion of staff, should be part of any long-term planning. These are perhaps not the first things to consider when creating a new laboratory, but they should be thought about at some stage, like as part of an annual business review process.

Note: In addition to seed testing training, staff need health and safety training in to meet national regulations. Even if not aiming for ISTA accreditation, managers need knowledge and training in basic quality assurance (QA) and management.

To start a laboratory and ensure its effective management, analysts need skills in seed testing, and managers need skills in both seed testing and business management. It is essential to hire people who wish to stay longer than a few years since building up expertise in seed testing from zero takes time. Finding ways to encourage staff loyalty should be considered, such as job security, good salaries, annual bonuses, health care, a sense of team and belonging, career progression, etc.

3.2 Planning for staffing

Where might one find people trained in seed testing? The vacancies can perhaps be advertised, but there is not a worldwide surplus of seed analysts needing a job. Unless an established seed analyst training system in the country exists, people will need to be trained from the beginning. Is there local expertise to do that? Will the help of a consultant be needed? Will an already established ISTA accredited seed testing laboratory in a neighbouring country be needed to help with the training? In the latter case, it is best to look for accredited laboratories with a common language to facilitate communication during training. These options all involve considerable investments in time and money. See section 3.4 of this chapter for ideas on training.

3.3 How many seed analysts are needed?

In *Project Seed Laboratory 2000–5000* (van der Burg *et al*., 1983), the authors stated that a laboratory doing other seed determination (OSD), analytical purity and germination testing for 2000 samples of mixed species needs four analysts. For a laboratory testing 5000 mixed species samples, seven analysts were needed, plus one administration person and a person to head the laboratory. Their planning and estimates were also based on needing to staff the laboratory during the peak 3 months of annual testing where, for a 5000-sample laboratory, they may need to test about 28 samples per day.

Another way to estimate the testing needs is to determine how long it takes to receive, prepare, test, report, file and store samples. This approach may also help estimate how much to charge customers for a seed testing service. The fee for seed testing will need to include staff time, consumable costs, building overheads, depreciation, etc., and will be influenced by the funding. The laboratory may be supported by the state, a parent company or be entirely self-funded, needing to invoice for work at the full economic rates (see Chapter 14: Budgeting).

The testing time estimated in Table 3.1 is approximate, rounded and varies depending on staff experience and established QA requirements. Extra tests will be needed for occasional retesting, verification of equipment, stock solutions and storage conditions, and conducting internal and external Proficiency Testing. The values in Table 3.1 estimate the efficiency of one person for a 500-sample laboratory, only testing large-seeded species and not accounting for peak testing periods or the risk that comes with relying on just one person. As mentioned in section 3.1, having at least three people in the laboratory is more prudent. The overall approach taken in Table 3.1 with the inbuilt assumptions of what a working year produces gives a similar result to the estimates in the 1983 publication.

Note: Equipment needs are covered in the individual testing chapters in this handbook and in the seed testing equipment and consumables checklists in Chapter 13.

Table 3.1. Estimated sample preparation, testing and reporting times for the different tests on large-seeded crop species, grass species and other difficult crop species

Task	Time estimates (minutes)	
	Large-seeded crop species	Grass species and other difficult species
Sample receiving	5–10	5–10
Working sample preparation (mixing and dividing)	10	15
Percentage analytical purity testing	40	60
Other seed determination and seed ID (OSD)	30	120
Thousand-seed weight (TSW) testing	15	15
Germination testing: • preparation of substrates • planting	15	25
Germination assessment	45	45
Moisture testing	30	30
Worksheet checking, result calculation and reporting	5–10	5–10
Results authorization, issuance and invoicing	5–10	5–10
Seed analyst testing time (minutes)	*200–215*	*325–340*
Other time per sample (minutes)	30	30
Total sample processing, testing and reporting time (minutes)	*230–245*	*355–370*
Additional time needed[a]		
Linked to analysis[b] • preparation of analyses (setting climate rooms, checking balances, preparing labels, carrying samples) • maintenance of equipment • cleaning of benches and equipment • checking supplies, ordering, sample reception	Percentage of total time (estimated at 10%)	Percentage of total time (estimated at 10%)
Staff management[c] • internal meetings • organization of work • training • safety • time for staff breaks • vacations (percentage of total time) • preparation to start work upon arrival and to leave work	Percentage of total time (estimated at 20%)	Percentage of total time (estimated at 20%)

Note(s):

[a]The lower part of the table provides some estimates that add 10–30 percent on to the total working time per staff team needed and depends on legislation, internal management rules, staff number, etc.

[b]Significant time must be added to the time strictly dedicated to the analysis, e.g. preparation, cleaning, staff laboratory life (discussion and coordination).

[c]Additional time for staff management will vary with internal regulations or national legislation.

Some of the tasks included in the time estimates in Table 3.1 could be done by seed analysts or by another administration person. It is recommended that the signing and authorization of test results be done by a senior analyst or the head of the laboratory; they can be the same or a different person from the senior analyst. When a laboratory has less experienced analysts or people in training, their results should be double-checked by experienced and authorized colleagues. As the laboratory staff increases in size, the number of administrators needed to help with human resources like recruiting, salary payments, invoicing, etc., may need to increase. Alternatively, some seed analysts will need to be involved in some of these tasks. Using an integrated computer and laboratory information management system (LIMS) to save time and staff performance is also a consideration. Maintenance of a QA system, related documentation and compliance with accreditation standards brings huge responsibilities. An extra staff member in addition to the head of the laboratory to fulfil these functions is a good idea.

Overall estimates of staff number needed are included in Table 3.2. A 2000-sample laboratory is estimated to need four seed analysts, the same as in the 1983 publication. For a 5000-sample laboratory testing mixed crop types with a similar ratio of mixed crop types used in the 1983 publication, the laboratory requires eight analysts compared to seven in the 1983 publication. Temporary staff may still be needed in peak periods. Other testing scenarios and staff needs when QA is a laboratory requirement are also shown in Table 3.2.

Table 3.2. Estimates of staff number needed based on the testing times from Table 3.1 and 1500 h per working year for one full-time equivalent (FTE) person[a]

Samples per year	Seed analysts	Laboratory head and administration staff	Quality assurance role	Total FTEs needed
500 large-seeded crop species with moisture and thousand-seed weight testing	2	Analysts also do this	0 or 1[c]	2 or 3[c]
1000 large-seeded crop species	2	Analysts also do this	0 or 1[c]	2 or 3[c]
2000 mixed crop types (50:50 large-seeded species to grass crop mix)	4	1	0 or 1[c]	5
5000 mixed crop species (75:25 large-seeded species to grass crop mix)[b]	8	2	0 or 1[c]	10 or 11
5000 mixed crop species (50:50 large-seeded species to grass crop mix)	10	2	1	13
5000 grass species	18	3	1	22

Note(s):

[a]i.e. 40 weeks per year × 5 days per week × 7.5 h per day (the number of 40 weeks allows for holidays, sick time and other lost time per year per person).

[b]This ratio of large-seeded crop species to more complex species to test is similar to the testing ratio used in staff number estimates in *Project Seed Laboratory 2000–5000* (van der Burg *et al*., 1983).

[c]If the laboratory is planning to become ISTA accredited, to fulfil both the testing and QA requirements the minimum is likely to be at least three people.

In a larger laboratory, it makes sense to split the workload between sample receipt, mixing and dividing, media preparation, analytical purity testing, germination testing, supervision, administration, purchasing, etc. Training all staff members in all the testing needed at the laboratory gives the best flexibility in planning for testing during peaks, to cover work during holidays or if staff are absent for other reasons. It is also a good idea to provide job rotation during the day or week, to avoid people sitting all day doing the same task, providing a healthier work environment and increasing job satisfaction. Investing in digital systems and other equipment solutions will also improve work efficiency, reducing staff number to levels lower than those shown in Table 3.2, especially for the larger testing number of grass species. Achieving 400–500 samples per analyst per year may then be possible. The decisions made based on Chapter 2 of this handbook are essential and will influence the type and size of the laboratory and staffing levels.

3.4 Training needs

If there are no existing staff members that can provide in-lab training for new staff, other training opportunities like ISTA workshops, self-study of publications, web-based learning or consultancies may exist. University courses in seed science or technology might be available, and FAO supports member countries in capacity-building activities for seed testing. See the Bibliography of this handbook for a list of useful references and resources. The different ISTA technical handbooks are useful resources for training and provide details on the International Rules for Seed Testing (ISTA Rules). The testing chapters in this handbook provide guidance and tips on testing. Remember to make use of expertise from within the ISTA accredited laboratory network.

Some countries may require seed analysts to be licensed or meet minimum education requirements. In these countries, the national seed authority, ministry of agriculture or official governmental or state seed testing laboratory will often provide structured training courses, and require both public and private seed analysts to pass theoretical and practical examinations before issuing them a licence to work in or run a seed testing laboratory. See the ISTA website for a suggested analyst training programme.[1]

[1] www.seedtest.org/en/technical-committees/seed-analyst-training-603.html

Chapter 4: Buildings and workflow

4.1 General requirements

A seed testing laboratory should be designed to be easily accessible for sample delivery and to allow an efficient sample workflow (Figure 4.1). Ideally, it should be established on the ground floor, but this may not always be possible. If on more than one floor, a lift (elevator) will need to be added to the design with sufficient capacity to move people and equipment between floors. Local climate conditions and building regulations will affect the overall building construction and available building materials. Ventilation and insulation may be required or recommended depending on the local situation and therefore cannot be considered in this handbook. Energy efficiency and insulation should be considered and proper planning to meet local building requirements may be needed.

Essential building requirements include the following:

- reliable supply of electricity, heating/cooling and water;
- sewage connection or sewage disposal system;
- phone landline and internet;
- area for reception of samples and consumables/ equipment;
- work space in a building to accommodate testing and personal needs, including sample reception, offices, different laboratory rooms, sample storage, cleaning room, lunch/ staff meeting rooms and toilets (restrooms);
- functional layout to allow efficient sample handling, via a logical one-way movement of sample workflow, if possible;
- adequate illumination and ventilation;
- ventilation/fume exhaust for staff working with treated seeds and chemicals;
- wall insulation, such as for cold storage or a self-contained walk-in germination room; and
- option to extend the building in case of increased staff number or more test methods.

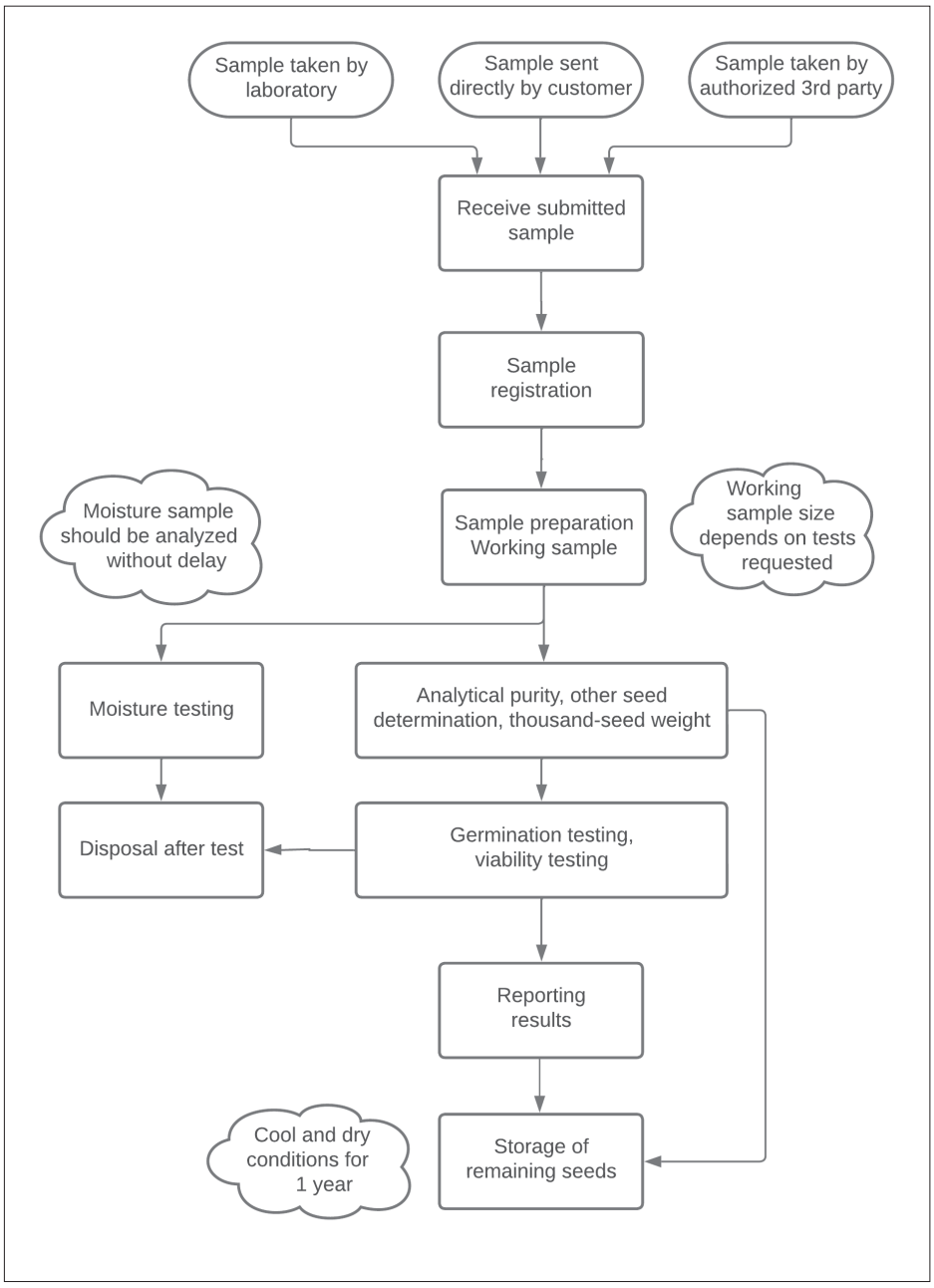


Figure 4.1. Generic sample process workflow for tasks within a seed testing laboratory building

Note: When designing and building walk-in germination rooms, assistance from specialist companies and engineers is highly recommended to ensure adequate insulation, cooling, uniformity of temperature and to avoid any possible issues of damp, water supply, drainage, etc.

Safety tips: Consider restricting eating and drinking in the entire seed laboratory if handling treated seed, or allocate a separate room equipped with fume hoods and additional ventilation to test treated seed, where eating and drinking are prohibited.

4.2 Workflow and building layout for a laboratory conducting standard methods

The layout of a seed testing laboratory can be roughly divided to reflect the different functions of testing and administration. Supporting rooms such as staff and storage rooms should be included in the floor plan to avoid long distances to walk or to carry materials. See Figure 4.2 and Figure 4.3 for examples of possible layouts. The exact sizes and required surface area will depend on staff number and laboratory tests. The entrance to the laboratory should be controlled so that access to the laboratory and administration rooms is only permitted to authorized persons. One way is to have a security guard at the laboratory entrance, but a less expensive alternative is establishing electronic key card access to the laboratory with a separate reception area for sample drop-off and visitors.

Note: If you are building a seed laboratory for the first time or repurposing existing buildings, make sure you visit or contact an existing accredited seed laboratory to get an idea of the space you might need and different options and ideas. If a physical tour is not possible you might be able to arrange a virtual tour.

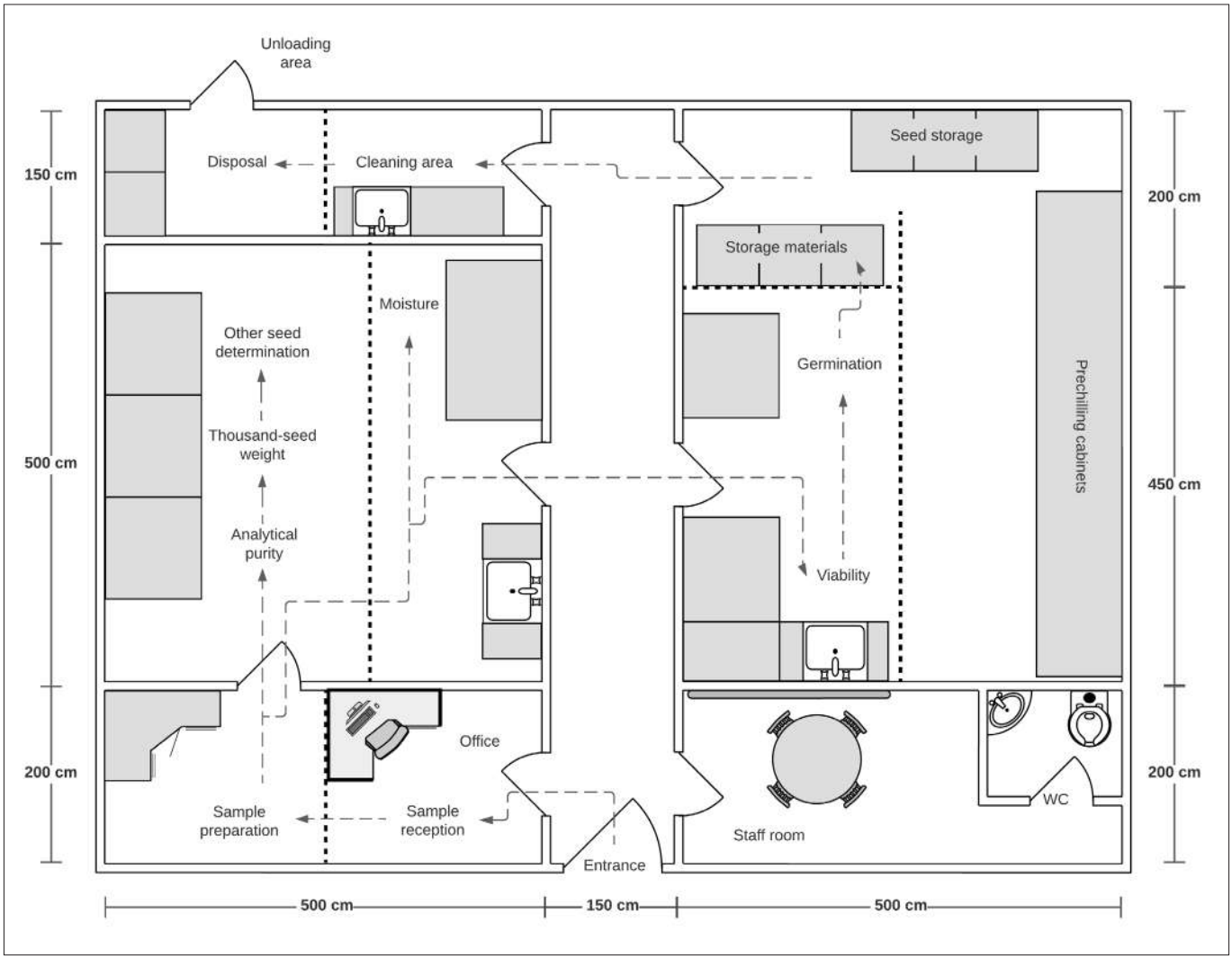


Figure 4.2. Possible building layout and sample workflow in a small three-person seed testing laboratory (100 m^2)

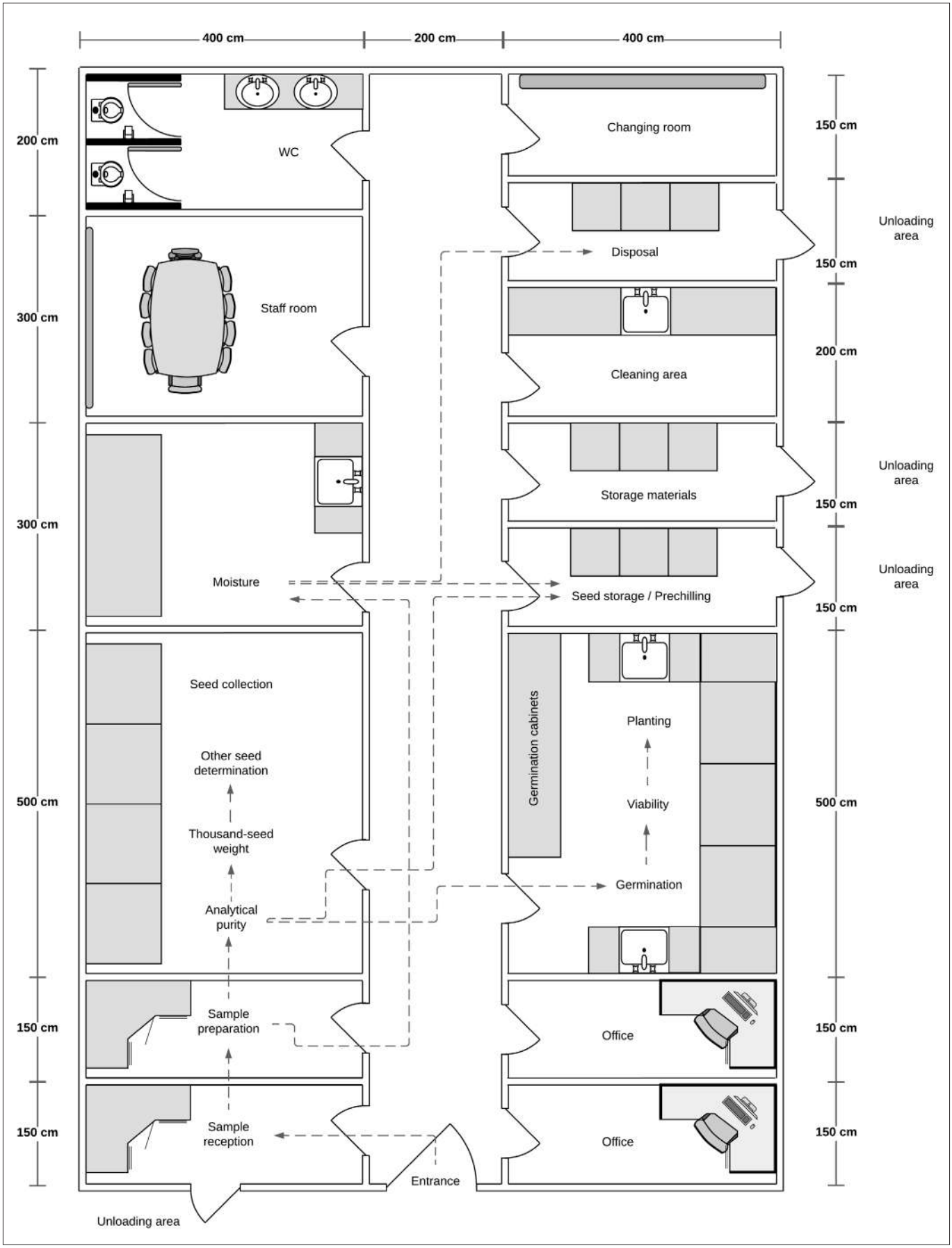


Figure 4.3. Generic room designs and room arrangements for a larger (160 m^2) seed testing laboratory to allow a one-way sample flow

The submitted samples arrive at the laboratory by post, courier, or are delivered personally by the sampler, farmer or an authorized person. It is advisable to assess the conditions samples are exposed to when sent by post or courier, as a long journey and high temperatures may adversely affect germination results. A general unloading area allows consumables and samples to be easily transported to the sample reception area where the sample registration (including confirmation of all requested tests) takes place. The incoming samples should be processed as soon as possible. If there is a high workload and the samples need to be stored before analysis, they should be kept under cool and dry conditions. At the stage of sample reception, different verifications are done to ensure the sample is suitable for the scope of analysis requested, e.g. test requests and sample details are complete, there is sufficient sample weight, and the sample container is sealed and intact. It should also be noted if the sample was sent by a certified sampler or not, as that will change the options and details included in the final test report.

Safety tips: Consider height-adjustable worktables to avoid staff sitting for too long in the same position and to allow adjustment for working in a sitting or standing position.

If the initial requirements are met, the submitted sample will be mixed and divided to prepare a working sample for the different tests. The moisture test sample is not mixed or divided at this stage and will stay in the moisture-proof container waiting to be analysed. Normally, the next step is to conduct the analytical purity analysis and other seed determination (OSD). The moisture test can be performed in parallel. A thousand-seed weight (TSW) analysis can be done with the pure seed fraction. The pure seeds determined during the analytical purity analysis are typically used for a germination analysis and tetrazolium (TEZ) test. Pure seeds can be taken independently from the submitted sample if, for example, a TSW, germination or TEZ test is requested but no analytical purity analysis. In all cases, the pure seeds need to be determined by a qualified analyst before starting the tests. The remaining sample, the analytical purity fractions and the other seeds of the OSD are kept in a cool and dry storeroom. The laboratory defines the retention period for stored samples; a storage time of at least 1 year from the sample reception day is recommended. Storage allows access to seeds for a retest in case of a customer query or complaint about the sample.

QA tips: Define the safe storage period for moisture and germination samples based on repeated tests for selected storage samples.

The seeds used for moisture analysis (oven-dried), seedlings from germination tests and cut seeds from a viability test (TEZ test) are disposed of as waste. Special chemical disposal may be required for treated seeds and the remaining TEZ solution.

Safety tips: Plan to safely dispose of waste such as old seed samples, treated seed, used germination paper and sand, seedlings, chemicals, etc. Remember that the amount of sand to be disposed of may be significant and might need a separate disposal system because of its weight.

4.3 Workflow and building layout for a laboratory conducting additional specialized tests

4.3.1 Seed health (disease) testing

Additional and separate rooms are necessary for the workflow of a seed health (disease) testing laboratory. These could be housed in a different building, a building extension on the same floor or on another floor. Separate rooms are required to prepare the samples; to house fume hoods, clean areas for media preparation (sterile and/or laminar flow cabinets), an autoclave to sterilize media; and to dispose of contaminated seed. Media preparation rooms can be small but additional space for controlled temperature incubators, fridges and freezers, and storage of media, reference material and samples will be needed. Equipment will often need to be duplicated for seed disease testing to avoid the risk of cross-contamination, for example, mixing and dividing equipment, and germinators. The size of the rooms will depend on the number of samples and different diseases to be analysed. If polymerase chain reaction (PCR) disease testing is planned, further separate rooms will be needed (see also section 4.3.2 for genetically modified organism, GMO, testing). A one-way flow is essential to avoid cross-contamination of samples, keep diseased samples contained and maintain clean areas. An autoclave can be used for the preparation of substrates and the sterilization of wastes before disposal. Figure 4.4 shows that the autoclave can be accessed in two ways.

Possible rooms and a sample workflow for a seed health (disease) testing laboratory may include:

- separate room for the preparation of the samples for analysis;
- separate room with an autoclave for sterile media preparation;
- separate room(s) for where the analysis can be performed;
- space for sterile and laminar flow cabinets;
- space within a room for fridges, freezers and incubators;
- separate room for sample storage;
- additional rooms as office space for report writing or other administrative tasks, for quality assurance (QA) and document storage; and
- additional meeting room(s) for staff or visitors.

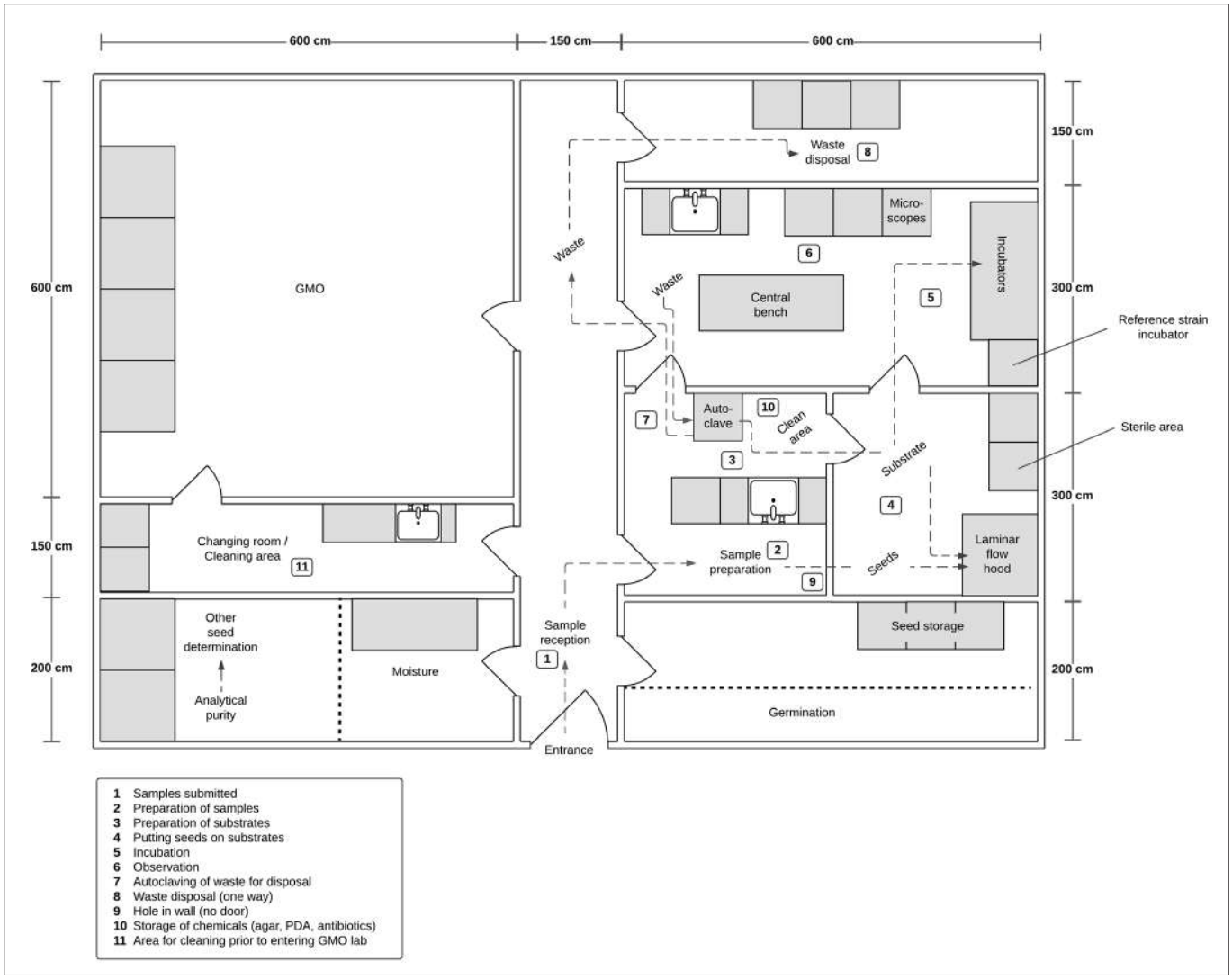


Figure 4.4. Additional module in a seed testing laboratory layout for seed health (disease) testing with a one-way workflow to avoid contamination

4.3.2 Polymerase chain reaction / genetically modified organism testing

It is crucial to have separate rooms for the workflow of a PCR/GMO testing laboratory. The rooms can be small (depending on the number of samples to be analysed), but it is essential to have a one-way flow to avoid any cross-contamination of samples. Each room must be kept at a very high standard of housekeeping and cleaning to prevent DNA spillage and possible false positive results. The submitted sample must be processed into a working sample with a mixing and dividing principle followed by sample grinding. It is best to designate one room to grind the samples at the greatest distance from the PCR/GMO analysis rooms, such as on a different floor within the same building or in a separate building. A possible layout with a one-way workflow is shown in Figure 4.5.

Possible rooms and a sample workflow for a PCR/GMO testing laboratory may include:

- one room for grinding the samples;
- a separate room for the preparation of the samples for analysis;
- a separate room for DNA extraction or other kinds of analysis like protein-based variety testing;
- space within a room for preparation of the mixes used for assays; and
- a separate room for the instruments used for DNA-based methods (e.g. PCR machines) where the data analysis could also be done.

Note: The reagents used to make the mixes must be stored and managed in a space where the samples are never present, like a separate laminar flow or ultraviolet cabinet.

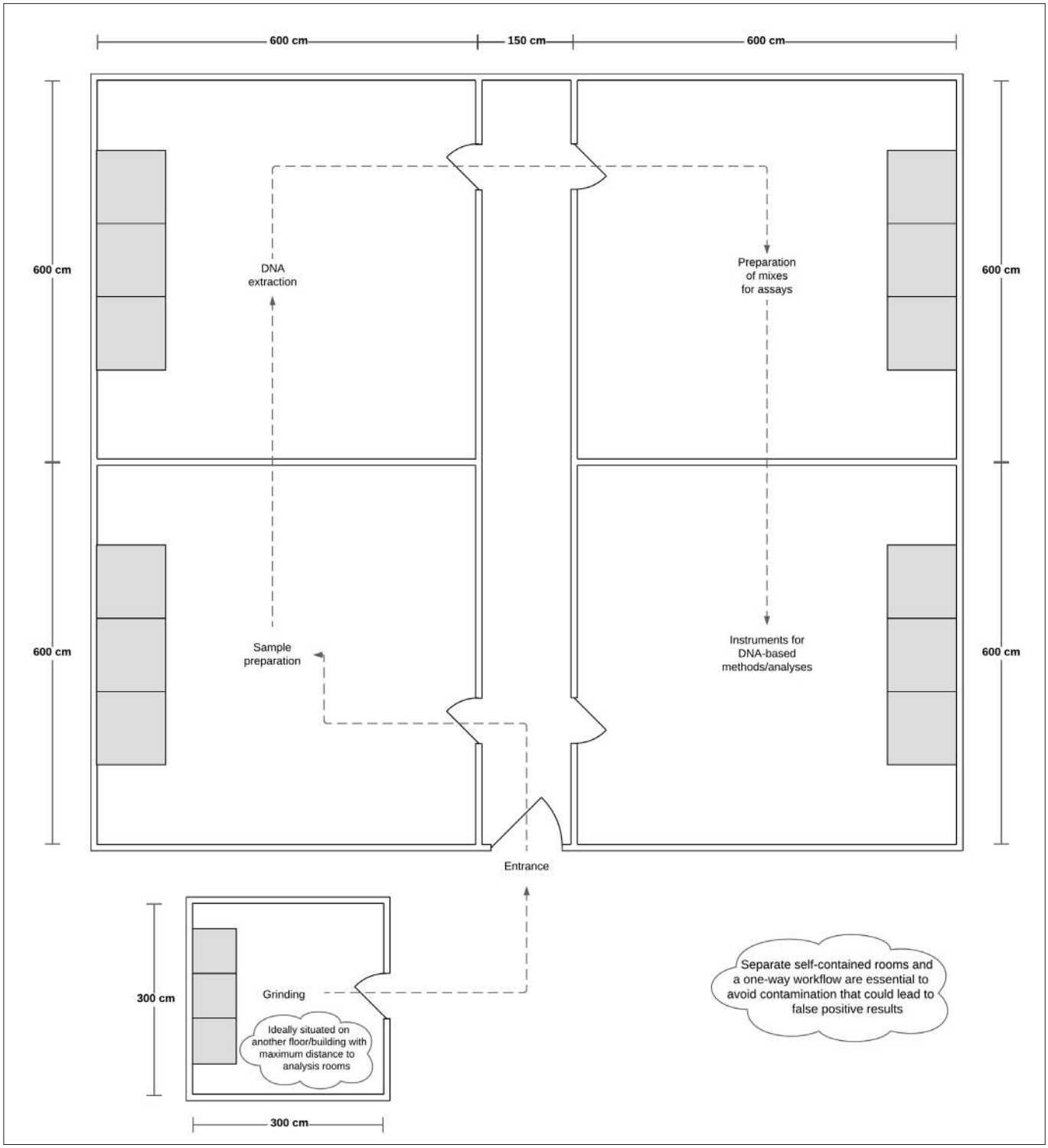


Figure 4.5. Additional seed laboratory module for polymerase chain reaction / genetically modified organism (PCR/GMO) testing

4.3.3 Variety testing

More advanced or specialized seed testing laboratories may be involved in variety testing using electrophoretic, molecular or other techniques. The rooms for variety testing using molecular techniques also need to be clean, but it is not necessary to keep to such a high standard as for PCR/GMO testing. The same generic design can be used for variety testing as suggested for PCR/GMO testing (see Figure 4.5).

A possible workflow for variety testing may include:

- one room for the preparation of the samples and to house the machines to grind the seeds;
- one room for the preparation of the samples for analysis, e.g. protein or DNA extraction; and
- one room for the analysis, e.g. preparation of the gels, electrophoresis or DNA testing and data analysis.

Chapter 5: Quality assurance

5.1 Basic principles

A small laboratory may not initially require a quality assurance (QA) or quality management system. However, it will need one as the laboratory grows or transitions to an ISTA accredited laboratsory, or one that complies with national seed accreditation systems. Therefore, it is good to establish and operate a QA system appropriate to the type, range and volume of work performed from the beginning. The QA system must ensure that the required degree of accuracy and precision is achieved, deficiencies are detected, appropriate corrective actions are taken and the actions are recorded. The laboratory QA system must be documented in a regularly updated quality manual (Q-manual) available to the laboratory staff and must include or refer to the supporting procedures, including standard operating procedures (SOP) and work instructions (WI). The Q-manual must outline the structure of the documentation used in the quality system. The overall quality objectives (Q-objectives) must be documented in a quality policy (Q-policy) statement. The Q-policy statement must be concise and issued under the chief executive's authority. The roles and responsibilities of technical management, the quality manager and the testing and sampling staff must be defined in the Q-manual. For more details and examples, see the Accreditation section of the ISTA website.[1]

5.1.1 Structure and format of quality documentation

The quality documentation (Q-documentation) must reflect all laboratory activities and may consist of the following three levels, also referred to as a 'document hierarchy' (see Figure 5.1).

1. Q-manual and annexes or appendices: A Q-manual's purpose (often 10–30 pages) is to outline the general policies and procedures for staff, customers, accreditation bodies and legal bodies to provide an overview of the laboratory's QA system.
2. Documented QA system and technical procedures: The SOPs concisely describe standard procedures to provide enough information for staff to conduct the work concerned. The volume of work depends on the size of the laboratory, number of tests, number and qualification of staff and kind of equipment in use.
3. Other QA documents: The WIs provide additional details for standard procedures, e.g. species-related information on a specific test method, how to programme the equipment, etc. Forms, checklists and reports related to a standard procedure should also be provided where appropriate.

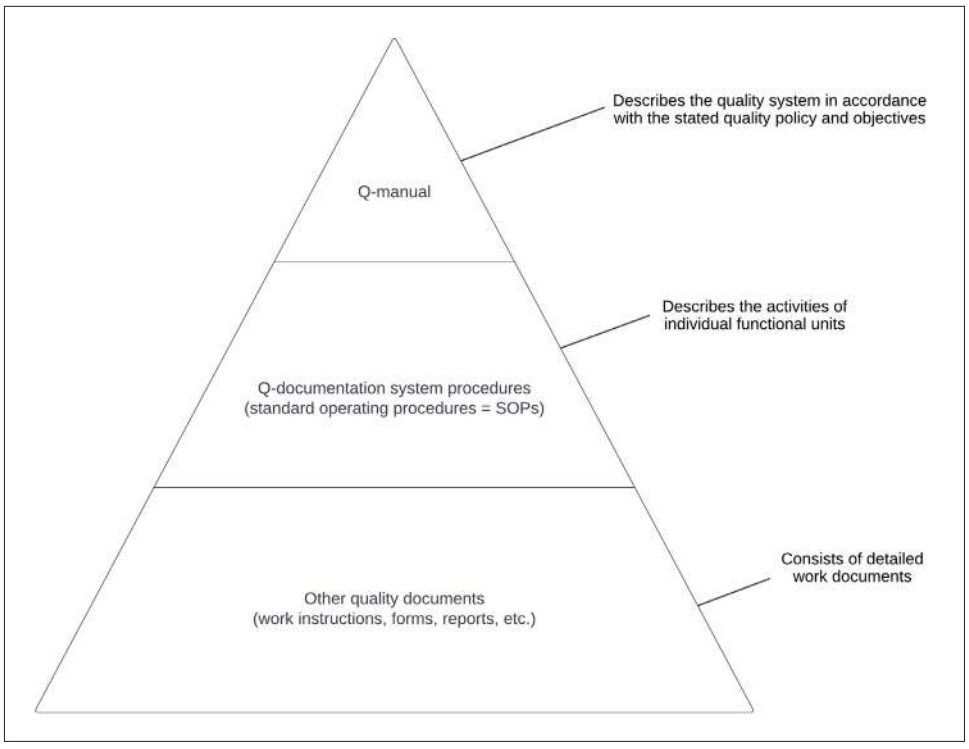


Figure 5.1. Structure of the quality documentation

[1] www.seedtest.org/en/accreditation.html

There is no required format for the Q-documentation; however, it should convey accurately, completely and concisely the Q-policy, Q-objectives and governing laboratory procedures. Q-documentation can be in hard or digital/soft copy.

5.1.2 Preparation of a quality manual

Competent personnel (a group or an individual) must be appointed and tasked with establishing the QA system documentation, including the Q-manual. The appointee(s) is (are) responsible for the following tasks:

- obtaining data on the actual state of the QA system;
- planning the documentation system/structure;
- collecting and compiling existing documentation and requesting additional documentation where necessary;
- reviewing the documentation to ensure clarity, suitability and proper structure;
- developing a distribution policy;
- incorporating the necessary changes;
- acting as a contact in all matters of the QA system;
- ensuring that the QA system is consistently implemented and followed; and
- ensuring continual improvement of the QA system.

The Q-manual may be developed and used by an organization for purposes including, but not limited to the following:

- communicating the laboratory's Q-policy, procedures and requirements;
- describing the QA system;
- providing a documented basis for auditing a QA system;
- providing continuity of the QA system and its requirements during changing circumstances (must be updated regularly);
- training personnel in the QA system requirements and methods of compliance; and
- presenting the QA system for external purposes, such as demonstrating compliance with the respective accreditation standard.

The items to be included in the Q-manual are covered in the following sections.

Scope and field of application

Clearly state for which activities/departments of the laboratory the Q-manual is applicable. For example, all the laboratory's seed sampling and testing work may be included in the scope, or research work may be excluded from the scope.

Table of contents

Indicate titles of chapters/sections and subchapters/subsections. The numbering or coding system of sections, subsections, pages, figures, exhibits, diagrams, tables, worksheets, etc., should be clear and logical.

Abbreviations

Provide definitions and explanations of abbreviations specific to the laboratory.

Quality policy and quality objectives

In this section of the Q-manual, state the intentions and objectives of the laboratory. The section should also describe how the Q-policy is made known to and understood by all employees and how it is implemented, maintained and improved at all levels.

Note: A description of the activities of the seed testing laboratory/sampling entity is not a substitute for the quality policy (Q-policy) statement. A sampling entity is an organization taking care of seed sampling only and not testing. See the ISTA website (www.seedtest.org/en/accreditation.html) for more details about this option and how it can fit into the ISTA accreditation system.

The Q-objectives are determined based on the Q-policy. Q-objectives are quantifiable (current value, target value, time period) to facilitate a target/actual value comparison. The Q-objectives need not necessarily be included in the Q-manual. A reference to the document where they are to be found, for instance, an annual plan, would be sufficient. Determination and verification of achievement of the Q-objectives are to be made at least annually in the management review or other relevant planning documents.

Standard operating procedures, including work instructions

Provide SOPs for those operations of the Q-manual that need to be described in detail. The SOPs serve the staff members as a practical working aid for daily use. They should be compiled so that they can be used as a 'how to do it' method, like a cooking recipe. It is recommended to keep the SOPs concise, exact, and to the point and they should represent the practice by using simple wording, pictures and flow charts. SOPs may be supplemented by WIs, describing single aspects in even more detail or by giving examples.

SOPs should be provided for, but not be limited to, the following subjects.

System SOPs:
- document structure and control procedure;
- sample receipt and registration;
- issuance of testing reports (if relevant);
- training of new and experienced laboratory staff (for sampling and testing activities), including authorization/recognition, the procedure of warning, and suspension and withdrawal of authorization;
- internal audit procedure;
- dealing with complaints;
- nonconforming work and corrective action procedure;
- preventive action procedure (if relevant);
- purchasing of services and supplies;
- management review procedure; and
- quality control procedure (e.g. monitoring by check sampling and check testing).

Technical SOPs:
- maintenance, repairs, control, calibration and/or verification for each item or group of equipment (e.g. balances, working thermometers, pH meters, seed blower, grinder, moisture oven, germinators, dividers, etc.);
- storage and disposal of samples;
- control and disposal of chemicals;
- maintenance and description of reference seed collections and how verification of species is accomplished (including a list of species); and
- testing of new batches of germination substrates.

Testing SOPs/WIs:
- sampling of seed lots;
- mixing and dividing of samples in seed warehouse/laboratory; and
- methodology for each test within the laboratory's scope (e.g. analytical purity, germination test, determination of other seeds by number, determination of moisture content, testing of coated seed, etc.).

Note: The general policy and procedures already described in the quality manual (Q-manual) should not be mentioned again in the standard operating procedures (SOP), to avoid redundancy and the possible risk of inconsistency.

A flow chart is particularly helpful in visualizing a process and representing the essential elements of a given procedure on a single page. It may be supplemented by explanatory notes or be itself the summary to a textual description. By consensus, a minimal number of symbols is used, facilitating the generic application of the SOP and making it a tool that is easily understood (see Figure 5.2).

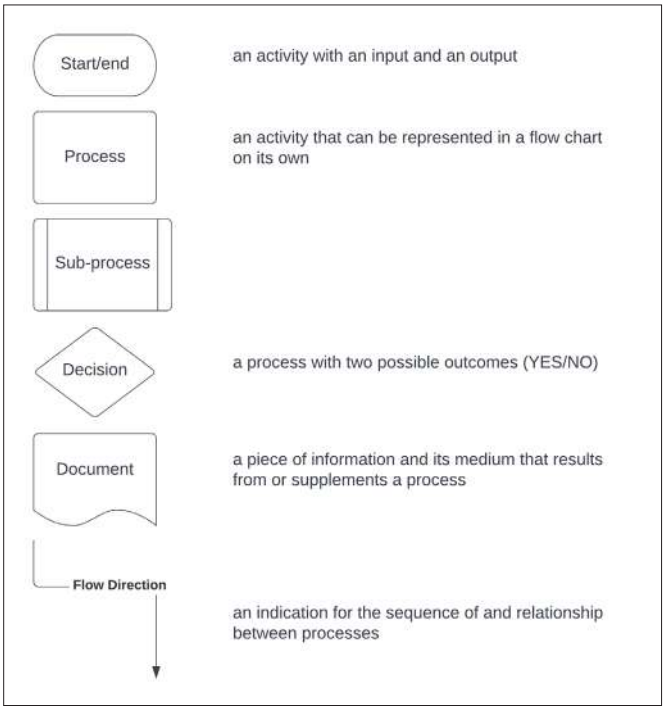


Figure 5.2. Symbols for a process flow chart

Organization and management description

In this section of the Q-manual, the laboratory should describe the organization's high-level structure. This may be shown with an organizational chart, where responsibilities, authorities and the interrelationship structure are included. It puts the laboratory in the overall context within a larger organization and shows the organizational structure within the laboratory. Ideally, it illustrates sections/departments (staff administration, financial department, etc.), functions or positions (technical manager, quality manager, etc.), how sampling is linked to the laboratory, and how the samples flow through the laboratory, including sample reception/registration, testing sections and issuance of testing reports.

5.2 Document control

The laboratory must establish and maintain procedures to control all documents that form part of its QA system (internal and external), such as: (i) testing or sampling methods; (ii) reference documents (seed law and regulatory documents that provide a legal framework); (iii) other normative documents like minimum seed standards and rules for regional and international seed trade; (iv) lists of prohibited, noxious and tolerated weeds per crop in different countries and regions; and (v) equipment manuals. All documents made available to the staff in the laboratory must be reviewed and approved for use by authorized staff before issue. A master list or an equivalent document control procedure identifying the current revision status and distribution of documents in

the QA system should be established and be readily available to preclude the use of invalid or obsolete documents. QA system documents generated by the laboratory must be uniquely identified. Such identification should include the date of issue or revision identification, page numbering, the total number of pages or a mark to signify the end of the documents, and the issuing authority/authorities. Changes to documents must be reviewed and approved by the same function that performed the original review unless specifically designated otherwise. Procedures should be established to describe how document changes are made and controlled.

5.2.1 Description of quality assurance system documentation

An explanation of the structure of the Q-documentation should be provided, e.g. how many levels, what kinds of documents are used (Q-manual, SOPs, WIs if applicable, forms, logbooks, checklists, etc.), the format (digital/soft or hard copy), and so forth. The description should ensure that the user understands how the documentation is to be used. There should be a description of the document identification system (e.g. 'SOP 01' or 'SOP A01'; 'Q' for quality document, 'S' for standard operating procedure, 'A' for appendix, 'W' for worksheet, 'R' for external reference document; or 'A' for administration, and 'F1' for the form number 1).

5.2.2 Document control procedure

A document control procedure aims to guarantee that each page of the Q-documentation is identifiable and attributable. Document issue and change controls are essential to ensure that the content of the Q-documentation is properly authorized. The document control system defines how suggestions for changes in the documents can be made, who decides on necessary amendments, and the time frame in which changes are expected (periodicity of revision). Create a form where suggestions/revisions are noted and brought to the attention of the person responsible for the revision.

Each page of the Q-documentation should at least contain the following:

- name/title of the document;
- page number;
- revision status;
- version number (or 'valid from' date); and
- control indication such as '*This is a controlled document*' or '*This document is not included in the document review system*'.

Note: Number the pages of each Q-manual chapter or SOP separately, rather than numbering the pages consecutively for the whole document. The format must be as 'page X of Y' instead of only 'page X'.

A description should also be given about how external documents are controlled. The distribution concept outlines the addressees/recipients for controlled or uncontrolled document copies. If documents are distributed electronically or by mail, an explanation must be given on how the laboratory/sampling entity ensures that the new versions are received. This may be done by listing the documents on a master list of controlled documents (or documents matrix).

A master list of controlled documents should at least contain the following:

- document name and code;
- version number of current documents (or 'valid from' date);
- approval date;
- recipients (can be a person or a place, e.g. room number);
- person responsible for the distribution of new or revised documents; and
- retrieval of obsolete versions.

5.3 Laboratory premises

The environment in which the laboratory tests are undertaken must not invalidate the test results or adversely affect measurement accuracy. The testing premises must be protected as required from adverse conditions such as extreme temperatures, dust, moisture, steam, vibration, electromagnetic disturbance/interference, and should be maintained accordingly. They must be sufficiently spacious to limit the risk of damage or danger and allow staff to make practical and precise movements. The laboratory should have the equipment and energy sources needed for the testing. When required for particular tests, the laboratory must be equipped with appropriate devices to monitor environmental conditions. There should be an effective separation between neighbouring areas with incompatible activities. Measures must be taken to prevent cross-contamination between samples and between tests, and ensure good laboratory housekeeping. A brief description of how this is done should be included in the Q-documentation. The premises should be described, including a floor plan. If applicable, provisions taken against excessive temperatures, moisture and vibration (e.g. unstable workbenches and tables) should be mentioned in the documentation, as well as measures taken to protect the staff in terms of health and safety. The Q-documentation should describe the appropriate internal regulations for access and use of the laboratory premises by staff during working hours and off-time. How entry of external persons (staff of other departments/premises, clients or other visitors) is controlled must also be described in the Q-documentation; it should ensure that external persons are not left unattended on the premises. The Q-document should also describe the sample storage system before and after testing (i.e. where, how and how long the samples are stored, and which components of the samples are stored); control/

treatment and recording of pests and diseases; control and recording of temperature and if necessary, relative humidity; and the procedure for disposal of the stored samples (e.g. treated versus untreated seeds).

5.4 Equipment and consumables relevant to laboratory activities

5.4.1 Provision and maintenance of equipment

Laboratory staff and samplers must have access to all items of equipment required for the correct performance of sampling and testing for which the laboratory is accredited. Machines in the laboratory must be run appropriately by authorized staff. Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the equipment manufacturer) should be readily available for use by the appropriate laboratory staff. Equipment and its software used for testing and sampling must be capable of achieving the accuracy required and should comply with specifications relevant to the tests concerned.

There must be documented SOPs for operating, maintaining, calibrating and monitoring sampling and testing equipment. Whenever practicable, all equipment under the control of the laboratory and requiring calibration should be labelled, coded or otherwise identified to indicate the calibration status, including the date when last calibrated and the date or expiration criteria when re-calibration is required. All equipment must be properly maintained to ensure protection from corrosion and other causes of deterioration. Any equipment subjected to overloading or mishandling, giving suspect results, or has been shown by calibration or otherwise to be defective should be taken out of service and clearly labelled until it has been repaired and shown by calibration/verification or test to be performing its function satisfactorily. Each piece of equipment and its software used for testing must be uniquely identified when practicable and significant to the result.

5.4.2 Calibration, reference and testing materials

All sampling, measuring and testing equipment, for which this is possible, should be adequately calibrated before being placed into service and calibrated/verified regularly afterwards. A logbook or worksheet should be kept in which the results of each calibration, service and repairs are recorded. Calibration and servicing of equipment must be performed according to an established programme. The overall programme of calibration/verification of equipment must be designed and operated to ensure that, wherever applicable, measurements made in the laboratory are traceable to national and international measurement standards. Reference to records kept on these measures should be provided. A general description of the calibration/verification programme is included in the Q-documentation.

Appropriate calibration samples, reference materials and reference standards of measurement must be held by the laboratory and be used for calibration and reference purposes only. Where possible, they should be traceable to SI (= Système International) units of measurement or to certified reference materials. Examples include calibration samples for seed blowers, standard buffer solutions for pH meters, calibration weights for balances and reference collections of seed. Calibration samples for the seed blowers are provided by arrangement with the ISTA Secretariat.

The laboratory/sampling entity must examine and describe the effect of defective equipment on any previous tests and should state how it withdraws and reissues ISTA Certificates where faulty results are suspected. The laboratory must have procedures for safe handling, transport, storage and use of reference standards and reference materials to prevent contamination or deterioration of samples and to protect their integrity. A general description of maintenance, servicing, labelling, handling and repairing of testing and measuring equipment is included in the Q-documentation. Records of maintenance, servicing and repairs of each item of equipment may be stored with the respective item or in an appropriate place.

Descriptions of the general procedures for dealing with cases where any item of equipment has been subjected to overloading or mishandling, where it gives suspect results or has been shown by calibration or otherwise to be defective, should also be present in the Q-documentation. General procedures include, but are not limited to, taking out of service, labelling, advising the responsible person and returning to service after repair (i.e. functioning check, calibration).

The sampling, testing and measuring equipment may be listed as a table in the Q-documentation. Columns with the following specifications should be included:

- kind of equipment (e.g. balance, soil divider, calibration weight for balances);
- date of purchase/acquisition;
- manufacturer;
- unique serial and/or inventory number;
- range of measurement (e.g. thermometer: 0–70 °C; balance: 0.001–50.000 g; moisture oven: 100–150 °C) and precision (e.g. sieve: 0.50 mm mesh size; working thermometer: accurate to 0.1 °C);
- internal check or verification interval (e.g. daily, weekly, monthly) and external calibration, if certified reference standards of measurement are used (e.g. yearly, biennially);
- maintenance interval;
- room number and/or location (e.g. analytical purity section, room no. 125); and
- reference document number (SOP, logbook, user manual).

5.4.3 Purchasing services and supplies

Services and supplies that affect the quality of sampling and testing must be identified. The laboratory should have a policy and procedures for selecting and purchasing services and supplies that affect the quality of the tests, and for the purchase, reception and storage of reagents and laboratory consumables relevant for the tests. The laboratory should ensure that purchased supplies (e.g. germination substrate/media) that affect the quality of tests, are not used until they have been verified and comply with the standard specifications or requirements defined in the specific test method. Critical services such as external calibration of weights needed for balances must also be checked to verify their compliance with the standard requirements. A general description of the procedure for selecting and purchasing services and supplies and their records must be included in the Q-documentation.

The purchase documents for items that can affect the quality of laboratory output should contain information describing the services and supplies ordered. These documents must be reviewed and approved for technical content prior to release. The laboratory should evaluate suppliers of critical consumables and services that affect the quality of testing and sampling and must maintain records of the evaluations and list those approved. An explanation of how and when the suppliers are evaluated and approved (acceptance criteria) should be included.

5.5 Laboratory staff

Laboratory staff and samplers must have the necessary education, training, technical knowledge, demonstrated skills and experience for their assigned functions. The laboratory should use personnel employed by or contracted to the laboratory. Where contracted and additional technical and key support personnel are employed, the laboratory must ensure that such personnel are supervised and competent, and work following the laboratory's QA system.

There should be a job description for each laboratory staff member and sampler. A job description should include an outline of the key tasks and the required level of education, training, technical knowledge and experience. The laboratory must provide adequate supervision of testing staff and samplers, including trainees, by persons familiar with methods and procedures, the purpose of each test and the assessment of results.

The laboratory management should consider formulating goals concerning the laboratory personnel's education, training and skills. Specific personnel should be appointed to perform particular types of work. The laboratory should ensure the availability of records of the relevant appointments, educational and professional qualifications, training, skills and experience of all technical personnel, including contracted personnel.

The laboratory must consider including the following aspects in the Q-documentation:

- a description of responsibilities and authorizations of the laboratory functions and positions (e.g. head of the laboratory, technical manager, quality manager, supervisors, technicians/analysts/samplers, administrative personnel, trainees, etc.);
- a staff matrix showing responsibilities and suitable deputies;
- a description of the general policy concerning internal and external training (i.e. workshops, instruction, on-the-job training, etc.), including information on how the laboratory management determines individual training needs; under what criteria (e.g. based on annual performance appraisals); on what basis is the training plan set; how the training needs are identified and recorded; and how training events are recorded; and
- a description of a general training procedure for new staff members, supplemented by a more detailed SOP describing the training programme, acceptance criteria and approval of new staff members for independent work.

5.6 Methods and procedures for sampling and testing

For sampling and testing purposes, the laboratory staff must adhere to the respective methods and procedures as described in their QA system. The laboratory should ensure that instructions and reference data relevant to the laboratory's work are up to date and readily available to staff. Documents must be written with as much detail as necessary to allow staff to perform their tasks. All calculations and data transfers should be subject to appropriate systematic checks. When computers or automated equipment are used for the acquisition, processing, recording, reporting, storage or retrieval of test data, the laboratory must ensure that the computer software is validated as being adequate for use and that data protection is operating in suitable conditions to maintain the integrity of test and calibration results.

5.6.1 Sampling

The integration of sampling in the laboratory's organization and management is described in the Q-documentation and may be supplemented by an organizational chart. A list of authorized samplers must be maintained to demonstrate the provisions in place to ensure the independence of sampling or samplers if company-based samplers are used. Usually, only the authorized samplers are allowed to sample the lots for which an official testing report is issued. The laboratory must provide a list of authorized/recognized samplers and their affiliations (e.g. name of the ISTA sampler, authoriza-

tion number, status, specimen signature/initials, unique identification). In the case of seed processing plants with a built-in automatic sampler, a list of authorized automatic samplers (name and address of the owner, authorization number, unique identification) and the ISTA samplers responsible for the automatic sampler must be included. A description of automatic sampler authorization and the monitoring process is provided according to the ISTA requirement described in the 'Protocol for the approval of automatic seed samplers'.[2]

What are the requirements to obtain, maintain and suspend the formal authorization as a sampler? This part of the Q-documentation should describe procedures and criteria on how the ISTA samplers are authorized and how their authorization is maintained, i.e. initial training, refreshing training and monitoring, such as internal auditing and/or check sampling. The frequency, scope and responsibilities of training and monitoring activities (SOPs of authorization, training programme, monitoring programme) should be indicated. The laboratory should also specify the sampling procedures applied, i.e. national seed regulations or ISTA sampling procedures, including the latest revision date. When both national and ISTA requirements are described in the same SOP, a clear differentiation must be made. A description of the seed lot identification system should ideally be included to ensure that each seed sample is traceable to the respective seed lot by a unique seed lot identification. This is unnecessary for some customers, but it will affect details provided on the analysis report, such as the difference between issuing an advisory in-house test report or an Orange or Blue ISTA Certificate. The laboratory should describe how it handles the samples, including unique identification, labelling, transport, storage and disposal. Records must be kept on samples showing any unusual condition.

5.6.2 Scope of testing

Applied test methods such as national seed regulations and other methods, including the latest revision date, should be specified. A clear differentiation must be made when both national and internal tests are described in the same SOP. The laboratory's SOPs and WIs on testing must be up to date and readily available to staff. They should be written in as much detail as necessary to allow staff to perform their tasks. The staff must be trained following a systematic plan, and re-training should be undertaken when needed or where changes to SOPs are necessary.

5.6.3 Process management (workflows)

Workflows of all relevant laboratory procedures and tests can be depicted in the Q-documentation, preferably through flow charts to show the steps sequentially from sample entry to reporting of results.

5.7 Test reports

The results of each test or series of tests conducted by the laboratory should be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions. If a sample is sent by a customer that is not an authorized sampler, the report should clearly mention that it refers to the quality of the seed in the sample and not to the seed lot as a whole. For domestic needs, the results can be on a report designed by the laboratory or may be specified by national requirements. For reporting on ISTA Certificates, the laboratory must be accredited by ISTA for the test methods reported, and reporting must follow the procedures in Chapter 1 (ISTA Certificates) of the *International Rules for Seed Testing* (ISTA Rules). See the ISTA website for an interactive ISTA Certificate Learning Tool.[3]

5.8 Quality control procedures

5.8.1 Staff monitoring

The QA system must define and document quality control procedures specific to seed lot identification, sampling arrangements and laboratory testing procedures. These may include check sampling, check testing and other monitoring programmes. This monitoring should be planned and reviewed (responsibilities, frequency of monitoring, way of evaluating results, thresholds and means to follow up). The resulting data should be recorded so that trends can be detected and, where practicable, statistical techniques applied to review the results.

Check sampling is used to monitor the reproducibility of results, wherein the seed lot is sampled by different seed samplers. The monitoring must be done on all crop groups for which the laboratory holds accreditation. Check sampling is one tool to monitor samplers but is not an absolute requirement. Auditing and/or re-examinations are other acceptable ways to monitor samplers (see also Chapter 6: Sampling, in this handbook and Chapter 10 in the *ISTA Handbook on Seed Sampling*).

Check testing is another monitoring of reproducibility of results where different employees test the same sample. This monitoring must be done on all tests and crop groups for which the laboratory holds accreditation. Check testing is one tool to monitor analysts but is not an absolute requirement. Conducting internal and external Proficiency Tests (PT), and/or re-examinations are other acceptable ways to monitor analysts.

An explanation should be given in the Q-documentation for these procedures, describing how the results are recorded and evaluated. The Q-documentation should describe how possible trends are identified, including a systematic follow-up on the performance of the testing and sampling staff over time. It can also describe the actions to be taken when a trend is detected.

[2] www.seedtest.org/api/rm/48N2CF2Q5CRYT5F/tcom-p-03-protocolfortheapprovalofautomaticseedsam-1.pdf
[3] www.learn-ista.org

5.8.2 Control of nonconforming testing and sampling work

The laboratory should have a policy and procedures that must be implemented when any aspect of its sampling, testing or reporting does not conform to its own procedures or the agreed requirements of the client. The policy and procedures must ensure that:

- the responsibilities and authorities for the management of nonconforming work are designated, and actions (including halting work and withholding of test reports, as necessary) are defined and taken when nonconforming work is identified;
- an evaluation of the significance of the nonconforming work is made;
- corrections are made immediately, together with any decision about the acceptability of the nonconforming work;
- where necessary, the client is notified, and work is recalled; and
- the responsibility for authorizing the resumption of work is defined.

Where the evaluation indicates that the nonconforming work could recur or that there is doubt about the compliance of the laboratory's operations with its own policies and procedures, the corrective action procedures given must be promptly followed.

5.8.3 Complaints and corrective actions

The laboratory must establish a policy and procedures to deal with complaints and corrective actions. It must designate appropriate authorities for implementing corrective actions when nonconforming work or deviations from the policies and procedures in the QA system, or technical operations, have been identified. The procedure for corrective action should start with an investigation to determine the root causes of the problem. Once the possible root causes have been identified, the laboratory must establish corrective actions to eliminate the problem and prevent recurrence. The laboratory must document and implement any required changes resulting from corrective action investigations. After implementing the corrective actions, the laboratory should monitor the results to ensure that the corrective actions taken have been effective. The laboratory must define and document arrangements for dealing with complaints and should take corrective action whenever complaints are identified.

5.8.4 Action to address risk and opportunities

The laboratory should consider a procedure to evaluate risks, make risk-based decisions, review any business or improvement opportunities and act accordingly. QA system records must be kept and can be documented in the laboratory's periodic management review.

5.9 Tools to monitor laboratory quality assurance system and competence

5.9.1 Internal audits

Periodically, the laboratory must perform internal audits of its activities according to a predetermined schedule and procedure that reflects the activities to be audited. The internal audit programme should address all elements of the QA system, including testing and sampling activities. It can be divided according to different activities (e.g. system aspects, technical aspects, equipment) and can be performed in-house by trained laboratory staff. Audits must be performed so that they verify the laboratory's continual compliance with the established requirements and its own quality system. The auditors should have the necessary education and skills and must take the necessary steps and respect principles before, during and after the audit to accomplish a successful audit. The area of activity audited, the audit findings and the resulting corrective actions should be recorded. Follow-up audit activities must verify and record the implementation and effectiveness of the corrective actions taken. Additional audits should be held in case of any doubts about the laboratory's compliance with its own policies and procedures or its compliance with the ISTA Accreditation Standard, if applicable. The laboratory must ensure that the appropriate areas of activity are audited as soon as possible.

5.9.2 Reviews by management

Under a predetermined schedule and procedure, the laboratory's executive management must periodically review the laboratory's QA system, and testing and sampling activities, to ensure their continued effectiveness and introduce any necessary changes or improvements. The management should evaluate the progress made on objectives and tasks raised in previous meetings and establish new ones. Due dates and responsible persons should be agreed upon, and records must be kept.

5.9.3 Continual improvement

The laboratory should strive to continually improve procedures and efficiency through risk-based decisions and cost–benefit considerations. Any laboratory staff can contribute to improvements and it is best to incorporate this input into the laboratory culture.

5.10 Recording and archiving

The Q-documentation should include descriptions of the records used and completed by the laboratory staff, such as:

- sampling application form;
- sampling report;
- laboratory work cards, including records of original observations, calculations and derived data;
- equipment logbooks, including calibration data, maintenance and repairs of equipment;
- records on monitoring activities such as auditing, check sampling and check testing results;
- management review reports;
- training;
- staff performance appraisals; and
- test reports.

The procedures followed to check test results should be present in the Q-documentation and the relevant technical SOP. The person undertaking these checks and the stage at which test results are checked on, allowed tolerances, completeness, correctness and accuracy, should be recorded. The Q-documentation should specify responsibilities and authorization to make corrections on the computer (e.g. password protection) and laboratory work cards. It must include a description of how to record and protect data (inscriptions made using an indelible pen) and how to proceed if mistakes need correction (e.g. on the laboratory work cards, records and the computer). The laboratory should describe the verification process for updated computer software and other working tools electronically stored (e.g. checks of correct calculations and tolerance, where applicable). A description of the concept of retaining computerized data (backup frequency, media) and ensuring that data backups remain legible if computer software is changed, is provided in the Q-documentation. The laboratory archiving system should ensure that documents are kept and are legible for at least 6 years (for ISTA accredited laboratories) or longer in some national systems. For electronic archiving systems, it is necessary to check that there is no automatic deletion set in the system. For paper archiving systems, it is necessary to check the type of printing to ensure it lasts for 6 years or more. A table is recommended to report all relevant archive information, including type of document, intermediate/final depository, retention time, date and means of disposal.

Chapter 6: Sampling

6.1 Why proper sampling is essential for seed testing laboratories

Sampling is of fundamental importance in assessing the quality of a seed lot. One of the first things people are taught when attending ISTA workshops on sampling is that the test result is only as good as the sample taken. ISTA's emphasis on sampling is reflected in the dedicated chapter on sampling (Chapter 2) in the *International Rules for Seed Testing* (ISTA Rules). In addition, only samples taken by trained and authorized samplers may be used for testing for the issuance of an ISTA Orange International Certificate (OIC). OICs are recognized for seed trade by most countries and are often used in seed sale negotiations, especially where international seed movement is involved. An OIC is often a prerequisite in seed imports. It ensures an unbroken chain of traceability – from sampling to issuing the test results. In cases where the sampling process is not considered essential, or the ISTA Rules do not make provision for the sampling (such as for seed mixtures), or the sample was taken by a person not accredited/authorized by an ISTA accredited laboratory, an ISTA Blue International Certificate (BIC) can be issued. Seed testing laboratories that are not ISTA accredited can issue their own analysis report but should state whether the sampling was under the control of the laboratory. The end user would then be aware of the greater risk and uncertainty of the test results if the sampling process was not controlled and subject to standardized methods. If seed laboratories are not ISTA accredited, they should state on the analysis reports whether the test results reflect the entire seed lot or just the sample.

Note: The ISTA Orange International Certificate (OIC) ensures traceability of the test results to the seed lot by using ISTA samplers to take and submit the sample to the laboratory. The ISTA Blue International Certificate (BIC) only ensures the traceability of the test results to the submitted sample, not to the seed lot.

In a national seed certification system where sampling is regulated and controlled, the test results will be more reliable than in a system with unregulated sampling. It is true that there is uncertainty in any system, and although rare, people can take shortcuts or perpetrate fraud in both regulated and non-regulated sampling and testing systems. There is a greater risk of uncertainty where sampling is not regulated. Seed certification is built on underlying quality assurance (QA) principles and traceability from the field to the processed lot through sampling and testing. A country's or company's reputation and trust in the quality of the seeds traded can take a long time to build but can be quickly lost. Worldwide, both officials and companies work together to help maintain a high level of trust in seed sampling and testing. This cooperative approach using science and international standardized methods to underpin the needs of quality seed production and supply, helps ensure the marketing of a quality-assured product, encouraging repeat business for the seed trade.

Even if a laboratory is not planning to issue OICs, sampling is still important to ensure the test result represents the true quality of a seed lot and meets the end users' needs. When establishing a seed testing laboratory, how the samples are taken and submitted to the testing laboratory and who is authorized to take the samples, are key components of the planning and subsequent management of the laboratory. A network of trained samplers under the authority of the seed testing laboratory or as a separate sampling-only entity should be part of an overall seed certification or similar quality control system, whether country- or company-driven. The number of samplers needed will depend on the requirements discussed in Chapter 2 (Deciding what is needed for a seed testing laboratory) and Chapter 3 (Staffing) of this handbook.

6.2 Sampling of seed lots

Specific sampling procedures may exist for national certification or phytosanitary purposes. If nothing is established, Chapter 2 of the ISTA Rules[1] and the *ISTA Handbook on Seed Sampling* provide an excellent system to adopt. In addition to sampling methods and procedures, Chapter 2 of the ISTA Rules stipulates the submitted and working sample sizes and the maximum seed lot sizes (ISTA Rules Table 2C) for all the species covered by the ISTA Rules. These maximum seed lot sizes are mirrored in the Organisation for Economic Co-operation and Development (OECD) Varietal Seed Certification Schemes and in the regulatory document of the seed legislation in some countries. Creating a uniquely identifiable seed lot for use in sampling and testing helps ensure traceability. The unique seed lot identification could be derived from a country- or company-based system. The unique identification is the essence of a QA process. It is therefore well established in seed certification programmes globally and embedded in ISTA procedures. Even if ISTA accreditation is not the final goal, these are all good processes to consider and follow when starting a new seed testing laboratory.

[1] www.seedtest.org/en/publications/international-rules-seed-testing-1168.html (free download)

QA tips:

- Download and use the ISTA Sampling App (www.seedtest.org/en/services-header/tools/bulking-sampling-committee/ista-sampling-calculator.html) to determine the minimum number of primary samples to be taken from a seed lot.
- Watch the ISTA sampling training videos (ISTA Bulking and Sampling Technical Committee documents on the ISTA website, www.seedtest.org/en/services-header/documents/technical-committees-documents.html).
- Ensure all bags are accessible for sampling, and none are hidden in a stack. It is good practice to inform the seed processing plant before sampling.
- Make sure bags are clearly marked and have labels.
- Identify bags you want to take primary samples from with a marker pen before starting to sample, and count them to ensure the minimum number is taken.
- Sample stacked seed bags from the bottom up to avoid any loose seed falling from bags higher up into your sample container.
- Make sure your sample collection container is free from static.
- Only use verified moisture containers for moisture sample submission.
- Do not send an underweight, unlabelled or unsealed sample to the testing laboratory.
- Remember to send the sampling documentation to the laboratory that links the seed lot to the submitted sample.

Seed lots for planting purposes are assumed to be heterogeneous even though they are often well-mixed as a result of processing. For example, for a seed lot of *Zea mays,* the maximum ISTA and OECD seed lot size is 40 000 kg, and the minimum submitted sample size is 1 kg (ISTA Rules Table 2C). If the 40 000 kg *Z. mays* seed lot had been packaged for sale into 25 kg bags, there would be 1600 bags. From the 1600 bags, a minimum of 30 primary samples is needed to create the composite sample (ISTA Rules Table 2A), and then from that, the 1 kg submitted sample needed for testing is obtained. If the 40 000 kg seed lot of *Z. mays* was packaged for sale into 500 kg bags there would be 80 bags. From the 80 bags, a minimum (rounded up) of 58 primary samples is needed to create the composite sample (ISTA Rules Table 2B), and then from that, the 1 kg submitted sample needed for testing is obtained. The apparent discrepancy between the minimum number of primary samples for the same mass that must be taken from the 25 kg bags and those that must be taken from the 500 kg bags is based on statistical considerations. The distribution of impurities in different sized containers may vary. In the 500 kg bags, the probability that the sampler would sample the impurities in the same ratio as what occurs overall in the seed lot is less than in the smaller bags because of the larger volume. Therefore, the number of primary samples is adjusted upwards to increase the chances. The calculation of the minimum number of primary samples for the smaller containers (ISTA Rules Table 2A) is based on the number of containers, whereas for the large containers and seed streams (ISTA Rules Table 2B), it is based on the total mass of the lot.

Figure 6.1 provides a schematic representation of the sampling process using primary samples to produce first a composite sample, then a submitted sample, and finally, the working samples for testing in the laboratory. The 1 kg working sample in this example is only one-40 000th of the seed lot and therefore, needs to be representative of the quality of the whole lot.

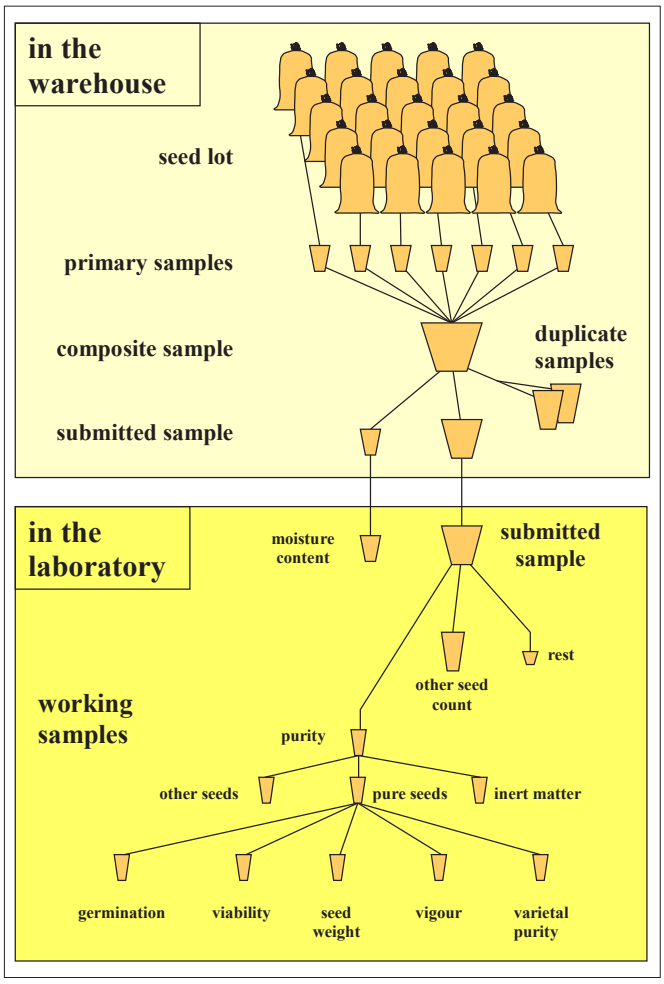


Figure 6.1. A schematic flow diagram illustrating at which point in the sampling process the primary, composite, submitted and working samples are obtained

For example, a small farm-based seed producer with just thirty 25 kg bags can conduct sampling systematically using ISTA sampling principles. Thirty 25 kg bags require a minimum of 15 primary samples to be taken randomly at different positions from 15 different bags in the lot, which are then combined, reduced (if necessary) and then submitted as one sample for testing. In cases where there is only one bag of seed, ISTA prescribes that three primary samples must be taken from the one bag. The three primary samples should be taken at different positions (i.e. from the top, middle and bottom of the bag). This systematic approach is much better than taking just a few handfuls of seed from the top of one bag or a scoop from a large heap of seed stored on the farm or in the warehouse (Figure 6.2). If seeds are packed in small sachets or packets, the entire unit is taken as a sample, and the number of units to be sent to the laboratory are described in Chapter 2 of the ISTA Rules (section 2.5.1.2).

The tables in Chapter 2 of the ISTA Rules provide the minimum number of primary samples needed in the different sampling scenarios affected by seed lot weight and container size.

Figure 6.2. Seed storage in a warehouse: **a** grass seed warehouse, Denmark; **b** pallets stacked with 25 kg bags; **c** large bags stacked; **d** large bag and double-sleeved sampling stick (images courtesy of ISTA)

6.3 Manual sampling

Like other laboratory staff, samplers need to be trained and have access to suitable equipment. Types of sampling equipment and guidance on the most appropriate methods to use are given in the ISTA Rules Chapter 2 and the *ISTA Handbook on Seed Sampling*. Also, view ISTA online videos in the document section of the ISTA Bulking and Sampling Technical Committee's webpages on the ISTA website. A range of sampling triers and sticks for bag sampling are shown in Figure 6.3. Figure 6.4 gives examples of stick and cargo samplers used for large open bags, bags with impenetrable walls, bins or large wooden boxes. Sampling by hand is also an option for all species and is indeed the only method for some very chaffy species and large-seeded pulses (legumes) that probes may damage.

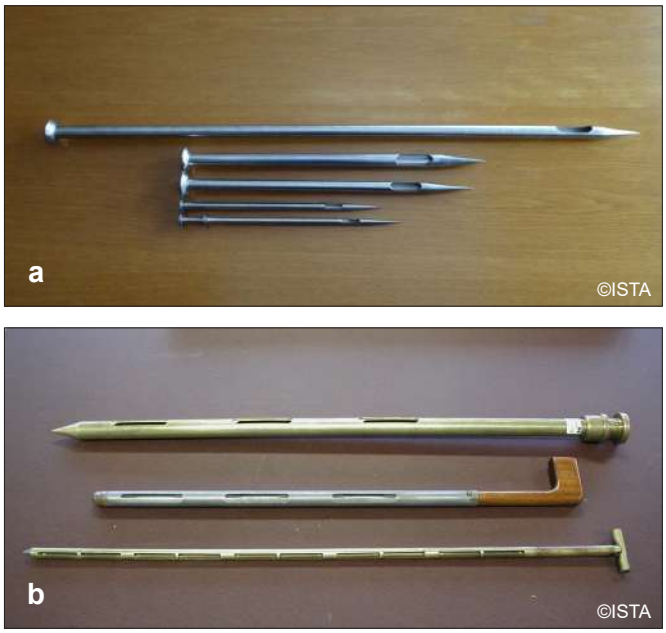


Figure 6.3. Collections of different sized sampling triers for sampling of bags: **a** set of Nobbe triers; **b** stick triers (images courtesy of ISTA)

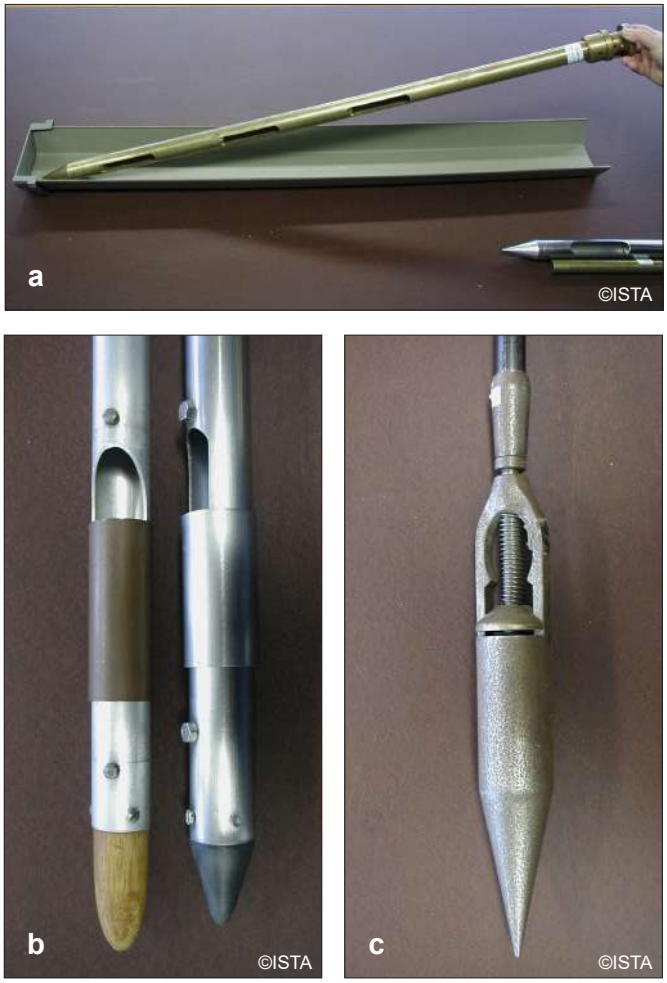


Figure 6.4. Samplers used for large open bags and containers with impenetrable walls: **a** stick trier with partitions and a special receiving pan; **b** sleeve-type cargo sampler closed by a collar; **c** spring-pressed valve type of cargo sampler (images courtesy of ISTA)

Having learned how to use the sampling equipment, the correct minimum number of primary samples then needs to be taken to provide a representative composite sample for testing. The composite sample may be labelled, sealed and sent for testing without any further action by the sampler. However, if the composite sample is too large, it can be reduced to a smaller submitted sample (Figure 6.1). The ISTA requirements for the minimum submitted sample size are defined in Table 2C (Parts 1, 2 and 3) of the ISTA Rules. Part 1 of this table lists 332 agricultural and vegetable species, Part 2 lists 236 tree and shrub species, and Part 3 lists 355 flower, spice, herb and medicinal species traded internationally. The ISTA submitted sample sizes are the minimum weight needed for the 'basic' ISTA accredited laboratory tests; but the sampler may need to submit larger samples, if the same sample is required for multiple tests or for other purposes, such as post-control varietal testing under the OECD Seed Schemes or to provide duplicate samples to be stored for company or QA purposes. Submitted samples for testing seed moisture content should be obtained from the same composite sample but packed immediately in a separate moisture-proof container. If the whole composite sample is used as the submitted sample and seed moisture testing is also requested, the entire composite sample must be packed in a moisture-proof container. This is not recommended for seed with a very high seed moisture content, as it may adversely influence the

germination capacity. All submitted samples for moisture testing should be taken according to the ISTA criteria established in Chapter 2 of the ISTA Rules (section 2.5.1.5.2).

On the ISTA website, there is a very helpful free-to-download ISTA Sampling Calculator Application (ISTA Sampling App) that can be used in multiple languages, providing samplers with the correct minimum number of primary samples needed for coated or uncoated seed, for manual or automatic sampling, for different seed lot and container sizes. In addition, the App checks to see if the species being sampled is listed in Table 2C of the ISTA Rules (if not there, it is not included in the ISTA Rules). Inclusion in this table is a prerequisite to allow issuing of an OIC and ensures that the maximum seed lot size (with the allowed amount plus 5 percent tolerance) is not exceeded. The App also provides the minimum submitted and working sample sizes for the species being sampled and information regarding the pure seed definition of the species concerned.

Whether sampling in a warehouse, a seed production facility or a farm, safety is important and should be the number one priority. Always follow national safety recommendations and be aware of the risk of stacked seed lots falling on samplers. Sampling should only be done at a height that can be reached without overreaching, i.e. not by standing on a ladder or another seed lot. The seed company needs to be made aware that bags or pallets may need to be moved to allow safe sampling from all parts of the seed lot. Equipment and forklift trucks may operate in warehouses, and samplers must be mindful of additional safety risks. Other risks involve sampling treated seeds, inhaling plant dust and debris, or the presence of rodents or other animals. In some areas of the world, hand sampling could pose an additional risk if a venomous creature like a scorpion, snake or large spider has found a comfortable home in an open bin or a bag of seed.

Safety tips:
- Sampling can be dangerous.
- Sample triers have sharp points; be careful carrying and using them.
- Sampling is generally conducted in a warehouse with moving equipment, e.g. forklift trucks. Wear high visibility clothing, safety shoes, safety helmets and glasses, as appropriate.
- Extra lighting for taking samples might be needed.
- Do not climb on stacked seed bags to sample them.
- Sample safely with your feet on the ground.
- When hand sampling from open bins or bags in some countries, be aware of venomous creatures.
- Always follow national and company safety guidelines.

6.4 Automatic sampling

Automatic seed samplers installed in a processing plant need to meet established criteria. If correctly installed, operated and maintained, these will give more consistent samples than manual probe or hand sampling. The laboratory receiving samples is responsible not only for the training of samplers but also for the approval of suitable automatic sampling equipment. For traceability, a register of approved location, coded equipment and the ability to trace back to the automatic sampling equipment used on an individual seed lot are essential in an established seed testing laboratory following a QA-based system. Figure 6.5 depicts an example of an automatic sampler. The various designs that meet the ISTA requirements are discussed in more detail in the *ISTA Handbook on Seed Sampling*.

The ISTA website and the document section of the Bulking and Sampling Technical Committee's webpages stipulate a protocol for approving automatic seed sampling equipment. The protocol provides a checklist to ensure the equipment complies with the requirements, including how to take samples across the whole cross-section of the seed stream after the completion of processing and treatments and just before seed bagging. The automatic sampler must also be set to take the minimum number of primary samples as calculated using Table 2B of the ISTA Rules. This can be done by using the ISTA Sampling App, which will also provide the sampling interval to which the timing device must be set to obtain the required number of primary samples. To this end, regular maintenance and record-keeping must be done.

Figure 6.5. An automatic sampler of the moving beak type, with the collection of a composite sample into a plastic tube (image courtesy of ISTA)

The automatic sampling process produces a composite sample which, as for manual sampling, can be labelled, sealed and sent for testing without any further action by the sampler. If needed, the composite sample can be reduced to a submitted sample (Figure 6.1), the minimum sample size of which is defined in Table 2C of the ISTA Rules.

6.5 Sample reception in the laboratory

Once the laboratory receives the representative seed sample sent by the seed sampler (Figure 6.6), seed testing can start. The following chapters describe the basic tests, all of which begin by mixing and dividing the submitted sample to produce a working sample (Figure 6.1). The first test is usually analytical purity.

Figure 6.6. Labelled sample bags and moisture samples received by the laboratory: **a** cloth container (image courtesy of ISTA accredited laboratory GB04); **b** submitted sample bags from ISTA accredited laboratory CH01; **c** paper sample bags from ISTA accredited laboratory DE19; **d** soft plastic/polyethylene bags (image courtesy of ISTA); **e** rigid plastic container, more suitable for moisture samples (image courtesy of ISTA accredited laboratory GB04)

Chapter 7: Analytical purity, other seed determination and thousand-seed weight testing

7.1 Working sample handling, mixing and dividing

Typically, the submitted sample must be reduced to a smaller amount (the working sample weight). This allows testing in a reasonable amount of time and following testing rules, such as the *International Rules for Seed Testing* (ISTA Rules). Although there is a compromise between detection and duration of testing, the reduction into a working sample weight (as specified in the ISTA Rules Table 2C) must be standardized. The overall objective of following standardized methods is to reduce the submitted sample to a working sample while maintaining the homogeneity of the sample. The working sample can then still represent the quality of the whole seed lot from the original sampling action.

For analytical purity and other seed determination (OSD) the submitted sample is mixed and divided down to a working sample size. The working sample for a purity analysis should be at least 2500 seeds, and for the OSD, at least 25 000 seeds. There is a maximum limit of 1000 g tested for OSD. The working sample sizes are prescribed in Chapter 2 (Sampling) of the ISTA Rules. If both tests are requested by the client, the laboratory can decide to take two independent working samples, one for the purity analysis and one for the OSD (e.g. 20 g and 200 g, respectively), or the analytical purity working sample is an extraction of the OSD working weight (e.g. 20 g and 180 g, respectively). For other tests like germination, viability and the thousand-seed weight (TSW) test, the pure seeds can be used after the analytical purity test, or pure seed can be taken from the submitted sample. For the moisture analysis, a separate sample is submitted and stored in a moisture-proof container; it is not divided before preparation for the moisture analysis (see Chapter 10: Moisture testing, in this handbook).

There are different standardized methods to mix and divide the seed sample depending on the laboratory's preferences and the seed characteristics. Manual methods or different types of mixing and dividing equipment can be used.

7.1.1 Mechanical dividers

Each sample needs to be mixed and recombined by passing through the divider at least twice and, if necessary, three times. The dividing process then starts, where the sample is reduced by passing the seed repeatedly through a divider and removing parts on each working step (continual halving).

Conical divider (Boerner type)

The seed is placed in the hopper (Figure 7.1) and released by moving a slide gate located in the hopper throat. The product is evenly dispersed over a cone with pockets. After the initial separation, the seed is re-joined into two separate chutes, which empty out of the bottom hopper.

Figure 7.1. Boerner type conical divider (image courtesy of ISTA accredited laboratory IN39)

Soil divider (riffle divider)

This divider type is widely used in seed testing stations (Figure 7.2). The divider consists of a hopper with about 18 channels which need to be wide enough that the crop seeds and common impurities pass freely through the channels into the two receiving pans. Some riffles have attached and hinged 'tipping' pans.

Figure 7.2. Soil (riffle) dividers: **a** non-tipping type (left) and tipping type (right); **b** detail of non-tipping type riffle divider (images courtesy of ISTA accredited laboratory CA08)

Centrifugal divider (Gamet type)

The seed is passed through the top hopper/funnel onto a shallow cup or spinner (Figure 7.3). With the rotation of the spinner (electric motor), the seed is thrown out by centrifugal force and falls downwards into two channels. With the help of stationary baffles, the seed falls in equal parts into the two collecting containers under the two exit tubes/spouts. It is essential to place the divider on a level surface to obtain accurate dividing results.

Figure 7.3. Gamet type centrifugal divider (image courtesy of ISTA accredited laboratory CA08)

Rotary divider

The rotary divider is powered by an electric motor and achieves both mixing and dividing in one operation. The seed is poured into a hopper, the motor turned on and the crown unit rotates (at approximately 100 rpm). The seeds then flow through the inlet cylinder of the six to ten attached subsample containers.

Variable sample divider

The variable sample divider is powered by an electric motor and achieves both mixing and dividing in one operation. The seed is poured into a hopper which flows to a rotating tube (approximately 40 rpm). The seed is mixed and divided by further hoppers within the device. The seeds are collected in two or more collection pans (inside or outside the hopper).

7.1.2 Verification of mechanical dividers

Before putting any divider into use, an initial check must be performed to ensure the equipment is fit for purpose. After the initial check, a regular check should be introduced, addressing the following queries:

- Is the divider placed on a levelled, clean table?
- Is the divider suitable for the analysed crops (free-flowing)?
- Are any seeds bouncing out or getting stuck in the divider parts?
- Is the seed sample separated into equal portions?
- Are the components homogeneously distributed in the subsamples? (This can be checked with a prepared verification sample of a chaffy and non-chaffy species.)

7.1.3 Manual dividing

During all mixing and dividing steps, it is necessary to take measures to ensure that the sample has no content loss and that no cross-contamination occurs. The work area should be clean. The divider must be cleaned and checked for remaining seeds or chaff before processing the next sample.

Spoon method

The spoon method is used for species with seeds smaller than *Triticum aestivum* subsp. *aestivum*; seeds susceptible to mechanical damage such as species like *Arachis*, *Glycine* and *Phaseolus*; and tree genera *Abies*, *Cedrus* and *Pseudotsuga*. The method can also be used to obtain working samples for seed health testing. After preliminary mixing of the seed, the sample is evenly poured over a tray. With a straight-edged spoon and a spatula, at least five randomly selected portions are taken as the working sample to test.

Hand halving method

The hand halving method can be used for chaffy seeds (e.g. *Echinochloa*, *Gossypium*) and for easily damaged and fragile seeds like *Arachis*, *Glycine* and *Phaseolus*. The method can also be performed for certain tree and shrub species. The sample is poured on a smooth, clean surface and thoroughly mixed with a spatula. The following steps are then followed:

1. The sample is divided into two portions.
2. The two subsamples are divided again into four subsamples.
3. The four subsamples are divided into eight subsamples.
4. From the eight subsamples, every second sample is selected and recombined into a working sample.

7.1.4 Precision of working sample and balances

The sample and the fractions need to be weighed, calculated and reported to a certain precision. The minimum number of decimal places to use for the working sample is presented in Table 7.1, which is sourced from section 3.5.1 of the ISTA Rules.

Table 7.1. Minimum number of decimal places to use for the working sample

Weight of working sample or subsample (g)	Minimum number of decimal places
Less than 1.000	4
1.000–9.999	3
10.00–99.99	2
100.0–999.9	1
1000 or more	0

Source: **ISTA.** 2022. *International Rules for Seed Testing*. Wallisellen, Switzerland, International Seed Testing Association, section 3.5.1.

Safety tips: If the laboratory is dealing with pelleted or treated seeds, it is necessary to protect the operator from harmful dust and fumes during all working steps. The following protection measures can be applied to ensure the safety of the staff:

- Have a designated workstation or room for handling treated seeds, if possible.
- Use personal protective equipment (PPE) to avoid any potential health issues from treated seed dust or debris.
- Wear a laboratory coat, gloves, a dust-/fume-repellent face mask and safety glasses/goggles.
- Work on treated seed in a well-ventilated room or under a fume/dust extraction hood during all processing steps of the seed testing.
- Be careful when washing off seed treatments or coatings to detect and identify the seeds. Note: Washed seeds are not usually used in the germination test.
- Clean all residue from working surfaces and equipment after completing the testing process.

7.2 Equipment for analysis

The equipment for the preparation of working samples and for conducting seed analysis is split into two categories:

- *essential equipment* (**EE**), which should be available in most, if not all, seed testing laboratories; or
- *advanced equipment* (**AE**), which is more expensive and/or needed for specialized testing.

7.2.1 Divider EE

A divider is needed to prepare a working sample and should be located on a clean and levelled table. Different types are described in section 7.1.1 of this chapter. Depending on the type, an electric power source may be required. Additional equipment for dividers may include:

- brush, to clean the divider **EE**
- bubble level, to help level the divider **EE**
- vacuum cleaner and/or air pressure, to clean efficiently and remove dust/debris **AE**

7.2.2 Balances and working/reference weights EE

Several different balances (Figure 7.4), accurate to different numbers of decimal places and able to weigh alternative maximum weights, are needed to perform sampling, sample preparation, analytical purity, OSD, TSW, moisture analysis, and weighing of chemicals (e.g. tetrazolium salt, gibberellic acid). The laboratory will also need different balances depending on the commonly tested species and the scope of methods. If a laboratory works mainly with large seeds (e.g. *Zea mays*), a balance capacity of more than 1000 g and a balance accuracy of zero or one decimal place must be used. For small seeds (e.g. *Thymus vulgaris*), the working sample for analytical purity is 0.5 g and for OSD is 5 g, so an analytical balance (four decimal places) should be used. Working/reference verification/calibration weights must be available to check the balances regularly (Table 7.2). The working/reference weight should be used on a predefined schedule to verify the precision of the balance. A tolerance level between the balance and the working/reference weight of 1/1000 can be set as acceptable, e.g. with a 50.00 g reference weight, the tolerance of the balance could be +/−0.04 g from 49.96 to 50.04 g; for a 5.000 g reference weight, from 4.996 to 5.004 g.

An external provider (often a contracted unit of the balance manufacturer) should be used to undertake an annual maintenance and calibration service and provide a certificate for the verification of the balance accuracy/performance. The balances (Figure 7.4) must be installed on a clean and levelled surface, with easy access to an electrical supply. Ideally, they should be placed on a weighing table with an anti-vibration surface. The weighing tables need to have a solid construction, e.g. concrete pillars, a marble plate or materials which do not transmit vibration. An airflow coming from open windows, doors, ventilation, or people regularly walking past the balances should be avoided.

QA tips: Anti-vibration solutions are needed for balances. Different types of construction for balance tables are possible, e.g. concrete. Avoid wooden tables with slim wooden legs. Check the suitability of a balance table and its location by placing the high precision balance on the table, then press down on the table or knock it lightly with a small hammer to check if the weight indicated changes.

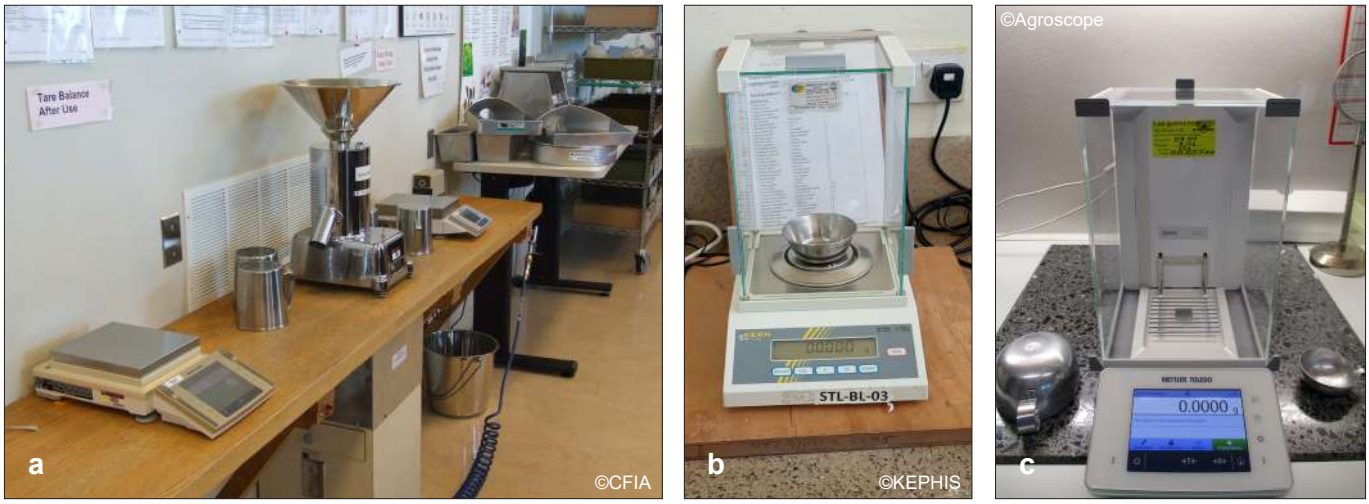


Figure 7.4. a Balance area at ISTA accredited laboratory CA08; **b** and **c** four-decimal place analytical balances (images courtesy of ISTA accredited laboratories KE01 and CH01)

Table 7.2. Balance purpose and check/weights

Working sample weight range	Purpose	Precision of balance required	Example reading on balance	Suitable working/reference weights
1000 g and more	Sampling, sample reception, sample preparation, analytical purity, other seed determination (OSD)	0 decimal places	1005 g	100 g 1000 g
100.0–999.9 g	Sampling, sample preparation, analytical purity, OSD, thousand-seed weight (TSW)	1 decimal place	500.1 g	100.0 g 500.0 g
10.00–99.99 g	Sampling, sample preparation, analytical purity, OSD, TSW, chemicals	2 decimal places	50.54 g	10.00 g 50.00 g
1.000–9.999 g	Sample preparation, analytical purity, OSD, TSW, moisture analysis, chemicals	3 decimal places	5.293 g	1.000 g 5.000 g
Less than 1 g	Sample preparation, analytical purity, TSW, chemicals	4 decimal places	0.6785 g	0.5000 g 1.0000 g

7.2.3 Seed blower AE

A seed blower (Figure 7.5) is mandatory for some grass species (e.g. *Poa pratensis*, *Dactylis glomerata*) to separate the working sample into light and heavy fractions. This pre-sorts the working samples when testing these species and reduces the manual analysis time required for both *Poa* and *Dactylis*. A blower can also be used to aid the testing for any species but is not mandatory. The blower needs to be calibrated with a reference sample (available for purchase from the ISTA Secretariat, ista.office@ista.ch) and can be verified for daily use using an anemometer (wind speed meter).

Figure 7.5. Seed blower (image courtesy of ISTA accredited laboratory ZA01)

7.2.4 Sieves EE

To help in testing efficiency and to reduce testing time, a first separation during the analytical purity and OSD can be done using individual sieves or a set/series of sieves with different mesh sizes (Figure 7.6).

Figure 7.6. Sieves of different mesh sizes (image courtesy of ISTA accredited laboratory UG02)

7.2.5 Magnifiers

Hand lens EE

Hand lenses with a 3–10× magnification (see Figure 7.7a) are required. The objective diameter should be at least 5 cm.

Illuminated magnifier EE

The magnifier (circular or rectangular) is integrated with a light source and can be adjusted in height and angle (see Figure 7.7b). Magnification in illuminated magnifiers is often quoted as 'dioptre'. Both 3 dioptre (×1.75 magnification) and 5 dioptre (×2.25 magnification) lenses are suitable for routine use, depending on the size of the seed being tested.

Stereo binocular microscopes AE

The stereo binocular microscope (see Figure 7.7c) can provide a higher (up to ×10) magnification to help identify single seeds or perform a purity analysis with small seeds. This microscope provides a three-dimensional view and a good depth of field. The option to have the microscope on a swing arm can be useful if the analysis workspace is limited. Digital cameras can be added to some microscopes to take pictures. Even mobile (cell) phone cameras can be used with adapters.

Digital microscope AE

With the magnification of a digital microscope (see Figure 7.7d), a purity analysis or OSD can be performed while the seeds are seen and evaluated on a screen. The image is two-dimensional, but a greater depth of field can be achieved by using stacking software. There are ergonomic benefits of using these microscopes. Digital microscopes also allow easier picture taking and sharing for reference during testing and for training; some can be linked to the internet to allow remote sharing of live images. The digital microscope usually has a built-in computer and display monitor in addition to the optical parts.

Figure 7.7. Magnifiers for seed analysis: **a** hand lenses and a mobile phone (to take a photograph that can be enlarged); **b** illuminated magnifier; **c** stereo binocular microscope with camera; **d** digital microscope (images courtesy of ISTA accredited laboratories CA08, ZA01 and IN39)

7.2.6 Flat spatulas (spikes), tweezers, scrapers, needles EE

Each analyst needs this basic equipment to work on analytical purity and OSD testing (Figure 7.8).

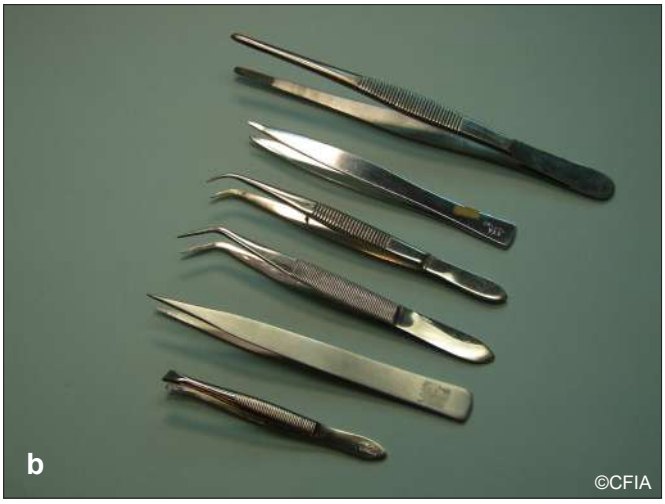


Figure 7.8. Flat spatulas (spikes) (**a**) and tweezers (**b**) (images courtesy of ISTA accredited laboratory CA08)

7.2.7 Half funnel/pan and containers EE

A half funnel/spouted pan is required for transferring seed samples into different containers. Beakers and glass or metal containers (ideally with lids) are used to separate and weigh the different seed fractions during the purity analysis and OSD (Figure 7.9).

7.2.8 Tins/covers EE

If a working sample is being processed and must be left out for a certain time (e.g. during a staff break), a cover such as a cake tin or a hood should be used to protect the working sample from any alteration.

Figure 7.9. Containers for purity analysis and other seed determination: **a** beakers, glass, metal and a wooden tray; **b** larger containers (images courtesy of ISTA accredited laboratory CA08)

7.2.9 Calculator/computer EE

A calculator or computer can be used for recording and calculating the test results. If possible, integrating these into a laboratory information management system (LIMS) could standardize or formulate the reporting and data enquiry.

7.2.10 Seed herbarium as a reference source EE

Depending on the seed testing scope and laboratory capability, essential seed specimens must be available as a testing reference for both analytical purity and OSD. A seed herbarium (Figure 7.10) should be established as a reference collection for seed identification. There is a limited number of seed collections on the market to purchase; therefore, a laboratory will usually need to establish its own seed collection through years of accumulation. A seed collection of a species must include typical seeds as well as variations of seeds in size, shape, maturity, colours, shading, and different types of dispersal units or forms to capture the full range of how the species could appear during the analysis. With a self-established seed collection, an experienced botanist needs to confirm or verify the correctness of the species. The seed collection should be stored with protection from sunlight and other sources of deterioration or damage (e.g. heat, humidity, fungus, insects and rodents) and regularly checked for those influences.

For reporting needs, the scientific names of the species should be checked and updated regularly or cross-referenced for any changes. Aside from the indication on the specimens stored in plastic containers, glass tubes or boxes, a list of updates on species and specimen history should be maintained in digital format (preferred) or hard copy. The seed collection is extremely valuable and must be protected from cross-contamination during usage. However, the staff should have easy access to use the collection during the seed testing analysis. Although a separate working herbarium or an individual analyst's small reference collection can be used, the maintenance must be the same as for a larger seed herbarium, with verification, updates and avoiding contamination.

QA tips: Verified seed specimens as reference material are essential for reliable seed ID. The *ISTA Universal List of Species* provides a minimum suggested list of species needed by a seed laboratory.

Figure 7.10. Seed herbarium collections (images courtesy of ISTA accredited laboratories CH01, CA08 and KE01)

7.2.11 Ergonomic seats or tables

The analysis in seed testing is often static, and the staff members remain in one position for a long time. Ergonomic seats will support and protect against damage to the back and neck. Adjustable height tables can achieve a comfortable height for workstations for different members of staff and their working positions.

7.2.12 Analyst work area

A curved cutout in the table gives a large and comfortable working space (Figure 7.11). The tables can have an integrated diaphanoscope, allowing transmitted light to help analyse certain seeds, especially grass species. Diaphanoscopes can also be purchased separately or be self-built, often utilizing a 12-volt light source (Figure 7.11d and f). The surface of the table should be hard, non-reflective and scratch-free. The material used for the table should be free from static effects. Natural light is best, but any good lighting is essential. A suitable brightness of artificial light (bright but not too bright, to avoid eye strain) should be available to analysts.

Safety tips: Consider ergonomics issues (e.g. repetitive injuries) for staff.
- Make use of sit/stand benches.
- Take regular stretch breaks.
- Stop and focus eyes on different objects to avoid eye strain.
- Use digital microscopes to avoid neck strain.
- Make sure the analysts are well positioned to avoid possible back and neck injury.

Figure 7.11. Work areas for analytical purity at ISTA accredited laboratories: **a** laboratory IN39; **b** laboratory UG02; **c** laboratory KE01; **d** desktop diaphanoscope at laboratory KE01; **e** laboratory ZA01; **f** analytical purity workbench at laboratory DE19

7.3 Analytical purity

The objective of the percent purity analysis is to separate a working sample (at least 2500 seeds) into the three fractions or components of pure seeds, other seeds (weeds, other crops) and inert matter (e.g. chaff, stones, broken seeds). The purity analysis result is reported as a percentage for each fraction and to a precision of one decimal place. Before the purity analysis begins, the working sample weight is recorded (on a worksheet or digital data system). The other seeds found during the analysis must be identified and reported using the latest scientific names listed in Table 2C of the ISTA Rules and in the *ISTA List of Stabilised Plant Names*. For example, common wheat is reported as *Triticum aestivum* subsp. *aestivum* and soybean as *Glycine max*. Where it is impossible to determine the species with certainty based on seed characteristics, reporting must be done to the most precise taxon possible, e.g. *Poa* spp. The kind of inert matter is described depending on the content found, e.g. pieces of seed units half or less than the original size, seed units with no seed, stems, fungal bodies, soil, stones, etc. A precise description of the pure seed and the other fractions (other seeds and inert matter) is published in the ISTA Rules and the *ISTA Handbook on Pure Seed Definitions*.

The analytical purity working sample is spread onto the worktable, a slate or diaphanoscope. Each seed and every particle are visually analysed individually by moving single seeds with a spatula or tweezers from the working sample to another pile of already analysed seeds. 'Other seeds' are species other than the declared crop species of the working sample. Other seeds and inert matter are removed and kept separately. If the analytical purity sample has a high content of inert matter, sieves can be used to conduct a first rough separation before doing the individual analysis of seeds. The criteria to identify the fractions and seeds are based on external morphological appearance (shape, size, colour, glossiness, surface texture, structural features). By using a transmitted light source, light or empty seeds/caryopses can be identified. All other seeds and inert matter particles present are removed, leaving the pure seed. As such, the separation results in the three components (Figure 7.12). Each component is weighed as a single unit (Figure 7.13) and recorded on the analytical purity working card or in a LIMS. The gain or loss from the initial working sample must be no more than 5 percent. Otherwise, a new working sample must be prepared and analysed.

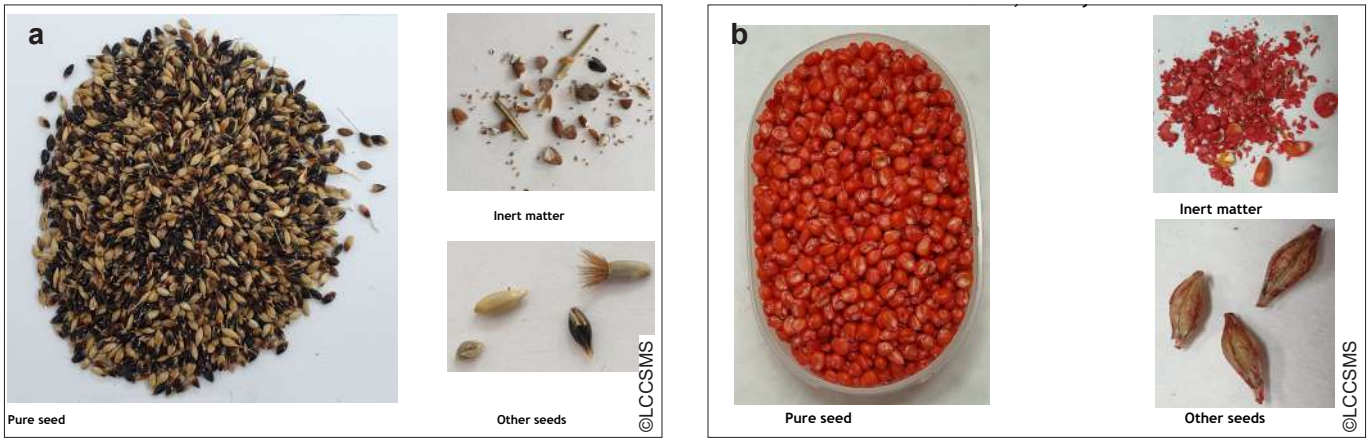


Figure 7.12. Analytical purity tests showing the three fractions produced (pure seed, other seeds and inert matter) for *Sorghum bicolor* subsp. *drummondii* (**a**) and treated *Zea mays* (**b**) (images courtesy of ISTA accredited laboratory RO05)

Figure 7.13. Analytical purity fractions ready for weighing (image courtesy of ISTA accredited laboratory CH01)

For reporting the results of the purity analysis on a test certificate, the percentages of pure seeds, other seeds (scientific name according to the *ISTA List of Stabilised Plant Names*) and inert matter found in the working sample must be included. If no other seeds or inert matter are found, this must be indicated as '0.0 percent'. It can be difficult to identify some other seeds. Essential requirements to ensure the accuracy of the testing results include training, consultation with experienced seed analysts, and use of the laboratory's reference seed collection or digital reference seed collections. Depending on the seed size, magnifiers, like hand lenses, illuminated magnifiers, and stereo binocular or digital microscopes are necessary tools. Ideal light and brightness conditions should be provided for the purity analysis and OSD. A workplace near a window is ideal for utilizing natural lighting. In addition, supplementary artificial light devices should be installed at every workplace. After the analysis, the other seeds and inert matter can be stored in small paper bags or envelopes. There must be an unambiguous link (e.g. unique sample number) between the sample identification and the stored fractions. The other seeds need to be stored in controlled conditions for a defined time period (see Chapter 11: Sample storage, of this handbook).

7.4 Other seed determination (seed identification)

The objective of the OSD is to analyse a working sample of the crop species stated by the client/applicant of at least 25 000 seeds and detect other species in the sample. The number of seeds of species other than the crop species stated is counted and reported with their scientific names.

The OSD can be performed as a *complete test* – all other species are identified, counted and removed from a working sample of at least 25 000 seeds. There is also an option to conduct a *limited test* where only predefined species (e.g. species categorized as noxious in certain countries) are counted and removed. The test could be limited, e.g. to the species defined by the country regulations or the customer, such as *Avena fatua*, *Cuscuta* spp., *Rumex* spp. The test specification *reduced test* means that fewer than 25 000 seeds are analysed, but all other species are identified, counted and removed. This is usually only done on small seed lots or expensive seeds. The *reduced-limited test* is used if fewer than 25 000 seeds in the working sample are analysed for predefined species only (see 'limited test'). If a species analysed is difficult to identify from other species, the limited test can be performed on one-fifth of the prescribed working sample. The type of test used for the OSD must be reported on the analysis certificate (see Chapter 1 of the current ISTA Rules on how to complete ISTA Certificates). Even if a laboratory is not using ISTA Certificates to report their results, a similar reporting method is useful for consistency with internationally agreed seed testing methods.

For an OSD analysis, the working sample is first weighed and recorded (on a worksheet or digital data system). The OSD sample is then spread on the work area, ideally on a surface or pad of contrasting colour (e.g. coloured paper, glass or desk mat). This is helpful to better see the morphology of single seeds. Each seed is visually analysed individually by moving single seeds with a spatula or tweezers from the working sample to another pile of already analysed seeds. Seeds of species other than the declared crop species of the working sample are removed and kept separately. The other seeds found during the analysis must be identified and reported to the highest taxon possible, such as species or subspecies level, using the latest scientific names listed in Table 2C of the ISTA Rules and the *ISTA List of Stabilised Plant Names*. The criteria to identify the seeds are based on external appearance (shape, size, colour, glossiness, surface texture, structure). Depending on the seed size, magnifiers like hand lenses, illuminated magnifiers, and stereo binocular or digital microscopes are helpful and necessary tools.

Certain details must be included for reporting the OSD results on a test certificate, such as:

- the weight of the working sample;
- the kind of test conducted (i.e. complete, limited, reduced, reduced-limited);
- the number and scientific names of the other species found in the working sample reported; and
- if no other seeds are found, a statement indicating so (i.e. 'No seeds of ____ species were found in ___ g of seed examined' if following Chapter 1 of the ISTA Rules for reporting. Other options are: 'Other seeds: 0', or 'No other seeds found').

After the analysis, the other seeds can be stored in small paper bags or envelopes for a defined period in controlled conditions (see Chapter 11 of this handbook). There must be an unambiguous link (e.g. unique sample number) between the sample identification and the stored fractions.

7.5 Thousand-seed weight test

The TSW test is a common analysis usually performed after the analytical purity test. The results of the TSW, together with the germination rate, give important information to end users and allow them to calculate how many seeds per hectare are needed to be sown for different establishment rates. The TSW can vary widely depending on the growing conditions within and between seasons. Within species (e.g. beans), a wide range of TSW results can be obtained.

The TSW analysis can be performed with different methods:

- counting the whole pure seed fraction (manually, Figure 7.14, or with a seed counter, Figure 7.15); or
- counting eight replicates of 100 seeds (manually).

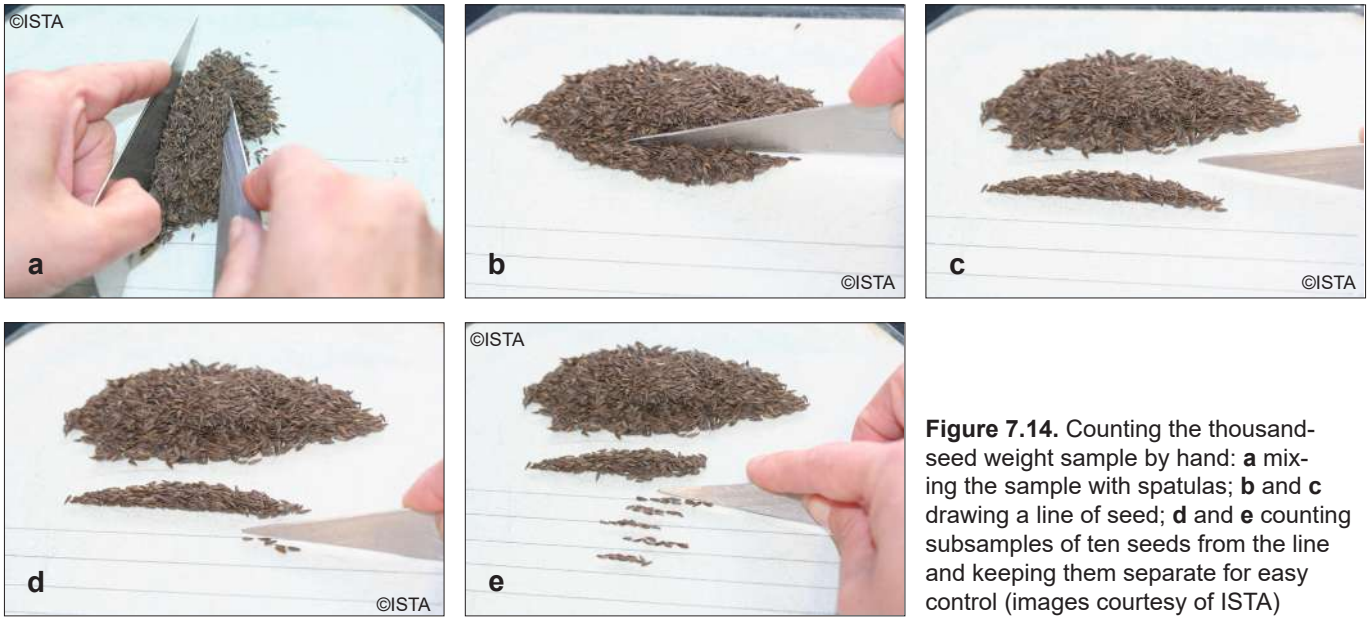


Figure 7.14. Counting the thousand-seed weight sample by hand: **a** mixing the sample with spatulas; **b** and **c** drawing a line of seed; **d** and **e** counting subsamples of ten seeds from the line and keeping them separate for easy control (images courtesy of ISTA)

Figure 7.15. An example of a seed counting machine (image courtesy of ISTA accredited laboratory CH01)

As described in section 7.3 of this chapter, the number of seeds in the working sample for analytical purity should be at least 2500 seeds. For an efficient workflow, the TSW analysis should be performed after the analytical purity test, where the fractions of pure seed, other seeds and inert matter are already separated. However, this is not obligatory; the analysis can also be done as a single test, but the seeds must be identified as pure seeds before starting the TSW test. It is recommended to do the TSW test immediately after obtaining the pure seed fraction and as soon as possible on receipt of the sample. If the seed is exposed to the ambient conditions in the laboratory, the seed weight could be influenced by dry or humid air. In some situations, to mitigate samples losing weight after receipt in the laboratory, a subsample can be stored in a moisture-proof container, or the TSW working sample can be taken from samples submitted for the moisture test.

7.5.1 Method description for counting whole pure seed fraction

Manual counting: The number of seeds in the pure seed fraction is counted and recorded. The working sample is weighed using a balance. The accuracy of the balance and number of decimal places is described in Table 10A of the ISTA Rules and included here in Table 7.3 for easy reference.

Counting machine: The pure seed fraction is poured into the counting machine as described in the manufacturer's instructions. The number of seeds displayed on the counter is recorded. The working sample is weighed using a balance. The accuracy of the balance and number of decimal places is described in Table 10A of the ISTA Rules (Table 7.3).

Table 7.3. Number of decimal places for weighing, calculating and reporting for the thousand-seed weight test

Minimum working sample for purity analysis for the species being analysed, according to Table 2C, column 4 (g)	Minimum number of decimal places for weighing and calculation		Number of decimal places for reporting both methods (10.5.2.1 and 10.5.2.2)
	Counting the whole pure seed fraction (10.5.2.1)	Counting replicates (10.5.2.2)	
Less than 1.000	4	4	4
1.000 – 9.999	3	4	3
10.00 – 99.99	2	3	2
100.0 – 999.9	1	2	1
1000 or more	0	1	0

Source: **ISTA.** 2022. *International Rules for Seed Testing*. Wallisellen, Switzerland, International Seed Testing Association, Table 10A.

7.5.2 Verification of a seed counting machine and regular checks

It is not mandatory to have a seed counting machine (Figure 7.15), but if a laboratory purchases one, certain verifications must be made to check if the device is fit for seed testing. A seed counter requires the following:

- an electric supply;
- a dry, clean and level work area; and
- a balance with a sufficient number of decimal places.

An initial fit-for-use check and regular verification checks using reference samples need to be done, for example:

- Check the different top units for different sizes of seeds. The counter should be free-flowing with the capability to count single seeds.
- Verify the counting of different species by hand and by machine to check the accuracy of the device; a tolerance between the two methods is allowed (e.g. an allowed difference of up to five seeds).
- Regular checks should also be done with reference samples of different species for which a TSW test is regularly done in the laboratory. The number of seeds should initially be checked by the hand counting method and regularly afterwards to avoid a loss or gain of seeds; a tolerance between the two methods is allowed (as defined by the laboratory).

7.5.3 Method description for counting eight replicates

Manual counting: The pure seed fraction is mixed thoroughly with a spatula, and eight replicates, each of 100 seeds, are counted randomly (see Figure 7.14). Each replicate is weighed using a balance and recorded. The accuracy of the balance and number of decimal places is described in Table 7.3 (sourced from Table 10A of the ISTA Rules).

7.5.4 Calculation and expression of thousand-seed weight results

The results of the TSW test are expressed according to sections 10.5.2 and 10.6 of the ISTA Rules (Table 7.4).

Note: Precision in the number of decimal places required while counting, weighing, calculating and reporting the results is described in the ISTA Rules Table 10A, and is also included as Table 7.3 in this chapter for easy reference.

Table 7.4. Calculation and expression of results for thousand-seed weight analysis performed by two methods

Formula for counting the whole pure seed fraction (manually or with seed counter)	Formulae for counting eight replicates of 100 seeds (manually)
$\text{Weight of 1000 seeds} = \frac{\text{Sample weight}}{\text{Number of seeds counted}} \times 1000$	*Calculation 1* of variance, standard deviation and coefficient of variation
	Variance $\text{Variance} = \frac{N \sum x^2 - (\sum x)^2}{N(N-1)}$ x = weight of each replicate in grams N = number of replicates Σ = sum of
	Standard deviation $s = \sqrt{\text{Variance}}$
	Coefficient $\text{Coefficient of variation} = \frac{s}{\bar{x}} \times 100$ $\bar{x}$ = mean weight of 100 seeds
	The *coefficient of variation* should not exceed: *6.0* for chaffy seeds *4.0* for other seeds If the coefficient of variation exceeds whichever of these limits is appropriate, count and weigh an additional eight replicates and calculate the standard deviation for the 16 replicates. Discard any replicate that diverges from the mean by more than twice the standard deviation as calculated.
	Calculation 2 of the average weight of 1000 seeds from the weight of eight or more 100-seed replicates
	$\text{Weight of 1000 seeds} = \frac{\sum \text{Weight of 100 seed replicates}}{\text{Number of 100 seed replicates}} \times 10$

Source: **ISTA.** 2022. *International Rules for Seed Testing*. Wallisellen, Switzerland, International Seed Testing Association, Chapter 10.

Chapter 8: Germination testing

8.1 Germination test aims

> The object of the germination test is to determine the germination potential of a seed lot...
>
> Germination of a seed in an ISTA test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in the field.
>
> The germination percentage… indicates the proportion by number of seeds which have produced seedlings classified as normal under the conditions and within the period specified… [in the current ISTA Rules].
>
> (ISTA, 2022, Chapter 5)

The definitions of 'percent germination' (see above) given in Chapter 5 of the *International Rules for Seed Testing* (ISTA Rules) are used worldwide in national regulations, and by seed companies, end users and stakeholders involved in seed trade and exchange. In the context of this handbook, the aim is to provide potential laboratories with tools to fit their needs (see Chapter 2 of this handbook, which aims to define the laboratory's needs). Therefore, an ISTA accredited laboratory (advanced and expert seed testing laboratory) will have to implement and strictly follow the definitions and the methods described in the ISTA Rules. Contrastingly, in a laboratory that aims only to inform clients/stakeholders about the germination capacity of samples for local use (standard seed testing laboratory), the germination tests can be modified from the ISTA methods. Likewise, if the requirement is only to advise the seed lot processing plant (basic seed testing laboratory), then testing fewer seeds or for shorter durations to facilitate quicker germination results could be an alternative solution. This chapter covers these various approaches.

Deviations and simplifications of a 'germinated seedling' can be used for in-house needs, such as in the early evaluation of seedlings or early counts of germinated seeds. Any in-house method modification should be documented, and the staff should be made aware of when to accept seedlings under these simplified criteria. For germination to occur, seeds need to be placed in favourable environmental conditions (temperature, water supply and a suitable growing media/substrate). Additional conditions such as light for growth, dormancy breaking treatments or ways to limit the development of pathogens that can interfere with the test result may be needed. The laboratory needs specific equipment, materials, and consumables to provide the seeds with these essential conditions.

Note 1: Early counts of germination are described in the ISTA Rules (Chapter 5). These should not be confused with the 'radicle emergence test', which is a vigour test. The control of environmental conditions for this vigour test is more strict than for a germination test and is not described in this handbook. See Chapter 15 (Seed vigour testing) of the ISTA Rules for more details.

The plans and workflow of the germination laboratory are dealt with in Chapter 4 (Buildings and workflow) of this handbook; therefore, there is no further discussion of the aspect in this chapter. For specific details on how to execute the germination test and how to evaluate the seedlings, refer to the ISTA Rules and the *ISTA Handbook on Seedling Evaluation*. This chapter reviews the descriptions of equipment, materials, and consumables needed for germination tests based on the ISTA definitions. For ISTA accredited laboratories, the requirements discussed must always comply with the ISTA Rules and the ISTA Accreditation Standard.

8.2 Working sample preparation

8.2.1 Pure seed

An ISTA germination test uses pure seeds taken from the pure seed fraction of the analytical purity test. It is important to remember that during the analytical purity test, broken seeds are classified as 'inert matter' if they are half or less of the original size and classified as 'pure seed' if they are more than half (50 percent) of the original size. Any seed that was classified as pure must have the same chance of being picked for the germination test. Pure seeds are randomly selected from the pure seed fraction, which can include broken seeds, meaning that the biggest and best seeds are not always selected. In cases where an analytical purity test is not done, pure seeds need to be taken from a working sample. Once again, pure seeds need to be selected and may contain broken seeds that are more than half (50 percent) of the original seed size.

To have a reproducible test representing the seed lot quality, the laboratory must follow strict criteria to define which seeds should be planted. It is strongly recommended to follow the ISTA definition of 'pure seeds' given in Chapter 3 (The purity analysis) of the ISTA Rules. The planting of pure seeds only requires some basic training and very little equipment (see Chapter 7 of this handbook for details about working sample preparation and pure seed definitions).

8.2.2 Size of working sample

The best compromise between work accuracy and quantity to get a reliable, repeatable and reproducible result is to test 400 seeds (4 × 100 seed replicates) as indicated in the ISTA Rules. Under the ISTA Rules, it is possible to test two replicates of 100 seeds or, in the case of very expensive seeds, only 100 seeds, but that is the exception. For other needs such as in-house testing quality control with a requirement for cheaper but less precise tests, 200 or even only 100 seeds can also be considered. The tests using fewer seeds can give acceptable results, but the amount of work, rapidity of tests, aim of the test and/or client expectations are also taken into account; it is not just about the precision of the test. The variability between replicates is checked for tolerance when calculating the test result. If outside the tolerance limits, the test needs to be repeated. The germination tolerance tables in the ISTA Rules are based on the statistical binomial law applied to percentages, which is the basis of the calculation of germination results to provide repeatability and reproducibility (Figure 8.1).

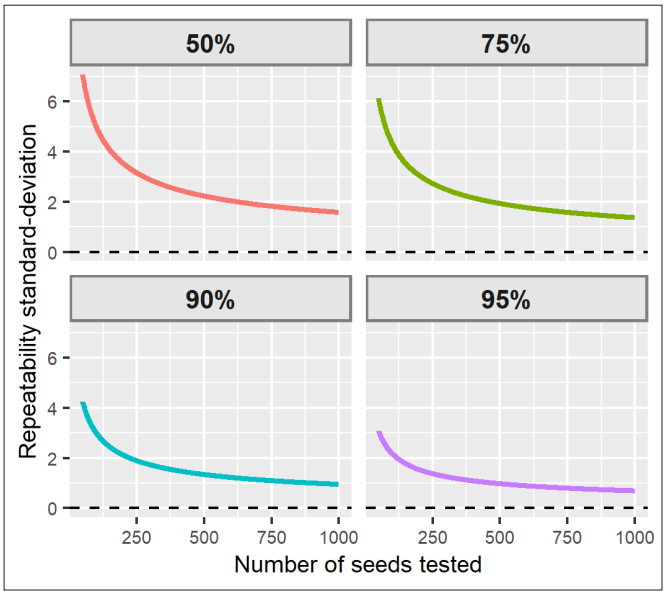


Figure 8.1. Repeatability standard-deviation of a germination result as a function of the number of tested seeds and for four germination levels

8.3 Essential equipment, materials and consumables

This section describes the equipment and materials that have major impact on the seed laboratory building plans, e.g. size of the rooms, needs for electric power, water supply or ventilation. It then discusses considerations for other equipment and consumables essential for the germination test but not affecting the building size. The main steps of a germination test, e.g. preparation of the test, planting, incubation and growth, seedling evaluation, calculations and reporting of the results, are all covered.

8.3.1 Germination devices (germinators)

Controlled-temperature germination devices (germinators) are needed to provide the specific temperature(s) required for incubation to promote germination and early growth. These can be walk-in germination rooms, cabinets or tables.

General incubation requirements for germination

The germination test is influenced by the environmental conditions, including temperature provided to the seed, free water, aeration (oxygen), light or darkness, and the absence of any adverse factor (toxic chemicals and compounds, diseases), as detailed in the current ISTA Rules, Chapter 5. The germinators must provide the best conditions to the seeds to achieve the maximum potential of germination as required in an ISTA germination test (standard method). The principle for a germination test is to put seed in the best possible conditions to promote the maximum possible germination and to evaluate seedlings so that only those expected to produce a normal plant in the field will be counted as 'normal seedlings'. In this way, a germination test estimates the maximum potential of a seed lot to produce good seedlings in the field if conditions are right. As for other tests, the sample should be taken following the ISTA sampling procedures.

Temperature

After years of research, ISTA has defined the best temperatures for germinating seeds for an extensive list of species. The temperatures given by the ISTA Rules are necessary for good results and require an accurate temperature adjustment within limits of +/−2 °C. For alternating temperatures (e.g. 20<=>30 °C, with 20 °C for 16 h and 30 °C for 8 h per day), a relatively quick temperature change must be achieved in less than 3 h. Germination cabinets meeting these requirements usually have sufficient capacity for cooling and heating to achieve a quick temperature adjustment when the doors are opened.

The ISTA Rules propose different temperature regimes according to the species being tested. It is advisable to choose the best range of temperatures that will suit most of the species tested in your laboratory to minimize the number of germinators needed. The most commonly used temperatures are 20 °C, 25 °C, and an alternating temperature of 20<=>30 °C. If you have low numbers of tests for species that require a large range of temperatures, you may consider whether this is worth the additional cost of building or of purchasing specific germinators. Alternatively, you may wish to restrict the number of species in your testing scope.

Laboratories with lower accuracy needs, e.g. those only requiring quick informative tests during seed processing or after harvest, could use temperatures outside the ISTA accuracy range of +/−2 °C. This has the effect of reducing the accuracy of the equipment and cost, but would not allow the use of the equipment for ISTA testing or achieving future ISTA accreditation. However, it is still important to avoid large or long variations in temperature.

Water

Free water needs to be available to the seeds. The relative humidity (RH) of the air surrounding the seeds must be kept near 100 percent to avoid excessive drying of the media/substrate during the test. This level must be maintained during the long periods of constant temperature and the relatively short times of change from one temperature to another when using alternating temperature cycles. To maintain a high level of RH around the seeds, it is preferable to cover each germination box than to set a 100 percent-RH level in the germinators. A high level of moisture inside the germinator can damage the electrical equipment and stimulate the growth of moulds, ultimately needing more cleaning and maintenance. Tight waterproof covers or plastic bags are a good solution (Figure 8.2). Plastic bags are cheaper but cannot be easily reused, adding cost and not being environmentally friendly. Rolled towels can be packed in large, closed plastic containers that can be stacked, also optimizing the volumes used. It is important to ensure that water is of good quality to avoid having any chemicals interfering with germination.

Light

For a few species, light is inhibitory to germination (see the ISTA Rules for specific test methods on a per species basis). For other species, light is not an absolute requirement, e.g. the species where the rolled towel method is used. Where light is needed, it can be provided from fluorescent tubes or LED lights, with a relatively low emission in the far red and a high special emission in the red region, equivalent to between 3000 K (neutral white) to 4000 K (cool white). However, light for germination is advised for producing more sturdy seedlings, allowing easier and more correct evaluation. In addition, the red emission of the white fluorescent tubes has a dormancy breaking effect. Lamps should be installed so that illumination is as uniform as possible between 3000–4000 K. Precautions should be taken to ensure that the starter and the chokes producing heat are positioned properly so that they do not affect the control of the germination temperature and RH. LED lamps have a longer lifespan, and the wavelength is known and controlled. Some LED lamps use a 12-V intensity which avoids the risk of electric shock. Light can cause a 'greenhouse effect' through the plastic covers of the germination boxes; care should be taken to check the temperature at the seed level and to adjust the temperature setting of the germinator accordingly.

Aeration

Gas exchange should be possible to keep the composition of the atmosphere surrounding the seed approximately normal (continuous supply of oxygen and removal of such gases as carbon dioxide). However, experience has shown that artificial, forced ventilation does dry out the tests.

Containers

Dimensions of the media/substrate containers should be such that maximum use of the equipment is achieved. However, spacing between seeds and between tests should not be so close that it hampers the germination process or allows the undue spread of any seed-borne disease.

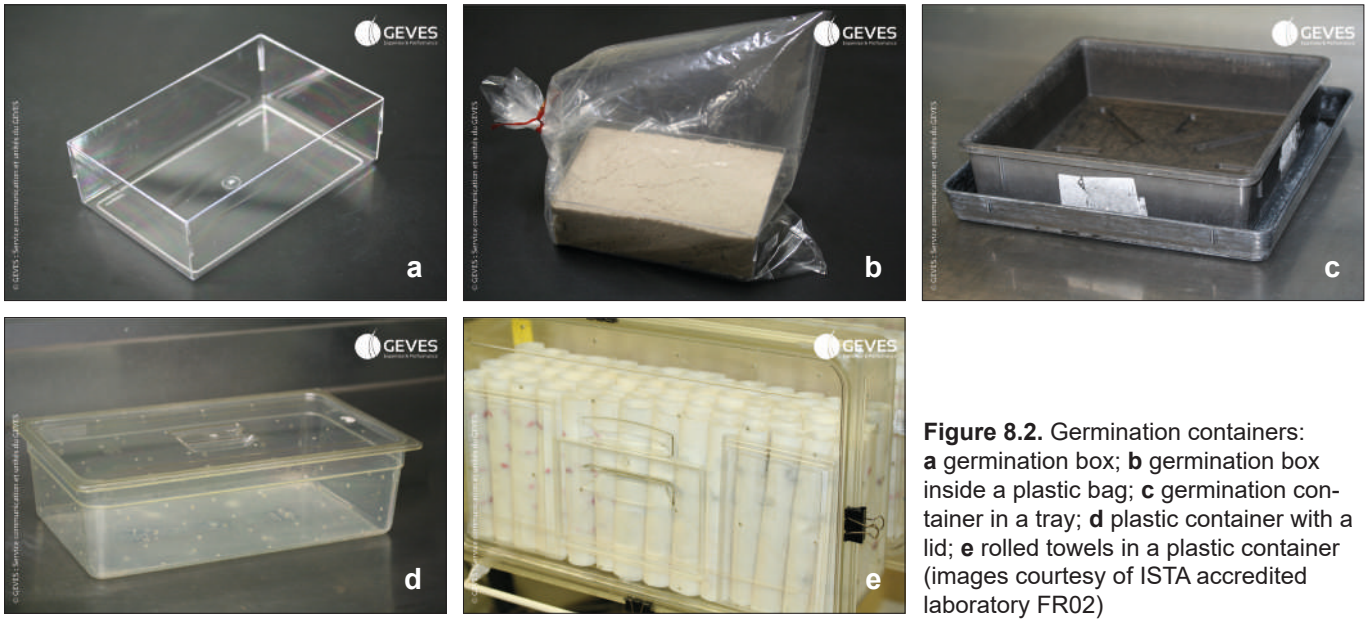


Figure 8.2. Germination containers: **a** germination box; **b** germination box inside a plastic bag; **c** germination container in a tray; **d** plastic container with a lid; **e** rolled towels in a plastic container (images courtesy of ISTA accredited laboratory FR02)

How to define the type of germinator needed

Germination rooms (insulated and temperature-controlled walk-in rooms), smaller controlled-temperature cabinets or germination tables can be used for germination testing. See the following section, Description and characteristics of germinators, for details of the different types of germinators available. It is important, especially when deciding on room sizes, to define the best choice for the laboratory's needs; it may be that a combination of different germinators is required. When planning for walk-in germination rooms, the choice of insulation material is important, and this should be protected from moisture penetration to avoid it losing its function.

To select the best germination device that fits your needs, consider the following (see Table 8.1):

- *Species and size of seeds* – These are often linked to the germination media/substrate and are important criteria to define the type of germinator that you need. Walk-in germination rooms are well adapted to the needs of large-seeded species (cereals: wheat, maize; pulses: vegetable beans, faba/broad beans; some agricultural species: sunflower) that are planted in sand, organic growing media or rolled towels. Small seeds need less space, and their specific requirements can be met using smaller germination cabinets. Indeed, grasses, some vegetables and some flowers germinate well in small germination cabinets with a cooling system or on germination tables that provide high RH. Such tables also provide a constant supply of water for imbibition and growth but are often more expensive, and the number of suppliers is limited.
- *Number of tests with same environmental conditions (temperature, light)* – Walk-in germination rooms are designed for many tests performed in similar temperature and light conditions. They will generally be set up at commonly used temperatures such as a constant 20 °C or 25 °C, or an alternating temperature of 20<=>30 °C (20 °C for 16 h and 30 °C for 8 h in every 24-h cycle). They are designed for large laboratories with a small or large scope of species. Germination cabinets can also be suitable for large or small numbers of species, but the purchase of several cabinets may be required. Combined with the need for different temperatures, a range of cabinets may give the laboratory versatility to handle variations in the quantity of analyses or temperatures or provide fallback solutions in case of maintenance or breakdown.
- *Media/substrate and related containers used* – Containers with sand or organic growing media need more space and stronger shelves and are perhaps more appropriate for walk-in rooms than for small germination cabinets. Rolled paper towel tests or tests using sand or organic growing media cannot be used in germination 'wet' cabinets or on germination tables.
  'Top of paper' (TP) and 'pleated paper' (PP) methods can be used in all kinds of germinators but are better media/substrate for germination cabinets where the number of shelves can be increased to optimize the number of tests per square metre. Paper (not rolled) is the only media/substrate that can be used on germination tables.
- *Maintenance of equipment* – This depends on the manufacturer. Walk-in rooms built by local companies may be easier and cheaper to maintain, but it can be more difficult to ensure homogeneity of the temperature. Germination cabinets are smaller and they usually more easily achieve the required homogeneity of temperature and light. Germination tables generally need specialized maintenance contracts with external companies or trained staff to do the maintenance.
- *Quality checks* – In a quality assurance (QA) system framework, such checks need careful attention. QA checks using temperature profiles in walk-in rooms are more complex, but these checks are usually only performed once, typically before the rooms are placed into service, to ensure the rooms meet the design specifications and are fit for purpose (see QA tips 3). Temperature probes are needed for daily checks on each piece of equipment. These daily checks are more efficiently conducted for larger walk-in rooms or large cabinets than several small ones.
- *Productivity and number of analyses per square metre* – These aspects require consideration for temperature-controlled rooms. The best ratios are for walk-in rooms and dry/wet germination cabinets. In walk-in rooms, the number of analyses can be increased when using trolleys with customizable shelves to adapt to the size of the seeds and seedlings, as in the cabinets. On the contrary, germination tables with a single level have a very low ratio of analyses per square metre and the ground space (square metreage used) is not optimized.
- *Versatility and flexibility* – These are important criteria for laboratories with a large scope of species and methods. Again, germination cabinets are the best option compared to walk-in rooms or germination tables. Several small or medium cabinets offer more flexibility and opportunities to test at different temperatures, optimizing the occupation of the space, and provide the ability to switch them off during the low season. New cabinets can be purchased to meet new client requests for testing. Costs of the different equipment and their ongoing maintenance also need to be considered.

Table 8.1. Criteria to help select a germination device

Criteria		Germination device[a]			
		Walk-in room	Dry cabinet	Wet cabinet	Table
Species	• All species	++	+++	~	~
	• Large-seeded species (cereals, etc.)	+++	++	~	~
	• Small-seeded species (grasses, pulses, vegetables, etc.)	++	+++	++	++
Number of tests	• Large number (more than 50 per week)	+++	+++	+	~
	• Small number (fewer than 50 per week)	~	+++	+++	+++
Temperature	• 1–3 temperatures (e.g. 20 °C, 25 °C)	+++	+++	++	++
	• More than 3 temperatures (e.g. 15 °C, 20 °C, 25 °C, 20<=>30 °C)	~	+++	++	++
Media/substrate	• Sand, organic growing media	+++	++	~	~
	• Rolled towels	+++	++	~	~
	• Paper	++	+++	+++	+++
Water	• In media/substrate (with lids, covers)	+++	+++	~	+++
	• In environment (relative humidity, without lid)	~	~	+++	+
Maintenance	• Low cost, in-house, easy	+++	++	+	+
Total[b]	*(Estimated versatility = sum of +)*	*27*	*32*	*17*	*17*

Note(s):
[a]Germination devices are scored as 'preferred' (+++), 'good' (++), 'suitable' (+) or 'not suitable' (~).
[b]Estimates are based on experience but can vary depending on preferred methods, species to be tested, equipment available locally, expertise of staff for building and maintenance.

Note 2: One box is often around 14 × 17 × 4.5 cm for testing on paper or sand, and larger containers are often around 30 × 30 cm for large seeds tested on sand or organic growing media. To calculate a germinator's capacity for testing, assume one container contains 100 seeds (cereals), 50 seeds (maize) or 25 seeds (vegetable beans, faba/broad beans). Containers using 'top of paper' (TP) or 'pleated paper' (PP) methods need less space (smaller boxes) for 50 to 100 seeds. Large boxes can contain up to 50 rolled towels with 50 to 100 seeds in each.

Determine the initial surface needed for one test (400 seeds), which will depend on the media/substrate used and the seed size. Then calculate how many tests can be placed in the germination room. Depending on the species/size of seeds, each box can take one or two replicates or fewer, meaning that the rooms can accommodate various numbers of test, that can easily be calculated.

Example: Assume a germination room has a total capacity of 16 m² (8 shelves of 2 m² each, e.g. arranged in two rows of four shelves separated by a central corridor).

- The room can accommodate a maximum of 660 small containers (14 × 17 cm) for germination tests on paper. It can contain 165 samples of four replicates of 100 seeds (e.g. grasses) or 80 samples of eight replicates of 50 seeds (e.g. watermelon).
- The room can accommodate a maximum of 170 large containers (30 × 30 cm) for germination tests on sand or organic growing media. It can contain 42 samples of four replicates of 100 seeds (e.g. cereals, rice), 21 samples of eight replicates of 50 seeds (e.g. maize), or 10 samples of 16 replicates of 25 seeds (vegetable beans, faba/broad beans).

QA tips 3: *Controlling temperature in germination rooms, cabinets and tables.*
Temperature profiles (i.e. temperature readings taken in several different locations in a germinator) are used to check the homogeneity of the temperature in all areas of the germinator simultaneously. Germination tests should not be placed where the required temperature is not reached (including the tolerances of +/−2 °C). The temperature profile is based on measurement of the temperature in nine different parts of the germination device: front top, middle and bottom (each left, middle and right); middle top, middle and bottom (each left, middle and right); back top, middle and bottom (each left, middle and right). If alternating temperatures are used, checking the temperature profiles and successful transition period for these regimes is also necessary. Any temperature records are compared to the set temperatures to determine if adjustments are needed.

Temperature probes (thermometers, probes, data loggers) are required for this process. Simple glass–liquid thermometers can be used, but the thermometers need to be moved to different places for readings to be taken at frequent time intervals. Glass–liquid thermometers are relatively cheap, but they are less accurate than electronic probes and need more staff time. Thermometers are a good compromise for laboratories with a low budget for investment and maintenance, when staff time is available. Data loggers are easier to manipulate, more accurate, versatile, easier to download data from and transfer to a computer, can be connected to an alarm system, need less staff time, but have a significantly higher initial cost. Data loggers with probes are the best option when affordable.

Daily temperature control: Aim to check the temperature provided to the seeds. Measuring twice daily is advised as a minimum. With an accuracy of +/−0.5 °C, glass–liquid thermometers are fit for purpose but require analysts to record the temperature twice per day. Max/min glass–liquid thermometers can be used to record possible variations outside the recording periods (i.e. night, weekend).

Description and characteristics of germinators

Walk-in germination rooms

Germination rooms (Figure 8.3 and Figure 8.4) have a large capacity for tests and are specially built to control the room temperature and, optionally, the RH. Most walk-in rooms cannot be moved as they are part of the building in which they are contained. The rooms can be built in the laboratory building or another building nearby. The isolation, aeration and ventilation of these rooms are part of the existing building. Other walk-in rooms are free-standing and self-contained, available for purchase and to be used for germination testing. Typically, these free-standing rooms are prefabricated units that can be assembled within an existing interior room or warehouse, with or without climate control. They have their own climate control system and are not dependent upon the climate control of the building where these units are housed. If walk-in germination rooms are needed, dedicated space in the building should be allocated at the very beginning of the building planning process. For building and insulating the walls, ceiling and floor, see Chapter 4 of this handbook. It is possible to equip the room with large shelves and vertical or horizontal lighting. As large numbers of analyses are placed daily or weekly in these rooms, trolleys on which germination boxes, plates or containers (e.g. with rolled towels) can be placed and transported can replace shelves. Light can be controlled so that only sections of the room being used are switched on.

Controlling temperature to meet the ISTA requirements of +/−2 °C of variation in all parts of the room can be challenging (see QA tips 3 on temperature profiles). It is important to achieve adequate air movement within the walk-in germination room using fans to help ensure there is little or no temperature stratification within the unit. Fans provided in the rooms by the manufacturer can sometimes be inadequate to properly circulate the air inside the rooms. Opening the door of the room too often can significantly change the homogeneity of the temperature. Cooling systems are required to mitigate light and the side-effect of increased local heating.

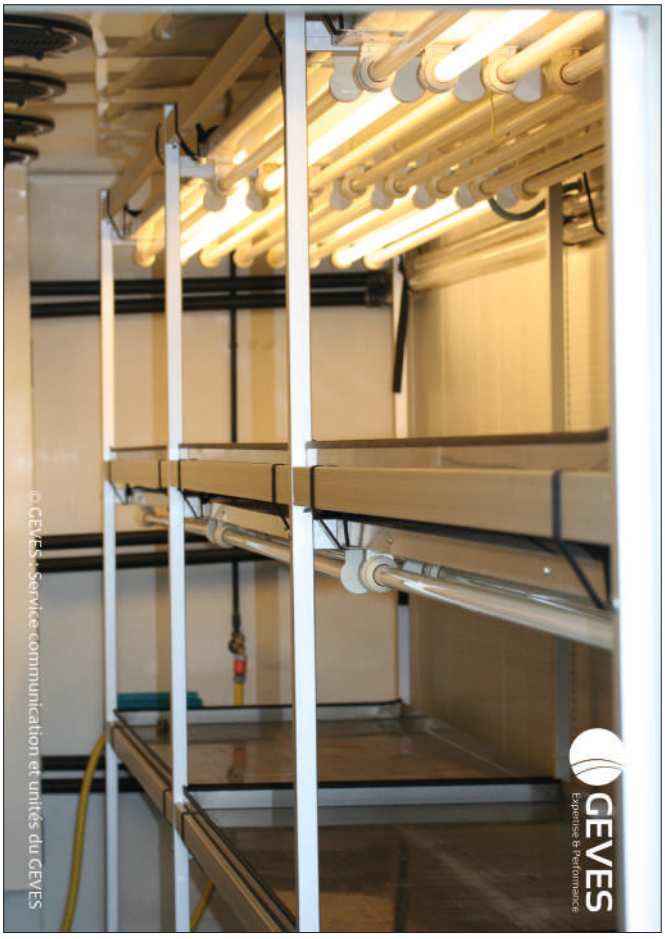


Figure 8.3. Climatic walk-in germination room with shelves (image courtesy of ISTA accredited laboratory FR02)

Figure 8.4. Examples of walk-in germination rooms: **a** with shelves; **b** material growing on shelves; **c** with trolleys (images courtesy of ISTA accredited laboratories UG02, IT01 and KE01)

To fulfil building requirements for a walk-in germination room, consider the following:

- Define the room size (inside and outside as the walls are affected by the thickness of insulation materials).
- Identify the kinds of tests to be performed, the media/substrate (paper, sand, organic growing media) and the containers to be used (plates, boxes, containers, with or without covers).
- Determine the testing capacity, i.e. number of tests (see Note 2).
- Specify the internal equipment (fixed shelves or trolleys and the space between shelves/trolleys).
- Identify the range of temperatures needed, the tolerances and the homogeneity of temperature requested.
- Ensure sufficient electrical power and a power generator to guarantee continuity in power supply to maintain temperature control.
- Consider the light levels (periodicity, intensity, wavelength) and kinds of lamps (LED, fluorescence).
- Consider remote control of lighting and heating.
- Consider aeration ventilation (holes in the walls, fans in the ceiling).
- Specify easy-to-clean surfaces and floor drains for cleaning.

Note 4: Before building, the exact size of a walk-in germination room (inside walls) should be calculated, according to the surface needed for one replicate of 100, 50 or 25 seeds per box, to optimize the number of boxes per square metre and avoid unused areas. If possible, it is recommended to design the germination room with more than one heating/cooling unit to ensure continued service, should one fail during the busy testing season.

Depending on each country's weather, some germination rooms may spend more time heating to 20 or 25 °C while others may spend more time cooling to reach 20 or 25 °C.

Note 5: Wheeled trolleys are mobile and provide an alternative to shelves. They are ergonomic, making it easier for staff to move the germination boxes or trays to the germination room or bring the seedlings into the laboratory for evaluation. The number of shelves on a trolley can easily be adjusted to the species tested. Trolleys are often modified to be fully enclosed to mitigate moisture loss. The front and/or back panels are often clear to allow light penetration to the samples within the trolley. Trolleys make it easy to empty the room for cleaning and are themselves easy to clean. In terms of investment and operating costs, it is advisable to compare the cost of trolleys with that of shelves.

Germination cabinets

Cabinets are stand-alone pieces of equipment, usually on wheels for easy movement. They are generally manufactured by specialized companies and are not built in-house. Cabinets may be 'wet' or 'dry' and can be equipped for either constant or both constant and alternating temperatures (see Figure 8.5 and Figure 8.6 for examples of both types). Contrary to wet cabinets, the tests in dry cabinets must be covered to prevent drying out during the germination period. When using filter paper as a media/substrate, the wet cabinet can hold many more samples than the dry cabinet, where all tests have to be germinated in containers. Germination cabinets require maintenance and may be considered more specialized than germination rooms. A cabinet with a water-cooled cooling unit needs a continuous supply of clean water. This is also the case where the temperature and RH conditioning are regulated via water. Consequently, guaranteeing a continuous water supply should be accounted for when choosing a cabinet. Otherwise, the choice should be a cabinet with temperature and RH control independent of a continuous water supply.

To fulfil purchase requirements for a germination cabinet, consider the following:

- Allocate a room to house the germination cabinets. The room usually needs to be air-conditioned to avoid the cooling compressor of the cabinets being overworked. The room requirements should be defined with or without air conditioning (cooling) when planning the building. Note: The compressor of a germinator, fridge or freezer generates about 1 kW of heat when working.
- Identify the kinds of tests to be performed, the media/substrate (paper, sand, organic growing media, agar) and the containers to be used (plates, boxes, containers, with or without covers).
- Choose between a wet or dry cabinet. Consider that, for wet cabinets, a supply of clean water is needed, plus a way to remove the wastewater.
- Determine the testing capacity, i.e. number of tests (see Note 2).
- Specify the internal equipment (space between shelves).
- Identify the range of temperatures needed, the tolerances and the homogeneity of temperature requested.
- Ensure sufficient electrical power and a power generator to guarantee continuity in power supply to maintain temperature control.
- Consider the light levels (periodicity, intensity, wavelength) and kinds of lamps (LED, fluorescence).
- Consider automatic control of lighting and heating.

Figure 8.5. 'Dry' germination cabinets: **a** in germination room; **b** with door open and showing probes; **c** with soybean seedlings (images courtesy of ISTA accredited laboratory CA08)

Figure 8.6. 'Wet' germination cabinet: **a** door closed; **b** door opened and light on; **c** inside view of shelves (images courtesy of ISTA accredited laboratory GB04)

Germination tables

These tables consist of a germination plate upon which filter paper beds are placed. The most known type of germination table is the Jacobsen apparatus (or Copenhagen tank) (Figure 8.7). The seed beds are kept continuously moist through a paper wick (Figure 8.8), extending from the seed bed through slots or holes in the germination plate into the underlying water bath. To prevent drying out, the paper bed is covered with a bell jar (Figure 8.9), provided with a hole allowing for ventilation without undue evaporation. The temperature is controlled directly by conditioning the germination plate or indirectly by heating/cooling the water in the water bath. The water in the water bath may have to be replaced occasionally or when it becomes dirty. However, the water used for temperature conditioning need not be replenished because it runs in a closed circuit. When air-cooled, this system makes the apparatus independent of a continuous water supply. The tables can be set to provide constant or alternating temperatures between 5 °C and 35 °C.

To fulfil purchase requirements for a germination table, consider the following:

- Allocate a room for the germination table. In warm regions, the room may need to be air-conditioned to avoid large differences in temperature between the media/substrate and the atmosphere, which would increase evaporation at the seed level. The room requirements should be defined with or without air conditioning (cooling) when planning the building.
- Identify the kinds of tests to be performed (paper only) and the kinds/sizes of bell jars to cover the tests.
- Ensure the supply of clean water and a way to remove the wastewater.
- Determine the heating and cooling equipment capacity to guarantee the accurate regulation of the water temperature.
- Determine the testing capacity, i.e. number of tests (see Note 2).
- Identify the range of temperatures needed, the tolerances and the homogeneity of temperature requested.
- Ensure sufficient electrical power and a power generator to guarantee continuity in power supply to maintain temperature control.
- Consider the light levels (periodicity, intensity, wavelength) and kinds of lamps (LED, fluorescence).
- Consider automatic control of lighting and heating.

Figure 8.7. Germination table, Copenhagen tank type (image courtesy of ISTA accredited laboratory GB04)

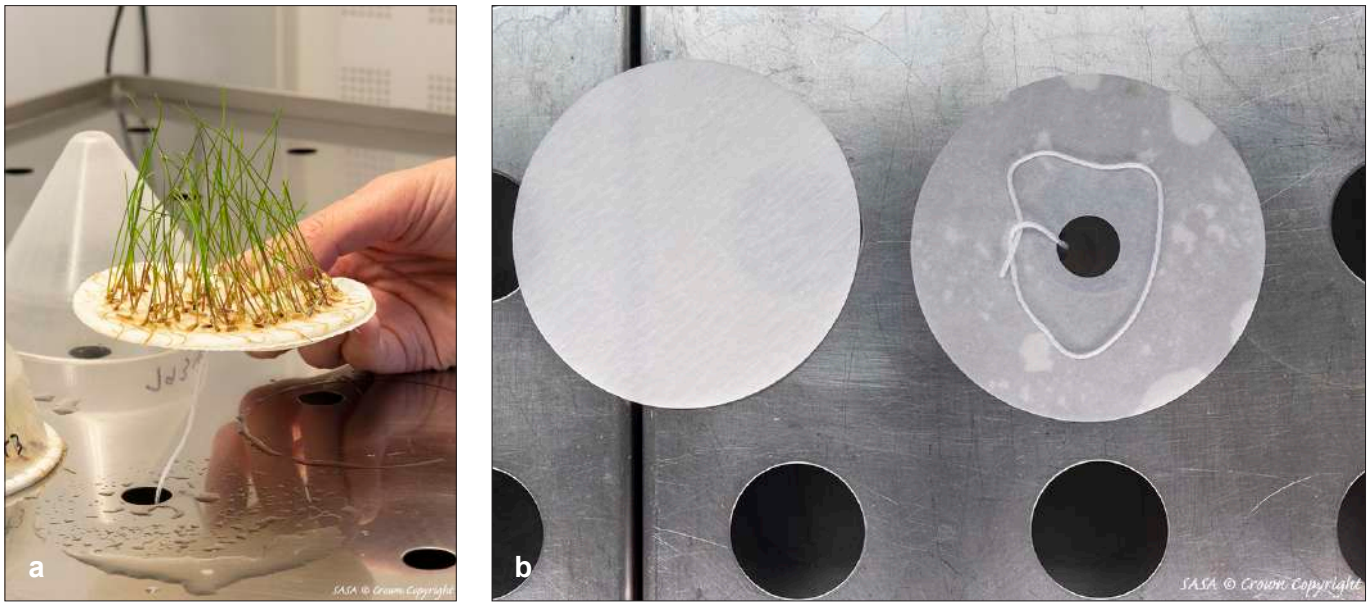


Figure 8.8. a Seedlings, wick and paper on a germination table; **b** top view of wick and paper (images courtesy of ISTA accredited laboratory GB04)

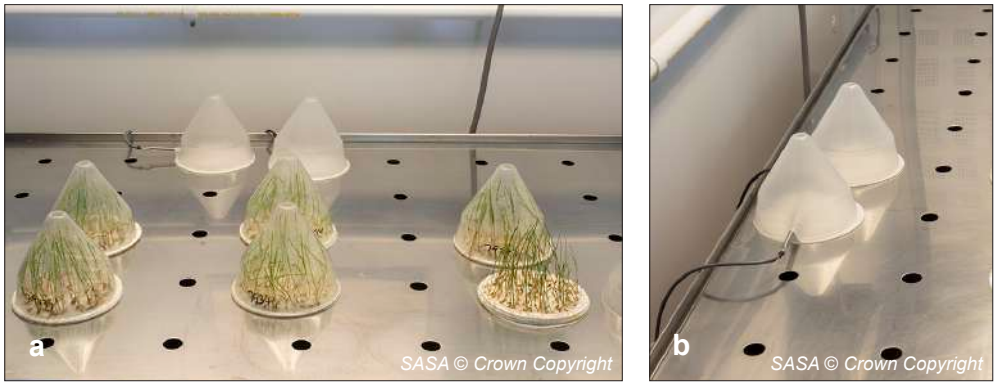


Figure 8.9. a Bell jars to prevent tests drying out on a germination table; **b** with temperature probe (images courtesy of ISTA accredited laboratory GB04)

Capacity of germination equipment required

The capacity or quantity of germination equipment needed depends on:

- the kinds of equipment chosen;
- the number of samples that must be processed in the peak season;
- the kinds of seeds to be dealt with in the peak season;
- the germination methods used (i.e. temperature, media/substrate, germination period, possible pre-treatment for breaking dormancy); and
- the effective dimension of the equipment in relation to the space each test replicate takes.

Note 6: The number of germination cabinets you need is calculated based on the expected number of samples × the number of replicates of 100 seeds × the surface area of one replicate. To calculate the global capacity of the laboratory, the test duration is an important factor.

Example: A laboratory receives 10 samples per workday (5 workdays a week) of *Sorghum bicolor* subsp. *bicolor* (10-day germination test period) during 1 month. This equals a total of 50 samples per working week (from Monday to Friday = 50; Saturday and Sunday = 0). Then, another 10 samples arrive each day on the next Monday, Tuesday and Wednesday (another 30 samples in total), before the tests are finished at day number 10 and before the first 10 samples (previous Monday) are taken out of the germinator. Therefore, the space needed is for 80 samples submitted in a 10-day period.

The total germinator space needed can be calculated as 80 samples × 4 replicates of 100 seeds each sample = 320 germination boxes of 100 seeds. If the surface area of each box is 0.21 m × 0.15 m = 0.0315 m², the total need is 0.0315 m² × 320 boxes. This equates to a 10 m² cabinet capacity needed to meet the testing requirements at a defined temperature for *Sorghum* sp.: one walk-in room of 10 m² capacity; or two germination cabinets (based on a cabinet with five shelves of 1 m² for each shelf); or one 10 m² germination table.

The same calculation can be made for several combined species with different durations of germination, number of seeds per replicate, and similar or different temperatures. Room and cabinet heights also need to be considered for volume (m^3) calculation.

For many species, it is also necessary to have a specific room or cabinet for breaking dormancy (pre-chilling at temperatures of 5 to 10 °C) and a temperature-controlled room to store samples after testing (see Chapter 11: Sample storage, of this handbook). Extra germination cabinets for research purposes are an additional need for those laboratories that plan to extend their scope of species or do other germination or vigour-based research.

8.3.2 Germination growing media/substrate

Seeds need to be placed in or on media/substrate to germinate. The kinds of growing media/substrate and their quality requirements are detailed in the current ISTA Rules, Chapter 5. There are three main kinds of growing media/substrate: paper, sand and organic growing media. A combination of these media/substrates is also possible (Figure 8.10), e.g. paper covered with sand.

The definition of growing media in the ISTA Rules (Chapter 5) states:

> Growing media used for germination tests are products which provide sufficient pore space for air and water, for the growth of the root system and for contact with solutions (water) needed for plant growth. With paper as the base medium, any combination of growing media prescribed… [by the ISTA Rules] for that species is allowed, provided that each growing medium is verified and meets the specifications prescribed… [by the ISTA Rules].
>
> (ISTA, 2022, Chapter 5)

Regarding water retention, the growing medium should "hold sufficient water to provide continuous movement of water to the seeds and seedlings… The water content of the growing medium should be adjusted to correspond to the needs of the species being tested, based on the maximum water-holding capacity of the medium. The water retention is then expressed as a percentage of the maximum retention" (ISTA, 2022).

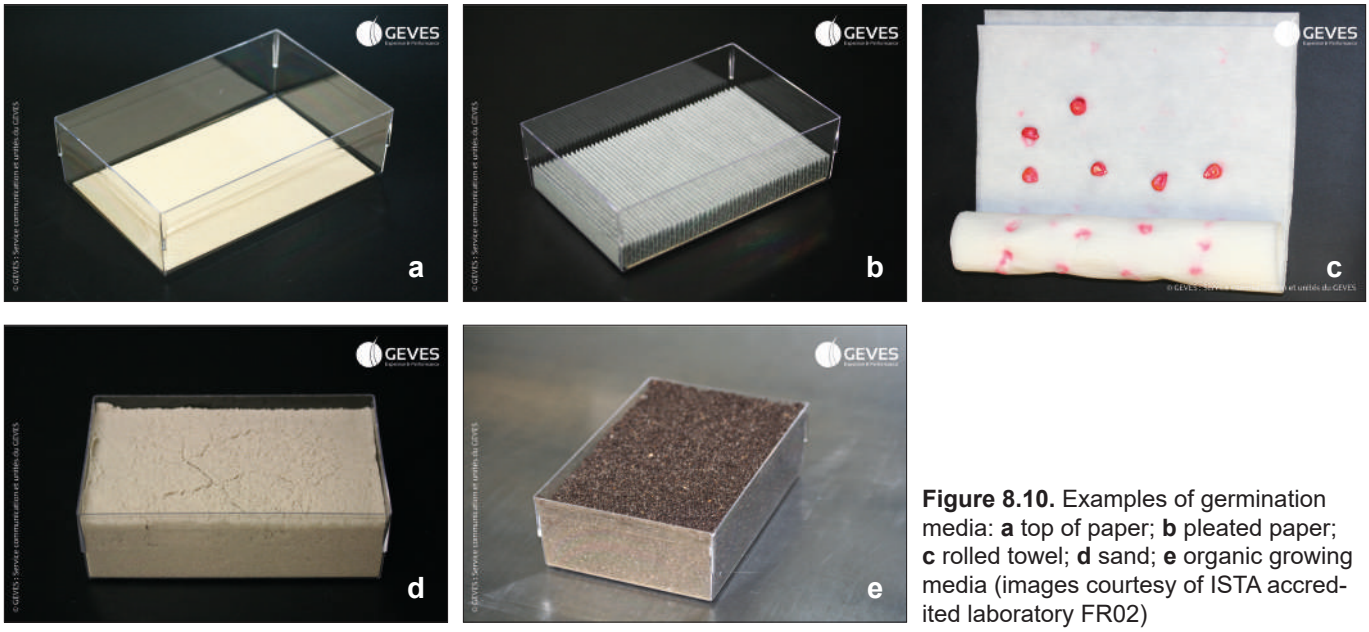


Figure 8.10. Examples of germination media: **a** top of paper; **b** pleated paper; **c** rolled towel; **d** sand; **e** organic growing media (images courtesy of ISTA accredited laboratory FR02)

The ISTA Rules (ISTA, 2022, Chapter 5) also state that:

- pH (6.0–7.5) should be checked in the media/substrate;
- conductivity should be no more than 40 millisiemens per metre;
- the media/substrate should be clean and free from toxicity; and
- the media/substrate should be used only once.

For laboratories that may have difficulties 'to check all the specifications or to get growing media from suppliers with the requested specifications', the ISTA Rules say that "it is permissible to replace the measure of conductivity [and pH] with biological tests such as phytotoxicity" (ISTA, 2022). Examples of media/substrate quality controls are given in the I*STA Handbook on Seedling Evaluation*.

The growing media/substrate should be stored in dry, secured places to avoid damage as a result of humidity, moulds or pests. Paper can be stored in the laboratory, whereas sand or organic growing media can be either inside or outside, as long as it is in a protected area with easy access for delivery and retrieval.

Note 7: Laboratories with a limited budget or that have difficulties getting supplies of the requested growing media/substrate can source their own, provided that the specifications are met. Sand can be collected from local rivers or deserts if permissible, provided it is heated in an oven before use to eliminate any possible microorganisms that may affect germination. Organic growing media may be purchased in local garden centres, and paper media or towels can be purchased locally at lower prices, such as in a supermarket.

Safety tips 8: *Disposal of growing media/substrate wastes after germination tests.*
The used growing media/substrate may represent large amounts of heavy wastes (sand or organic growing media) or wastes full of seeds potentially contaminated by unchecked/unknown diseases or carrying chemicals from seed treatment. It may be useful to have a protocol for waste disposal that should fulfil the local or national regulations and the laboratory policies on environmental management.

Note 9: *Germination of treated and coated seeds.*
Testing germination of treated and coated seeds presents several constraints:

- The laboratory may need to be adapted to ensure safety, e.g. better air extraction and ventilation in the planting rooms, over the benches. Personal protective equipment (PPE) may be necessary, e.g. laboratory coats, gloves, masks, safety glasses.
- The media/substrate may need to be adapted to adsorb the chemicals, to prevent phytotoxicity on seedlings and reduce the risk of erroneous test results. For this purpose, paper can be replaced by sand or organic growing media.
- The seed-coats of coated seed may have specific water absorption properties. The amount of water provided to coated seeds may need to be adjusted to eliminate conditions that are either too dry or too wet during germination testing.

8.3.3 Planting equipment

Planting by hand

Planting by hand is the most common method for sampling and sowing pure seeds to conduct a germination test (Figure 8.11). This method requires very little expensive equipment or materials but is time-consuming. Staff should receive training to avoid selecting the largest seeds.

The equipment needed for manual planting includes:

- tweezers, which can be protected with plastic in case of fragile small seeds;
- small containers free from electrostatic, in which the working sample can be placed before sowing (Note: It is best to avoid taking the seeds directly from paper bags or envelopes as the seeds could become stratified/layered by manipulation and therefore segregated by size);
- gloves;
- waterproof pens/pencils; and
- labels.

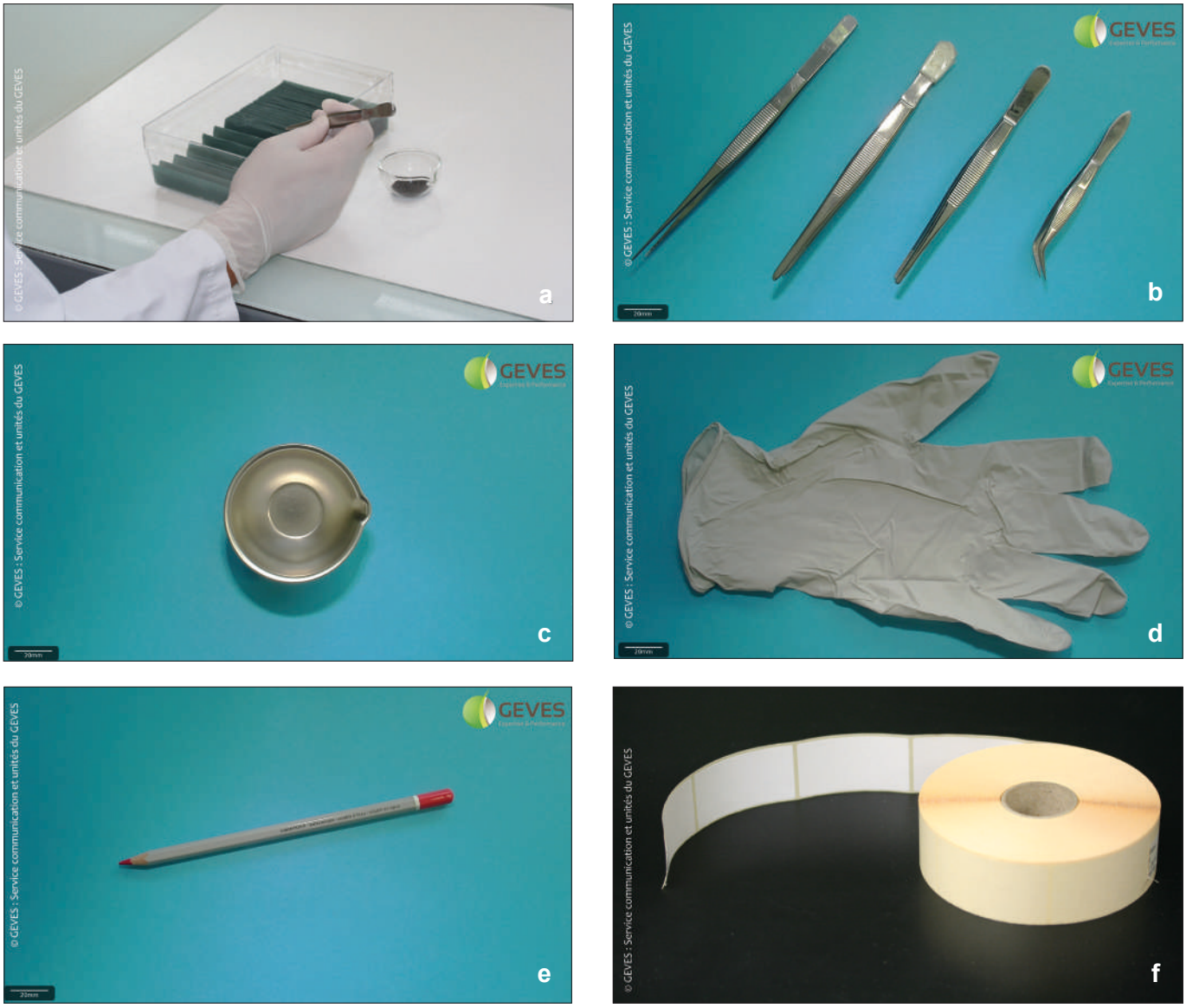


Figure 8.11. Small equipment for planting germination tests by hand (**a**): **b** tweezers; **c** container; **d** gloves; **e** waterproof pencil; **f** labels (images courtesy of ISTA accredited laboratory FR02)

Counting devices

For some kinds of seeds, counting devices have replaced time-consuming counting and sowing by hand. In general, provided that the seeds are not too small, some types of smooth, non-hairy and round to elliptical seeds can be satisfactorily arranged ('counted') into a grid pattern on these devices and then transferred to the germination media/substrate. There are two basic types of counting device:

- *Vacuum counting heads*, e.g. for seeds of the size of cereals (wheat and rice) and *Brassica* species.
- *Counting boards*, often used for counting maize, lupins, peas, beans and cereals, when there is no vacuum counting head available. Counting boards are relatively easy to make locally. They do not need power or a vacuum.

Counting device requirements

- The size of the vacuum counting head or counting board must correspond with that of the germination media/substrate and/or the size of the germination test replicate container. Seeds must be spaced regularly and distantly to prevent the spread of infection if disease is present on the seed.
- The seeds are counted in units of 25, 50 or 100, so that the germination test results can be easily computed and checked for their agreement with the appropriate ISTA tolerance table.
- In counting, seed segregation must be avoided. How the seeds are put on the counting head or board can determine whether segregation will occur. The rolling of the seed should be avoided as much as possible. The seed should be carefully poured out on different spots of the head or board to facilitate spread over the whole surface. The vacuum head should never be placed into the seed, as the lighter seeds will be selected.
- Because counting heads and boards are difficult to clean, using different heads and boards for chemically treated seeds is advisable.

QA tips 10: Check counting equipment to ensure that it does not influence the test results. Subsamples of 100 seeds can be taken successively from a pure seed working sample until the whole working sample is sampled. Each subsample is then weighed and/or germinated. The results of the weighing and/or germination tests for each subsample can be expressed on a graph to check any possible trends caused from the selection of seeds. Mixtures of different coloured seeds of the same species can also be used to detect selection. Other statistical analyses may be performed.

Vacuum counting heads

The dimensions of the head (Figure 8.12) will depend on the size of the germination boxes or filter paper but should always be fractionally smaller (e.g. for squares and rectangles: 0.75 cm less in length and width; for circles: 0.75 cm less in diameter). The diameter of the holes in the counting head varies with the size of the seed, e.g. 1.1 mm for seeds of the size of cereals; 0.3 mm for seeds of the size of *Brassica* species.

Some deviation in hole size is acceptable because the effect also depends on the strength of the vacuum. The counting head should have an edge to prevent the seeds from rolling off. The edge should be interrupted for some length to remove the surplus seed. Good results can be obtained by using a high-power vacuum cleaner for suction; however, the noise generated may be a nuisance. A dedicated vacuum compressor can be used, but these tend to make even more noise and need to be housed where the noise is not a problem, with the vacuum lines piped to where the vacuum is needed in the building.

Safety tips 11: *Use of vacuum counting heads for treated seeds.*
A vacuum cleaner exhaust may release fine dust and vapours from the chemical seed treatment into the atmosphere. Care should be taken to channel the exhaust air from the vacuum cleaner to the outside, or the vacuum can be placed outside but away from open windows or air intakes.

The general instructions to use a vacuum counting head are as follows:

1. Place the counting head horizontally with the holes upwards.
2. Close the valve and bring seeds onto the head with the vacuum turned off.
3. Switch on the vacuum and remove the excess seeds.
4. Check that there is one seed on each hole.
5. Turn the counting head over, release the vacuum and drop the seeds onto the media/substrate.

Figure 8.12. Vacuum counting heads: **a** typical head for planting; **b** various heads for different sizes of seeds and blotters; **c** vacuum planter for cereals; **d** head with large-sized holes for cereal seeds; **e** head with seeds attached (images a–c courtesy of ISTA; images d and e courtesy of ISTA accredited laboratory FR02)

Counting boards

Counting boards (Figure 8.13) are designed for large-seeded species like peas, beans or maize. They do not need any power and can be made in-house to suit the size of the germination containers used. The dimensions of the boards depend on the media/substrate, though the length and width should be about 0.75 cm less than the seed container or planting surface. The diameter of the holes in the board will vary and depend on the kind of seed tested but must allow the largest seed of a sample to pass through. The top and bottom layers have 25, 50 or 100 holes, and their number and size depend on the seed size and the dimensions of the seed container or planting surface. The bottom layer is movable with the same number and diameter of holes as the top and, until moved, is offset from the holes in the top layer. When the top layer of the board has been filled, the bottom layer is positioned so that the holes in the top and bottom layers correspond; the seeds will then fall onto the germination media/substrate. The counting board should have an edge on all sides to prevent seeds from rolling off but have a gap/space on one edge to discard the surplus seed easily.

Figure 8.13. Using a counting board: **a** filling the seed into the 50 holes and removing the excess seeds; **b** lifting the board away from the pre-wetted paper towel after the seeds have been released by pulling back the lower part of the board and allowing the seeds to remain on the towel; **c** the 50 seeds are evenly spaced on the single layer of wet paper towel ready to have another layer of wet paper added on top, before folding and rolling to complete preparation of one of eight replicates of 50 seeds for a 400-seed germination test (images courtesy of ISTA accredited laboratory CA08)

Other equipment for germination

Containers

- For germination tests using the TP or PP methods, reusable opaque or translucent plastic containers can be used to facilitate cleaning (see Figure 8.14 for examples). The size can vary and be adapted to the seed size. It may also be possible, for example, to pack four containers (14 × 17 × 4.5 cm) in a tray (30 × 35 cm). The container can be provided with a tight-fitting, high-transparency cover (e.g. 2 cm to 10 cm high depending on the size of the seedlings) for use under dry conditions (e.g. germination rooms, dry cabinets). Plastic bags are a cheap alternative to hard plastic lids or covers.
- For vertical rolled towel tests, plastic bags can be used to line the container. Rolled towels are placed inside the plastic bag, and the top of the bag is closed to stop moisture loss during the germination test.
- For germination tests in sand (e.g. for wheat, rice, maize and pulses), the same containers as for paper tests can be used (14 × 17 × 4.5 cm) with fewer seeds in each. The holding container or tray can also be larger, like those used in gardening or horticulture (e.g. 30 × 30 × 5 cm), with a tight-fitting, high-transparency cover/lid (e.g. 9–15 cm high) for use under dry conditions (e.g. germination rooms, dry cabinets), or without a cover/lid in wet germination cabinets or wet rooms with controlled RH.
- For germination tests using the PP method, a sheet of paper can be wrapped around the pleated paper.
- To prevent drying, the seed beds on the germination table can be covered with a bell jar and provided with a small hole permitting ventilation without undue evaporation. The bell jar should not completely cover the seed bed but leave a small edge of the paper exposed, allowing some evaporation. This ensures the transport of possible toxic and coloured substances to the edges of the paper.

Note 12: *Optimizing the space for germination incubation.*
Use containers and lids that can be piled or stacked to minimize the space occupied in the germination device. Placing the containers as sets of four on trolleys or trays facilitates packing and transport.

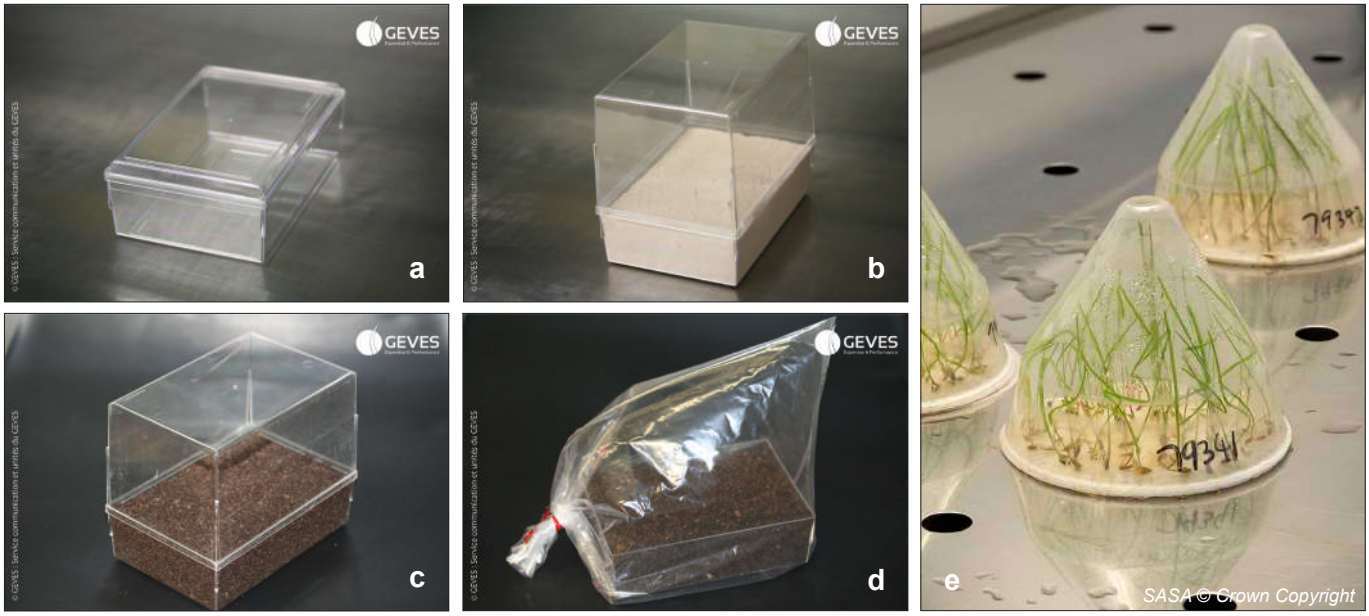


Figure 8.14. Examples of germination containers and covers: **a** plastic box with a transparent lid; **b** container with sand and a tall transparent lid; **c** container with organic growing media and a tall transparent lid; **d** plastic box with an inflated plastic bag as a cover; **e** bell jars (images a–d courtesy of ISTA accredited laboratory FR02; image e courtesy of ISTA accredited laboratory GB04)

Rakes and scrapers

Small rakes and scrapers (Figure 8.15) are used for loosening and smoothing the sand. The same type of scraper can be used for cereals and pulses, but for small seeds sown more shallowly, such as onions (0.5 cm depth), the scraper must not be too deep.

For cereals or pulses, the boxes are filled with sand to such a height that, after using the long side of the scraper to take away the excess sand, a seed bed layer of about 2.5 cm thickness is left. This layer is loosened with the rake and the seeds are sown. They are then covered with carefully raked sand while avoiding touching the seeds. Excess sand is then removed with the short side of the scraper. The resulting structure allows good gas exchange. For cereals and pulses, sowing depth should be 1.0–1.5 cm.

Figure 8.15. Use of a scraper (detail, bottom) to ensure a constant layer of substrate (images courtesy of ISTA accredited laboratory FR02)

Mixer

Use a small concrete mixer to mix sand with water to ensure the right moisture level for germination.

Spatulas

Metal or plastic spatulas can be used for counting seeds.

Cleaning equipment

Disinfection products and waste bags will be needed to clean the planting area and the benches after seedling evaluation.

Containers (bins) with wheels

Containers are used to store and eliminate the wastes at the end of the germination tests (sand, organic growing media, paper, seedlings).

8.3.4 Evaluation of seedlings, calculations and records of results

Evaluating the seedlings at the end of a germination test needs very little equipment (tweezers, gloves, pens, hard copies of worksheets/working cards). See Chapter 12 (Working forms and reports of analysis) of this handbook for worksheet examples. It is beneficial and sometimes required to use an illuminated magnifier to evaluate small seedlings more accurately and efficiently. The magnification strength is typically ×2 or ×3 but could be higher if desired. Magnification is very helpful with the more detailed evaluation of, for example, *Lactuca sativa* seedlings exhibiting necrosis or *Glycine max* seedlings exhibiting lesions. If affordable, a camera combined with a monitor or a computer is useful for both creating QA or research records and taking images of normal and abnormal seedlings as reference material for training analysts. Calculators and/or computers may be used to calculate percentages and check the tolerances, but all calculations can easily be made by hand. Analysts must receive training in germination testing based on the ISTA Rules and the *ISTA Handbook on Seedling Evaluation*.

Note 13: *ISTA tools and publications for germination tests.*
Tools have been developed by the ISTA Germination Committee and supported by the Statistics Committee, such as the calculation of water-retention capacity of media/substrate, check of cleanliness/phytotoxicity and pH measurement. These can be found in the *ISTA Handbook on Seedling Evaluation* and on the ISTA website (www.seedtest.org/en/services-header/tools/germination-committee/germination-toolbox.html).

Chapter 9: Viability (tetrazolium) testing

9.1 Viability (tetrazolium) testing method overview

The objective of the topographical tetrazolium (TEZ) test is to quickly estimate the potential germination of a seed sample, and also to determine if ungerminated seeds at the end of a germination test are dead or dormant. If 5 percent or more fresh seeds are identified after a germination test, a viability test should be done. In that case, any viable seeds are reported as fresh seeds, while the non-viable seeds are reported as dead seeds as part of the germination test results form/worksheet (see the *International Rules for Seed Testing*, ISTA Rules, sections 1.5.2.6 and 5.6.5.3).

The TEZ test determines if seeds are viable and have the potential to germinate at a later stage. The solution ingredient is 2,3,5-triphenyl tetrazolium chloride/bromide, which, when imbibed into the cells, reveals the reduction processes in living cells. The living cells of a seed embryo stain red while the dead cells appear unstained (Figure 9.1). Non-living tissue, like the endosperm of cereals, does not stain red.

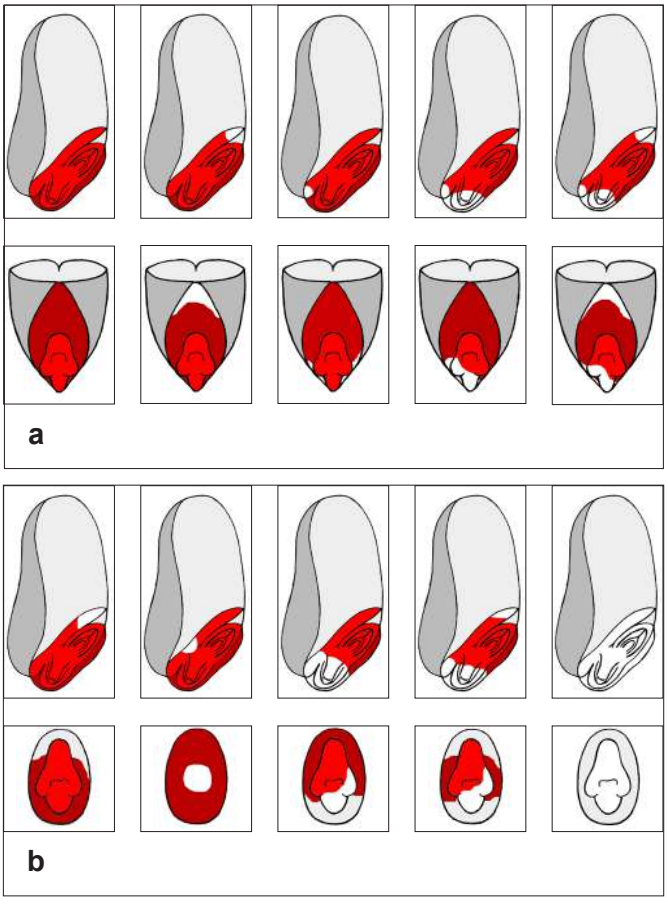


Figure 9.1. Viable (**a**) and non-viable (**b**) seeds stained with tetrazolium solution (2,3,5-triphenyl tetrazolium chloride/bromide)

9.2 Working sample

Following the ISTA Rules, a TEZ test is conducted on four replicates of 100 pure seeds, but fewer seeds and/or fewer replicates can be used as advisory tests. If an analytical purity test has been performed before the TEZ test, the pure seeds from this analysis are used. Otherwise, the pure seed needed must be taken separately from a subsample of the submitted sample. In this case, the sample must be mixed (see Chapter 7, section 7.1 of this handbook), and four replicates of 100 pure seeds are counted out for use (randomly taken, not selected). Preparing five additional spare seeds for each replicate is recommended in case the preparation of some seeds fails.

The TEZ test is undertaken when there is a need to estimate the germination potential of a seed lot in a short time; the whole test is completed in hours rather than days. Before staining, the seeds need to be pre-moistened with water (at about 20 °C, room temperature). This can be done by soaking in water or by placing seed on or between wet paper. More details on pre-moistening duration and preparation steps for each species are described in the ISTA Rules (Chapter 6: The topographical tetrazolium test) and the *ISTA Working Sheets on Tetrazolium Testing*. The moistened seeds are easier to prepare (cutting) and allow the colour to be more even, thus facilitating the evaluation. For many species the seed needs to be cut or the seed-coat pierced, punctured or removed so that the tissue of the seed embryo is exposed and the TEZ solution can easily penetrate. During seed preparation, the analyst must ensure the seed does not dry out. The cutting of all 400 seeds can take a considerable amount of time. As each seed is cut it can be placed into a water container. However, the preparation steps should be completed as quickly as possible so that living cells are not damaged. The specific guidelines for seed preparation, including the cutting, piercing and excision of the embryos or removal of the seed-coat, are listed for the species included in the ISTA Rules (Chapter 6) and in the *ISTA Working Sheets on Tetrazolium Testing*.

After the seeds are prepared, each replicate can be placed into a container, and the TEZ solution can be added to cover the seeds. If single seeds are floating on the surface, they can be pushed down with forceps. The containers with the seed and TEZ solution are kept in the dark in an incubator, cabinet, germinator or oven at 30 °C +/−2 °C for the staining period specified in the ISTA Rules. If there is any risk of evaporation, the container must be covered with a lid and aluminium foil can be used to inhibit light exposure. To check the reliability of a test result, the average percentage of the replicates is calculated to the nearest whole number and compared with Table 6B of the ISTA Rules (reproduced as Table 9.1 here). The result is reliable if the difference between the highest and lowest replicate does not exceed the

indicated tolerance. Maximum tolerated ranges for replicate differences are the same as for germination tests. The results are reported as percent viability with the details of the exact method used if it varied from the prescribed TEZ solution concentration or staining temperature of 30 °C.

QA tips: When reporting on ISTA Certificates (see ISTA Rules, section 1.5.2.8), remember to record and report the test conditions used if the exact conditions listed in the ISTA Rules, Chapter 6, are not followed.

Table 9.1. Maximum tolerated range between four replicates of 100 seeds in one tetrazolium test (two-way test at 2.5 percent significance level)

Average viability (%)		Maximum range
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93–94	7–8	10
91–92	9–10	11
89–90	11–12	12
87–88	13–14	13
84–86	15–17	14
81–83	18–20	15
78–80	21–23	16
73–77	24–28	17
67–72	29–34	18
56–66	35–45	19
51–55	46–50	20

Source: **ISTA.** 2022. *International Rules for Seed Testing*. Wallisellen, Switzerland, International Seed Testing Association, Table 6B.

9.3 Materials and equipment

9.3.1 Tetrazolium salt

The TEZ salt 2,3,5-triphenyl tetrazolium chloride/bromide is used with a 1 percent concentration dissolved in distilled/deionized water or buffer at pH 6.5–7.5. Lower concentrations of TEZ salt are permissible, but the staining time needs to be prolonged in this case. The TEZ salt should be stored in dry, cool and dark conditions.

Safety tips: Follow chemical safety sheets when using tetrazolium salts and solutions.

9.3.2 Buffer solution

It is recommended to prepare the TEZ solution in phosphate buffer solution to achieve the correct pH range. Two solutions are prepared with distilled/deionized water:

Solution 1: Dissolve 9.078 g KH_2PO_4 in 1000 ml distilled/deionized water

Solution 2: Dissolve 9.472 g Na_2HPO_4 in 1000 ml distilled/deionized water

Two parts of Solution 1 are mixed with three parts of Solution 2; the pH must be between 6.5 and 7.5. The correct amount of TEZ salt is then dissolved in this buffer solution.

9.3.3 Tetrazolium solution

To produce a 1 percent TEZ solution in 1000 ml buffer or distilled/deionized water, 10 g of TEZ salt is carefully weighed and mixed, using a laboratory magnetic stirrer if available. The pH of the solution must be between 6.5 and 7.5. The solution needs to be stored in dark and cool conditions (5–10 °C) where it can be kept for up to 1 year. If stored for longer, an efficacy test must be conducted to prove that the solution is still viable. It is good to use a control sample of live seeds for each batch of samples during routine testing.

9.3.4 Tools for manipulation

Seeds often need to be cut, pierced or peeled. A sharp scalpel, needles and forceps can be used for these activities. All cutting equipment must be sharp; otherwise, bruises in the inner seed will complicate the assessment of the stained parts.

Safety tips: Be careful not to accidentally cut yourself when using sharp scalpels, needles and forceps. Wear eye protection to avoid eye injury by 'flying' seeds when applying pressure to cut them.

Preparation of hard kernels like tree nuts will require cutting pliers, hammers or a hacksaw to open the nuts and a vice to hold them safely. Safety glasses are recommended when breaking open tree nuts.

9.3.5 Containers

Suitable containers should be heat- and chemical-resistant and easy to clean, ideally with a lid. Small glass beakers with aluminium foil lids can be used.

9.3.6 Staining environment

For the staining time with the TEZ solution, the seed/solution container must be placed in a controlled temperature environment at 30 °C +/−2 °C in the dark. This can be an incubator, oven, cabinet or germinator without illumination. If there is a risk of evaporation affecting the concentration of the TEZ solution, the containers must be covered.

9.3.7 Sieves

At the end of the staining period, the seeds need to be removed from the TEZ solution, and the solution disposed of properly. This can be done by straining the seed and TEZ solution through a mesh sieve or strainer. The seeds are then rinsed/washed under running water, to stop the staining process. The stained seeds must then be covered with water (tap water is suitable) to prevent them from drying out until they are evaluated.

9.3.8 Magnification

Depending on the size of the seeds, a magnifier (with or without illumination) or a stereo binocular microscope should be used to allow for precise preparation (cutting, piercing, peeling) and assessment of the stained seeds (Figure 9.2).

Figure 9.2. Stained seed being examined under a stereo binocular microscope and a beaker of seeds ready for examination as part of the viability (tetrazolium) test (image courtesy of ISTA accredited laboratory CA08)

9.4 Assessing viability

To assess viability, the stained parts of every single seed need to be evaluated. Depending on the species, different essential parts of the seed must be stained red to rate the seed as viable. The germination and viability (TEZ) values can be closely aligned but in certain circumstances the viability test can yield higher results. This is because the staining caused by the TEZ solution may include seeds as viable that would not be counted as normal seedlings in a germination test as a result of fungal diseases, dormancy issues or some mechanical damages. Detailed descriptions of the evaluation of viable seeds are available in the ISTA Rules (Chapter 6) and in the *ISTA Working Sheets on Tetrazolium Testing*.

9.5 Method verification

9.5.1 Quality assurance checks

To check the suitability of the TEZ solution, the following quality assurance (QA) checks should be done:

- Record the batch numbers of the chemicals and check expiry dates. If the expiry date has passed and the salt looks good (still powdery), a seed lot of known viability can be tested to determine if the TEZ salt still reacts and causes staining.
- Check that the pH of the TEZ solution is between 6.5 and 7.5.
- Record the TEZ solution's production date and expiry date on the bottle.
- Compare the efficacy of the newly prepared solution alongside a residue of the old solution by preparing and staining 100 seeds in both solutions. Evaluate the seeds, and if the staining quality and results are equivalent (small tolerance allowed), the solution is fit for use.

QA tips: Use control samples of known viability to allow comparisons of old and new stock solutions or as part of ongoing testing to ensure living tissues are stained red. This is only necessary when using a new batch of tetrazolium salt. If it is the same salt, only the pH of the new solution has to be checked.

9.5.2 Controlled temperature environment

The environment used must be checked periodically to ensure conforming with the allowed temperature range of +/−2 °C for the staining period at 30 °C. Cabinets used for other purposes can be employed, temperatures re-set if needed and monitored during the test. Staining can take place between 20 °C and 40 °C, but 30 °C is optimal.

Chapter 10: Moisture testing

10.1 Moisture testing methods

The objective of the moisture analysis is to determine the moisture content of a seed sample with an oven method or a moisture meter (non-destructive rapid test). The moisture analysis is a standard method that customers often request from seed testing laboratories. The percent moisture content of seeds is on a fresh weight basis and can influence the germination, vigour, storage capability, processing attributes and weight of a seed lot. In some countries, seed may need to be below a moisture content threshold before it is sold.

The more precise method is to dry a sample of known weight in an oven to drive off the free water, then re-weigh the sample and determine the weight loss, which is then calculated as the percent moisture (water) content on a fresh weight basis. The ISTA oven method uses a low- (103 °C) or high- (130 °C) temperature drying method, depending on whether the species has a high or low oil content. The drying time and the need for grinding also vary for different species. For reporting on an ISTA Certificate, see the specific test method requirements for the different species listed in Chapter 9 (Determination of moisture content) of the *International Rules for Seed Testing* (ISTA Rules).

Electronic moisture meters estimate the amount of water in a bulk of seed by passing electricity through and measuring the electric conductivity of the bulk. Their accuracy is acceptable at normal ranges of moisture content (10–15 percent) and decreases in seed samples that are too dry or too wet. Near-infrared (NIR) or near-infrared transmittance (NIT) moisture meters measure the absorbance and reflectance of light to determine the moisture levels. The more light that is absorbed, the higher the moisture content. The light that is reflected back to the device is converted using an algorithm to provide an accurate reading.

A comparison of the two methods for moisture testing conducted by laboratories following the ISTA Rules is shown in Table 10.1, with advantages and disadvantages for each method.

Table 10.1. Comparison of the two moisture content methods that can be used if following the ISTA *International Rules for Seed Testing*

	Moisture testing in oven	Moisture testing with moisture meter
Overview	Seeds are prepared (grinding or no grinding), weighed and placed in a controlled-temperature drying oven. After a defined time, the samples are cooled down and weighed again. The difference in weight is reported as percent moisture content. The process can take between 1 h and 17 h, plus preparation time, or longer if pre-drying is required.	The seed moisture content is measured by a moisture meter. This process is mainly non-destructive and the measurement is done within a few minutes.
Advantages	Precise method; 17 h at 103 °C is the ISTA reference method for moisture testing.	Measurements in a few minutes.
Disadvantages	Longer duration. Laboratory verification for the grinder and the oven are required on a regular basis.	Ongoing external calibrations and laboratory verification are required; potentially higher initial and ongoing costs. Precision decreases at extreme moisture contents, i.e. when seed is too dry or too wet.

To ensure accurate results, the seed sample for some species must be ground before determining the moisture content. This can affect both oven and moisture meter methods; for example, the glumes of chaffy seed may act as insulation against electric conductivity and produce readings of moisture content below the real value. Equipment for moisture testing using the oven method or the moisture meter method is shown in Figure 10.1.

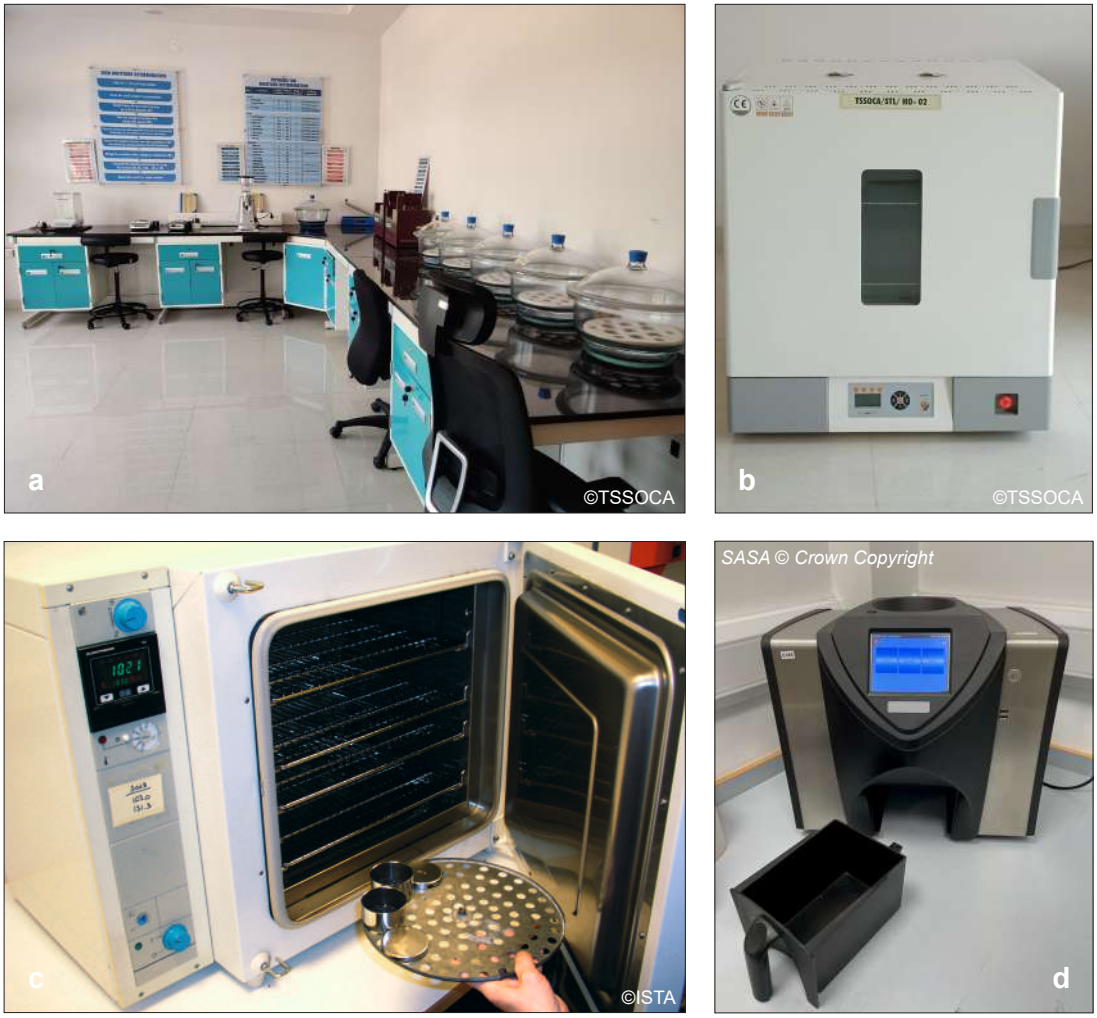


Figure 10.1. Moisture testing equipment: **a–b** moisture room and hot air oven (images courtesy of ISTA accredited laboratory IN39); **c** samples being placed in oven with containers open (image courtesy of ISTA); **d** example of a moisture meter (image courtesy of ISTA accredited laboratory GB04)

10.1.1 Submitted working samples

After obtaining the composite seed sample, the submitted moisture sample must immediately be taken because there is a risk that the room's atmospheric humidity might influence the seed sample's moisture content (gain or loss). Table 10.2 shows appropriate submitted sample sizes for moisture testing, according to the moisture method used.

Table 10.2. Minimum submitted sample sizes for moisture testing

Moisture testing method	Submitted sample size (g)
Oven method – no grinding or cutting Moisture meter	50.00
Oven method – fine and coarse grinding	100.00

To take the submitted sample, the composite sample is mixed once with a spoon or a divider, then seeds are removed with a spoon or scoop from at least three positions in the sample, and placed into a waterproof container (Figure 10.2) for transport from the sampling site to the laboratory. Suitable containers are bags made of laminated foil plastic/polyethylene that can be sealed airtight (e.g. with tape or by heat sealing). Another option is to use glass or plastic/polyethylene containers with an airtight top (see Figure 6.6e). After filling the container, air must be released, or in the case of a glass jar, it must be filled fully.

Note: Transporting samples for moisture content testing can be challenging. Air must be excluded from the sample: if the moisture content of the seed sample is too high under sealed aerated conditions, the bulk of seed may begin a process of increased respiration that will alter the results. In hot countries or during hot summer periods, samples may need to be transported quickly from the sampling location to the laboratory. Refrigerated or cooled transport methods may also be considered.

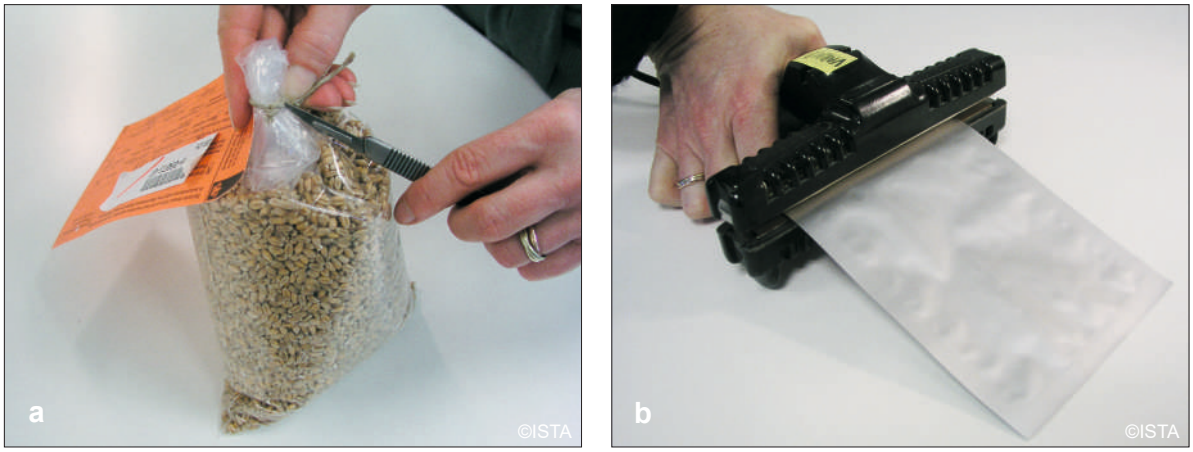


Figure 10.2. Moisture-proof bags for submitted moisture samples: **a** plastic bag with seal; **b** foil bag with heat sealing (see also Figure 6.6e for a rigid plastic container with an airtight top) (images courtesy of ISTA)

To verify the suitability of a submitted moisture sample container, the following checks must be done:

- Is the container suitable for the volume of seeds (different thousand-seed weights) and easy to fill?
- Can it be sealed easily, even when sampling outside the laboratory?
- Is it waterproof? To simulate the time from sample creation to delivery in the laboratory, fill ten containers from a new batch of containers with water, weigh them, store the containers under normal room conditions, and weigh the containers again after 1 day, 3 days, 5 days, 10 days, or longer if needed. The difference between the original and final weights for each container must be 0.2 percent or less during the simulated delivery periods tested, for the batch of containers to be considered suitable.

The submitted moisture sample should be delivered and analysed in the laboratory as soon as possible.

10.1.2 Working sample

To prepare the working sample, the submitted sample is opened and stirred with a spoon, or the original container's opening is placed against the opening of a similar container, and the seed is poured back and forth between the two containers. This process should be completed in no longer than 1 min.

The weight of the working sample (whole seeds) or replicate (cut or ground seed) is dependent on the diameter of the drying containers used, as follows:

- diameter greater than 5 cm and less than 8 cm: 4.5 +/−0.5 g; or
- diameter greater than 8 cm: 10.0 +/−1.0 g.

The seeds or ground material should be evenly distributed in the container with a maximum mass per unit area of 0.3 g/cm^2.

10.2 Oven method process and equipment

QA tips: Remember to verify equipment as fit for use before using to test samples.

If no grinding is required, two replicates weighing 4.5 g (+/−0.5 g) each must be taken with a spoon from at least three different positions in the submitted sample and placed into suitable pre-weighed containers. This working step should be performed in less than 30 s per replicate to avoid moisture loss.

For the grinding process, the submitted sample is mixed, and a part of it is ground in a grinding mill with the setting for fine or coarse grinding, depending on the species. The grinding process should be limited to 2 min. After grinding, the mixing and subsampling for two replicates is done according to the sample preparation above.

Large tree seeds and those with a hard seed-coat (e.g. Fabaceae) or those with high oil content, could be cut into pieces smaller than 7 mm instead of being ground with a mill. The cutting must be done on at least ten intact seeds from the working sample to have approximately 5 g for each of the two replicates. The cutting process must take no longer than 4 min. After cutting, the pieces are recombined, mixed and weighed in the containers.

Note: Depending on the grinding mill used, it is possible to grind hard seeds and partly oily seeds (e.g. *Moringa oleifera*).

A flow chart of the oven method for moisture testing is presented in Figure 10.3. To comply with ISTA accreditation, the laboratory needs a grinder, sieves, an oven, drying containers and desiccators, as well as quality assurance (QA) calibration and verification processes for the oven method.

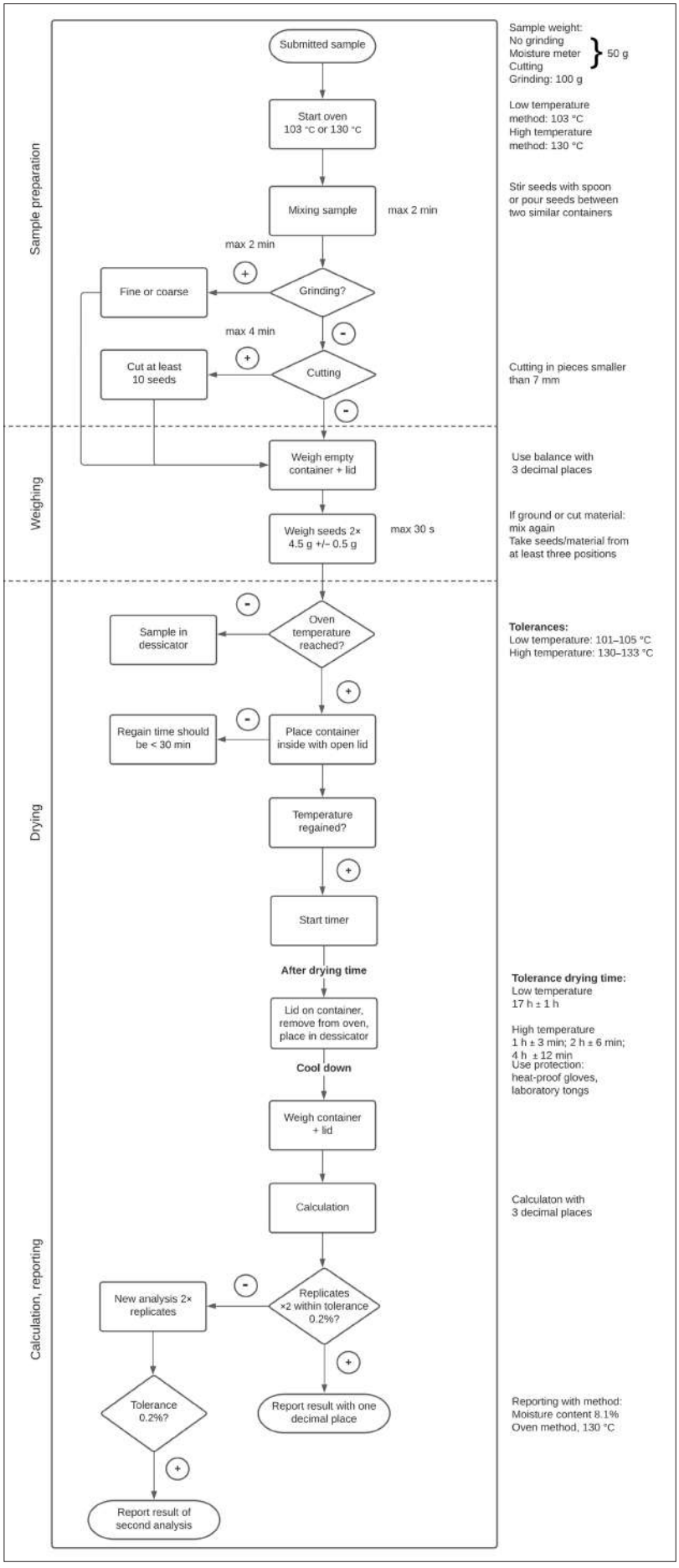


Figure 10.3. Flow chart of the oven method for moisture analysis

10.2.1 Grinding mill

The grinding mill (Figure 10.4) must consist of materials that do not absorb moisture and are easy to clean. The grinding needs to be done rapidly and uniformly, and the mill should have as little dead space as possible and not develop too much heat during the grinding process. The mill needs to be adjustable to obtain different fine and coarse grinding particles, or different mills are required to conduct fine and coarse grinding.

Note: See Chapter 9 of the ISTA Rules to find out which seed species need to be ground before moisture testing.

Particle size for *fine* grinding:

- At least 50 percent must be smaller than 0.50 mm, i.e. pass through a 0.50 mm mesh sieve.
- A maximum of 10 percent can be bigger than 1.00 mm, i.e. can remain on a 1.00 mm mesh sieve.

Particle size for *coarse* grinding:

- At least 50 percent must be smaller than 4.00 mm, i.e. pass through a 4.00 mm mesh sieve.
- A maximum of 55 percent can be smaller than 2.00 mm, i.e. pass through a 2.00 mm mesh sieve.
- After every grinding process, the mill needs to be cleaned to avoid any contamination with residues from the former ground material. To clean the grinding mill, a brush, a vacuum cleaner and/or air pressure can be used.

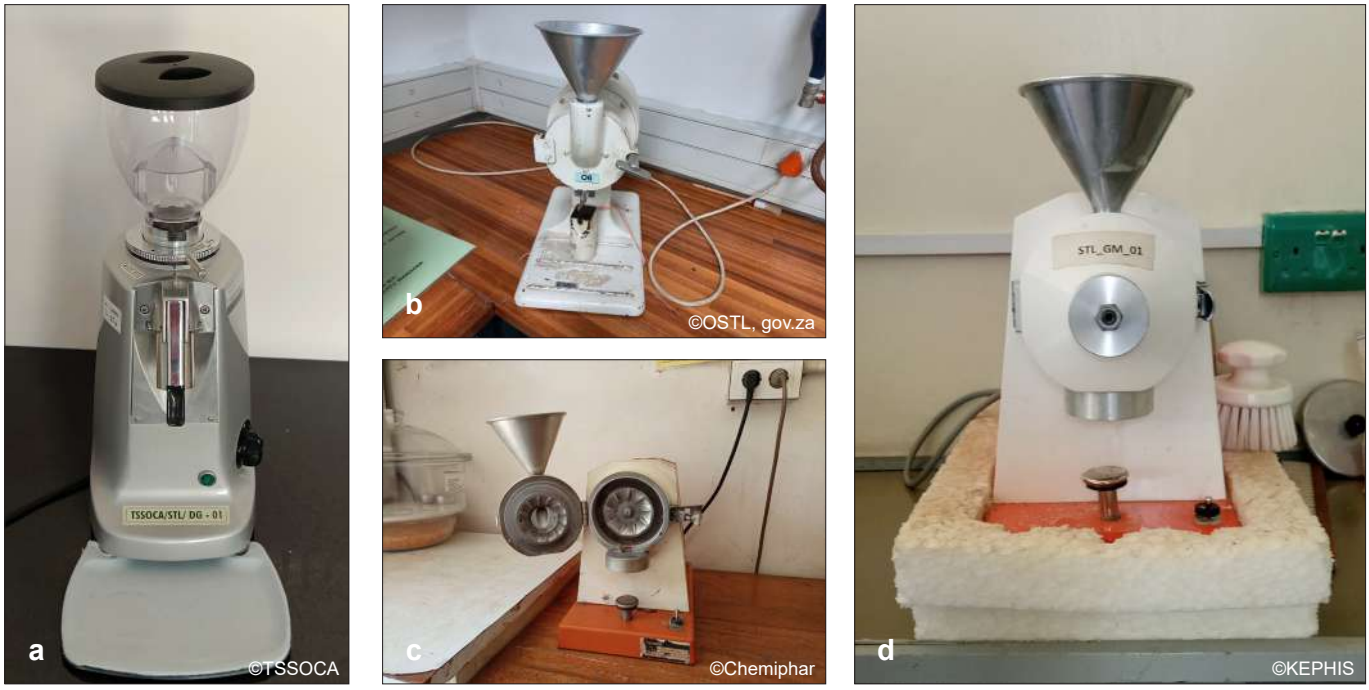


Figure 10.4. Grinders for preparation of seed moisture samples: **a** disc grinder; **b** adjustable course and fine grinder; **c–d** seed grinders (images courtesy of ISTA accredited laboratories IN39, ZA01, UG02 and KE01)

10.2.2 Sieves

Sieves can be purchased separately and should be stackable with a bottom collection pan. To verify the particle size of the grinding mills, the laboratory needs different wire sieves, preferably from a manufacturer which can provide a certificate for the precision of the sieves. If both fine and coarse grinding are required as part of the testing, sieves with meshes of 0.50 mm, 1.00 mm, 2.00 mm and 4.00 mm will be needed, as well as a collection pan.

10.2.3 Electrically heated oven

The moisture oven must be electrically heated, have sufficient insulation, a vent to allow the moist air to leave, and a fan for the uniform temperature distribution in the oven. The oven must be capable of maintaining 103 °C for low-temperature drying for a period of at least 17 h and 130 °C for high-temperature drying for a period of at least 4 h, on all shelves used. After placing the samples in the oven, the oven must reach the set temperature within 30 min.

The tolerances for the temperatures are:

- *Low-temperature method* requires 103 °C, but the temperature range can be 101–105 °C. Best to set the oven at 103 °C.
- *High-temperature method* requires 130 °C, but the temperature range can be 130–133 °C. Best to set the oven at 131 °C.

To check the suitability of the drying oven, two verifications must be done, on first purchase and at regular intervals after purchase. Measurements should be conducted with a reference thermometer on all oven shelves in different positions (front, middle, back in all dimensions). Reference thermometers with a wire (to enable reading the temperature without opening the door) and data loggers are most suitable for verification. All temperatures should be within the set tolerances.

To perform the ventilation test (ISTA Rules, section 9.2.4.2), the maximum number of samples that fit or will be used in the oven must be prepared at the same time. Suitable samples are then dried using the low- (17 h at 103 °C) or high-temperature method (using a species with a 2 h test duration only at 130 °C), then removed from the oven and allowed to cool in a desiccator before weighing and calculating the moisture content. The same samples are then re-dried (2 h low-temperature method or 1 h high-temperature method) with the containers in the same position as for the first drying period and allowed to cool in a desiccator before weighing. The percent moisture content is recalculated. For individual samples, the variations in moisture content must not differ by more than 0.15 percent. This check must be performed using a species that requires a high temperature and a drying time less than or equal to 2 h. The same species can be used for high and low-temperature methods.

10.2.4 Containers

The container for the drying process must be heat-resistant and not moisture absorbent. Glass or metal containers with a lid (e.g. tins) are suitable (Figure 10.5).

Figure 10.5. Containers used for oven drying of samples (image courtesy of ISTA accredited laboratory SN01)

10.2.5 Desiccator

A desiccator is needed to allow the samples in the hot drying containers to cool without altering the moisture content before re-weighing (Figure 10.6). It can also be used if there is an unexpected waiting time during sample preparation. The lower part of the desiccator is filled with a desiccant (e.g. silica gel, activated aluminium) and a screen/shelf. Many desiccants require activation in 130 °C or higher (depending on the desiccant) for up to 24 h before use and then regularly afterwards. They can be self-indicating by a colour change which shows when reactivation is required. If the lid does not seal the container, the lid's outer rim, where it is connected with the main container of the desiccator, can be covered with a thin layer of Vaseline (petroleum jelly) or laboratory grease to secure it.

Figure 10.6. A desiccator for cooling samples after oven drying (image courtesy of ISTA accredited laboratory IN39)

10.2.6 Balance

For the measurements and calculations during the moisture analysis, a three-decimal place balance (Figure 10.7) able to weigh up to at least 50 g, if not more, is required to allow for the 10 g sample weight plus the weight of the drying containers. The three-decimal place balance needs to be accurate to at least +/−0.001 g.

Figure 10.7. Balance and containers for sample weighing (image courtesy of ISTA)

10.2.7 Cutting tools

Cutting is required for some species, e.g. *Arachis hypogaea*, *Ricinus communis* and certain tree and shrub species. Cutting tools can be a knife, a scalpel or pruning secateurs.

10.2.8 Safety equipment

If testing for moisture is conducted on treated seeds, the high temperature may alter the chemicals applied to the treated seeds and create toxic vapours in the room. Special safety measures such as extra room ventilation, more frequent air extraction and personal safety equipment (e.g. safety mask) may be needed.

Safety tips: Wear heat-resistant gloves and use laboratory tongs/pliers when handling hot containers from the oven.

10.3 Moisture meter method process and equipment

Measuring moisture using moisture meters is a rapid test method. Different types of moisture meters exist (e.g. NIR or NIT technology). They must be calibrated before the first use and regularly during routine use to ensure proper functioning. If testing to verify a moisture meter is needed for reporting on an ISTA Certificate, a comparison with the reference oven method in the same laboratory can be used. In other situations, an external calibration service, a verified reference sample or a comparison to the oven method can be applied. The process is explained in detail in Chapter 9 of the ISTA Rules (section 9.3.1.5.1) and the *ISTA Handbook on Moisture Determination*.

10.4 Test results

For the oven moisture determination method, a calculation is done to obtain the percent moisture content. The calculation is performed with three decimal places. The moisture content is then reported with only one decimal place for the final result. Between the two replicates, a maximum difference of 0.2 percent is allowed and can be reported. If the difference is higher, a retest must be done.

Calculation:

$$\frac{Loss\ of\ weight}{Initial\ weight} \times 100 = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where
M_1 is the weight in grams of the container and its lid;
M_2 is the weight in grams of the container, its lid and the seeds before drying; and
M_3 is the weight in grams of the container, its lid and the seeds after drying.

The moisture reading taken with a moisture meter can be recorded to more than one decimal place but must only be reported to the nearest 0.1 percent, as for the oven determination method. The correct rounding procedure is included in the *ISTA Handbook on Moisture Determination*. It is essential to follow this guide to avoid errors when reporting moisture test results.

Chapter 11: Sample storage

11.1 Storage before testing

Seed with orthodox storage characteristics is easily transported in its dry state, but it is worth remembering that it is alive, or at least it should be as it needs to grow when planted. Therefore, seed samples taken for testing must be stored under suitable conditions (e.g. ambient, often around 20 °C and relative humidity, RH, of less than 60 percent) before testing, so that general seed quality is not adversely affected. To facilitate this, when samples are taken and packaged into suitable containers (see Figure 11.1 and Figure 6.6) and undergoing transit, they are not exposed to extremes of heat, cold or high humidity. See the other chapters in this handbook for needs specific to the different tests; for example, moisture-proof containers for moisture testing. Testing can usually start soon after the samples arrive at the seed testing laboratory. If the start of testing needs to be delayed for several days or weeks, sample storage prior to testing needs to be considered under a controlled environment that would delay seed respiration and ageing.

QA tips: Seed is alive. Mishandling between sampling and testing, e.g. high temperatures, high humidity or mechanical damage, could adversely affect test results and would no longer represent the quality of the seed lots.

Figure 11.1. Storage systems after testing: **a** cotton bags; **b** paper bags; **c** plastic bags for moisture testing to prevent any water loss; **d** mixed container types (images courtesy of ISTA)

Storage conditions vary depending on the tests performed:

- Components from an analytical purity or other seed determination test can be stored under various conditions, provided that the components' morphology and external physical characteristics are kept intact.
- Temperature and RH are important parameters for the samples in germination or viability (tetrazolium) testing.
- Moisture levels can change over a short period.
- Temperature and container types are important criteria to consider.

If live insects or mites are found during testing, samples should be moved to a −20 °C freezer. Even if the seed samples are not sufficiently dried before freezing, the low temperature kills the insects and prevents them from contaminating other samples. The samples could be left at about −20 °C until disposal or for a minimum of 2 weeks and then monitored for insect or mite activity.

11.2 Storage after testing

After testing, the samples must also be stored under suitable conditions for several reasons. Some countries may have legal requirements to retain samples for a set period. If there are no regulations, it is advisable to keep them for at least 2 months after the end of the planting season to cover any retesting that might be needed in case of end user complaints. After that time, another sample would need to be taken from any remaining seed if a complaint was made about the seed lot.

It is good general practice to store the tested sample and the components of the analytical purity test (e.g. pure seeds, inert matter, other seeds found) for a defined period in case the customer has any enquiry after the testing is reported. This is also a requirement if the laboratory is ISTA accredited. The ISTA Accreditation Standard requires storage under conditions that maintain the quality of the seed sample to allow for retesting or any other follow-up for at least 12 months. For example, the stored sample would allow investigation if, on arrival in the importing country, the germination was very low, or if a weed species was found but not reported in the initial analysis.

The need to go to the sample again after testing is rare, and it is acceptable to define that, for example, moisture test quality deteriorates under local storage conditions after 3 months, so that the moisture sample would be disposed of after 3 months from results issuance. The same principle could also be applied for analytical purity and germination testing.

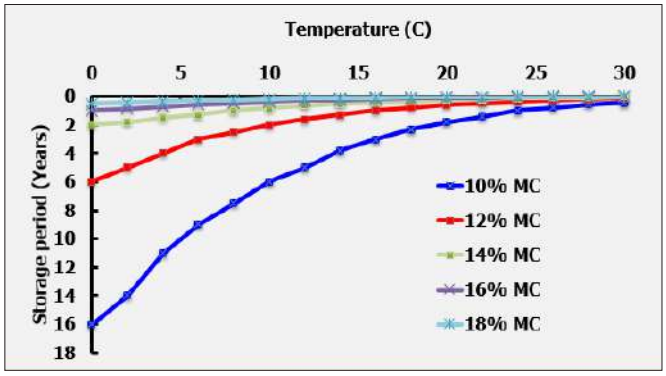
11.3 Suitable storage conditions

Suitable storage conditions will vary depending on the defined storage period, geographic location and time of year. In some regions, controlled temperature and RH storage will be required. In all regions, protection from pests (e.g. insects, mites, rodents) is needed. Pest control may require preventive traps (insects or rodents) or regular fumigation.

Safety tips: Insect infestation can pass from one sample to another. Fumigation of rooms or chemical treatment of walls by a professional or licenced person is necessary. Applying chemical products inside a closed building may harm human health, so staff safety needs to be considered.

For some parts of the world, dehumidification might also be needed. In other areas, the local conditions can be dry but hot, so only temperature would be a concern, or cool and dry (e.g. a basement in some locations), so neither temperature nor RH would be a concern. Storage duration is directly linked to the seed moisture content of the sample received. Storage temperature and RH combine to affect the longevity of samples in storage (see Figure 11.2). For most species, based on general feedback from laboratory audits, a temperature of less than 15 °C and a RH of less than 50 percent are suitable to maintain germination/viability for at least 1 year of storage. Other combinations of temperature and RH may also be suitable. The temperature and, if possible, the RH should be monitored to see what conditions can be achieved; a moisture or humidity indicator could be placed in the storage room. Meteorological records, if available, can be used to determine the annual local ambient conditions. This can help with planning and decisions about possible storage under ambient conditions. If the RH is low in the storage area, moisture loss effects of samples stored for germination should also be considered.

QA tips: Only the temperature needs to be monitored in a long-term storage room, provided the stored samples have a low or standard moisture content. A storage temperature of 15 °C is usually safe for cereal samples at 12 percent moisture content or less, legumes at 10 percent or less, and oilseeds like *Brassica* species at 8 percent or less for at least 1 year. Care is needed with oily seeds since viability can often decline faster at higher storage temperatures.



Source: Adapted from **Ellis, R.H & Roberts, E.H.** 1980. The influence of temperature and moisture on seed viability period in barley (*Hordeum distichum* L.). *Annals of Botany*, 45(1): 31–37.

Figure 11.2. An example of how storage moisture and temperatures affect the speed of loss in viability of barley (*Hordeum distichum* L.) seed samples that were dried to a range of different moisture contents (MC) prior to storage

Storage in original sample containers is acceptable (e.g. paper, cotton bags, aluminium foil bags, glass containers or plastic). However, care must be taken if using airtight or plastic containers, especially if the seed moisture content is high. For seeds with high moisture content stored in plastic, the temperature can increase in the sample, and any surface fungi, bacteria or insects present can start to grow and become a problem. With respiration and excess surface water in long-term storage, the seed can easily become mouldy or start germinating (Figure 11.3).

QA tips:

- Keep storage areas clean, tidy and pest-proof (from insects, mites and rodents). Fumigate if necessary and if safe to do so.
- Use unique sample numbers and make labelling obvious so it is easy to find samples.
- Keep a sample inventory with records on sample ID, date received and disposal date.
- Store samples in consecutive order to make sample tracking and disposal easier.
- Maintain a record of the temperature and, if possible, relative humidity (RH) of the storage room. Record any extreme conditions that might affect the storage environment.
- Plan to retest stored samples to detect any significant loss in germination capacity.
- Maintain records of any quality control checks made.

Figure 11.3. Examples of storage pests: **a** bean weevil; **b** saw-toothed grain beetle; **c** saw-toothed grain beetle larvae (images courtesy of Cereal Research Centre, AAFC)

11.4 Size of storage area

The size of the storage area needs to be considered based on the annual sample testing numbers and any planned expansion of testing. The area should be organized to allow an easy workflow when adding new samples and disposing of old ones.

Note: Ten 1 kg samples occupy a storage space of about 0.35 m x 0.45 m x 0.25 m.

To help in planning the size of the storage area needed in a new laboratory and to allow the exchange of old and new samples, it is a good idea to include twice the space needed for one annual cycle of samples. Shelving or labelled boxes/containers will facilitate easy sample retrieval and maximize efficient use of the storage area (see Figure 11.1). To ensure staff health and safety, remember not to overload boxes and make them too heavy for lifting. The use of closed but not airtight plastic sample storage containers can be a good solution as they can limit the development of insects and cross-contamination. Such containers can be more easily stacked on themselves and on shelves.

11.5 Disposal of samples

Maintain a storage and record-keeping system and establish a procedure to keep track of the dates the samples were stored and disposed of. Identify areas to discard the seeds or contact waste companies, accounting for possible risks of spreading seeds that could be infected with pathogens, contaminated with noxious seeds, or treated with chemicals. Incineration of seed samples after testing is another possibility, where available.

Chapter 12: Working forms and reports of analysis

12.1 Working forms

Seed laboratories need ways to capture raw data, complete calculations where necessary, record results and report rounded results to the correct number of decimal places. In other words, it is important to know what was done, by whom and when. Systems using worksheets or forms that create permanent and traceable records of the tested sample can be paper-based or digital/computer-based. Depending on a laboratory's work processes, the working forms can be entirely paper-based, partially paper-based (e.g. lot and client information and reporting are digital, while working cards are on paper), or fully digitalized, including entering and retaining the results.

As a general requirement, it is necessary that at sample receipt, a unique/individual sample number is generated for and allocated to each sample (e.g. 21-0005 may be the fifth sample received in the year 2021). For uniformity and clarity, the laboratory can define how the year is designated; for example, the calendar year 2021 can be shown as 21, or the work year from 1 April 2020 to 31 March 2021 can be shown as 2020/21, or the seed year could span the period from 1 July 2020 to 30 June 2021. Use the sample number for the sample request, working cards, reported results, storage and to track disposal. In effect, the unique number will connect all information and work done on a sample, including original observations, data and calculations recorded; it provides a traceable audit trail. Using a digital barcoded system might help in this process.

For all work processes such as sample registration, preparation, analysis, reporting and storage, the person conducting the activity must also be traceable. A known name, staff number, and approved initial or staff signature can be handwritten on forms or added with a controlled stamp or digital signature, so critical parts in the different testing stages can be tracked. The dates for the sampling request, sampling report, registration, analyses and storage/disposal also need to be noted.

QA tips: Do not use pencil, white-out or correction fluid on worksheets. Any errors must be crossed out but do not obliterate the change, and remember to initial and/or date any changes. Quality assurance (QA) is about *traceability* and *transparency*.

State on the worksheet/working card all equipment/devices used to work on a sample and that have a critical influence on the test result, especially if several pieces of each type of equipment are present in the laboratory, e.g. which trier, riffle divider, balance, germinator, etc. To help track and report the devices in a traceable and uniform way, maintain a list of equipment with unique laboratory numbers. If using a digital system, this could be incorporated into dropdown lists to avoid typographical errors when completing worksheets, but take care that the correct item is selected. Incorporate on the worksheets/working cards the tolerances or acceptance limits allowed for use, e.g. 'the pH for the media needs to be within the range pH 6.0 to 7.5'.

The ISTA Seed Sampling Template gives the basis for a sampling form (see Figure 12.1). The use of this template is not limited to ISTA accredited laboratories and is not an ISTA requirement. When combined with a sample and seed lot testing request from the customer to the sampling laboratory (see Figure 12.2 as an example), a sampling form supplies all the necessary information for reporting the results and invoicing the customer. Using standardized templates and forms with suitable explanations or any important sampling or testing needs can help avoid misunderstandings between customers and the laboratory.

SEED SAMPLING TEMPLATE

Template N°:

ISTA Accredited Sampling Entity/Laboratory

Name, code and address of sampling and issuing entity/laboratory	
Sampling site (place and country)	

Seed lot and seed sample

Name and address of the applicant requesting sampling	
Species (Botanical name)	
Cultivar*	
Category*	
Seal of the lot (e.g. stitched label, metal seal) and/or under which authority the seed lot is sealed	
Marks of the lot	
Seed treatment	
Type of container*	
Number of containers	
Size/weight of the containers*	
Size/weight of the lot	
Seal of the sample (e.g. type of seal, seal number)	
Sample containers (total number, types)	
Sampling date (yyyy/mm/dd)	YYYY/MM/DD

Tests requested

Name and code* of testing laboratory					
Select test from dropdown list (click on NR)	NR	NR	NR	NR	NR
Other test/s, please specify:					

Additional information*

Please specify any other relevant information:	

Seed Sampler

Name (typed)	Signature, if applicable

DECLARATION OF THE SIGNATORY

I confirm that sampling and sealing have been carried out in accordance with the current International Rules for Seed Testing of the International Seed Testing Association (ISTA).

	YYYY/MM/DD	
Place and country	Date	Signature/name and position of the signatory

Optional information is identified by '*'. If optional information is not provided, enter 'NR' in the relevant box (NR=Not Reported).

Source: **ISTA.** 2023. Technical Committees Documents. In: *ISTA*. Cited 1 June 2023. www.seedtest.org/en/services-header/documents/technical-committees-documents.html

Figure 12.1. ISTA Seed Sampling Template

REQUEST FOR SAMPLING AND TESTING		
Applicant name:		
Applicant address:		
Applicant email:	**Applicant phone number:**	
Is sampling required?	**□ Yes**	**□ No**
If sampling is required at a different location provide details:		
Crop details		
Species:	Variety:	
Seed lot weight (kg):	Number of containers:	Container weight (kg):
Seed lot ID number:		
Testing requested (tick as applicable)		
□ % Analytical purity	□ % Germination	□ % Moisture □ Thousand-seed weight
□ Other seed determination	Complete test looking for all other species:	□ Yes □ No
□ Limited test for a specific list of other species only (list species):		
□ Disease test (provide details):		
□ Other (e.g. GMO, vigour):		
Notes or any special testing required, e.g. to meet contract specifications or country requirements		
Provide details:		
Reports of analysis (tick boxes that apply)		
□ ISTA OIC □ ISTA BIC	If ISTA certificates required, how many duplicates?	
□ Domestic certificates □ Other:		
Certificates to be sent by: □ Email □ Mail □ Courier		
Applicant's statement		
I, the undersigned as the applicant, understand and accept that it is the applicant's responsibility to fully state the sampling and testing specifications requested and that the laboratory will test, issue the reports of analysis and invoice according to the application. Any changes in the required testing will be amended by the applicant in writing or by email.		
Applicant name:	Signature:	Date:

Form number and version number: **Issue authorized by:**
Form effective from date: (dd/mm/yyyy)

Figure 12.2. Example of a request for sampling and/or testing from the client to the laboratory

As a minimum, the laboratory must state the following data for traceability and sample/lot identification on the worksheets/working cards:

- unique registration number (allocated at sample receipt and creating a unique and traceable link to the sample, the customer testing request and its associated paperwork and seed lot number if applicable);
- unique number/code of the equipment used;
- analyst performing the tests (e.g. name, initials or signature, as defined in the quality assurance, QA, system);
- person verifying the results (e.g. test supervisor's name, staff number, initials or signature, as defined in the QA system); and
- other information considered necessary by the laboratory (e.g. lot number, variety, year of production and treatment).

A worksheet/working card may contain all tests that can be performed in the laboratory or may have a separate form for each test. The worksheets shown in Figures 12.3 to 12.6 are based on examples from an ISTA accredited laboratory for combined analytical purity testing and other seed determination (Figure 12.3), thousand-seed weight testing (Figure 12.4), moisture testing (Figure 12.5) and germination testing (Figure 12.6).

QA tips: Laboratories initially do not need to have all the quality assurance (QA) fields or data on worksheets that accredited laboratories must do. However, it is good to know what might be needed later.

Where applicable, circle options of yes, no or test type

Balance ID number:

Divider ID number:

Sample chemically treated: Yes No

Sample ID:

Species under analysis:

% ANALYTICAL PURITY TEST

Working sample weight at start: **g**

Description		**g**	**%**	**Rounded to 1 dp for reporting (%)**
	Pure seed			
Other seeds				
Inert matter				
Working sample weight at end:				
% loss start to end				
% loss not more than 5%			**Yes No**	

OTHER SEEDS BY NUMBER DETERMINATION

	Number in sample weight:		
Test type: Complete Limited Reduced Reduced-Limited	_____g	_____g	___g
SCIENTIFIC NAME			

Analysed by:	**Date (dd/mm/yyyy):**	**Results verified by:**	**Date (dd/mm/yyyy):**

Form number and version number **Issue authorized by** **Form effective from date: (dd/mm/yyyy)**

Figure 12.3. Example of a worksheet needed in an ISTA accredited laboratory for analytical purity testing and other seed determination

THOUSAND-SEED WEIGHT (TSW) TEST			
Species under test:		**Sample number:**	
Balance number:			
Replicate	**Weight of 100 seeds (g)**	**Note:** Coefficient of variation must not exceed 6.0 for chaffy seeds or 4.0 for other seeds **Comments:**	
1			
2			
3			
4			
5			
6			
7			
8			
Coefficient of variation			
Average of replicates		**Analysed by:**	**Date (dd/mm/yyyy):**
TSW to report (to correct dp)		**Results verified by:**	**Date (dd/mm/yyyy):**

Form number and version number **Issue authorized by** **Form effective from date: (dd/mm/yyyy)**

Figure 12.4. Example of a thousand-seed weight testing worksheet needed in an ISTA accredited laboratory

% MOISTURE TEST						
Species under test:			**Sample number:**			
Balance ID number:						
Method:	°C		hours		Grinding / No grinding	
	Weight (g)		Weight container + sample (g)		Drying loss	
Replicate container number	Container	Sample	Before drying	After drying	g	%
Observations: Replicates in tolerance: Yes / No						
Analysed by:	**Date (dd/mm/yyyy):**		**Results verified by:**		**Date (dd/mm/yyyy):**	

Form number and version number **Issue authorized by** **Form effective from date: (dd/mm/yyyy)**

Figure 12.5. Example of a moisture testing worksheet needed in an ISTA accredited laboratory

% GERMINATION TEST

Species under test:				**Sample number:**					
Special treatments to break dormancy:									
Date into pre-chill	**Days in pre-chill**	**Date put to germinate**		**Germination temperature:**	**Germinator number:**	**Light or dark**			**Notes:**
	Normal after days	Normal after days	Normal after days	**Normal at test end days**	**Hard at test end**	**Fresh at test end**	**Abnormal at test end**	**Dead at test end**	
Replicates									
A									
B									
C									
D									
E									
F									
G									
H									
Mean									
Replicates in tolerance: Yes or No									
% to report after days									

Analysed by:	**Date (dd/mm/yyyy):**	**Results verified by:**	**Date (dd/mm/yyyy):**

Form number and version number **Issue authorized by** **Form effective from date: (dd/mm/yyyy)**

Figure 12.6. Example of a germination testing worksheet needed in an ISTA accredited laboratory

Forms are needed to record all data for the maintenance and calibration of equipment/devices. After the initial fit-for-use checks are made at purchase, regular calibration and/or in-house verifications should be done to ensure that equipment continually meets the requirements or tolerances. The laboratory defines the periodicity of any checks; for example, all balances are calibrated by an external provider annually but should be verified on first use or verified on a daily or weekly schedule thereafter. The staff member doing the maintenance, calibration or in-house verification must note all observations and data. An approval decision that the device is fit for use or has passed a routine verification check must be made. If the equipment fails in-house verification checks or annual calibration, it needs to be taken out of service and investigated for the failure. If the problem is linked to an equipment supplier failure or mechanical breakdown following a QA system approach, a root cause analysis would be needed. Action taken needs to be documented and recorded by the responsible person in the laboratory. For the period where the device requirements are not met, an 'out of service' sign should be attached to the equipment. See Figure 12.7 for an example of a periodic in-house balance verification worksheet.

CENTRAL LABORATORY FOR QUALITY OF SEED AND PLANTING MATERIAL

BALANCE VERIFICATION SHEET

TYPE: **SERIES:** **NUMBER:** **Verification Mass (VM):**

Year: **Tolerance:**

DATE																					
Weight 1																					
Weight 2																					
Weight 3																					
Analyst:																					

1 = 500.5																					**500.5**
VM = 500.0																					**500.0**
2 = 499.5																					**499.5**

VM = Verification Mass
1 = VM + Set tolerance
2 = VM − Set tolerance

1 interval = 0.1 g

Verification accepted by: DATE:

LCAIL-02-dc-01 Version: 02 Application date: 01.07.2010 Approval date: 01.07.2010

Figure 12.7. Example of a balance verification worksheet needed in an ISTA accredited laboratory

12.2 Reports of analysis

The reporting of results can differ between laboratories because of different local governmental requirements or customer needs. Use standardized formats whenever possible to help avoid misunderstandings between customers and end-users of the reports of analysis. For example, germination test results on an ISTA Certificate are separated into percent normal seedlings, percent abnormal seedlings, percent hard seeds, percent fresh seeds and percent dead seeds. Some people may define 'germination' to meet a certification standard of percent normal seedlings + percent hard seeds, or even percent normal seedlings + percent hard seeds + percent fresh seeds. Indeed, others may report 'germination' based on a tetrazolium test, which is inaccurate as it is a viability test not a germination test, although indicative of the potential of seeds to germinate.

When reporting on an ISTA Certificate, the format is fixed, but some content is flexible and adaptable to customers' needs. This is fully explained in Chapter 1 (ISTA Certificates) of the *International Rules for Seed Testing* (ISTA Rules), providing an excellent basis for what should be included in a report of analysis. The ISTA position paper on the measurement of uncertainty in seed testing provides a background to the statistical validity of ISTA seed testing results. Examples of the most common tests are shown in Table 12.1 to Table 12.5 (Note: The content and data are fictional).

Note: Even if your laboratory is not ISTA accredited, avoid using a similar layout and colours on in-house test reports of analysis as in the ISTA Orange and Blue International Certificates.

Table 12.1. Client, seed lot, sample and sampling information for the report of analysis

Laboratory name and address	
Customer name and address	
Seed lot information	e.g. Seed lot identifier, seed lot size, bag label (tag) numbers, treatment, variety name, cultivar, name of sampler
Date of sampling	If useful or requested
Date of sample received at laboratory	If useful or requested
Unique laboratory sample number	Number generated from the laboratory, e.g. 21-0005
Statement of accuracy or responsibility	Any statement about the accuracy of testing or responsibility for use or interpretation of test results
Place and date of results issuance	
Name or signature of authorized or responsible person	Signature (manual or digital)

Table 12.2. Example of a percentage analytical purity (reported to one decimal place) for the report of analysis

Analytical purity		
Pure seeds (%)	**Inert matter (%)**	**Other seeds (%)**
99.7	0.1	0.2
Kind of inert matter found	Stones, stems, broken seeds	
Other seeds found	*Chenopodium album*, *Rumex acetosa*, *Setaria glauca*	

Table 12.3. Example of other seed determination (seed ID) for the report of analysis

Other seed determination	
Other seeds by number 'complete test' in 1005 g *Chenopodium album:* 3 *Rumex acetosa:* 4 *Setaria glauca:* 6	Depending on the request, the test could also be 'limited', 'reduced' or 'reduced-limited'; this should be stated in the report The weight of the working sample should also be reported

Table 12.4. Example of germination test data for the report of analysis

Germination analysis				
Normal seedlings (%)	**Abnormal seedlings (%)**	**Dead seeds (%)**	**Fresh seeds (%)**	**Hard seeds (%)**
82	12	6	0	0
Test duration (days)	8			
Substrate, germination temperature, any dormancy breakage applied	Sand, 25 °C Could be also 'top of paper' (TP) or an alternating temperature (e.g. 15<=>25 °C) Any pre-treatment for breaking dormancy (e.g. 5 days pre-chilling at 7 °C) should also be reported if applicable			

Table 12.5. Example of thousand-seed weight data for the report of analysis

Thousand-seed weight analysis	
Thousand-seed weight (counting replicates)	220.5 g
Thousand-seed weight (counting whole pure seed fraction)	220.5 g

12.3 System backups

All data must be protected from loss, whether paper-based or digital. The risk of data loss in a paper-based system could be from a fire or flood, whereas in a digital system from a computer failure or data hacking attack. Using metal filing cabinets for a paper-based system could help minimize risks; loss as a result of major catastrophic events cannot be eliminated entirely and could be a reason to move to a digital storage solution. The consideration is then whether to use local storage devices or a cloud-based system with sufficient protection like firewalls and daily backups on a different server. Data protection of client information, with limited access, should also be considered for both analysis data and payment details.

A storage/archive time of at least 6 years is advised for all data and documents, whether paper-based or digital. This may extend beyond 10 years in some countries or companies. For an ISTA accredited laboratory, 6 years is the minimum record retention period and is a QA requirement, i.e. two audit periods. Key records like those for original acceptance (fit-for-use) or analyst training and authorization may need to be kept longer than the 6-year minimum requirement; for example, 6 years after the equipment is no longer in use or after the analyst is no longer testing at the laboratory.

If using digital media, data stored 6 years ago must still be recoverable, accessible and readable when needed. Changes in digital operating systems or approved system upgrades need to be considered as part of planning so that files can still be accessed in the future. For example, an operating system change or a change of storage device could mean that old digital files (e.g. on floppy disk) can no longer be read by current machines/software. It is advisable, or perhaps required, to undertake an annual check to see if any digital records deleted in error can be recovered from any backup system used.

12.4 Invoicing for work

At some stage, the work of sampling and testing will need to be invoiced to the customer. This could be an invoice directly to a customer, or it may be an internal reallocation of costs within a governmental or company laboratory. Invoices should use the unique sample number and list the completed tests. To facilitate this, create a ‘testing charges’ sheet and include fees for sampling, sample handling, testing and reporting. The costs will vary with the species and the test(s) being requested. In most cases, the most expensive part of seed testing is staff time, so good records of the time taken to test the seed sample are useful from a planning and long-term sustainable business perspective. Invoicing for sampling and testing could be done separately and in advance or in arrears after testing. See Chapter 3 (Staffing) and Chapter 14 (Budgeting) of this handbook for further discussions linked to this topic.

Note: Invoicing for sampling work is important. Know how much it costs for a staff member to sample and test seed samples. Create a ‘testing charges’ sheet for customers.

Chapter 13: Seed testing equipment and consumables

ISTA and FAO do not endorse any product or company. Providing examples in the checklists included in this chapter or in figures or text throughout the handbook does not imply a recommendation to use that product.

Table 13.1 provides a checklist of the main equipment needed in a seed testing laboratory and includes the requirements for external calibration and in-house verification intervals, as well as suggested verification criteria. There are several worldwide and in-country seed-specific equipment suppliers. A web search will provide links to follow up. There are also supplier advertisements in the ISTA magazine *Seed Testing International* (links to past issues can be found on the ISTA website[1]). You could also contact other established seed testing laboratories in your region to discuss suitable suppliers.

Regarding electricity supply, decide whether you will need an electrical generator as a backup or for ongoing electricity needs. Equipment specifications should be provided to suppliers or checked before purchase, including the local voltage and plug socket design for electrical equipment, especially when buying from outside the country. Organize maintenance agreements with in-country calibration laboratories for balances and other equipment needing specialist calibration, and contract other experts, e.g. refrigeration engineers.

Table 13.2 provides a checklist and recommended quantities for the main pieces of equipment, small pieces of equipment and consumables needed in a seed testing laboratory. For a continuous supply of consumables, plan to overstock by 20 percent or order well before stocks are used up. Chemicals, germination paper and other consumables are available from generic scientific and office equipment supply companies in-country. Other laboratories or university departments in the region could be contacted for advice on suitable suppliers, if needed. Essential supplies must be fit for use; therefore, they need to be verified on-site upon receipt in case items are unsuitable and need to be returned. Keep a list of suitable suppliers for repeat ordering and add notes if equipment or supplies are not fit for use.

[1] www.seedtest.org/en/publications/seed-testinginternational-1171.html

Table 13.1. Checklist of the main equipment needed in a seed testing laboratory (lab) including requirements for external calibration and in-house verification intervals, and suggested verification criteria

Work area	Equipment	External calibration	Frequency of external calibration	In-house verification	Frequency of in-house verification	Suggested verification criteria (lab can define own where examples are given)
Sampling	Sampling triers: various sizes, crop group specific	N/A	–	Yes	Before every use	e.g. Clean, not damaged, not blocked
Sampling	Divider (optional)	Not calibrated, but the correct size and number of chutes selected at purchase	–	Yes	Annual or as defined by lab	e.g. 5% deviation between two collector pans, additional check if the distribution of seeds in a mixture is balanced
Sample reception	Laboratory information management system (LIMS) for sample receiving and other uses	IT support and protection	Ongoing	Backup	Daily/weekly	–
Sample preparation in lab	Dividers: different types for different crop groups	Not calibrated, but the correct size and number of chutes selected at purchase	–	Yes	Annual	e.g. 5% deviation between two collector pans, additional check if the distribution of seeds in a mixture is balanced
Analytical purity testing / other seed determination (OSD)	Balance (scale): 1-, 2-, 3- and 4-decimal place balances are needed depending on the species being tested	Yes	Annual, checked by a contractor with primary reference weights	Yes	Daily or on first use; weekly or monthly	Levelled, clean, working weight checks
Analytical purity testing / OSD	Reference seed collection (Herbarium)	New seeds need to come from a verified source	–	Yes	Change of scientific names, extensions	Check for deterioration, e.g. mould or insect damage
Analytical purity testing / OSD	Microscope: stereo and/or digital with suitable extra lights	N/A	–	Yes	Clean and maintain as required	e.g. Annual cleaning and checks on any measurement options for stereo or digital systems if critical to testing needs
Analytical purity testing / OSD	Diaphanoscope: built into work area or separate (grass species testing only); could be self-built (see Chapter 7)	N/A	–	Yes	Clean and maintain as required	e.g. Clean regularly and change lights as required
Analytical purity testing / OSD	Seed blower	N/A	–	Yes	Annual	ISTA calibration samples for *Poa pratensis* and *Dactylis glomerata*
Analytical purity testing / OSD	Calibration samples for seed blowers	Calibrated at purchase	–	Yes	Annual	ISTA calibration samples for *Poa pratensis* and *Dactylis glomerata* need to be checked as fit for continued use
Analytical purity testing / OSD	Anemometer for seed blowers	Calibrated at purchase	–	Yes	Annual	e.g. By comparison to a second certified anemometer

Note(s)
N/A = not applicable.

Work area	Equipment	External calibration	Frequency of external calibration	In-house verification	Frequency of in-house verification	Suggested verification criteria (lab can define own where examples are given)
Thousand-seed weight (TSW) determination	Seed counter	N/A	–	Yes	Annual or after maintenance	e.g. Replicates continually out of tolerance, comparison to counting boards, deviation of five seeds, hand counting versus machine counting with different seed sizes
Moisture testing	Grinder (coarse)	N/A	–	Yes	Annual	Check particle size with sieves: 50% > 4.0 mm; 45% > 2.0 mm
Moisture testing	Grinder (fine)	N/A	–	Yes	Annual	Check particle size with sieves: 50% < 0.5 mm; 90% < 1.0 mm
Moisture testing	Moisture oven	N/A	–	Yes	Annual	Temperature: 101–105 °C; 130–133 °C Regain temperature in less than 30 min after inserting samples Ventilation test: with full capacity of samples, redrying; difference between results of first and second drying less than 0.15%
Moisture testing	Desiccator	N/A	–	Yes	Annual or when required	Drying at 130 °C
Moisture testing	Calibrated sieves for particle size checks	Calibrated at purchase	–	–	If damaged, or as defined by lab	Check older sieves for signs of wear by comparing hole sizes and using reference samples Reduce wear by only using specific sieves for any size-critical calibration checks and use other sieves for daily non-critical uses
Germination testing	Cabinets and walk-in rooms	N/A	–	Yes	Daily At first purchase then either annual or after maintenance	Temperature measurements maximum deviation +/−2 °C Temperature distribution within cabinet/room maximum deviation +/−2 °C Daily changeover for alternating temperatures within time limits
Germination testing	Reference thermometer	Yes	Annual or maximum every 3 years	–	–	–
Germination testing	Data loggers or working thermometers	Calibrated at purchase	–	Yes	Annual	Checks against primary reference thermometer
Germination testing	Vacuum counter	N/A	–	Yes	At first use and after repairs	Statistical comparison between germination or replicate weight results from hand counting versus vacuum counter
Germination testing	Substrate checks (see Chapter 8 for procedures)	N/A	–	Yes	After each delivery	Media and water pH: 6.0–7.5 Phytotoxicity test Water-holding capacity test
Viability testing	Tetrazolium (TEZ) solution	N/A	–	Yes	For each new solution prepared	pH and/or efficacy tests

Note(s)
N/A = not applicable.

Table 13.2. Checklist and recommended quantities for the main pieces of equipment, small pieces of equipment and consumables needed per staff member and/or in the seed testing laboratory (lab)

Work area	500 samples, limited species	5000 samples, wide range of species	Comments
All areas			
Suitable office space, tables, chairs, computer access, digital calculator, personal filing cabinets / storage units for the number of staff at the lab	1 set per person (2* per lab)	1 set per person (10* per lab)	–
Sampling			
Triers	2* per sampler	2* per sampler (20* per lab/sampling network)	–
Dividers	Useful but optional for samplers	Useful but optional for samplers	–
Sample bags: • paper/kraft • moisture-proof for moisture testing	 500 250	 5000 2500	Tear-resistant bags; bag capacity will be different for small- or large-seeded species
Sample sealing method	500	5000	–
Labels (when information is not directly written on bag)	1000	10 000	Labels can be pre-printed to capture required information and include logo of the organization/company
Administration and sample receiving			
Paper	Depends on local needs	Depends on local needs	–
Dating stamps	1 per person	1 per person (10* per lab)	–
Computer	1* per lab or per person	1* per lab or per person (10* per lab)	–
Laboratory information management system (LIMS), sample administration programme (SAP) or other digital systems	Optional	Optional	–
Filing cabinets	1* per lab or per person	1* per lab or per person (10* per lab)	–
Logbook for sample entry	1 per lab	1 per lab	If no digital system is used
Analytical purity testing / other seed determination (OSD)			
Purity analyst workstation	1 per analyst	1 per analyst (8* per lab)	Ergonomic benches and chairs should be considered
Seed reference collection	1 per lab	1 per lab	Minimum 130 species (based on *ISTA Universal List of Species*, see www.seedtest.org/en/services-header/tools/purity-committee/universal-list-species.html)
Dividers	1 per lab	2* per lab	–
Balance (scale): • 1- and 2-decimal place balances • 3- and 4-decimal place balances	 1 per lab Optional	 2* per lab 2* per lab	–
Balance table or stable weighing area	1 per lab	2* per lab	–
Set of check weights	1 set per lab	1 set per lab	–
Set of primary reference weights	Optional	1 set per lab	–

Note(s)
* indicates numbers that will change depending on the different species or crop groups tested, overall testing numbers, number of analysts and other people at the laboratory.

Work area	500 samples, limited species	5000 samples, wide range of species	Comments
Spatulas, tweezers, scalpels, dissecting needles for sorting through samples	1 set per analyst	1 set per analyst (8* per lab)	–
Straight-edged spoons for working sample preparation process	2 per lab	4 per lab	–
Extra desk lights	1 per analyst	1 per analyst (8* per lab)	–
Hand-held or illuminated magnifiers	1 per analyst	1 per analyst (8* per lab)	–
Microscope: stereo and/or digital with suitable extra lights	1 per analyst	1 per analyst (8* per lab)	–
Storage tubes and unit for seed collection	1 set per lab	1 set per lab	–
Large sample bags (paper, cotton)	Optional	Optional	–
Sieves to screen samples during testing	Optional	Optional	–
Small metal containers, watch glasses, Petri dishes, or other anti-static containers for storage of purity components during testing, storage of germination replicates, and for thousand-seed weight (TSW) determination when counting replicates method is used	10 per analyst (10–20 for lab)	10 per analyst (80–100 total for lab)	–
Envelopes or microtubes for purity component storage during and after testing	1000	10 000	–
Seed blower (grass species testing only)	Optional	1* per lab	–
Anemometer for seed blower	Optional	1* per lab	–
Diaphanoscope: built into the work area or separate	Optional	1* per lab	–
Labels (when necessary)	2000	20 000	May be needed on envelopes, germination containers, etc. during analysis; can be handwritten
Paper bags: • large for pure seeds • small for impurities if kept separate and for other seeds	 600 2 × 600	 6000 2 × 6000	–
Germination testing			
Germination work area	1 per lab	Shared area sufficient for 3–5 people at a time depending on workloads	–
Germinators: cabinets	2 per lab	5* per lab	–
Germinators: walk-in	Instead of cabinets or in addition*	Instead of cabinets or in addition*	–
Germinator for pre-chilling	1 per lab	1* per lab	–
Data loggers or check thermometers	6* (one per germinator, refrigerator, freezer, oven and storage area)	10* (one per germinator, refrigerator, freezer, oven and storage area)	–
Primary reference thermometer	1 per lab	1 per lab	–
Counting boards	Optional	Optional	–
Vacuum seed counter and vacuum compressor	Optional	Optional	–
Trays, shelves, covers	Sufficient for number of germinators	Sufficient for number of germinators	–
Boxes (plastic, paper)	200*	2000*	–

Note(s)
* indicates numbers that will change depending on the different species or crop groups tested, overall testing numbers, number of analysts and other people at the laboratory.

Work area	500 samples, limited species	5000 samples, wide range of species	Comments
Containers for rolled towel tests	10*	100*	–
Container or mixer for sand and organic growing media preparation	1 per lab	1* per lab	–
Germination paper to fit boxes/trays, or rolled towel, pleated paper or crepe paper	2000*	20 000*	'Top of paper' method (2 or 3 sheets per replicate), 'pleated paper' method (one sheet of top of paper plus one sheet of pleated paper per replicate)
Germination sand, organic growing media	Sufficient for test numbers	Sufficient for test numbers	–
Sieves, e.g. to sort through sand after testing for any missing seed	1 set per analyst	1 set per analyst (3* per lab)	–
Water still	Optional	Optional	–
Dishwashing machine	Optional	Optional	–
pH test strips or pH meter	1 set of test strips or 1 meter	1* set of test strips or 1 meter	–
Moisture testing			
Ventilated oven	1 per lab	2* per lab	–
Grinding mill	1 per lab	1* per lab	Suitable speed important
Calibrated set of sieves	1 set per lab	1* set per lab	–
Desiccator	1 per lab	2* per lab	–
Metal containers for samples	10*	20*	–
Heat-resistant gloves and/or handling tongs	1* per lab or per person	2* per lab or 1 per person	–
Electronic moisture meter	Optional	Optional	–
Thermometer with range up to 135 °C	1 per lab	1 per lab	One for each oven
Viability testing			
Refrigerator: chemical and solution storage	1 per lab	1* per lab	–
Tetrazolium (TEZ) chemical	Sufficient TEZ chemical for testing needs	Sufficient TEZ chemical for testing needs	–
Buffers	Optional	Optional	–
Spatulas, tweezers, scalpels, dissecting needles, small sieve	1 set per analyst	1 set per analyst (3* sets total per lab)	–
1 l (or larger capacity) glass stoppered container for stock solution	1 per lab	2* per lab	Dark glass bottles are preferable or can be covered in aluminium foil
Small beakers (approximately 50 ml capacity)	10*	30*	–
Sample storage			
Temperature-monitored room/area; exact size will vary with sample numbers tested	1*	1*	–
Freezer: sample storage for insect-infected samples only	1*	1*	–

Note(s)
* indicates numbers that will change depending on the different species or crop groups tested, overall testing numbers, number of analysts and other people at the laboratory.

Work area	500 samples, limited species	5000 samples, wide range of species	Comments
Personal and safety equipment			
Lab coats	2 per person	2 per person	Cotton, labelled and size-personalized
Disposable gloves (e.g. nitrile, single-use, non-sterile)	2* per person per workday	2* per person per workday	–
Safety glasses	1 pair per person	1 pair per person	–
Cleaning equipment (chemicals, wipes, etc.)	As necessary	As necessary	–
Fume exhaust system for working on treated seeds in different work areas	Depends on testing needs	Depends on testing needs	–

Note(s)
* indicates numbers that will change depending on the different species or crop groups tested, overall testing numbers, number of analysts and other people at the laboratory.

Chapter 14: Budgeting

This chapter highlights the main costs for a seed testing laboratory without providing detailed figures in different currencies, as regional costs and currency exchange rates vary significantly. Instead, we have provided details of the generic costs to consider and plan for when building and running a seed testing laboratory. The budget/costs can be split into three steps:

1. initial laboratory building costs, including the purchase of large equipment (original cost/investment);
2. managing the laboratory (operating budget); and
3. maintaining the laboratory (repair, renewal and extension).

14.1 Building the laboratory

14.1.1 Initial investment needed to establish the laboratory

The total budget for building a new laboratory should include the building, complete with furniture and large equipment. This can reach millions in any currency but will depend on the size of the laboratory planned. The initial budget is probably the easiest to secure but may take time to gain approval for as it is such a significant investment. Creating a laboratory is always part of a strategic programme where funds are allocated by the state, country, international organization or company. The balance in the budgeting is to adjust the building size to the available funds without sacrificing the technical needs represented by the scope of species and the kinds of tests, which will affect the building size and the number of rooms required. In planning for the initial build, the future budget needed to operate and maintain the laboratory should be considered and adjusted as necessary.

The price of a square metre of an equipped laboratory can be several times that of a square metre of normal office space. Building companies in many but not all regions of the world should be able to estimate the cost of a square metre of laboratory space versus a square metre of office space. Check what is included in the estimates from a building company. For example, are the main services of power, water, ventilation, air exchange, air conditioning, phone, internet connection, local IT network, and furniture like fixed benches and sinks, included in the building costs? The costs of large and small pieces of free-standing laboratory equipment then need to be added in as a separate budget but still included in the initial build costs; otherwise, the laboratory will just be an empty building.

14.1.2 Three possible construction scenarios

1. The laboratory is built from new as a stand-alone building (Figure 14.1). Costs are for the land, the building itself and connections to the main services like water, power and drainage. It is recommended to carefully examine the availability of reliable power, running water, communication (phone, internet), road access and any possible climatic risks (e.g. hurricanes, floods). If environmental works for main services are required, additional costs must be calculated and included in the original budget.

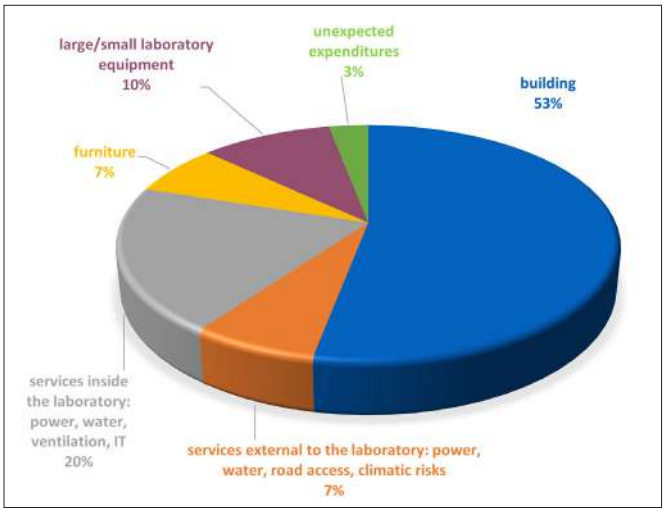


Figure 14.1. Laboratory created from new: building budget includes main areas of expenses and relative proportions of cost (based on estimations that can vary depending on the project and the location)

2. Installation of the laboratory in an existing building (Figure 14.2) may be less expensive. Nevertheless, in a seed testing laboratory, the ongoing flow of samples is a key element for good organization and to avoid mixing samples. See Chapter 4 (Buildings and workflow) of this handbook for ideas on workflow. Some rooms may require modifications and need to be equipped with ventilation, air exchange, air conditioning, vacuum equipment for sowing, reliable electric power, IT network, and water or waste disposal/management. Equipping an existing building may lead to additional costs that should be included in the project. If the building needs to be purchased before being transformed into a seed laboratory, the associated cost investment must also be added to the budget. Depending on the location, it may be more expensive to buy and transform an existing building than to build a new laboratory.

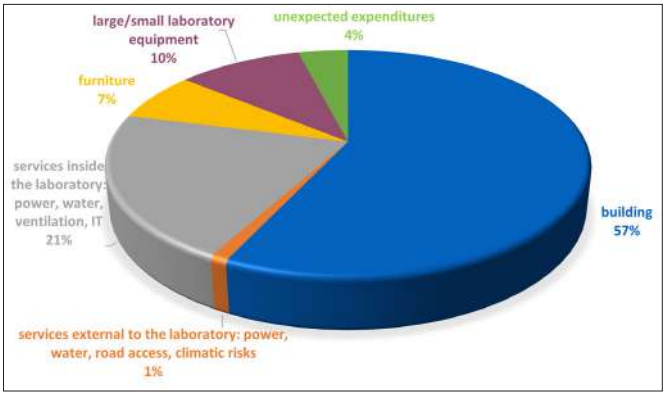


Figure 14.2. Laboratory installed in an existing building: building budget includes main areas of expenses and relative proportions of cost (based on estimations that can vary depending on the project and the location)

3. A small building or mobile unit could be a suitable option for a two- or three-person laboratory. The building needs space for an analyst to work, a germinator, a balance area, and so forth, but only enough to meet the needs of two or three people. The same main services are needed for a larger building, but costs may be considerably lower. For small laboratories there is the cost to retain at least one of the three skilled people to test the samples and multitask as sample receiver, analyst, quality assurance (QA) manager and administrator. Such is the case for several officially licenced or third-party seed testing laboratories working in various countries' national seed testing systems.

Note: A small (mobile) building is any kind of building made of one or a few rooms, e.g. a converted shipping container (Sea-Can) that can be placed on a trailer and moved. It can also be a small farm outbuilding or barn transformed into a laboratory. The building should be waterproof and have main essential services like a reliable power supply. Basic seed tests can be performed in limited space and with a minimum of equipment, the main requirement being competent staff.

14.1.3 Investments in furniture and equipment

Large and small laboratory equipment and furniture should be included in the original initial budget as it is challenging to equip empty rooms at a later stage. Do not forget there are ongoing equipment costs beyond the initial purchase and installation. The installation and set-up of large equipment, such as a walk-in germination room, requires the assistance of experts to design, commission and run the climatic system. We highly recommend that you negotiate the contracts for the commissioning and start-up of large equipment that might require staff training, and then consider ongoing maintenance and calibration. Any equipment purchase should specify a delivery date based on the completion of the new laboratory rooms and the necessary connections for power or water, to avoid equipment staying in boxes waiting for installation and warranties expiring unnecessarily. All these different aspects influence the budgets and need to be considered in detailed project planning, which can take a considerable amount of time.

Note: The development of software or a laboratory information management system (LIMS) to monitor and integrate the laboratory's activities, could benefit the traceability of the analyses, provide efficiency and standardize calculations, but initially is not a must. Paper-based systems work well, and we advise that it is better to establish the routine functioning of the laboratory before implementing an IT solution. Careful attention should be paid to evaluating the actual benefit of an IT solution in relation to the cost, perhaps even undertaking a separate cost–benefit analysis and project plan.

14.1.4 Investments to obtain essential services

Depending on the world location of the laboratory, it can be challenging to ensure reliable electric power and a running water supply. Even if the basic seed tests are quite robust and can support some environmental variations, some tests (germination, moisture) can be interrupted or adversely affected during a power cut. If this is a risk, use a backup electric generator to ensure a reliable, uninterrupted power supply. In some regions of the world, a secondary system to purify water may also be needed.

The local or national health and safety regulations are another area needing investment, but they are likely to vary worldwide. For a basic seed laboratory, the main areas to consider are the need to protect people against irritating dust from raw seeds, particularly cereal and grass seeds, and from treated seeds. Good ventilation, air exchange or filtration should be considered as part of the overall building costs. Including these in the initial building design may lower overall costs and improve efficiency.

14.2 Managing the laboratory (operating budget)

Getting the funds to install a new laboratory is a challenge, but it is a one-off budget. The most sensitive part of financial planning is the operating budget for managing the laboratory daily (Figure 14.3). The budget has to be reviewed and revised, and the funds sourced yearly. In the following sections, we propose three main areas (staffing, consumables and main services) of an operating budget. They are not based on an accounting system but are aimed at helping a laboratory identify the main operating cost centres.

Note: The budget needs to be a balance between income and expenditure. Income could be from subsidized funding from the government and/or from the sale of services and analyses. This will depend on the funding policy for the laboratory. Some governmental laboratories offer analyses for free or at reduced prices to farmers and clients, while others compete with other national laboratories for test income and charge at full economic rates for any testing. Company laboratories may include analysis costs as a part of seed production. Private third-party laboratories will need to balance costs and income to support a profitable business. These differences are why detailed budgeting is not included in this handbook.

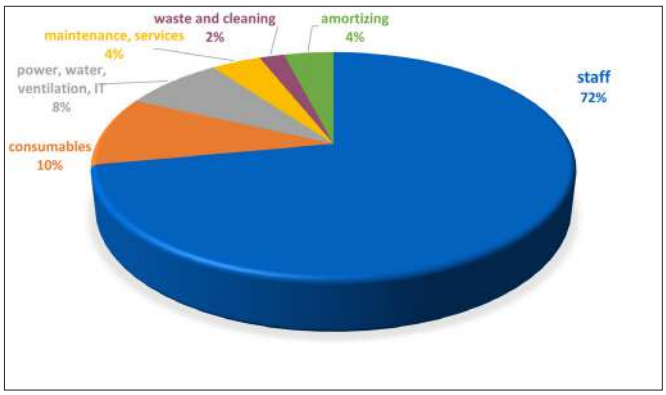


Figure 14.3. Operating budget: estimated proportions of the categories of costs

14.2.1 Staffing

A major part of the operating budget of seed test analyses is staff salaries. Permanent well-trained staff are an essential element and constitute the main cost in a seed testing laboratory. The time spent by the analysts to conduct the basic seed tests (analytical purity; other seed determination, OSD; thousand-seed weight, TSW; moisture; germination) is the main and significant cost compared to the ongoing equipment and consumables costs. The accuracy of the analyses depends on analyst competency, which means that people with qualifications are needed and may demand a higher salary rate. In the future, seed analyst functions may be replaced with IT solutions using machine vision, robotics and artificial intelligence (AI), but we are not there yet. Even if these options become available, trained people that understand seed testing principles will still be needed to work with the new machinery.

Staff numbers needed will vary depending on the testing scope of the laboratories and the number of samples to be tested seasonally or annually (see Chapter 3: Staffing). Often, but not always, governmental laboratories use state employees whose salary is paid by the government, so their salaries do not need to be included in the operational budget of the laboratory. In whatever way budgets are presented, salaries remain a key element. Temporary staff may be needed to address seasonal peaks of analyses, contributing to a significant proportion of the staffing budget. Training and qualification have an initial cost when new permanent and temporary people are employed, but ongoing training is also needed to maintain and develop the staff qualifications. An in-house technical training programme is an important part of additional staff costs, especially for laboratories aiming to apply for and maintain ISTA accreditation. About 10 percent or more of the staff time, and therefore salary costs, may be needed for ongoing training and qualification of staff.

National and international travel costs for staff training and meetings should be considered. A combination of in-person and virtual/remote meetings is necessary to build a support network and exchange experience with other people, organizations and laboratories.

Overall, it is estimated that staff costs are around 60–75 percent of the operating budget of a seed testing laboratory.

14.2.2 Consumables

In seed testing (basic tests), the consumables represent a small proportion of the cost of analyses, yet they are mandatory for completing the tests. The laboratory needs a budget to ensure a regular supply or sufficient stocks of the critical consumables before the start of the season for testing, e.g. labels, bags, envelopes, sand, germination paper, writing/printer paper, printer cartridges, blank ISTA Certificates (if ISTA accredited). Details and lists of supplies needed are included in the different testing chapters in this handbook, and checklists of equipment and consumables are included in Chapter 13 (Seed testing equipment and consumables).

- Sampling, analytical purity, OSD, TSW and moisture tests require consumables like labels, bags and other small equipment (tins, dishes, needles, spatulas, brushes, lenses).
- Germination testing needs more consumables such as labels, fresh substrate, boxes, trays, clean water, potassium nitrate (KNO_3), gibberellic acid (GA_3), brushes, needles, counting heads and trolleys, as well as equipment to check the quality of the environmental conditions, e.g. pH paper, thermometers, data loggers.
- Personal protective equipment (PPE) for health and safety needs (e.g. gloves, masks, laboratory coats) also need to be budgeted for and can represent a significant part of the ongoing costs of analyses.
- Office and analysis consumables (e.g. logbooks, printer paper, labels, pre-printed forms, pens, calculators) can all contribute to the costs.

14.2.3 Main services

The supply of electric power, natural gas or propane (if used for heating), water, sewage disposal, and phone/internet connection are usually based on contracts with an external provider but may not be in more remote locations. In some regions of the world, laboratories may need to be heated or cooled, depending on the season, and the humidity adjusted. In all regions, a reliable electricity supply is essential to run the controlled-temperature equipment needed in germination tests and the drying ovens for the moisture test.

Note: Green energy, solar equipment, windmills and watermills can provide alternatives to the current standard power supply. The laboratory may aim to be carbon neutral, or may simply need to overcome local deficiencies in power supply.

14.2.4 Cleaning and waste management

Laboratory activities produce waste, the elimination and disposal of which needs to meet local regulations. Depending on the location, the laboratory can manage this by itself or contract an external service provider to remove any waste material such as treated seed, genetically modified organism (GMO) seed, used germination substrates, used PPE gloves and masks, etc. Laboratory coats will require routine cleaning and can be dealt with by the laboratory or a contracted laundry service. All these activities should be included in the operating budget.

14.2.5 External costs

Memberships, accreditation, participation in Proficiency Tests (PT) and maintenance of seed reference collections are all costs to be considered. A basic seed testing laboratory may reduce some of these costs, such as external PTs, by voluntary membership in laboratory networks. Nevertheless, laboratories seeking ISTA accreditation will have to become members of ISTA and later pay accreditation and audit fees. ISTA membership costs are published on the ISTA website.[1]

14.3 Maintenance of building and equipment and controls

We identify three areas of maintenance:

- The *buildings maintenance* includes all kinds of maintenance and repair (electric plugs and sockets, wires, sinks, pipes, furniture), as well as painting, waterproofing and maintaining supplies. It also includes the schedule of extensions to conduct more analyses or to extend the scope of tests (seed health, GMO, vigour, variety tests) that may require new rooms, new equipment and more staff.
- The *maintenance of equipment* is essential to guarantee good performance and the reliability of the analyses. Daily control and maintenance can be easily done by the laboratory staff for some equipment, but regular controls and repairs need to be contracted; for example, make use of local refrigeration maintenance companies to ensure any air conditioning needed in rooms and germinators is maintained or repaired as necessary. Other smaller equipment will also need regular in-house or contracted maintenance/calibration, e.g. balances, seed blowers, grinders, incubators, germinators, ovens, thermometers, microscopes, data loggers, laboratory software, etc. Laboratories that seek accreditation will have additional costs for the external or internal calibration of some of the critical equipment.
- *Controls on installation and regular maintenance* of specific equipment may be imposed by local safety regulations, to prevent risks of accidents, electric shocks or risks from electric power fires. In some regions of the world these controls may provide protection against major environmental risks such as floods or hurricanes. The total cost of the controls will vary according to the risks and the local regulations.

Budgeting for maintenance is included in the operating budget as an amortizing or depreciation budget. Amortizing is a way of estimating the life expectancy of a building or large pieces of equipment and is part of future planning to inform decisions about when replacements might be needed. The amortizing/depreciation budget can be based on building and material depreciation, often calculated as the total cost of the building or equipment divided by the number of years of amortizing/depreciation. For example, building amortizing can be calculated for 15 up to 30 years, and laboratory equipment amortizing is around 5 years, but other durations can be decided based on the kind of equipment, local rules, and so forth. Plans like this can feed into an annual management review required for a QA-based system (see Chapter 5: Quality assurance). Remember that maintenance costs will start on day one with the opening of your laboratory and should not be delayed or forgotten.

Chapter 15: Region-specific considerations

The advice provided throughout this handbook has been made generic so that it can be applied to laboratories worldwide. Some region-specific and general considerations are discussed here.

Note: When purchasing electrical equipment from another country, check the voltage needed and plug supplied. Some equipment may need surge protectors.

15.1 Electrical equipment

When purchasing electrical equipment, ensure it will work with the local voltage (single-phase or three-phase) and frequency. Worldwide, the range for voltage is 110–127 and 220–240 V and a frequency of 50 or 60 Hz. Where possible, purchase smaller pieces of laboratory electrical equipment locally to meet the voltage needs. If purchased from another country, the plugs may need to be changed or an adapter used to allow connections to the electrical output / wall sockets (Figure 15.1).

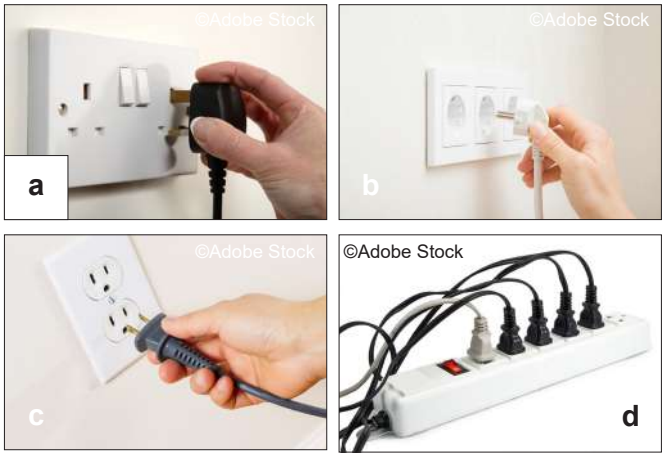


Figure 15.1. a–c Different types of plugs and their corresponding sockets; **d** plug board with multiple sockets (images courtesy of Adobe Stock)

Figure 15.2. Surge protector to protect electrical devices from voltage spikes in alternating current circuits (image courtesy of Adobe Stock)

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Some countries may have fluctuations in their electricity supply system. Such fluctuations may affect the normal functioning of electric equipment. It may be necessary to have a voltage regulator or surge protector for the whole seed laboratory or some pieces of equipment (Figure 15.2). A generator to supply electric power for emergency use could be considered in all regions, but in some regions it may be needed for everyday use (Figure 15.3).

Figure 15.3. a and **b** Backup generators may be necessary for continuous power supply (images courtesy of Adobe Stock)

15.2 Water supply

If a reliable continuous supply of clean water is an issue, then storage tanks (Figure 15.4) and/or ways to treat water to make it safe for use by staff, for mixing chemical solutions like tetrazolium or for use in germination tests may be needed. For example, use a water distillation system for smaller quantities of water. For larger quantities, storage tanks may need to be included within the building and kept in the dark to avoid algae formation. If water tanks are located outside, they will also need to be sealed to prevent animals from entering and contaminating the supply and aquatic insects from thriving.

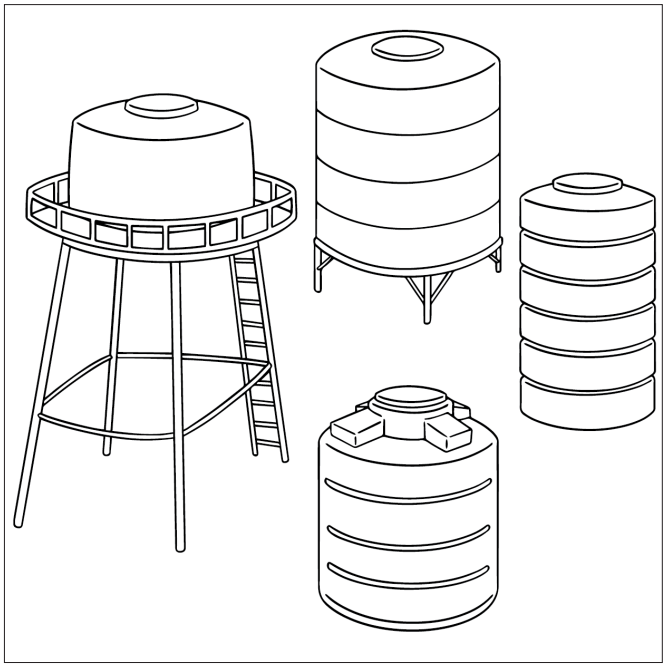

Figure 15.4. Water storage tanks

15.3 Refrigeration

Using local refrigeration engineers/technicians is always best for undertaking regular and preventive maintenance on compressors in air conditioning and dehumidification units. More specialized servicing may be required on germinators or other equipment, and the availability and cost should be considered before purchase, to allow the effective long-term use of the equipment. Another option is to have in-house personnel trained in specialized repair and maintenance of refrigeration equipment in collaboration with the supplier.

Note: The use of locally available authorized maintenance and repair contractors is recommended as one of the criteria to consider when purchasing equipment.

15.4 Balances and thermometers

Balances and thermometers need regular in-house verification checks while in use and annual calibration by a contractor from a calibration laboratory accredited to ISO 17025. The time intervals for calibration checks of primary reference material, e.g. calibrated balance weights or a reference thermometer, can be defined by the seed testing laboratory, but the risks of the equipment being out of calibration and how that could affect test accuracy need to be considered.

QA tips: You can verify your own equipment using a primary reference source but for calibration, use an ISO 17025 accredited calibration laboratory to do the work.

Small pieces of equipment can be sent away for calibration but need to be verified on return. For example, when using a primary reference thermometer to verify other in-house thermometers, send the primary reference thermometer away every 3 years for a calibration check/re-calibration but verify on return against an in-house thermometer to look for any gross errors. Then verify the in-house thermometers annually using the primary thermometer.

For balances, a certified set of primary reference weights should be available, but they only need to be assessed annually to verify the 'normal' check weights. These check weights are used to verify the balances on a daily/weekly basis as defined by the laboratory.

The same principles apply for other test methods that require calibrated pipettes, like polymerase chain reaction (PCR) tests.

Note: As well as regional considerations, specialized test methods like seed health checks, polymerase chain reaction (PCR), genetically modified organism (GMO) tests and vigour testing will need more space, extra rooms, specialized equipment and trained staff. See Chapter 4: Buildings and workflow.

15.5 Regional requirements

In the tropics and subtropics, buildings must be constructed to withstand the annual monsoon cycle. Other parts of the world experience high temperatures and humidity throughout the year, or their buildings must withstand annual shifts in temperature from +30 °C to −30 °C and fluctuations in humidity.

High levels of humidity may require dehumidification or different approaches to minimize the growth of moulds or the spread of insects within buildings, in sample storage or in the seed reference collections. Regular cleaning of surfaces and the use of insect traps are some possible solutions. Seeds can be placed in a freezer at about −20 °C for 2 days or more to kill insects and larvae.

Chapter 16: How to become an ISTA accredited laboratory or sampling entity

16.1 Why should a laboratory be accredited by ISTA?

At the beginning of the 20th century, the only seed testing laboratories were governmental/state laboratories put in charge of evaluating the quality of seed traded within and outside countries. In 1924, ISTA was created to help countries share their seed testing knowledge and harmonize their methods. Soon after, the seed industry, represented by the Seed Merchant Association (today the International Seed Federation, ISF), requested ISTA to create harmonized test reports for worldwide trade, now known as ISTA International Certificates. The ISTA International Certificates guarantee that a laboratory undertakes analyses following the ISTA harmonized seed testing methods, the *International Rules for Seed Testing* (ISTA Rules). There were initially three certificate colours for three different trading situations, but now only two exist. The Orange International Certificate (OIC) guarantees full traceability from the seed lot to the test results, whereas the Blue International Certificate (BIC) is a sample certificate not linked to a seed lot. The now-abandoned Green International Certificate represented seed lots located and sampled in a country different from the analysing country. All certificates fulfilled the needs of the international seed trade.

In 1995, ISTA developed a quality assurance (QA) programme to sustain the recognition of the competencies of its member laboratories to apply the ISTA Rules and report the results of their tests on the ISTA Certificates. Since 2004, the accreditation has been opened to industry, seed companies, private third-party laboratories and governmental/state laboratories. ISTA accredited laboratories are the highest category of seed testing laboratories recognized by countries and distinct economies, the seed industry and international organizations (e.g. the Organisation for Economic Co-operation and Development, the International Union for the Protection of New Varieties of Plants, the International Seed Federation). ISTA accreditation is a requirement under some national regulations.

16.2 Accreditation definition

Accreditation is a formal recognition of technical competence to complete specific tasks. Becoming an ISTA accredited laboratory can be a goal for any size of laboratory. It may also be a requirement of a company or organization, a business-related decision or a need related to exporting seed lots in international trade. The ISTA Executive Committee grants ISTA accreditation if a candidate for accreditation complies with the ISTA Accreditation Standard requirements as approved under the ISTA Articles.

16.3 ISTA accredited memberships

Laboratory – an ISTA member accredited/authorized by ISTA for: (i) seed sampling and testing; or (ii) seed testing only.

Sampling entity – an ISTA member accredited/authorized by ISTA for seed sampling only.

16.4 ISTA accreditation process

The time between the application and granting of ISTA accreditation depends on the review of the submitted application form and documents, as well as the receipt of membership, audit and accreditation fee payments. For accreditation, the time needed depends strongly on the laboratory's experience and efforts. Laboratories with seed testing experience running a well-established QA system usually need less time to obtain accreditation, but it can still take 1–3 years. Obtaining membership without accreditation is usually quicker.

16.4.1 How to become an ISTA member laboratory or sampling entity

Complete the application form for Laboratory / Sampling Entity Membership[1] and send it to the ISTA Secretariat (ista.office@ista.ch). Once the application is accepted, the membership fee needs to be paid. The membership fee is annual, paid in advance and may change yearly.

The following benefits are included with membership:

- the latest edition of the ISTA Rules in electronic format;
- new ISTA publications, including ISTA technical handbooks;
- previously issued ISTA publications at the discounted membership cost;
- participation in the ISTA Proficiency Test (PT) programme; and
- priority registration and participation in ISTA workshops and other ISTA events for all members of the laboratory or sampling entity at a reduced price.

The time required to process the membership depends on the completeness of the submitted application form and accompanying documents, as well as receipt of payment.

16.4.2 Participation in the ISTA Proficiency Tests

The ISTA Accreditation and Technical Department, with the Proficiency Test Committee Chair and its members, annually create PTs for the 'standard' test methods. The PT creation is not only sample preparation but also involves creating a programme for 3 years, managing the database to include new PTs, collating heterogeneity tests, and managing information related to the PTs. Other ISTA Technical Committees also create PTs for seed health, genetically modified organism (GMO) and variety tests. The PTs for the standard test methods aim to include all the standard tests and all the ISTA crop groups over a 3-year cycle. The ISTA PT programme plan can be consulted on the ISTA website[2] and is sent to a laboratory when they become an ISTA member.

Standard PTs contain at least three samples of a declared crop species. Each PT round (set of samples) includes at least one requested test (analytical purity, other seed determination, germination, moisture, viability, vigour or thousand-seed weight), and PT rounds are sent to participants during the year. After PT sample preparation, homogeneity tests are performed, and targeted other seeds are added to the samples. All added seeds are made non-viable before inclusion in the samples.

The ISTA Secretariat dispatches samples to participating laboratories around the world. Participation is mandatory for laboratories accredited by ISTA for the designated test and species group and voluntary for other laboratories. Laboratories preparing to become ISTA accredited must successfully participate in at least one PT round, e.g. one PT set for the species and tests they are interested in including in their accreditation scope. Contact the ISTA Accreditation and Technical Department for more details on the specific PT requirements to become ISTA accredited.

The PT results are statistically analysed by ISTA, and a laboratory's performance is rated as A, B, C or BMP (below minimum performance). The overall results of accredited and non-accredited member laboratories are analysed separately and reported on the ISTA website. As well as comparisons of results, the overall reports include images of the different seed species added to samples and are very useful training resources for all laboratories. A detailed and confidential performance report is also sent to each participating laboratory. This information and detailed feedback allow underperforming laboratories to identify areas of improvement.

Note: The process for becoming ISTA accredited for sampling only does not require participation in any ISTA Proficiency Test (PT) rounds.

16.4.3 Application for ISTA accreditation

1. *Complete and submit the application form to become accredited* – This can be done once a laboratory or sampling entity has become an ISTA member. The form can

be obtained from the ISTA Accreditation and Technical Department (audit@ista.ch).
An important step in the application is to define the scope of accreditation that covers the field of competencies and needs of the laboratory. The scope of accreditation is based on crop groups defined by ISTA and tests described in the ISTA Rules, including the sampling of seed lots.

2. *Submit the table of contents of the quality documents* – The laboratory or sampling entity should send a list of documents, including a quality manual, standard operating procedures (SOP), work instructions (WI) and related forms.
3. *Provide the quality documentation to the ISTA Accreditation and Technical Department* – The documents must reflect that the candidate for accreditation has established and implemented a QA system in accordance with the requirements of the ISTA Accreditation Standard. If this document is in a language other than English, the applicant must translate it into English.
The preparation process covered in these first three steps can take up to 6 months.
4. *Successfully participate in at least one ISTA PT round* – Applicants should join a PT programme for the species and tests they want to include in their accreditation scope. The rating obtained must be 'A' or 'B'.

16.4.4 ISTA audit

The purpose of the audit is to verify that the audited organization operates in conformity with the requirements outlined in the ISTA Accreditation Standard and confirm that the organization's management system is suitable to ensure compliance with the ISTA requirements. The audit involves a review of the submitted quality documents, an evaluation of PT results and an on-site assessment. During the assessment, relevant aspects regarding staff, facilities and seed sampling and/or testing are evaluated. Once the assigned ISTA auditing team has reviewed the quality documentation and it is considered satisfactory, an on-site assessment date is then agreed on, and the invoice for the first audit visit is issued. The laboratory or sampling entity must pay at least the first instalment of the invoice, before the on-site assessment. The on-site assessment is conducted by an ISTA auditing team, usually consisting of two auditors: a system auditor and a technical auditor. The system auditor is the lead auditor and can be an ISTA Secretariat staff member or an ISTA contracted system auditor. The technical auditor is chosen for their expertise and familiarity with the seed testing undertaken in the laboratory or sampling entity being audited. In some cases, a third auditor may be invited as an extra expert in a specific area of accreditation (e.g. seed health tests, GMO tests). After the on-site assessment, the auditors will provide the laboratory or sampling entity with an audit report and findings, including any identified nonconformities. The nonconformities must be addressed within a defined time frame, and corrective actions must be submitted to the audit team for review. The correction period time is limited to a total of 6 months after the audit date.

16.4.5 Granting accreditation

Once all substantial nonconformities have been addressed, within 6 months or less, the recommendation to grant accreditation to the laboratory or sampling entity will be made to the ISTA Executive Committee. When accredited, the organization will be listed as an accredited laboratory or sampling entity on the ISTA website. After accreditation is granted, the ISTA accreditation fee must be paid. The laboratory must participate in the ISTA PT programme for the tests for which it holds accreditation once it is accredited.

An accredited laboratory will be able to order and issue ISTA International Certificates (Orange and Blue). The certificates can be ordered from the ISTA Secretariat (ista.office@ista.ch). The certificates are used in the international seed trade to inform buyers and sellers about seed quality. An OIC is issued when both lot sampling and sample testing are performed under the responsibility of an accredited laboratory, under the authority of different accredited laboratories, or between an accredited sampling entity and an accredited laboratory. The results reported on an OIC refer strictly to the lot as a whole at the time of sampling. A BIC is issued when sampling from the lot is not under the responsibility of an accredited laboratory or sampling entity. The accredited laboratory is responsible only for testing the sample as submitted. It is not responsible for the relationship between the sample and any seed lot from which it may have been derived. The results reported on a BIC refer strictly to the sample at the time of receipt.

Re-accreditation is on a 3-year cycle. Every 3 years, the accredited laboratory is evaluated for its performance by an on-site audit following a similar process as the initial audit.

16.4.6 Accreditation termination, suspension or withdrawal

Laboratory or sampling entity accreditation can either be voluntarily terminated or suspended by the laboratory or sampling entity, or suspended or withdrawn by ISTA for specified reasons. Withdrawal of accreditation by ISTA is normally only following a suspension. Termination, suspension or withdrawal of accreditation may be applied to all or some specific test methods or crop groups included in the laboratory's or sampling entity's scope of accreditation. Because of the requirements of the ISTA Rules, termination, suspension or withdrawal of accreditation for a test will automatically include termination, suspension or withdrawal of accreditation for all test methods which require the use of that test. In such cases, the ISTA Accreditation and Technical Department will notify the laboratory of other tests involved. Any recommendation for suspension, withdrawal or reinstatement of accreditation is formally agreed upon by the ISTA Executive Committee.

Bibliography

This handbook refers to the following sources or suggests them as further reading. They may be accessed in hard copy or online.

References

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ISTA. 2022. *International Rules for Seed Testing*. Wallisellen, Switzerland, International Seed Testing Association.

Relevant papers and books

Black, M., Bewley, J.D. & Halmer, P., eds. 2006. *The Encyclopedia of Seeds: science, technology and uses*. Wallingford, United Kingdom of Great Britain and Northern Ireland, CAB International. 828 pp.

Ellis, R.H & Roberts, E.H. 1980. The influence of temperature and moisture on seed viability period in barley (*Hordeum distichum* L.). *Annals of Botany*, 45(1): 31–37.

Marcos-Filho, J. 2015. *Seed Physiology of Cultivated Plants*. Piracicaba, Brazil, Abrates. 660 pp.

Martin, A.C. & Barkley, W.D. 2000. *Seed Identification Manual*. Caldwell, United States of America, The Blackburn Press. 221 pp.

ISTA tools and resources

International Seed Testing Association
www.seedtest.org

Sampling Calculator: www.sampling-calculator.seedtest.org
ISTA Certificate Learning Tool: www.learn-ista.org
Moisture Calculator: www.seedtest.org/en/services-header/tools/moisture-committee/moisture-calculator.html
Seed Health Toolbox: www.seedtest.org/en/services-header/tools/seed-health-committee/seed-health-toolbox.html
***ISTA List of Stabilised Plant Names*:** www.seedtest.org/en/services-header/tools/nomenclature-committee/stabilised-plant-names.html
***ISTA Universal List of Species*:** www.seedtest.org/en/services-header/tools/purity-committee/universal-list-species.html
Seed Analyst Training: www.seedtest.org/en/technical-committees/seed-analyst-training-603.html
'Protocol for the approval of automatic seed samplers': www.seedtest.org/api/rm/48N2CF2Q5CRYT5F/tcom-p-03-protocolfortheapprovalofautomaticseedsam-1.pdf

ISTA *International Rules for Seed Testing*:
www.seedtest.org/en/publications/international-rules-seed-testing-1168.html

ISTA *Seed Science and Technology* journal:
www.seedtest.org/en/publications/seed-science-technology-1169.html

ISTA handbooks (www.seedtest.org/en/publications/handbooks-1170.html):

- *ISTA Handbook on Flower Seed Testing*
- *ISTA Handbook on Seedling Evaluation*
- *ISTA Handbook on Moisture Determination*
- *Handbook for Home-made Equipment*
- *ISTA Handbook on Seed Sampling*
- *Seed Health Methods* (annex to Chapter 7 of the ISTA Rules)
- *Handbook on Statistics in Seed Testing*
- *ISTA Working Sheets on Tetrazolium Testing*
- *Handbook of Tree and Shrub Seed Testing*
- Variety handbooks (www.seedtest.org/en/handbooks-calibration-samples/variety-committee-product-1048.html)
- *Handbook of Vigour Test Methods*

FAO publications

FAO Plant Production and Protection Division (NSP)
www.fao.org/agriculture/crops/agp-home/en

The **FAO 'Seeds Toolkit'** is designed for small farmers and extension workers and is written in simple language with illustrations.

- Module 1 – Development of small-scale seed enterprises
  www.fao.org/3/ca1490en/ca1490en.pdf
- Module 2 – Seed processing: principles, equipment and practice
  www.fao.org/3/ca1491en/ca1491en.pdf
- Module 3 – Seed quality assurance
  www.fao.org/3/ca1492en/ca1492en.pdf
- Module 4 – Seed sector regulatory framework
  www.fao.org/3/ca1493en/ca1493en.pdf

- Module 5 – Seed marketing
  www.fao.org/3/ca1494en/ca1494en.pdf
- Module 6 – Seed storage
  www.fao.org/3/ca1495en/ca1495en.pdf

FAO. 2019. *Voluntary Guidelines for the Conservation and Sustainable Use of Farmers' Varieties/Landraces*. Rome, FAO. 115 pp. doi.org/10.4060/CA5601EN

FAO. 2014. *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (revised edn). Rome, FAO. 182 pp. www.fao.org/3/i3704e/i3704e.pdf

Seed-related associations

International Society for Seed Science (ISSS)
www.seedscisoc.org
- ISSS *Seed Science Research* journal: www.cambridge.org/core/journals/seed-science-research

Association of Official Seed Analysts (AOSA) / Society of Commercial Seed Technologists (SCST)
www.analyzeseeds.com
- *AOSA Rules for Testing Seeds*
- *AOSA Seed Technologist Training Manual*
- *Purity Testing Handbook*
- *Seed Moisture Testing Handbook*
- *Seed Vigor Testing Handbook*
- *Tetrazolium Testing Handbook*

International Seed Federation (ISF) www.worldseed.org

Organisation for Economic Co-operation and Development (OECD) Seed Schemes www.oecd.org/agriculture/seeds

International Union for the Protection of New Varieties of Plants (UPOV) www.upov.int/portal/index.html.en

World Farmers' Organisation www.wfo-oma.org

Online seed ID tools

Most of the websites below have images to help with regular seed identification and in training.

Seed images can be found on the ***ISTA Universal List of Species*** (see web link, left). Use **GRIN Taxonomy** from the US National Plant Germplasm System for nomenclature and be aware that scientific names are frequently updated on this site: www.npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch

Digital Plant Atlas www.plantatlas.eu

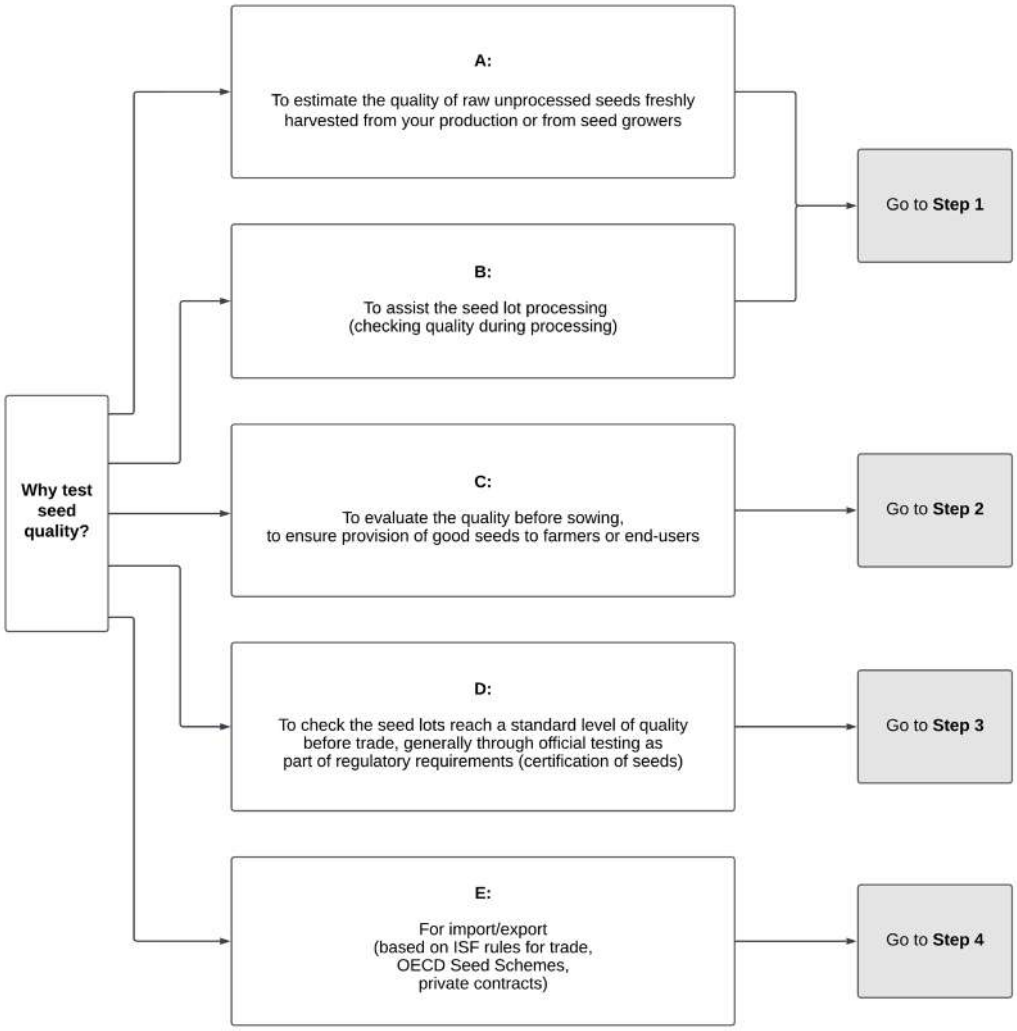
I.D.SEED GEVES www.geves.fr/tools/i-d-seed

International Seed Morphology Association (ISMA) www.idseed.org

Seed fact sheets are available from the **Italian Ministry of Agriculture** (www.crea.gov.it/web/difesa-e-certificazione/pubblicazioni-istituzionali-e-schede-tecniche), who also provide universal list pictures: www.crea.gov.it/web/difesa-e-certificazione/-/collezione-semi

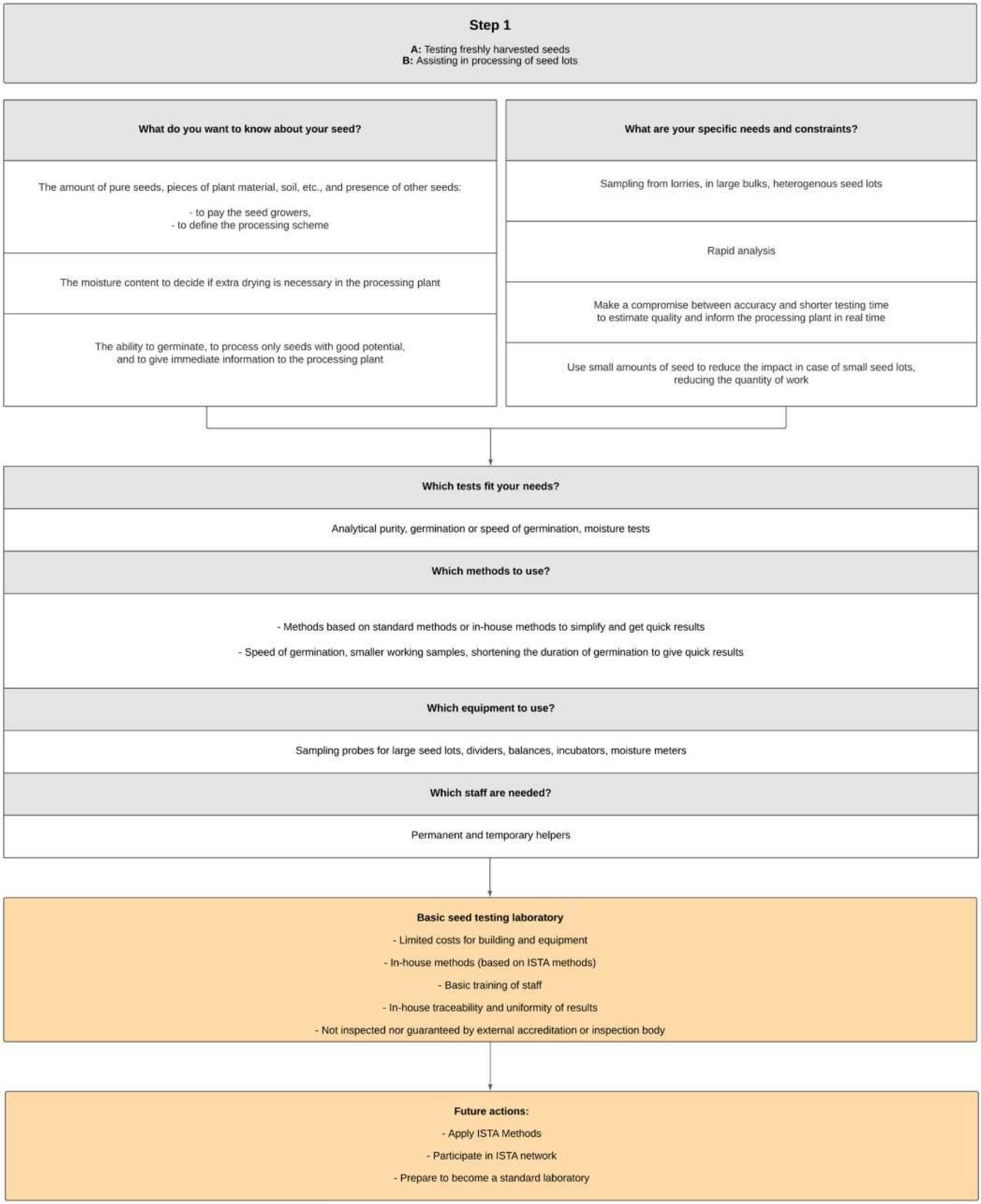
Appendix

A.1 Decision tree to define the needs of a seed testing laboratory



Note(s):
OECD = Organisation for Economic Co-operation and Development; ISF = International Seed Federation)

A.1 Decision tree to define the needs of a seed testing laboratory (Step 1)



A.1 Decision tree to define the needs of a seed testing laboratory (Step 2)

A.1 Decision tree to define the needs of a seed testing laboratory (Step 3)

Step 3

D: Check the seed lots reach a standard level of quality before trade, generally through official testing as part of regulations (certification of seeds)

What do you want to know about your seed?	What are your specific needs and constraints?
To guarantee that the seed lot reaches the minimum quality requirements for trade (e.g. certification)	Sampling to guarantee traceability to the end-user
To ensure traceability from the seed lot to the farmer or end-user	**Accuracy, reproductibility and uniformity of testing**
To guarantee scientific verification of the crop and identification of other species	Define the need for transport of samples
The ability to germinate and have optimum potential to develop in good soil conditions	**Follow national regulations**
The moisture content of the seed that will affect the weight for trade and maintaining viability during storage	**Guarantee that equipment is fit for purpose and controlled, and that the staff are competent**
Thousand-seed weight (TSW) to define sowing density	**Ensure sustainability of activities**

Which tests fit your needs?

- Sampling of seed lots, based on a network of trained and qualified samplers
- Analytical purity, other seed determination, ISTA germination, moisture tests, TSW

Which methods to use?

- ISTA methods for sampling and testing, or methods defined in the regulations
- Set up a quality assurance (QA) system

Which equipment to use?

- Sampling probes, dividers, balances, incubators, seed blowers (for some grasses)
- Moisture ovens and grinders
- Reference seed collection
- Have a system to calibrate the equipment
- Constant supply of material for testing (bags, boxes, germination substrates), electric power, water

Which staff are needed?

- Staff trained and qualified on standard methods (ISTA methods)
- Deputies for all main activities of staff
- Permanent qualified staff and helpers

Advanced seed testing laboratory

- Additional costs for calibration, verification and control of equipment
- QA system established
- Standard ISTA methods
- Qualified staff trained in using ISTA methods
- Reliability, traceability and uniformity of test results guaranteed
- Sampling network
- ISTA member (advised)

Future actions:

- Apply to become an ISTA accredited laboratory
- Participate in ISTA Proficiency Tests

A.1 Decision tree to define the needs of a seed testing laboratory (Step 4)

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