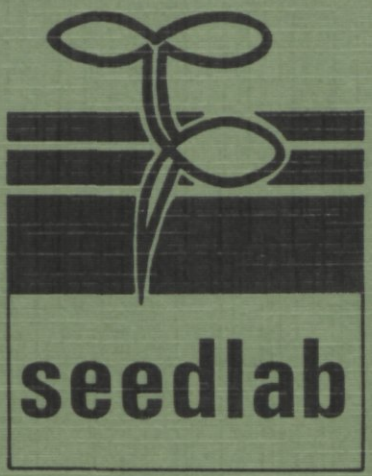
# project seed laboratory 2000-5000




## government seed testing station
## ministry of agriculture and fisheries
## netherlands

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# Project Seed Laboratory 2000–5000

(Second, revised edition)

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Contents



## Summary

A description is given of how a seed testing station in tropical or subtropical areas could be established. Two alternatives are described: Seedlab 2000, that can test about 2000 samples per year and Seedlab 5000 that can test at least 5000 samples per year. Directives and general considerations concerning the staffing, the organisation of the work, the lay-out of the building, and the equipment needed are given. Forty-six figures and two tables give an impression of the equipment and administrative forms used. Equipment which in the experience of the authors has been found suitable for the work is recommended, and detailed descriptions and company addresses are mentioned. Some equipment that is not commercially available is described and plans for construction are included. A list of books and journals for a basic seed testing library is appended.

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## Résumé

*Projet de laboratoire de semences 2000–5000*

On décrit comment une station d'essais de semences peut être établie dans les régions tropicales ou subtropicales. On décrit deux propositions: 'Seedlab 2000' qui peut analyser environ 2000 échantillons par an et 'Seedlab 5000' qui peut analyser au moins 5000 échantillons par an. On donne les principes directeurs et les considérations générales concernant le personnel, l'organisation du travail, la disposition du bâtiment et l'équipement nécessaires. 46 figures et deux tableaux donnent une idée de l'équipement et des documents administratifs utilisés. On recommande les équipements, qui d'après l'expérience des auteurs se sont révélés adaptés au travail et on fait état des descriptions détaillées et des adresses de compagnies. On décrit quelques matériels qui ne sont pas disponibles dans le commerce et leurs plans de construction sont inclus. Une liste de livres et de périodiques pour une bibliothèque de base en matière d'essais de semences est donnée en appendice.

## Zusammenfassung

*Das Projekt Saatgutlabor 2000–5000*

Es wird beschrieben, wie eine Saatgutprüfstelle in tropischen oder subtropischen Gebieten errichtet werden könnte. Dabei werden zwei Alternativen angeführt: Das Saatgutlabor 2000, welches ungefähr 2000 Proben pro Jahr prüfen kann, und das Saatgutlabor 5000, welches mindestens 5000 Proben pro Jahr untersuchen kann. Hinweise werden gegeben und Überlegungen angestellt hinsichtlich der Mitarbeiter, der Arbeitsorganisation, der Gebäudeplanung und der erforderlichen Geräteausrüstung. 46 Abbildungen und 2 Tabellen vermitteln einen Eindruck von der Einrichtung und den angewandten Verwaltungsformen. Ausrüstungsstücke, welche nach der Erfahrung der Autoren sich für die Arbeit als brachbar erwiesen haben, werden empfohlen sowie genaue Beschreibungen und Firmenanschriften aufgeführt. Geräte, welche auf Handelsbasis nicht erhältlich sind, werden beschrieben und Pläne für die Herstellung beigegeben. Als Anhang findet sich ein Verzeichnis von Büchern und Zeitschriften, die den Grundstock einer Bibliothek für eine Saatgutprüfstelle bilden.

## 1. Introduction

In 1979 the Government Seed Testing Station, Wageningen, Netherlands, published a paper called *Project Seed Laboratory 2000–5000* by M. Heuver, J. Bekendam, W. J. van der Burg and A. van Geffen, based on *Project Seed Laboratory 5000* (Proc. Int. Seed Test. Ass. 34 (1), 1969). It consisted of a text part, a binder with technical drawings and a binder with brochures of equipment. One hundred copies were distributed by FAO and another 100 copies by the Wageningen station. It described how a seed laboratory could be constructed, equipped, organised and administered. Alternatives were given for a laboratory that could test about 2000 samples per year and one that could handle at least 5000 samples per year. Certain equipment was recommended, based on the experience of the authors with it. Equipment produced by other manufacturers and used in other countries may however be as good. In this connection readers are advised to consult the Survey of Equipment and Supplies for Seed Testing (ISTA, 1981).

The need was felt to make it more widely available through a concise re-edition

in Seed Science and Technology. To this end the information on equipment is updated, much of the information from the binders is included in the text and figures, and recent experience of the authors in developing countries is included. However, one aspect is left out: seed health testing. The original publication gave only very limited information on the aspects of seed health testing and the equipment and training needed, and it was felt that the topic needed a paper of its own to be complete. Besides, the authors believe that seed laboratories of this kind, often located in countries with a developing seed industry, should start by investigating the basic quality aspects of the seed first: moisture, purity, germination.

The authors gratefully acknowledge the assistance of the following persons who have given their support either during the preparation of the original paper or this re-edition (in alphabetical order):
Dr. C. Anselme, C. Bense, Dr. W. P. Feistritzer, Prof. E. E. Hardin, Dr. M. J. Hill, Prof. L. Kåhre, D. B. MacKay, Dr. E. Madsen, Ing. A. Stuurman, J. H. B. Tonkin, C. Witte.

## 2. Staffing

### *2.1. General*

Seed testing requires an input of manpower that differs for each type of seed. A range of species therefore has to be allowed for in determining staff requirements. The presented estimate aims at the average requirements of a station in a tropical or subtropical region, dealing with 2000 or 5000 samples annually.

The work should be arranged in such a way that germination tests on all samples can be commenced within a reasonable time (24–48 hours) from receipt at the station. Intake of samples is rarely constant, neither in rate, nor in composition. For the laboratory testing 5000 samples annually a peak season lasting possibly three to five months can be expected, with an intake of 1750–3000 samples during that period. The station is expected to handle this peak expeditiously, with no more than a slight delay.

An example of possible staffing is given below. The number of analysts depends very much on the intake of samples in the peak period, the type of seed, the kind and number of analyses required and the experience of the staff.

### *2.2. Purity analysts*

An intake of 1750 samples in three months, each providing 21 working days, gives 28 samples per day. This means that for purity, each sample being subjected to duplicate analysis (two half working samples), 56 determinations per day have to be made. The time needed will then be:

| number of determinations | kind of seed | man hours |
|---|---|---|
| 14 × 2 | cereals, rice and maize | 9 |
| 7 × 2 | pulses | 4 |
| 7 × 2 | forage crops, including 'difficult' grasses | 19 |
| TOTAL 56 | | 32 |

Time must also be allowed for performing additional determinations whenever duplicate analyses are not sufficiently in agreement (this is checked by using the tolerance tables provided in the ISTA Rules).

If one analyst provides eight working hours a day, the purity section would be adequately staffed with four trained analysts. Staffing at this rate may sometimes lead to a backlog accumulating, but delays should not be alarming. Overstaffing is felt especially in the slack season as trained analysts cannot normally be laid off then.

One of the analysts should be delegated as responsible for the purity section.

*2.3. Germination analysts*

An intake of 28 samples per day means that 28 germination tests have to be carried out daily (not including a few tests that will appear not to be satisfactory for one reason or another and therefore have to be repeated). Although the amount of work involved depends on the kind of seed, variations are not as large as in purity analysis.

The work comprises:

- counting and planting 4 × 100 seeds, the substrate being moist paper, sand or soil;
- providing controlled environmental conditions for the tests;
- evaluating seedlings and results; most samples need several consecutive counts before the final result is obtained.

Three trained analysts should be able to handle 28 tests a day.

To supervise germination testing, including the flow of analysis, check on use and accuracy of equipment and maintain supplies one of the analysts should be placed in charge of the germination section.

*2.4. Moisture analysts & administrative staff*

All seven analysts should be trained in both aspects of analysis: purity and germination. One or more should also be trained to perform moisture determinations.

Unless calculation is carried out by an administrative clerk at least one analyst in each of the purity and the germination section should be trained to handle the calculation of test results.

In addition a seed laboratory needs administrative staff. This is even more so when an established laboratory also deals with seed certification, an activity that involves a considerable administrative burden. A starting seed laboratory however, should have at least one clerk/typist.

*2.5. Person-in-charge, training of staff*

Finally there will be a person in overall charge. It is essential that this person should have a good scientific background, preferably in botany or agronomy. He also needs at least a three months stay in another station with facilities and experience to provide him with adequate training. The analyst in charge of the purity or germination section should preferably have sufficient secondary education, if possible including professional education in agriculture. He or she should also receive training in another laboratory and attend international workshops from time to time. Such workshops are organised by or in co-operation with international organisation like FAO and ISTA and individual countries (e.g. the FAO-Norway Workshops and regional workshops as organised by the Seed Technology Centre of New Zealand, etc.). The analysts of the laboratory should receive training for an initial period of for instance two years which may be concluded with examinations.

*2.6. Total staff required*

To summarise, the 5000 laboratory should have the following staff:

1 superintendent
1 clerk/typist
7 analysts for moisture, purity and germination

---

9 persons in total

A smaller laboratory testing 2000 samples annually needs less staff. Four analysts qualified in both purity and germination testing could cope with this intake, one of them being given general responsibility for seeing that the work is done promptly and in accordance with the regulations.

**3. The work**

*3.1. Receipt and registration of submitted samples*

A submitted sample is a quantity of seed furnishing sufficient seed for the tests required. It is sent in a linen bag or other container. The submitted sample must be representative of the lot, containing the same constituents in the same proportion. How a representative sample is drawn from the lot and reduced to submitted sample size is prescribed in the ISTA Rules. On receipt, a clerk records particulars of the sample and tests requested, paying particular attention to any special requests.

The use of preprinted forms is recommended for entering the details to ensure accuracy and speed in issue of information by the station. For further detail see chapter 13.

*3.2. Purity test*

The object of the purity analysis is to determine:

– the composition by weight of the sample and by inference the composition of the

seed lot;
- the identity of the various species of seeds and other material in the sample.

The ISTA Rules prescribe:
- the size of a submitted sample;
- the size of a purity working sample, and how it should be taken from a submitted sample;
- how to separate a working sample into: pure seed
  other seed
  inert matter;
- how to evaluate test results, and how to gather additional evidence if needed;
- how to express and report the test results.

Moreover, the Rules recommend certain technical aids. If used with skill, these aids (see chapter 8) will reduce the time taken by purity analyses.

The purity test also provides the pure seed, which is essential for the germination test.

*3.3. Germination test*

A germination test provides information with respect to the field planting value of the seed. The ISTA Rules prescribe:
- number of seeds to be counted at random from the pure seed fraction;
- qualities and use of substrata: paper, sand and soil;
- directions for planting the seed;
- treatments for breaking dormancy;
- conditions of moisture, temperature and light that are optimal for growth and evaluation of the seedling;
- definitions by which seedlings are evaluated and rules for interpreting and reporting test results.

The Rules also recommend certain technical aids (see also chapter 9).

*3.4. Moisture test*

A moisture test provides information regarding the moisture content of the seed. The moisture content is a factor of economic importance because:
- too high a moisture content may result in loss of quality because of mould growth, ageing and increased insect damage;
- the proportion of water being paid for.

A sample of seed for a moisture test must be packed in a moisture proof container so that the sample cannot either dry out or absorb moisture between sampling and testing. In the ISTA Rules test methods are prescribed for each kind of seed.

*3.5. Various other items as prescribed and/or recommended in the ISTA Rules*

Purity tests for which dry and clean surroundings are essential, and moisture tests that would be badly affected by too high a relative humidity in the atmosphere, should be carried out away from the germination work, preferably in separate rooms.

Such rooms are provided in Seedlab 5000 (figure 1); Seedlab 2000 however has only separate germination and purity benches (figure 4). (Germination tests invariably generate much refuse in discarded seedlings, paper, sand, as well as water spilled on the working tables).

Other determinations which are usually carried out in the purity section are:
- number of weed and other crop seeds present, usually determined in 10 times the quantity of seed that is specified for purity; the seed acts of many countries specify maximum standards for weed and other crop seeds above which seed may not be imported;
- weight of 1000 seeds; this serves as a basis for estimating the weight needed to sow an area of land and can be indicative of the quality of the seed.

Other determinations which may be carried out in the germination section are:
- viability by means of the topographical tetrazolium test (see chapter 10).

The determination of genuineness of species or cultivar may be of interest. Techniques recommended in the ISTA Rules involve the examination of seeds and seedlings in the laboratory and plants in the greenhouse and in field plots.
The Rules also include a chapter dealing with International Certificates, reporting and tolerances. This is of particular interest to official laboratories that intend to join ISTA, and to issue International ISTA Certificates. Information on how to become an accredited ISTA station can be obtained through the ISTA Secretariat.

## 4. The building

### *4.1. General requirements*

In order to design a building that would provide the facilities for a simple laboratory for routine investigation of seed samples in developing countries, the special requirements of seed testing have been taken into consideration as well as the possible climatic conditions.

The following lay-out requirements have been considered:
1. a maximum of working space in a building of limited size;
2. an efficient and functional lay-out: the movement of samples through the laboratory should be logical;
3. central supervision;
4. adequate illumination and ventilation;
5. water supply and sewage concentrated to minimise plumbing work;
6. ease of extention of the building: allowance should be made for example for extending the administrative section of the building at a future date at moderate cost and effort and without seriously interfering with the structure of the building.

The points above influence both the lay-out of the building and the placing of furnishings and equipment within.

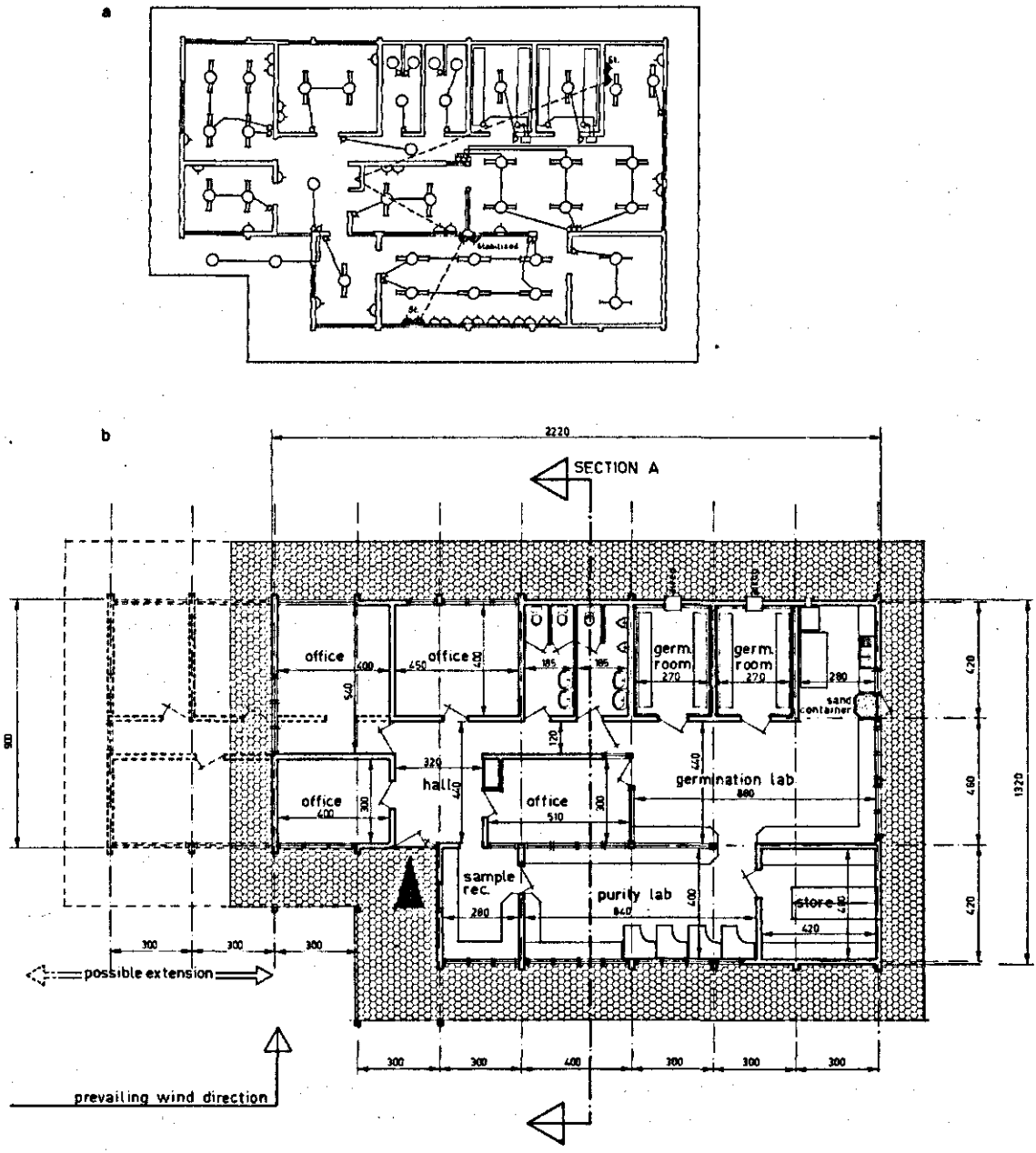
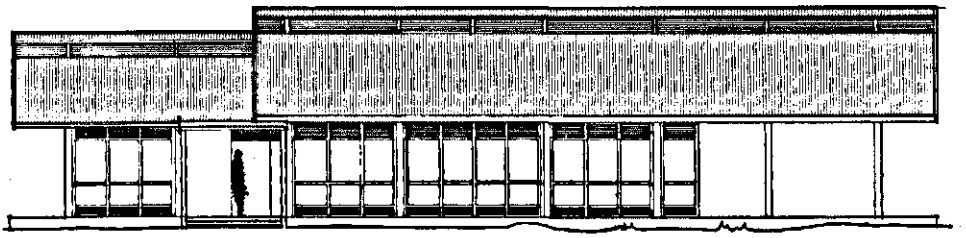



Figure 1. Seedlab 5000, wiring scheme and floor plan.
a. Electrical wiring scheme; dotted lines and hatched power outlets indicate stabilised power supply; b. floor plan, showing the possible extension that can be made, either during building or at a later stage; section A is presented in figure 2. Dimensions in cm.

a



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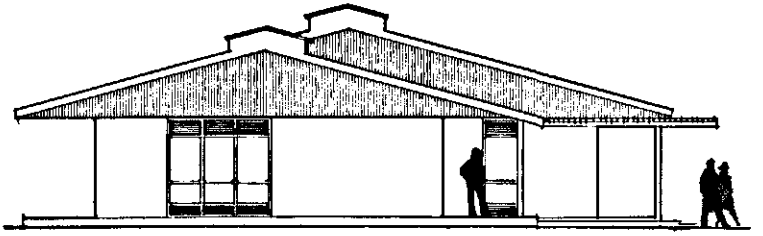

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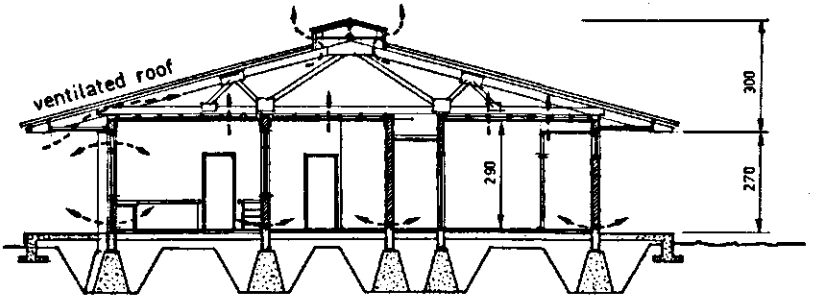



Figure 2. Seedlab 5000, elevations and section.
a. Front elevation; b. left elevation; c. section A as indicated in figure 1.b., showing the provisions for ventilation. Dimensions in cm.

*4.2. Lay-out*

The building is roughly divided into a laboratory section and an administrative section. If administrative activities increase or expand, the building can be adapted by enlarging it as indicated in figures 1 and 4.

The building has an overhanging roof providing shelter against direct sunlight and heavy rains; in addition it forces the rain water to run freely from the roof leaving a dry gallery around the building.

Entrance to the building is by a central hall, which is surrounded by offices and the sample reception area. From here samples are conveyed to the purity section. In order to make precise weighings a special vibration free weighing table is installed in the purity section. A voltage stabilised electrical supply may be essential for operating the sensitive balances and blowers. A long continuous working bench is fixed to one side with the purity tables opposite, facing the window for good illumination.

A storage room is provided with easy access from both purity and germination sections. The seed must be stored cool and dry and kept free from insects. The maximum temperature should be 15°–18 °C, so care must be taken in warm and humid climates to avoid condensation on the walls of the storage room (for example by adding insulation material to the walls). Chapter 9 gives details of wall construction.

In the germination section continuous working benches are provided. The space underneath these benches must be kept as free as possible, e.g. storage shelves underneath should be restricted and should not be in the way of analysts working at the bench. Cabinets on turnable wheels that fit under the bench are very convenient in this respect. In one corner there is a sink with an electric water heater (boiler) and an open brick container for sand, height about 50 cm and accessible from the outside for refilling. An electric concrete mixer can also be placed in this area for preparation of sand substrates. A voltage stabilised electrical supply may be essential and supply of clean water for cooling of the germination table and cabinet should be provided. If a cabinet or table with an air-cooled cooling system is chosen a ventilation opening in the wall is necessary.

An aluminium rack system is used in the germination rooms. This can be made following figure 20 or purchased ready made (see chapter 9). Every section has its own upright tubular fluorescent light which can be switched on individually from the front. The whole system is controlled by a programmable time-switch fitted on the outside of the germination room. For insulation directions see chapter 9.

The insulated doors may be bought from a firm specialising in this field, but could possibly be made locally of e.g. non-shrinking hardwood.

The supervisors' office is located centrally in the laboratory, with windows on three sides to give easy observation of all activities in the laboratory.

The office section is grouped around the hall and can also be used to accommodate visitors, or used as storeroom for stationery and equipment.

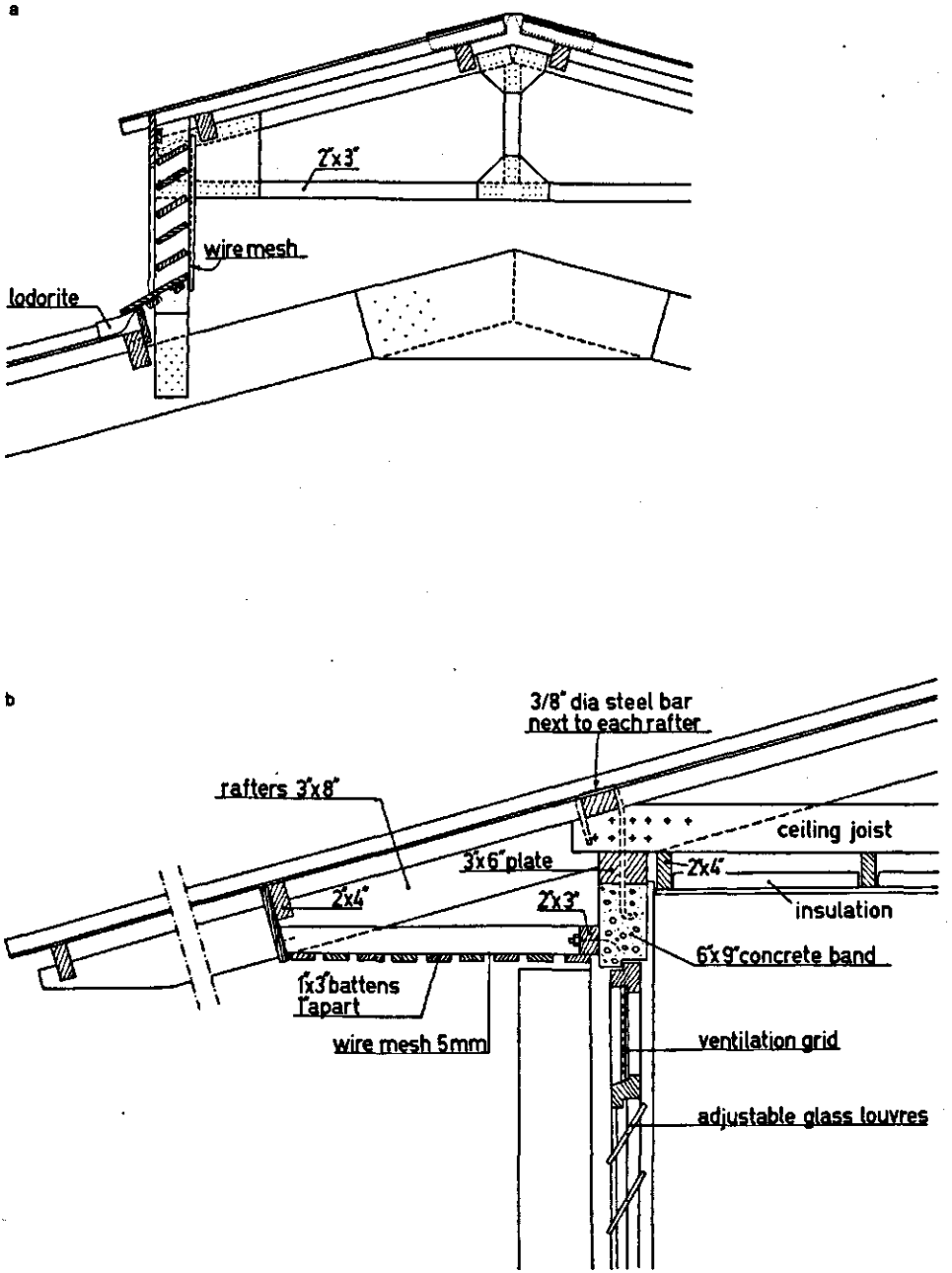



Figure 3. Seedlab 5000, roof construction.
a. Details of ventilation opening on top of roof; b. details of edge of roof showing ventilation opening with wire mesh. All dimensions in inches unless mentioned otherwise.

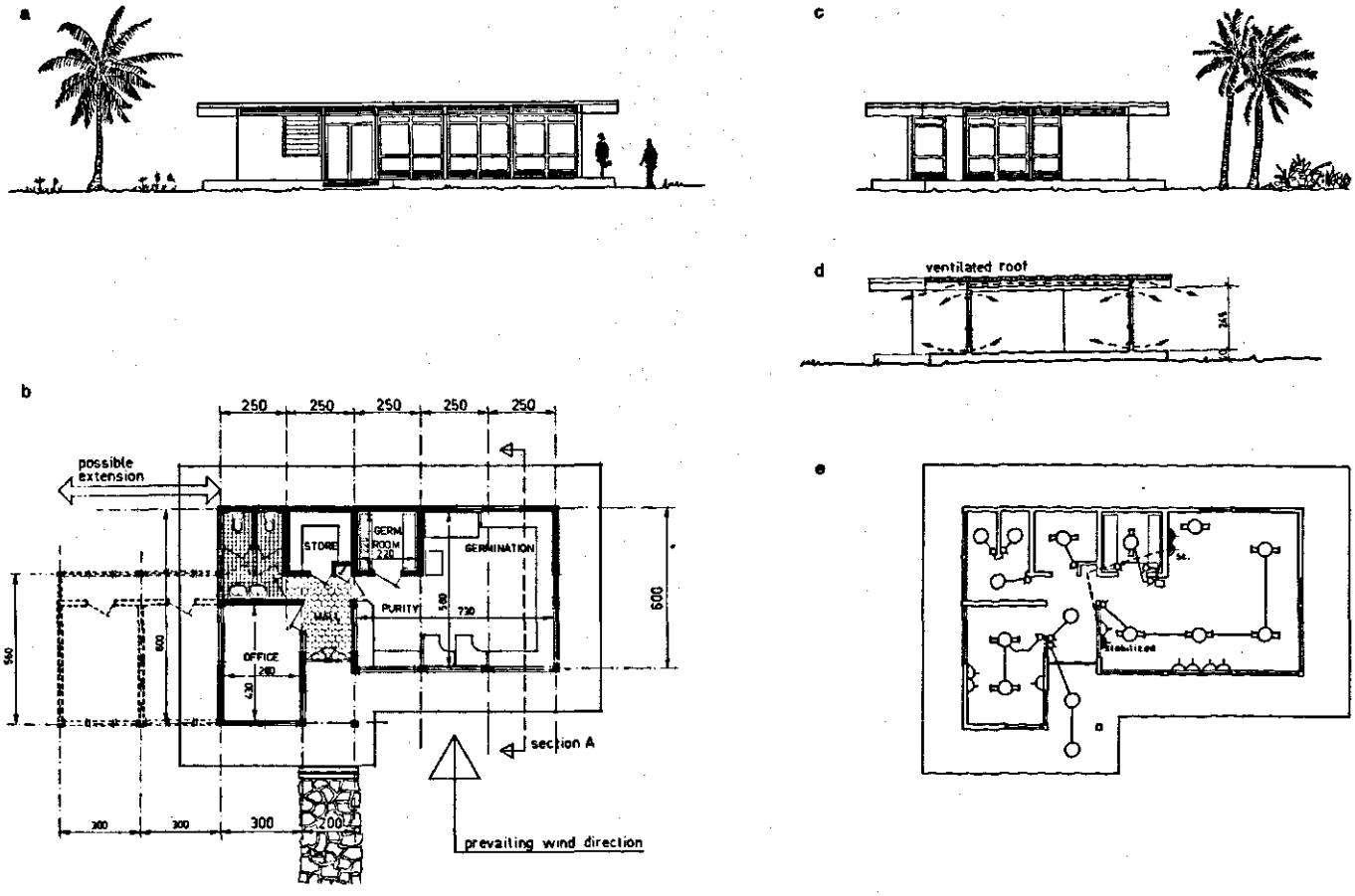



Figure 4. Seedlab 2000, elevations and floor plan.
a. Front elevation; b. floor plan showing possibility of extension; c. right elevation; d. section A of 4.b showing ventilation; e. electrical wiring scheme showing stabilised power supply. Dimensions in cm.

*4.3. Ventilation*

*Roof.* The laboratory has an overhanging roof to provide shadow.

- Seedlab 5000: The roof has an inclination of 15° and has a ventilation opening running lengthwise at the top. Just underneath the roof a ventilation opening is also provided, creating a chimney-effect that will ensure ventilation even in the absence of a breeze. The cooling effect of this ventilation system can be increased considerably by fixing aluminium foil at a distance of 3–8 cm below the roof-plating, leaving openings at the bottom and the top to allow ventilation. This design avoids any heat radiation from the roofing material to the air and building underneath. Ventilation openings are provided in the ceilings of rooms, so that air can move upwards to the top of the roof.

  The ventilation opening at the top and just underneath the roof should be covered with 4 mm to 8 mm square wire mesh.

  This type of roof is particularly suited to humid regions, whereas in dry climates a concrete roof, as used on Seedlab 2000 is satisfactory. In view of the size of Seedlab 5000 thermal effects on a concrete roof for this building should be taken into account and attention to detail in construction is essential.
- Seedlab 2000: This structure has a concrete roof, which should not pose any problems in view of the small dimensions of the building. This roof is also ventilated, the space directly underneath the roof having slits between the beams.

  With this construction it is very advantageous to add securely fixed plates of aluminium or another material, some 5–7 cm above the concrete, to ensure free air movement between the layers. This measure reduces thermal effects from the concrete roof considerably.

*Walls.* For the window-sections a standard size has been chosen in order to enable standardisation of most parts such as window panes, ventilation grids, etc. Prefabrication of these sections incorporating two ventilation openings in the window pane can be advantageous. Each section is composed of several modular parts, from bottom to top:

A – 30 cm, can be fitted with a variable or fixed ventilation opening, or be solid;
B – 60 cm, usually solid;
C – 120 cm, window, can be a louvre-type or two 60 cm opening frames (for the purity section it is advisable to keep windows closed);
D – 30 cm, can be fitted with a ventilation opening (variable or fixed) or an air-conditioned unit;
E – 5 cm, facia board.

The various ventilation openings should all be covered with mesh or mosquito mesh to stop the intrusion of insects, mice, etc. The ventilation openings at the bottom will ensure an effective cross-ventilation, usually considered very effective.

In the central office openings at the bottom of the walls and openings in the ceiling provide ventilation. In addition ceiling ventilators, working upwards, may be installed in the openings where considered desirable.

It is advisable to ventilate the toilets directly to the outside.

*4.4. Special installations*

For electric equipment such as balances, blowers and the germination equipment a voltage stabilised power supply may be required to ensure a stable voltage (figures 1a and 4e). Sockets should be located for easy use and it is preferable for those for stabilised power to have a different colour or design than those for non stabilised electric current.

In the purity section a weighing table with stone slab or a concrete working bench is required to provide a stable base for installing the balances. This can be purchased or be made on site; special attention should be paid to ensure a vibration-free construction (figure 11).

When handling treated seed an exhaust system must be used, especially when subdividing (submitted sample) and analysing a working sample for purity; also when counting and planting the seed for germination. For instance, a hood could be installed above the place where the seed is handled through which the dust and fumes are sucked off by means of a ventilator.

For the construction of the germination rooms see chapter 9.

Reinforced concrete columns and brick or concrete block walls are planned. If this proves to be too expensive, wood may be used. In that case the roof of Seedlab 2000 also has to be made of wood. All wood should be treated to improve its resistance to moisture, rot and insects.

Construction may be adapted to local circumstances, but it is imperative that the lay-out of the building remains unaltered, in order not to affect the efficiency of the work.

## 5. Sample reception

The main tasks of the office for sample reception are to register the submitted samples, provide them with an analysis number (= identification of the sample) and to decide what kinds of tests are required. Working sheets (analysis forms) are prepared for each test.

Most samples arrive at the laboratory by post. Preferably, applicants put their requirements on standard forms provided by the laboratory ('request forms', see paragraph 13.2).

Before starting to unpack a sample, it should be verified that both the sample and the tests requested meet with the conditions (such as those in the ISTA Rules) regarding identification, marking, sealing, packing and weight. Irregularities may be found, such as:

- the species or cultivar name on the request form is not the same as on the label of the sample;
- a moisture test has been requested, but a special moisture test sample has not been submitted;

– too much time appears to have elapsed since the sample was taken from the lot.

Such irregularities are recorded on the request form. The applicant or the sampler can be asked to provide additional information. All relevant details are recorded on the form. Preprinted forms of various types are an invaluable aid. Examples of such forms are given in chapter 13. Any information recorded on such forms is readily available to the laboratory.

The applicant's identity should never be shown on the analysis forms as it may bias the performance of an analyst.

Date of receipt and sample registration number are stamped on the analysis forms, the request form and the sample label. The use of a numbering machine and rubber stamps is recommended not only for numbering and for dating, but also for other items that appear frequently, such as codes and species names.

Blank forms, rubber stamps, scissors, glue, etc. should be stored readily available for use in the sample reception section. Stocks should be maintained at a level which will last a season. There should be ample room for temporary storage of incoming samples to leave working surfaces free for the safe handling of each sample and the preparation of the appropriate documents.

## 6. Administration centre (office)

Each sample, together with the set of forms that has been prepared in the sample reception area is passed to the administration centre. The superintendent checks the partly completed forms and distributes the samples and the forms to the appropriate sections that are to provide the required data. The cover form (see paragraph 13.3.), with the request form, is filed in an open tray, to be readily at hand for consultation. As soon as the other forms have been completed with the test results, they are filed in the cover form. This procedure is necessary in order to be able to provide interested parties (e.g. the sender of the sample) at all times with data on the progress of the different tests, and with provisional test results.

Calculation of the test results may either be carried out by the administration centre or by the leading analysts of the respective sections.

The purity test results are transformed into weight percentages with the aid of a calculating machine. The duplicate percentages are averaged and checked against ISTA tolerances. If they are not in tolerance, additional duplicate tests are prepared.

The germination test results are expressed in percentages based on number of seeds. If the test results are out of tolerance, the germination section performs a repeat test requiring further pure seed and another form.

As soon as all data have been obtained, the results can be forwarded to the applicant. Before that, a final check is made on the completeness and correctness of the data. Then the data are typed on a standard report form or certificate. Different types of standard forms are kept in stock in the office. When the results have been issued, the forms are filed in the cover form and put into the filing cabinet under 'concluded tests'. They are kept for a period (which may depend on legislation) after which they are disposed of.

The administration centre not only ensures the smooth running of the station, it also renders the best possible service to the applicants. This means that (preliminary) results are transmitted to the applicant whenever requested by telephone or by any other means.

## 7. Moisture unit

### *7.1. Introduction*

The object of a moisture analysis is to determine the moisture content of a seed lot at the time it was sampled. To that end, the sample must be handled in such a way that its initial moisture content is retained. Packed in a sealed moisture proof container (metal or plastic), it must be submitted to the station without delay and analysed directly upon arrival (see also paragraph 13.4.).

Methods for determining moisture in seed are:

A. Oven method. This is the common standard method. For detailed specifications see ISTA Rules. The principle of this method is the elimination of water from the seed by heat under precisely controlled conditions.
B. Quick methods. A variety of brands and types of rapid moisture meters are available. The quick test method should be calibrated or checked against the standard oven method, and is in general less accurate than the oven method.

### *7.2. Oven method*

The moisture analysis is carried out on independently drawn duplicate working samples, weighed with an accuracy of 1 mg. With the exception of cereals (two hours) and maize (four hours) most species are dried for one hour at 130 °C. Seeds containing oil are dried for 17 hours at 103 °C. Each empty container (non corrosive metal) is weighed, including its lid. The submitted sample is thoroughly mixed with a small spoon and two 5000 g portions are weighed directly into the containers. After weighing the containers with seed are placed in the oven which has already been heated to the drying temperature. The temperature in the oven drops when the samples are placed in it and therefore the drying period is counted from the moment the oven has regained the required temperature. At the end of the drying period the lids are placed on the containers and the containers are allowed to cool for 30–45 minutes in a desiccator (filled with e.g. silicagel) and then weighed again. All weighings should occur with an accuracy of three decimal places. The moisture content (M) is calculated to one decimal place by means of the formula:

$$M = \frac{M2\text{-}M3}{M2\text{-}M1} \times 100 = \frac{\text{loss of weight}}{\text{initial weight of seed}} \times 100$$

M1 = weight of empty container with lid
M2 = weight of container with lid and seed before drying
M3 = weight of container with lid and seed after drying and cooling

Where loss of weight can also be calculated as: weight of seed before drying – weight of seed after drying.

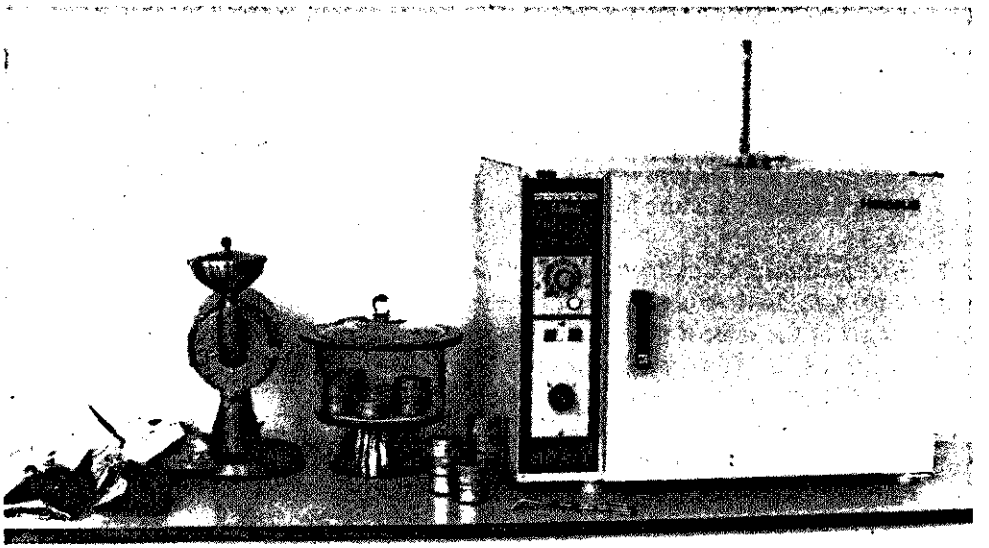


Figure 5. Moisture apparatus.
From left to right: moisture proof packed samples; grinding mill; desiccator; aluminium sample containers; pair of tongs; oven with thermometer inserted in top.

The duplicate result may not differ by more than 0.2% moisture content. Otherwise the analysis must be repeated.

*Grinding/pre-drying*. Some seed species must be ground (see ISTA Rules) before the actual moisture content is determined (cotton, rice, maize, cereals, sorghum, peas and beans). For cereal and cotton seeds fine grinding is necessary; at least 50% of the ground material shall pass through a wire sieve with meshes of 0.5 mm and not more than 10% shall remain on a wire sieve with meshes of 1.0 mm. For leguminous seeds coarse grinding is necessary; at least 50% of the ground material shall pass through a wire sieve with meshes of 2.0 mm. Adjust the grinding mill to obtain particles of the required dimensions. Grind a quantity of seed greater than that required for the test (about 20 g). For the seeds that have to be ground prior to drying, a retest should be made when the moisture content proves to be 17.0% or higher (for soya bean and rice these percentages are 10.0 and 13.0 respectively). But then the seed must be pre-dried before being ground. To that end two 50.00 g portions are weighed and placed on two open trays in the oven at 130 °C for 5–10 minutes (in the case of very moist seed, above 25.0% moisture content, the seed is spread in two open trays and dried at 70 °C for 2–5 hours, depending on the initial water content). The open trays are then placed for at least two hours in the laboratory and each of the duplicate quantities is weighed and a portion ground (e.g. 20 g). The ground material is then subjected

to a moisture test using the oven method. The moisture content (M) in the case of pre-drying is calculated according to the formula:

$$M = S_1 + S_2 - \frac{S_1 \times S_2}{100}$$

$S_1$ = percentage of moisture lost by pre-drying (stage 1)
$S_2$ = percentage of moisture lost by the oven method (stage 2)

*Moisture containers.* Containers should be of non corrosive metal (thickness approximately 0.5 mm) with side rounded at the base and a flat bottom, with loose fitting lids that are at the same time very flat in order to prevent loss of moisture. Suggested dimensions are: height 3 cm, diameter base 6 cm, so that there is not more than 0.3 g of material per $cm^2$. To ensure fitting of the lids, the rim of the container should be levelled by rubbing with an abrasive. Both lid and container should be provided with the same number to identify each sample after the drying period.

*Oven.* An electrically heated oven with adequate ventilation and thermostatic control which permits the temperature to be maintained at 130° ± 3°C. The heating capacity of the oven must be such that after preheating to a temperature of 130°C followed by opening and loading with containers, the oven will reach 130°C again within approximately 15 minutes.

*Balance.* A balance which weighs accurately in grams to three decimal places. Although the analytical balance of the weighing unit can be used, a more suitable type is the 'precision balance 2' (the intermediate type of table 1, see paragraph 8.2.).

*Grinding mill.* This must meet the following requirements:

a. It should be constructed of a material that cannot absorb moisture. Wood is not suitable.
b. It should be constructed in such a way that both seeds to be ground and the resulting ground material are protected as much as possible from the atmosphere of the room while grinding.
c. It should grind evenly, and should not be operated at such a high speed that the ground material is heated. Air currents that might cause loss of moisture must be reduced to a minimum.
d. It should be suited to large, small as well as hard seeds and the fineness of grinding should be adjustable; thorough cleaning should be easy.

*Sieves.* A set of three wire sieves and bottom receptacle should be available. The set shall have sieves of 0.5, 1.0 and 2.0 mm wire mesh.

*Desiccator.* The desiccator should have a thick metal or porcelain plate to speed cooling of the containers and seed. The bottom compartment has to be filled with a suitable

desiccant, e.g. silicagel coloured with cobalt chloride as indicator: as soon as the dark blue colour becomes pale pink, the desiccant should be reactivated by heating in the oven (up to 130 °C).

*A pair of crucible tongs.* To handle the (hot) containers.

### *7.3. Quick methods*

Various equipment has been designed to shorten the time taken for moisture determination. These quick methods may be classified by the two different principles used:

*7.3.1. Apparatus where the seed is heated directly by an infrared lamp and weighed by a built-in balance.* When seeds are heated by an infra-red lamp, heating of the material to higher temperatures is required than for the oven method. Most of these devices have a balance which continuously measures the loss of weight of the sample while being heated. The moisture percentage is usually read off from a direct reading scale. No calculations have to be made. The test can be finished in 10–15 minutes, depending on the kind of seed.


Figure 6. Quick moisture meter with infrared lamp.

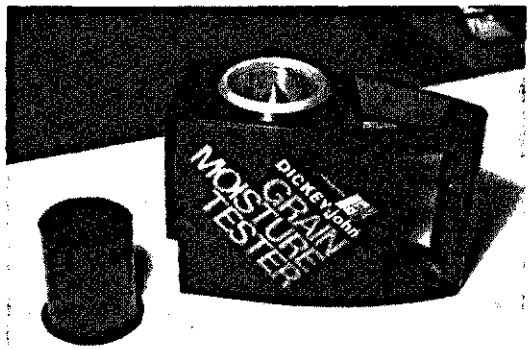



Figure 7. Portable, battery operated quick moisture meter.

*7.3.2. Electric moisture meters, where the moisture of the seed is directly determined by e.g. its conductivity.* Electric moisture meters are frequently used for a quick test. These meters do have a great advantage over all other methods in speed of use. The test can be completed in one minute.

For both types of quick meter each meter must be calibrated for each species and possibly each cultivar. The calibration should be carried out against the standard air oven method and the moisture determinations should be executed under standardised conditions. In general the reading of the meters is less precise than the result determined with the oven method and there may be as much as 1–2% difference.

Consequently, even calibrated meters are only suitable for approximate determination of the moisture content. The meters are especially suited for measuring moisture content in the field and during the process of seed drying and storage in the warehouse.

## 8. Purity section

The purity section makes tests on the seeds as received. The various functions are located in four units: subsampling, weighing, blowing and purity analysis.

*8.1. Subsampling unit*

Subsamples are taken from the submitted sample for the purity analysis. The object is to obtain a subsample (working sample) that is of the same composition as the submitted sample but will take less time to analyse.

The ISTA Rules contain detailed prescriptions for subsampling equipment and procedures. For taking a purity working sample (preferably two half working samples, independently drawn), the Wageningen station uses a combination of the mechanical dividing method and the spoon method.

This combination suits the size and the composition of almost any submitted sample, and moreover, enables the operator to work with optimal speed and efficiency.

The object of using a mechanical divider is to minimise any bias in the subsampling. Thorough mixing of the sample can be achieved by passing the entire sample through the divider several times before dividing off the portion required.

The object of further reducing the subsample, by means of the spoon method, is to get as close as possible to the prescribed minimum weight of the (half) working sample.

Various types of mechanical dividers are given in the ISTA Rules. The soil divider is recommended, consisting of the divider as such, and three pouring pans: A, B and C (figure 8).

In using the soil divider, the operator performs as follows:

- he empties the submitted sample bag into the pouring pan A;
- scatters the seed evenly in the pan;
- places the two receiving pans B and C alongside the soil divider;
- empties the pan A into the hopper, allowing the seed to flow at about equal rates along the entire length of the hopper, and to fill B and C each with one half of the submitted sample;
- replaces the pan B by the empty pan A;
- empties the pan B into the hopper, in the same way as previously with A, making equal parts (each 1/4) flow into C and A;
- replaces plan A by the empty pan B;
- empties pan A into the hopper, etc.

In that way a submitted sample is subjected to a series of halvings. The operator continues until a subsample which is five to ten times the prescribed weight of a (half)

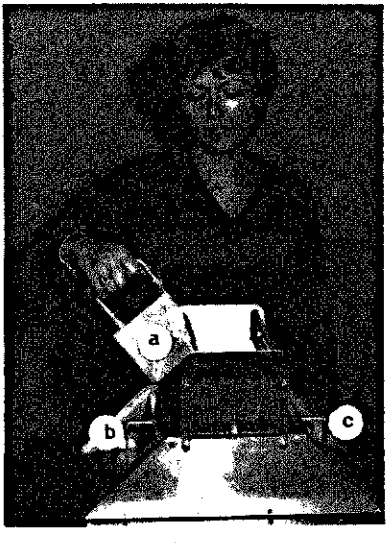



Figure 8. Soil divider.
The first of a series of halvings is shown.

working sample is obtained. Then the operator changes over to the spoon method, using the tools shown in figure 9.

The operator:

- pours the subsample evenly on a shallow tray, making it an even layer by pouring carefully with a side-to-side swing; shaking the tray is not permitted;
- with a spoon in one hand, a straight-edged spatula in the other, and using both, small portions are taken and transferred to the weighing beaker on a balance, carefully observing the increase of weight, and taking care that each spoonful is not more than one-tenth of the prescribed working sample weight, and that the spoonfuls are taken from all over the subsample. The latter precaution is to prevent a bias caused by possible segregation of the layer of seed. Segregation may occur both in horizontal and in vertical direction. Therefore, spoonfuls should be taken from all over the tray, and the spoon should be made to scrape over the bottom of the tray, i.e. the layer should not be skimmed, and the spoons should have a straight edge (figure 9);
- stops filling the weighing beaker as soon as the prescribed weight has been reached. The prescribed weight is a minimum and 5% over but not under the weight is acceptable. If it is over, the operator is not allowed to remove the surplus from the weighing beaker. It should either be left, even though the sample will take a longer time to analyse, or the process should be started over again. In the latter case, after emptying the weighing beaker back into the submitted sample bag, it can be refilled, with about 10 spoonfuls, from the material left in the shallow tray;
- writes the weight on a purity analysis form. This will serve as a check, when the working sample has been subjected to a purity analysis and the component weights have been added up. The initial weight and the sum of component weights may differ, the cause of which may not be obvious. If the difference is greater than a certain figure, a further test should be made. A suggested limit is 2% of the initial weight;
- transfers the working sample from the weighing beaker into a purity working sample container, and takes it to either the blowing unit or the purity unit, as required;
- returns the remainder of the submitted sample into the bag, either (a) to be stored in the case of a whole working sample, or (b) to provide for drawing independently the duplicate half working sample which may be needed to confirm the first test, when two duplicate half working samples are analysed.

*8.2. Weighing unit*

Balances intended for weighing samples, subsamples, fractions and components must meet certain requirements regarding precision. These are specified in the ISTA Rules, as follows:

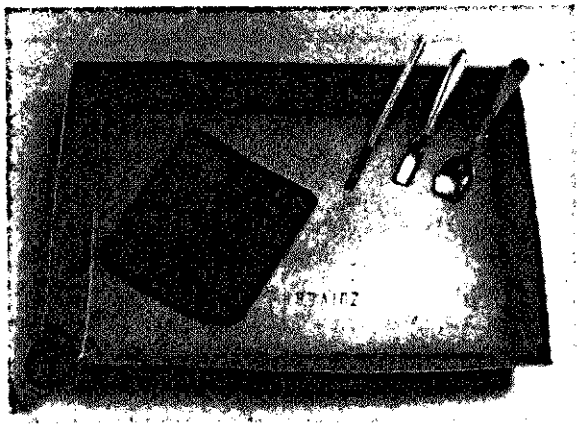


Figure 9. Tools for the spoon method.
Tray; straight-edged spatula; three straight-edged spoons.

| When the number of grams specified for the working sample is: | ...weigh the working sample and its components to the following number of decimal places: | Examples (g) |
|---|---|---|
| less than 1 | 4 | 0.8036 |
| 1 to 9.999 | 3 | 8.036 |
| 10 to 99.99 | 2 | 80.36 |
| 100 to 999.9 | 1 | 803.6 |
| 1000 or more | 0 | 8036 |

The whole range of weights between 0.5 and 1000 g can be determined with two balances: an analytical balance enabling 0.1 mg to be read accurately (capacity 160–200 g), and a precision balance with about 1 kg capacity, allowing 10 mg to be read accurately ('precision balance 1' in table 1).

If only two balances are available, congestion of work may occur in the peak season. Whenever congestion becomes a serious problem or when many samples have to be tested for moisture, a third balance should be available; this should be of a type intermediate to the other two ('precision balance 2'). All three balances should be of the direct reading type and provided with a tare mechanism.

*Balances* (figure 10). The choice of make of balances should mainly be based on the after-sales-service which can be offered by the manufacturer. A firm should be chosen which is able to offer a sound service contract. Servicing should preferably occur twice but at least once a year.

Table 1

| | Capacity (g) | | Readability (mg) |
|---|---|---|---|
| Analytical balance | 160 | ( 200) | 0.1 |
| Precision balance 1 | 1000 | (2000) | 10 |
| Precision balance 2 ('intermediate' type) | 160 | ( 220) | 1 |

*Weighing table* (figure 11). All balances should be placed on a weighing table. Such a table should consist of a stone slab (8 cm thick) resting on anti-vibration cushioning and supported by concrete or brick pillars. Weighing tables can be purchased from the balance manufacturers, but will usually have to be made locally. A concrete floor or a brick wall are needed at the place where the weighing table will be located.

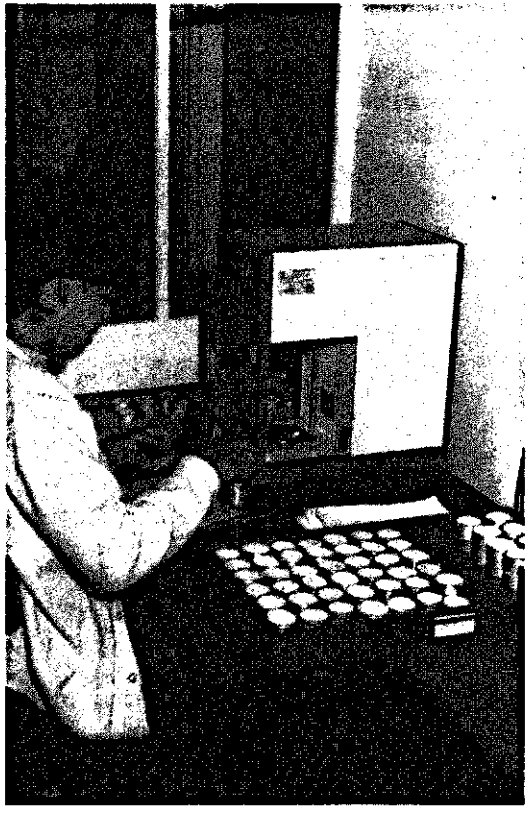


Figure 10. Analytical balance.

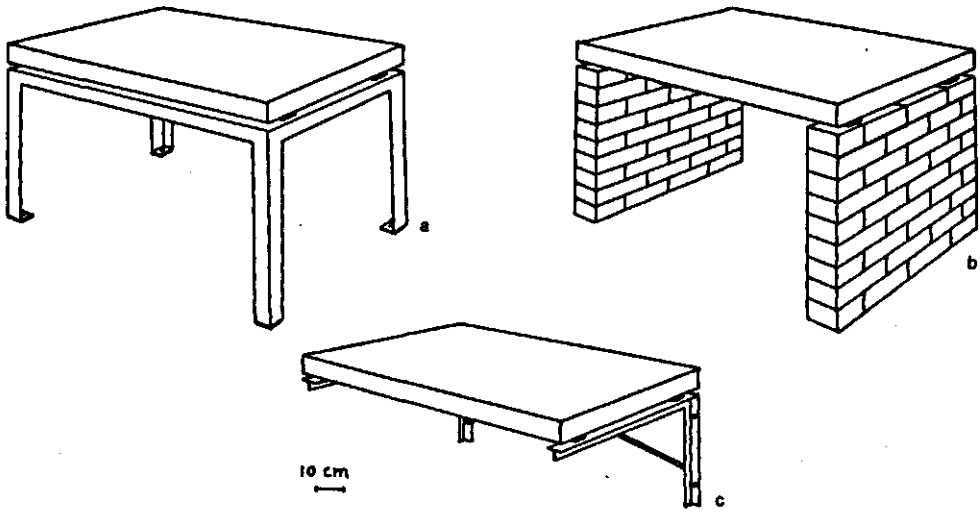



Figure 11. Weighing tables.
a + b. two possible constructions in case the table can be placed on the ground; c. alternative for a situation where no stable floor is present. Note the rubber blocks placed between the stone slab and the base.

*8.3. Blowing unit*

When grasses have to be tested, then each floret ('seed') has to be checked over a diaphanoscope for the presence or absence of a caryopsis (see purity unit). This work is very time consuming, and the labour costs of such an analysis would be much too high if it were not for the availability of blowers. The blower (figure 12) will separate the working sample into two fractions: a light and a heavy fraction. If the blower is set properly the heavy fraction will only contain florets with a caryopsis; the light fraction then will contain mostly empty florets but also some full ones. Only the light fraction has therefore to be checked and the full florets transferred to the heavy fraction, the remainder being inert matter. In this way at least 50% of the time spent on the analysis is saved.

For certain species ISTA has devised an alternative: the Uniform Blowing Method. To this end the ISTA Secretariat distributes calibration samples (+ instructions). The blower is set with this sample and after blowing the samples to be tested, the heavy fraction is considered to be full and the light fraction is considered to be empty. No checking over the diaphanoscope is needed. This, of course, saves even more time, and is also very benificial for the uniformity in results. The method is compulsory for *Poa pratensis* and *Dactylis glomerata*; it is recommended for *Chloris gayana*. It is the intention that more calibration samples should be prepared for the more difficult tropical range grasses: e.g. *Cenchrus ciliaris*, *Paspalum* spp., *Panicum maximum*. Blowing apparatus essentially consists of a centrifugal blower, the outlet of which is connected to the bottom end of a vertical tube of a few centimetres internal diameter and about half a metre length. A fine wire gauze, at the bottom end of the tube, retains

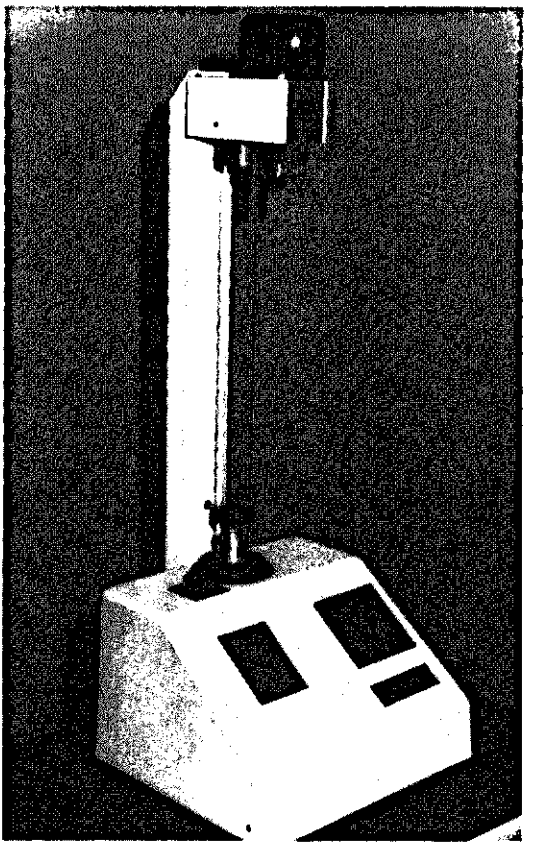


Figure 12. Seed blower.

the sample before it is blown, and also holds the resultant heavy fraction. Different arrangements exist to catch the light fraction. A valve allows the wind velocity to be set at a rate that has been found optimal for the kind of seed.

*8.4. Purity unit*

The object of the purity analysis is to determine: (a) the composition by weight of the sample being tested and by inference the composition of the seed lot, and (b) the identity of the various species of seed and inert particles constituting the sample. In accomplishing that objective, the sample is separated into three component parts: (1) pure seed, (2) inert matter, (3) other seed. Definitions of these categories can be found in the ISTA Rules. Detailed descriptions of all particles to be regarded as pure seed are given in the Pure Seed Definitions (see also: Handbook on Pure Seed Definitions).

The procedure for purity analysis (apart from subsampling, weighing and blowing), can be described as follows:

- The subsample (purity working sample) is spread on the working table, on either a 'slate' or a 'diaphanoscope' (see below);
- Each particle is judged individually, the criteria used being: external appearance (shape, size, colour, gloss, surface texture) and/or appearance in transmitted light;
- All other seeds and inert matter particles present are removed leaving the pure seed, the separations resulting in the three components mentioned above;
- Each component is weighed (in the weighing unit), the weights being entered on the purity form;
- Components may be retained for future reference although the pure seed will be sent to the germination unit, where 400 seeds of it will be used for the germination test.

An analyst must accomplish separations as prescribed in the ISTA Rules or as prescribed elsewhere. This means judging on sight and moving by hand each particle in the sample. As a purity working sample consists of more than 2000 particles, the amount of time required for carrying out the test is considerable. Therefore it is of great importance that the analyst is well trained and adequate equipment is available to reduce the time taken to a minimum.

Training should enable an analyst to:

1. identify all crop species and all weed species that occur frequently in the samples submitted to the laboratory; any gap in knowledge should be filled by consulting a suitable seed collection kept ready at hand;
2. put the available equipment to the right use;
3. be fully aware of the economic and other implications of test results.

The analyst's equipment should allow working with a minimum of effort and time, and also with a minimum of eye-strain and other tensions or stresses.

A purity working sample containing all three constituents equally and evenly dispersed throughout will take the longest to separate into three components, as every particle has to be isolated and moved individually. Conversely a sample consisting only of pure seed does not need any separation. The more a sample approaches homogeneity, the larger will be the average number of particles that can be shifted simultaneously, and the less will be the effort required. For that reason, whenever feasible, a working sample should be treated so that its components segregate as much as possible, before the actual analysis is commenced. Analysis of the fractions, one after the other, will take less time than the analysis of the sample as a whole. In addition to other suitable methods (see blowing unit) fractionating with a small sieve shaken manually is advantageous.

An optical aid, such as a magnifier or a binocular microscope, should allow samples of seed, spread on a flat surface, to be scanned rapidly. Rapid scanning requires (a) essential details to be definite, and (b) a sizable portion of the sample to be viewed

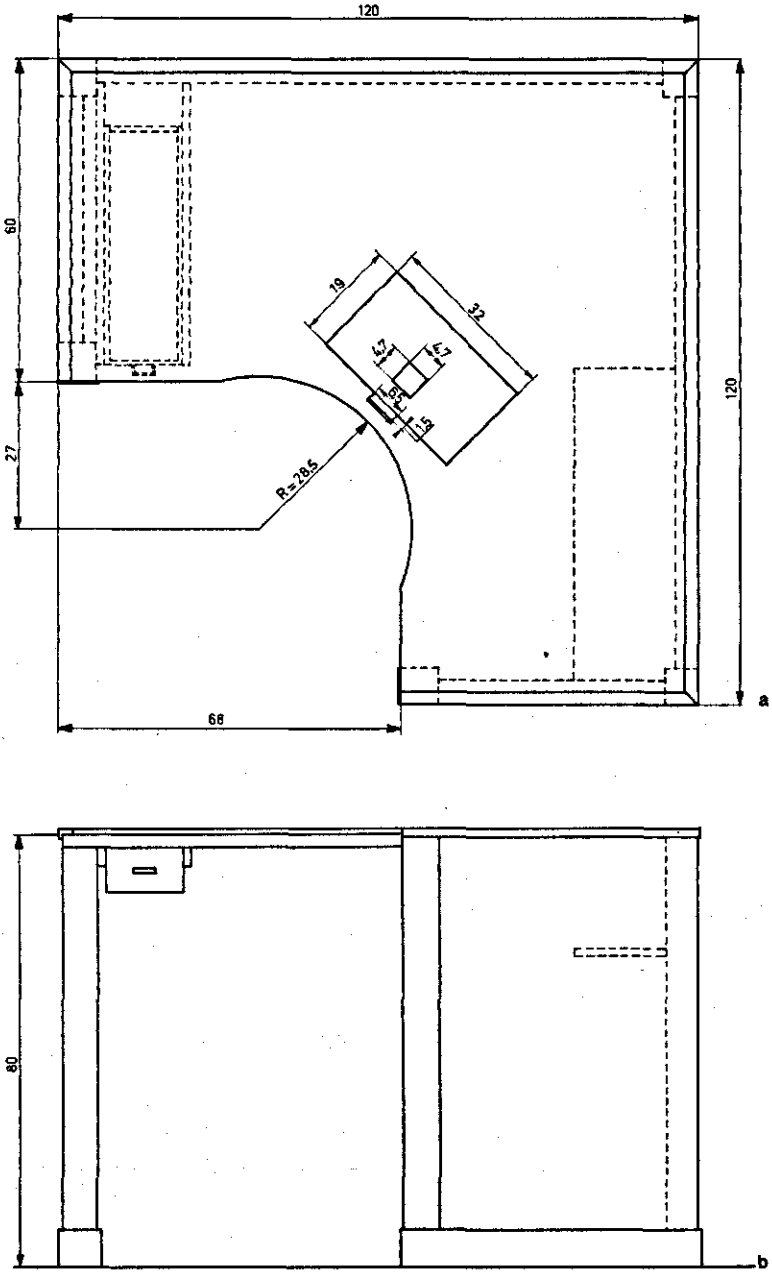



Figure 13. Purity working table.
a. top view showing round cut-out, frame for glass plate, square hole of diaphanoscope and oblong hole of small funnel; b. side view showing small drawer. Dimensions in cm.

at the same time. Experience shows that two magnifications, 3 × and 16 ×, are adequate for virtually all routine purity tests. A suitable mount or stand for the lens is very important; a hand lens (3 × magnification) is put to optimal use only if mounted on a stand, leaving both hands free for the work. The small working distance, the distance between the lens and the object, of many high-power magnifiers limits their usefulness in routine purity work; a lowpower (16 ×) microscope is therefore preferable.

*Working tables* (figures 13 and 16). The round cut-out in the table gives a large and comfortable working space (good support for the elbows). Each table can be provided with a built-in diaphanoscope, a device that is not commercially available, but enables seed to be judged in transmitted light. A hole of 4.7 × 4.7 cm is cut into the table top and this is covered by a thick glass plate that fits flush into the table top. Underneath a small 20 W (28 V) microscope lamp can be fitted. An additional funnel, fitted into the table is also recommended. Complete freestanding diaphanoscopes are also available.

*Incident light.* Although the laboratory windows may provide ample daylight, each table should be provided with a strong artificial light. Desk lamps with fluorescent daylight tubes are recommended. On each table a 'slate' (working board) can be laid over the glass plate of the diaphanoscope to judge in incident light, as shown in fig. 16.

A 'slate' may consist of a piece of hardboard 30 × 50 cm, sandwiched between hard plastic, one side dull black, the other side dull olivegreen. The hard plastic surface should be free from static (electric charges) and should be very smooth.

*Binocular microscope.* A magnification of 16 × is most convenient. The stand should be light-weight, so that the microscope is easy to handle; a stage that is open in front is recommended. In order to achieve this, it will be necessary to cut a section out of the front part of the ring stage commonly provided. Binocular microscopes on swing-arm stands can also be used, but usually this arrangement is found less convenient.

*Magnifier.* About 3 × magnification, object field diameter at least 5 cm, image perfectly aplanatic and sufficiently achromatic, i.e. without marginal blurring, distortion and discoloration; mounted on a light-weight base easy to handle and allowing unhampered manipulation to the seed sample on the table.

*Metal spatula, tweezers and scalpel needle* (figure 14). For moving seeds during the test.

*Seed collection* (figure 15). A cupboard for storing the main seed collection will be needed.

Metal units containing small plastic drawers are recommended. The seeds are put into labelled glass test tubes and stored in the drawers.

Figure 14. Various small instruments.
a. flat spatula for purity test and counting seeds for germination; b. scalpel needle for same; c. scraper for removing and levelling sand in germination containers; d. tweezers, drawn in two positions.

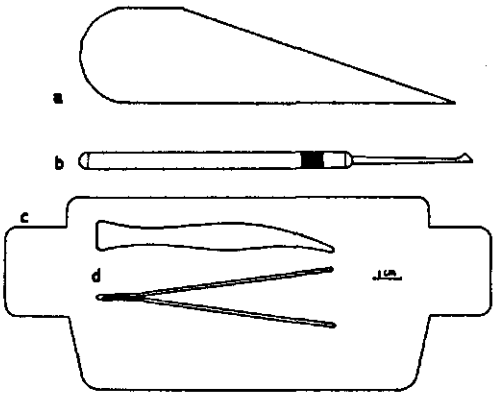



Figure 15. Main seed collection.

*Other instruments to be used* (figure 16).
- a 'half funnel', with the aid of which a sample of seed from the table can be directed into a small sample container;
- a set of watch glasses, used to cover seed fractions when the analyst is working on the sample; recommended diameters: 12, 10, 8 and 6 cm;
- aluminium containers; two sizes: 10 and 20 mm × 40 mm diameter respectively. The larger one may contain the pure seed fraction, while the smaller ones are designed for the other fractions. The lid of the containers should be well-fitting but should not be tight. The containers themselves should be 10 and 20 mm high respectively; the lid can be as shallow as 5 mm. The containers can be made of aluminium or another metal: plastic and cardboard are not recommended (see also figure 17);
- a small quick reference collection of crop seeds and weed seeds, in specimen tubes that are arranged in holes in a wooden block;
- the chair used should be especially designed for uninterrupted sitting at the bench;
- shelves or cupboard for temporarily storing purity components; its size depends on the intended use and on the number and size of sample components that are stored simultaneously.

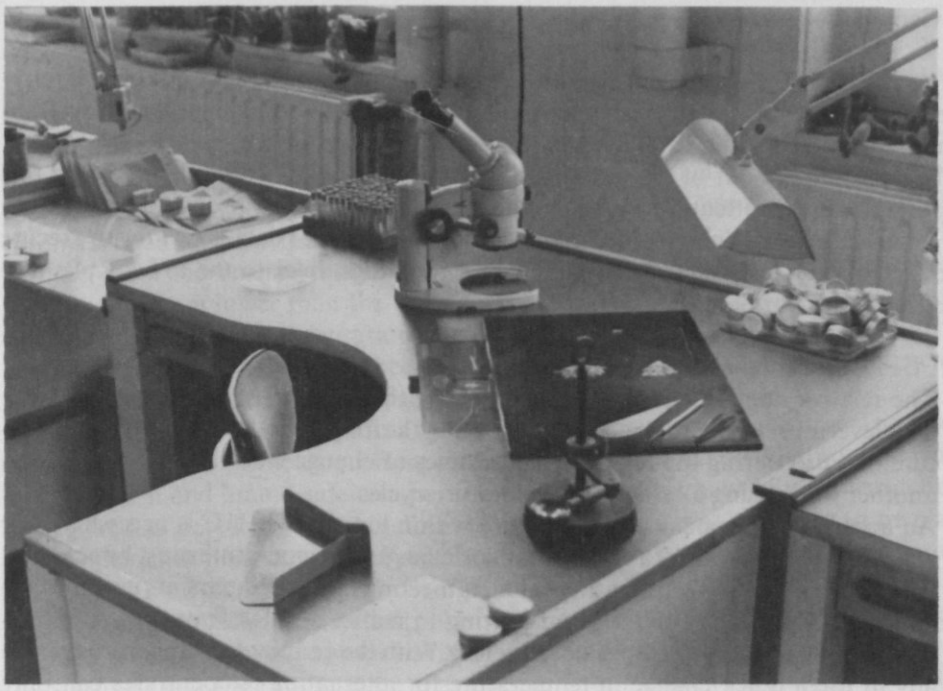


Figure 16. Purity working table and tools. For explanation see text.

Figure 17. Aluminium purity containers.

## 9. Germination section

In this chapter the equipment needed for germination is reviewed, and the requirements it must comply with are explained. Particulars of the equipment and firms from whom it can be obtained are given in chapter 14.

Because the lay-out of the germination laboratory is treated in chapter 4, in this chapter no further attention is given to this aspect. That applies also to administration of germination which is explained in detail in chapter 13. For details on how to execute the germination test and how to evaluate the seedlings, refer to the ISTA Rules and the Handbook for Seedling Evaluation.

### *9.1. General requirements for germination*

1. The relative humidity of the air surrounding the seeds must be kept near 100%. This level must be maintained not only during the long periods of constant temperature but also during the relatively short times of change from one temperature to another when using alternating temperature cycles.
2. An accurate adjustment of temperature within limits of $\pm$ 1 °C is necessary. For alternating temperatures a relatively quick change of temperature must be achieved in less than an hour. Germination cabinets meeting these requirements usually have sufficient capacity for cooling and heating to realize a quick temperature adjustment when the doors are repeatedly opened. With the germination apparatus generally in use a quick change in temperature for alternating temperatures can only be achieved when filterpaper is used as substrate. For germination tests in sand changes in temperature take comparatively rather long. However, tests in sand are as a rule made at constant temperatures.
3. A light source must be provided, using white fluorescent tubes, with a relatively low emission in the far red and a high special emission in the red region. The lamps should be installed in such a way that illumination is as uniform as possible and of an intensity of 750 to 1250 lx. Precautions should be taken that the starter and the chokes producing heat are positioned so that they do not affect the control of the germination temperature and humidity.
4. Gas exchange should be possible so as 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.
5. The dimensions of the substrate should be such that maximum use of the equipment is achieved. However, spacing between seeds and between tests should not hamper the germination process or cause undue spread of any seed-borne disease.

The most widely used apparatus in germination testing are the germination room, the germination cabinet and the germination table. Important, especially with regard to the room and cabinet type of germinator, is effective temperature insulation. Also important is that the insulation material should be protected from moisture penetration, making it lose its function.

*9.2. Germination rooms* (figure 19).
Rooms which are only conditioned for temperature (constant or alternating) by an air-conditioning unit are more and more commonly used now. Maintenance is relatively cheap and simple.

Control of light can be arranged in such a way that only those lights in the part of the room that is in use can be switched on. As mentioned already the rooms should be well temperature insulated. The purpose of insulation used in these controlled rooms is twofold. First, in order to assure the most efficient air-conditioning the inflow of heat from the outside should be reduced to a minimum. Similarly when outside temperatures are lower than the room temperature (at night) loss of heat should be minimised. Secondly, condensation of water on or (worse) inside the walls of the germination room has to be avoided, because this might well damage the wall construction, and cause the insulation material to lose its function.

Construction of walls with a low thermal conduction will solve the first. The second issue poses a more difficult problem. Ideally heat insulation should always be placed on the warm and humid side of a partition. However, this may cause constructional and practical problems and therefore the insulation material will have to be placed inside the germination rooms. Special care in design is then needed, the approach depending on the climate.

Between the insulation material (polystyrene, polyurethane, etc.) and the wall a layer of aluminium foil should always be fixed, in order to minimise heat radiation.

Starting from the presumption that outside temperature is around 35°C and the temperature inside the germination room needs to be 10°C, the following construction of the walls is needed (the examples are to be taken as a guideline):

| Maximum RH outside (%) | Thickness of insulation (cm) | Thickness of concrete wall (cm) |
|---|---|---|
| 70 | 5 | 15 |
| 77 | 5 | 10 |
| 85 | 10 | 10 |
| 90 | 5 | open wooden frame |
| 95 | 10 | open wooden frame |

In warm and humid climates (over 85% RH and around 35°C), the optimum solution is to replace the concrete wall by an open wooden frame ensuring sufficient ventilation on the outside (figure 18).

When the ceiling consists of a wooden construction a 5 cm thick layer of insulation material will be sufficient for most cases.

Rooms which will meet all these specifications may be difficult to build locally. In that case it is advisable to build rooms of prefabricated well insulated panels. Nearly all possible sizes are available (modular system). Such panels may be constructed according to the sandwich principle where the insulating material (e.g. polyurethane)

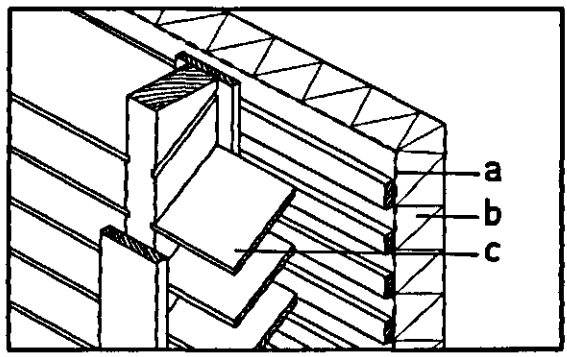


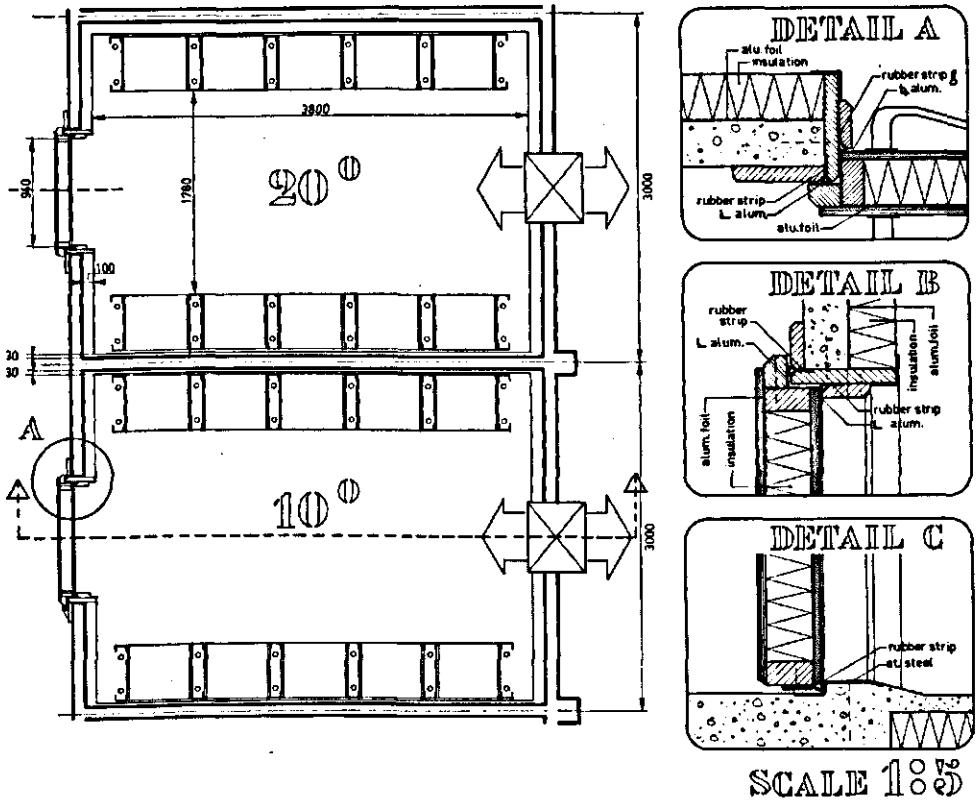

Figure 18. Open wooden frame.
Wall construction of germination room under warm (about 35°C) and humid (relative humidity over 85%) conditions. a. aluminium foil; b. insulation material; c. open wooden frame. See text.

is foamed between, for instance, enamelled steel plate. The panels can be delivered with varying thickness of the insulation material determined by the likely temperature differences between the room and outside.

Assuming the germination rooms in the 5000 samples laboratory have a maximum capacity of 560 containers for germination tests in sand, it can contain 140 samples of four replicates of 100 seeds (e.g. cereals, rice) or 70 samples of eight replicates of 50 seeds (e.g. maize). For containers see paragraph 9.8. If the 2000 sample laboratory has a germination room which will hold a maximum of 336 containers for germination tests in sand, it can contain 84 samples of cereals or 42 samples of maize. When using paper substrate and the plastic boxes from paragraph 9.8. (21 × 15 × 3 cm), the rooms of Seedlab 5000 and 2000 will hold 1680 and 1008 boxes respectively. Depending on the species each box can take one or two replicates, meaning that the rooms can accommodate 420 or 840 (Seedlab 5000) or 252 or 504 (Seedlab 2000) samples.

*9.3. Germination cabinets* (figures 21 and 22).
In the cabinet, as in the rooms, the seed can be germinated in darkness or light. However, light for germination is advised because more sturdy seedlings are produced and in many instances the seedlings are more easily and correctly evaluated. On top of these positive points the red emission of the white fluorescent tubes has a dormancy breaking effect (see paragraph 9.1.3.).

Cabinets may be 'wet' or 'dry' cabinets and be equipped for either constant temperatures or for both constant and alternating temperatures. Contrary to 'wet' cabinets, the tests in 'dry' cabinets must be covered against drying out during the germination period. When using filter paper substrate, the 'wet' cabinet can hold many more samples than the 'dry' cabinet where all tests have to be germinated in containers. Germination cabinets and also germination tables (see below) require more specialised maintenance when compared with germination rooms. With regard to cooling, when the cooling unit is water-cooled, the cabinet needs a continuous supply of clean water. This is also the case when the temperature and humidity conditioning is regulated via water. Consequently, when choosing a cabinet one should take into account



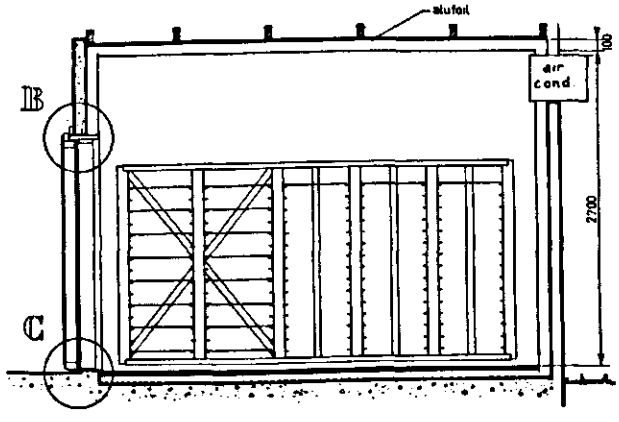




Figure 19. Germination rooms.
top left: floor plan of two germination rooms, showing arrangement of racks, fluorescent tubes, placing of doors and air-conditioners; bottom left: cross section of germination room along broken line as indicated in floor plan above; top right: details of door construction. Dimensions in mm.

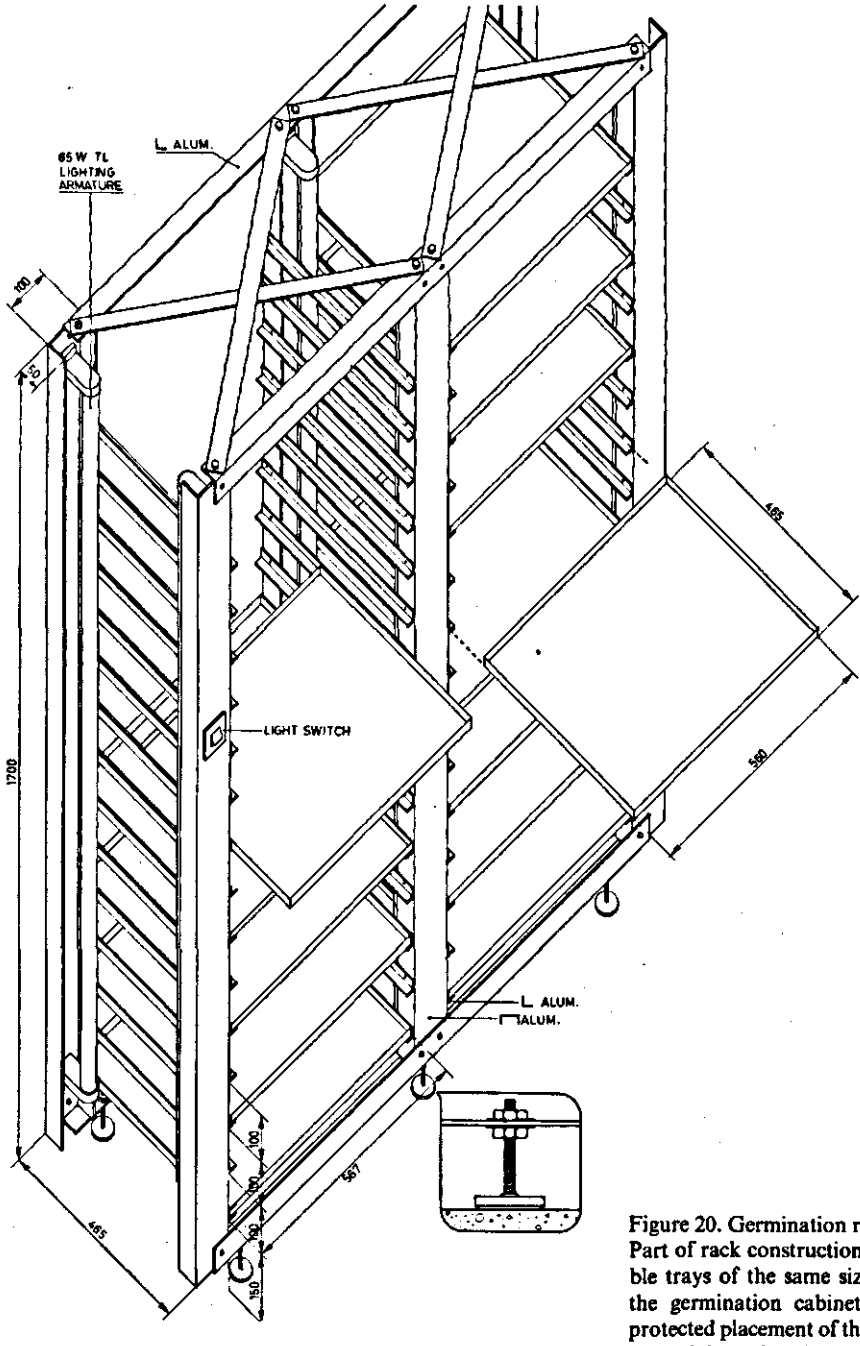



Figure 20. Germination racks.
Part of rack construction with movable trays of the same size as used in the germination cabinets. Note the protected placement of the fluorescent tubes. Dimensions in mm.

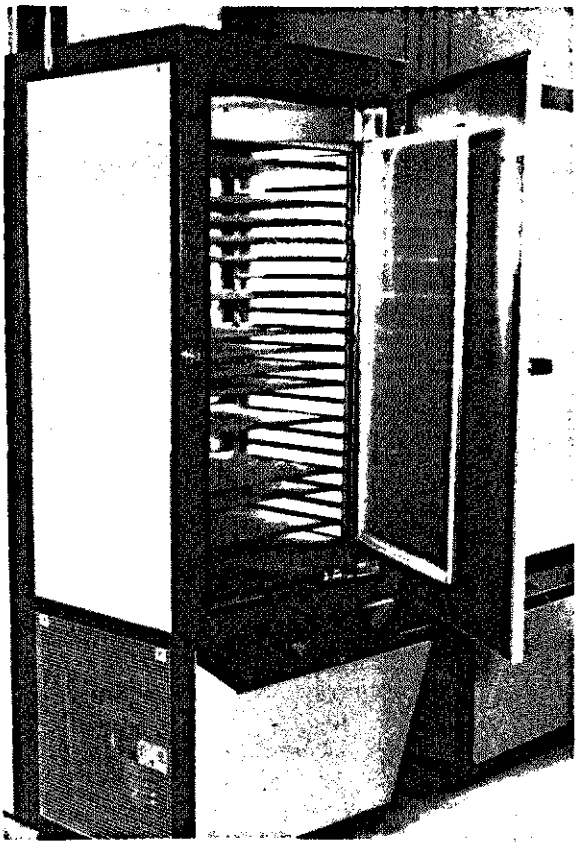


Figure 21. 'Wet' germination cabinet with light.

whether a continuous water supply can be guaranteed. Otherwise, the choice should be made for a cabinet which for its cooling and temperature/humidity conditioning is independent of a continuous water supply.

Except the Navep cabinet (see chapter 14) which is only air-cooled, all cabinets advised in this project can be delivered with either air or water cooling. The temperature range of the cabinets, for either constant or alternating temperature conditioning, is between 5° (0°)C and 35°C. They are well insulated and the walls are protected against penetration of moisture into the insulation material. However, in tropical and sub-tropical regions it may be wise to place the cabinets, especially those that are air-cooled, in an air-conditioned room (e.g. germination room) to extend the life of the cooling unit and to bring down the energy costs.

*9.4. Germination tables* (figure 23 and 24).

The germination table consists of a germination plate upon which filterpaper beds are placed. The seed beds are kept continuously moist by means of a paper wick (figure 24), which extends from the seed bed through slots or holes in the germination plate

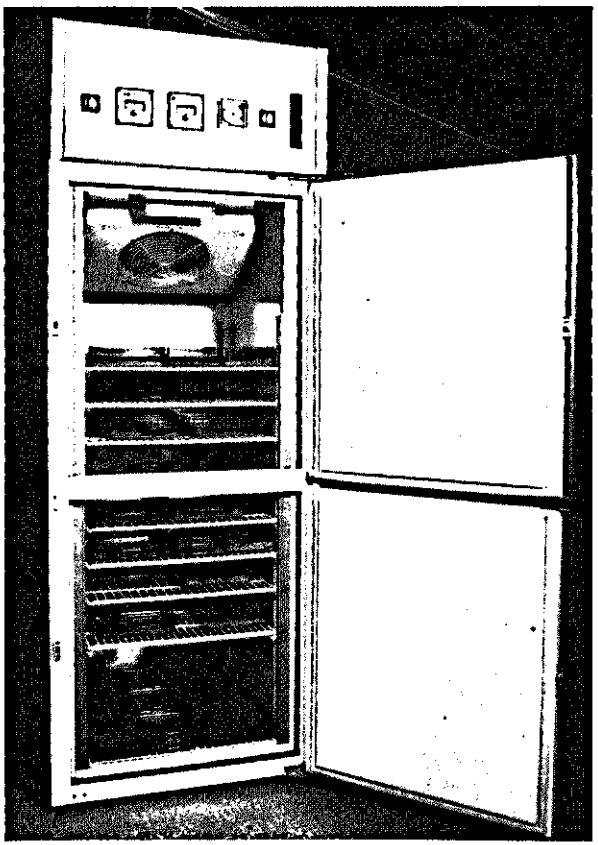


Figure 22. 'Dry' germination cabinet with light.

into the underlying water bath. To prevent drying out, the seed bed is covered with a bell jar provided with a hole allowing for ventilation without undue evaporation. The temperature is conditioned directly by conditioning the germination plate or indirectly by heating/cooling the water in the waterbath. The tables advised in this project have direct conditioning, either by the temperature controlled water running in a closed circuit through tubes attached underneath the germination plate or through tubes of stainless steel which make up the germination plate themselves. The water in the water bath may have to be replaced from time to time in case it has become 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 rather independent of a continuous supply of water. The tables may also be delivered water-cooled upon request. The tables are conditioned for constant and alternating temperatures between 5 °C and 35 °C.

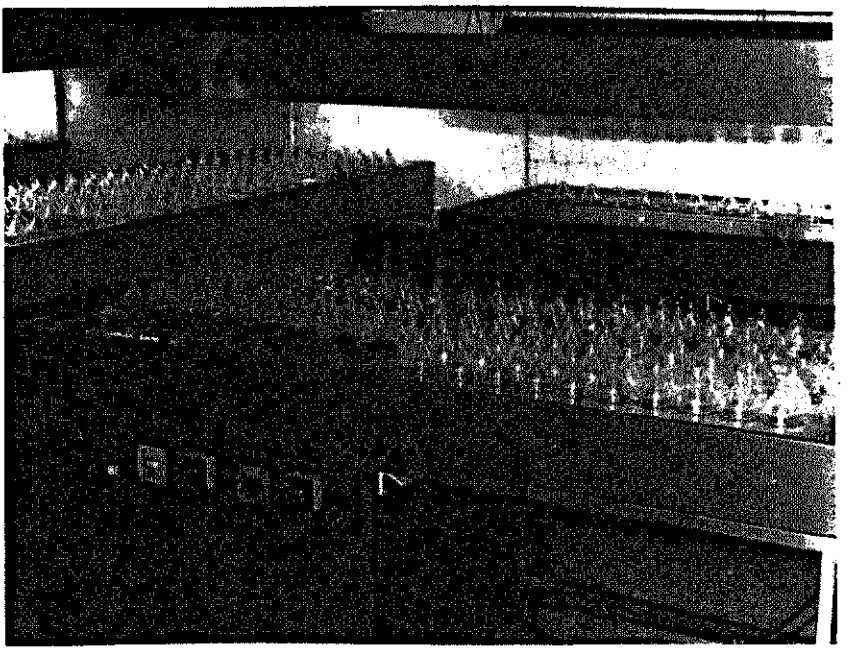


Figure 23. Germination table.

The quantity of germination equipment depends on the following:

a. the number of samples that must be investigated in the peak seasons;
b. the kinds of seed during that time;
c. the germination methods used (e.g. temperature, substrate, germination period, possible pretreatment for breaking dormancy);
d. effective dimensions of the equipment in relation to the space each test replicate takes (beds, containers).

For a 5000 sample laboratory with two germination rooms it may be necessary to have one germination table and at least one cabinet to prechill samples in order to break dormancy. However, when only cereals like wheat are tested, one of the rooms may be used for prechilling. For a 2000 sample laboratory equipped with one germination room, depending on the kind of species tested a cabinet for prechilling may be necessary, and also a cabinet with light or a germination table to extend the possible temperature regimes. Also for research purposes (e.g. methods) cabinets or germination tables are advised as a supplement to the germination rooms.

*9.5. Counting devices*

For some kinds of seeds counting devices have replaced time-consuming hand counting. In general, smooth, non hairy and round to elliptical seeds can be satisfactorily

Figure 24. Detail of germination table, showing water supply to the circle by means of a paper wick.

counted with these devices, provided that the seeds are not too small.

There are two basic types:

1. vacuum counting heads, e.g. for seeds of the size of
   a. cereals (e.g. wheat and rice)
   b. brassicas
2. counting boards, often used for counting maize, lupins, peas, beans and, when there is no vacuum-counting head available, also for cereals. Counting boards are relatively easy to make locally.

Requirements for counting devices

a. The size of the counting heads (vacuum counter) or counting boards must correspond with that of the germination substrate. It is important that seeds are spaced regularly and not too close to each other (in order to prevent spread of infection if disease is present on the seed).
b. The seeds are counted in units of 25, 50 or 100, so that the results of the germination tests can be easily computed and checked for their agreement with the appropriate ISTA tolerance table.

In counting segregation of the seeds must be avoided. The way in which the seeds are put on the counting head or board can determine whether segregation will occur or not. Rolling of the seed should be avoided as much as possible. The seed should

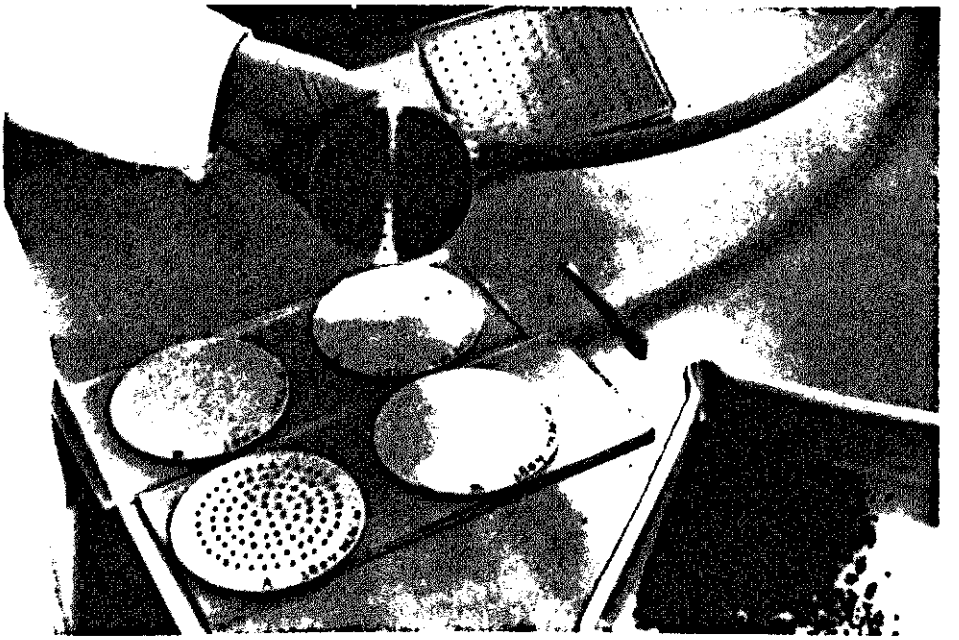


Figure 25. Vacuum counting heads.
Round head for filter paper circles and seeds of the size of brassica. In the background: rectangular head for larger seeds.

therefore be carefully poured out on different spots of the head or board, so that it easily spreads over its 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, it is advisable to use different heads and boards for chemically treated and untreated seeds respectively.

*9.5.1. Vacuum counting heads* (figure 25). Dimensions of the head depend on the size of the germination boxes or filterpaper, but should always be fractionally smaller. For squares and rectangles: 0.75 cm less in length and width; for circles: 0.75 cm less in diameter.

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 brassicas.

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. This edge should be interrupted for some length for removal of the surplus seed. Good results can be obtained by using a high-power vacuum-cleaner.

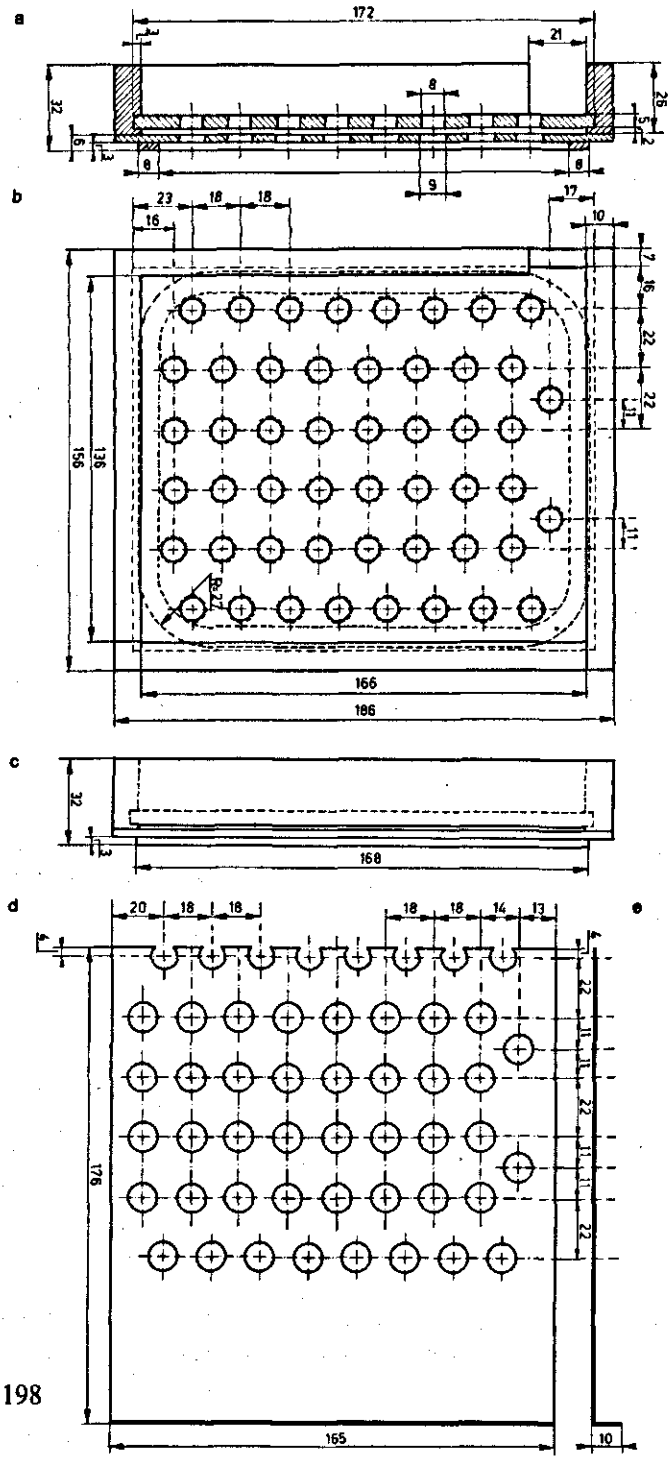



Figure 26. Counting board.
a. cross section; b. top view; c. side view; d. movable sleeve, top view; e. movable sleeve, side view. Dimensions in mm.

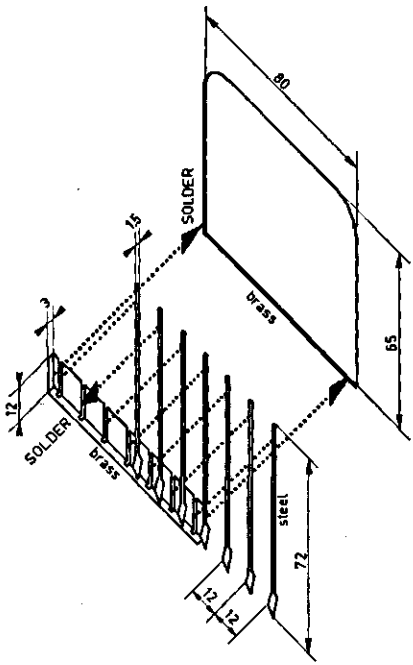



Figure 27. Assembly of germination rake. Dimensions in mm.

Use of vacuum counters:
- Place the counting head horizontally with the holes upwards;
- Close valve and bring seeds on head with the vacuum off;
- Apply vacuum and remove excess of seed;
- Check that there is one seed on each hole;
- Turn counting head over; release vacuum and drop seeds on substrate.

*9.5.2. Counting boards* (figures 26 and 30). The dimension of the boards depends on the substrate, though the length and width should be 0.75 cm less than the seed bed.

Diameter of the holes varies with the kind of seed (peas, beans, maize, etc.), but must allow the largest seed of a sample to pass through.

The bottom is provided with 25,50 or 100 holes, the number and size of these depending on the size of the seed and the dimensions of the seed bed. In the bottom is a movable sleeve with the same number and diameter of holes. When the sleeve is positioned so that the holes in bottom and sleeve correspond the seeds will fall onto the seed bed.

The counting board should have an edge on all sides, but interrupted at one place for removal of the surplus seed.

*9.6. Sand bunker*

Sand for germination tests can be stored in a bunker, which shall be made of concrete or bricks. Finishing has to be made smooth: e.g. with cement plaster or tiles. A provision for filling the bunker from outside is very convenient.

*9.7. Kitchen department*

For the cleaning of germination equipment and for preparing drinks for the laboratory staff, household kitchen equipment including a small refrigerator, two sinks, a stainless steel draining board and a water heater is needed.

*9.8. Further needs*

*Rakes and scrapers* (figures 27 and 14c). Small rakes and scrapers are used for loosening and smoothing the sand beds. At least two of each are required. For cereals and pulses the same type of scraper can be used, but for small seeds such as onions, which are sown more shallowly the scraper must not work as deep.

The method of use in the Government Seed Testing Station is explained below (see also figures 28 to 38).

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 sand that is carefully raked, 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; for onions 0.5 cm.

*Containers.* For germination tests in sand, e.g. for wheat, rice, maize and pulses. They measure 14 × 17 × 4.5 cm (figure 39), and can be provided with a tight fitting 9 cm high transparent cover for use under dry conditions (e.g. germination rooms).

For germination tests in pleated paper, e.g. for pelleted seeds and beets. They measure 21 × 15 × 3 cm (figure 40), and are used with paper substrata as recommended in chapter 14. The boxes can be stacked. Separate plastic lids are available.

*Bell jars.* To prevent drying out the seed beds on a germination table are covered with a bell jar, provided with a small hole permitting ventilation without undue evaporation. A bell jar should not completely cover the seed bed but leave a small edge exposed allowing some evaporation. This ensures transport of possible toxic and colour substances to the edges of the paper substrate.

*Filter paper.* A general requirement is that the filter paper used in germination tests is free from chemicals that are likely to affect seedling development. In the Dutch Station greyish round paper beds are used; this colour facilitates evaluation of the roots.

Two sizes, dependent on the size of the seed tested, are used, with diameters of 8 and 10 cm. The thickness of these papers is 0.6 mm (code no. T 300).

In the germinator the beds are placed on a layer of filter paper of the same size as the tray. This underlayer is white, and 0.5 mm thick (code no. ZH 1220).

In some instances folded papers of the envelope type are preferable. They are made of white paper folded to 26.5 × 23 cm. The thickness of this paper is about 0.3 mm (code no. ZH 1224). Pleated paper is used for germination of for instance beet seeds (code no. 3014, for the coverstrip no. 0858).

Almost all kinds of seeds referred to may be germinated either on paper (TP) or

Figure 28–38. Planting procedure in sand using a counting board.

Figure 28. Container is filled with moist sand and levelled with the long side of the scraper.

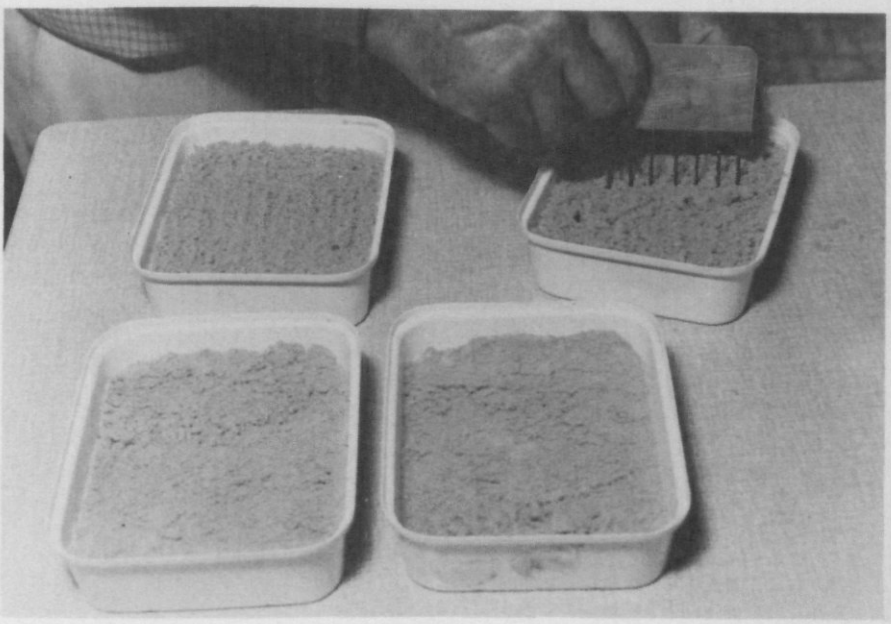


Figure 29. The seed bed is loosened with the rake.

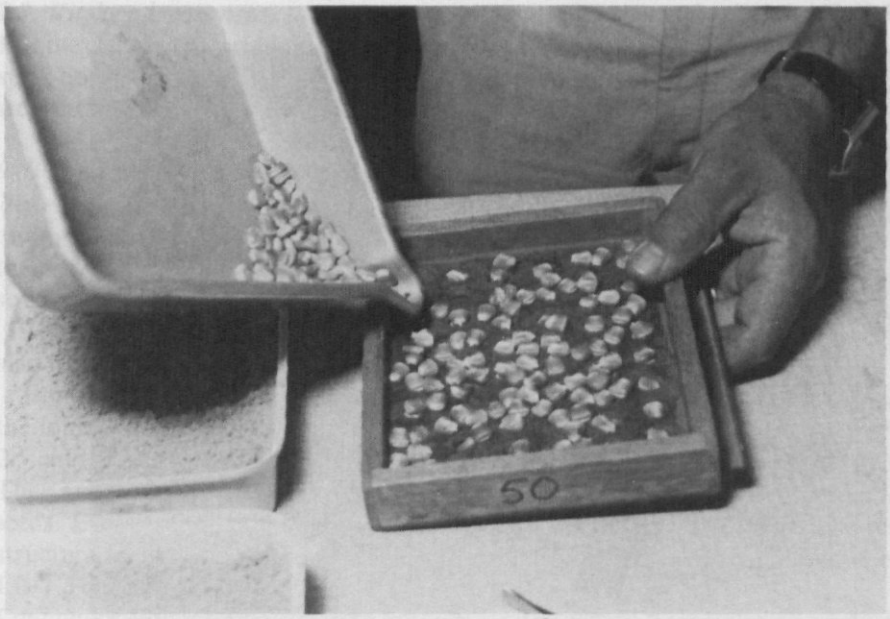



Figure 30. Counting board is evenly covered with seeds.

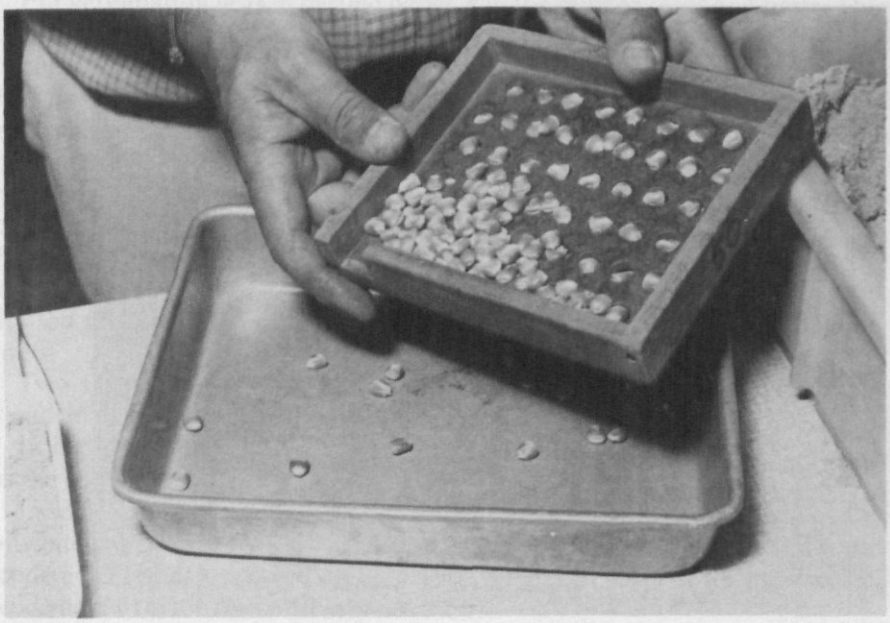


Figure 31. Surplus of seeds is shaken off.


Figure 32. The number of seeds is corrected with a tweezer.


Figure 33. Counting board is placed over seed bed and metal sleeve is pulled to release the seeds.

Figure 34. Planted seeds are carefully covered with loose moist sand.

Figure 35. The sand cover is loosened and levelled with metal rake, care being taken that seeds are not touched.

Figure 36. Excess of sand is removed with short side of the scraper.

Figure 37. Slips with analysis number are inserted in beds.

Figure 38. Four of the eight replicates ready to enter the germination apparatus.

Figure 39. One replicate in plastic container with transparent lid, ready for evaluation.

Figure 40. Shallow transparent containers for tests between paper; in this case pleated filter paper.

in sand (S). In the Dutch Station seeds of the size of wheat or larger are germinated in sand but smaller seeds are most often germinated on top of paper. Between paper methods have the disadvantage that the seeds easily make contact, increasing the danger of transfer of disease, and no light reaches the seedlings making them more subject to injury and rotting, and less easy to evaluate.

Whenever local paper is used comparative tests with other officially approved paper or with sand should be performed to check on the suitability of the paper.

*Sand.* In the Dutch Station white fine sand and river sand is used which does not need to be sterilised. In case the sand available is not free from injurious organisms it should be sterilised. The composition of the sand should be such that it passes a sieve with holes of approximately 1 mm diameter, but should not contain very fine particles like clay dust. After use the sand must either be discarded or it must be sieved, washed and sterilised. Sterilisation of sand can be performed at 150 °C for two hours in open steel pans. The sand should only be re-used a few times because of danger of accumulation of toxic substances.

*Watering bottles.* In general watering bottles are not used for moistening the germination substrate but can be used to give some extra water to filter pads to facilitate the removal of the seedlings.

*Tweezers* (figure 14.d). To handle small and tender seedlings short-armed tweezers should be used. To avoid injury the tweezer tips must not be too thin or sharp.

*Spatulas* (metal or plastic) for counting seeds.

*Needles* for evaluating seedlings, e.g. the growing point.
*Safety razor blades with holder* for scarifying or cutting seeds.
*Pre-drying oven.* This oven is used for breaking dormancy by keeping the dry seeds for a period at 35 °C before planting. The same oven can be used as needed for moisture testing.
*Mixer.* For mixing sand with water so that it contains the right amount for germination, the use of a small concrete mixer is recommended.
*Washing apparatus for beet seed.* Washing may be carried out in a special apparatus as explained below or just simply in cloth or nylon bags in running water as long as the requirements mentioned under 1 and 2 below are fulfilled.

The purpose of this equipment is to remove germination inhibitors from beet clusters. It must meet the following requirements:
1. the washing water must be regularly drawn off and replenished;
2. its temperature should be 25 °C constant;
3. the material must not be toxic nor corrosive.

*Binocular microscope.* The germination unit needs one binocular microscope with the same specification as for purity tests for closer examination of ungerminated seeds at the end of the test and for tetrazolium tests (see paragraph 8.4.).
*Chairs.* The chairs should be of an adjustable type to prevent tiredness as much as possible.

**10. Viability unit** (Tetrazolium)

The topographical tetrazolium method is a biochemical test to determine quickly the viability of a seed sample, but can also be applied to individual seeds that remain hard or fresh and ungerminated at the end of a germination test. The staining procedure should be executed in darkness at 30 °C. A small oven may be needed for this reason. Generally, depending on the species to be investigated, a 0.5% or 1.0% solution of 2, 3, 5-triphenyl-2H-tetrazolium chloride ('red tetrazolium') is used, obtained by dissolving 5 g or 10 g of the tetrazolium salt in 1000 ml of water with a pH ranging between 6.5 and 7. If the pH of the water is not in the neutral range the tetrazolium salt should be dissolved in a phosphate buffer solution. The tetrazolium solution must be stored in darkness.

Detailed information can be obtained from the ISTA Rules and the Tetrazolium Testing Handbook.

Remark: The results of tetrazolium and germination tests are generally in close agreement, but although properly conducted large discrepancies are possible. In tetrazolium testing only the embryo is evaluated, disregarding the influence of the outer structures of the seed (e.g. seed coat) which may influence the germination result (disease, dormancy). Besides, contrary to evaluation of the seedling in the germination test, not all abnormalities can be detected in the small embryo. Consequently, unless the seed is of a high quality, the tetrazolium test tends to give a higher result.

## 11. Storage room

As samples may need to be retested at some later date, for example in case of a dispute, it is necessary to have the availability of a storage room to keep the samples for about one year without losing quality.

To maintain germination capacity the seed must be stored at a temperature of about 18 °C and a relative humidity not higher than 65%. To meet these minimum conditions it may be necessary to use an air-conditioner. Precautions against damage by insects and rodents are imperative especially in tropical regions. The storage room must be made rodentproof and the seed stored in insectproof containers. Samples however that are already badly infected on arrival should be destroyed after testing because it is hardly possible to maintain the original quality during storage and because the risk of infection of other samples is too great.

## 12. Library

A convenient location should be chosen where books and scientific journals are placed with appropriate accommodation for consultation (see list of suggested books and journals, chapter 15).

## 13. Handling of analysis results

*13.1. Introduction*

In this chapter a system based on that used by the Government Seed Testing Station is explained. It consists of six forms: a cover form and five loose forms, all made from stiff paper.

The forms are numbered 1 to 6, and each has its special function.

Form 1: the folded cover form keeps all forms together, and is used for the calculation of the costs of the analyses.
Form 2: the request form used by the applicant for requesting analysis which is sent to the station together with the submitted sample.
Form 3: the moisture form used internally for the moisture analysis.
Form 4: the germination form used internally for the germination analysis.
Form 5: the purity form used internally for the purity analysis.
Form 6: the extra form used for all additional determinations (other seed by number, 1000 seeds weight, etc.).

In the upper right corner of all six forms space is provided for the analysis number. Upon arrival the sample is given a registration number in sequence of receipt. All forms will be given the same analysis or registration number. When the 5 forms are filed in the cover form in the right sequence 1 to 6, the six numbers can be seen at a glance. In this way it can easily be seen whether the 6 forms agree. If any of the

| *request number* | REQUEST FOR SAMPLE ANALYSIS | | | | | | *analysis number* 2 |
|---|---|---|---|---|---|---|---|
| copy of cert. to: | Information to be provided by applicant | certificates required | nation. | BIC | OIC | GIC | language |
| | | number of certificates | | | | | |
| | | mark required analysis → | purity | germ. | moist. | | rush anal. |
| signature of applicant | | name and address of applicant | | | | | |
| | | species, cultivar, generation, etc. | | | | | |
| copy of cert. to: | Information to be stated by (official) sampler | sampling and sealing agency | | | | | |
| | | official marks of lot | | | | | |
| | | official seal of lot | | | | | |
| | | weight of lot | | | | | |
| signature of sampler | | number of bags or other containers | date of sampling | | date of sending sample | | *date sample received |
| | | | | | | | |

N.B. applicant should keep a copy of the request form and refer to the request number when seeking information from the seed testing station

*to be completed by seed testing station

Figure 41. Form 2, Request form.
Used by applicant when submitting a sample for testing. For explanation see paragraph 13.2.

analyses are not required one or more of the forms can be omitted.

The forms are depicted in figures 41 to 46. The *italics* used at various places on these forms are not to be printed. The six forms are used as follows:

*13.2. The request form* (Form 2, figure 41)
This form should be used by the applicant or by the official sampler and should accompany the sample. Regular customers should be encouraged by the station to hold a stock of request forms. These forms should be provided with a pre-printed sequential number in the left corner. This number is not identical to the analysis number put in the right corner of the form. The number in the left corner helps the applicant to describe the sample when inquiring after preliminary results of tests. To this end the seed testing station keeps a record of all request numbers with their respective analysis numbers. The sender of the sample should retain a copy of the form. It is divided into three sections. The first (above the double line) contains the information provided by the applicant. This and the information stated by the official sampler (below the double line) provide the details for completion of ISTA certificates (Orange and Green International Seed Lot Certificates, abbreviated: O.I.C. and G.I.C.; the

SEEDLAB 2000–5000

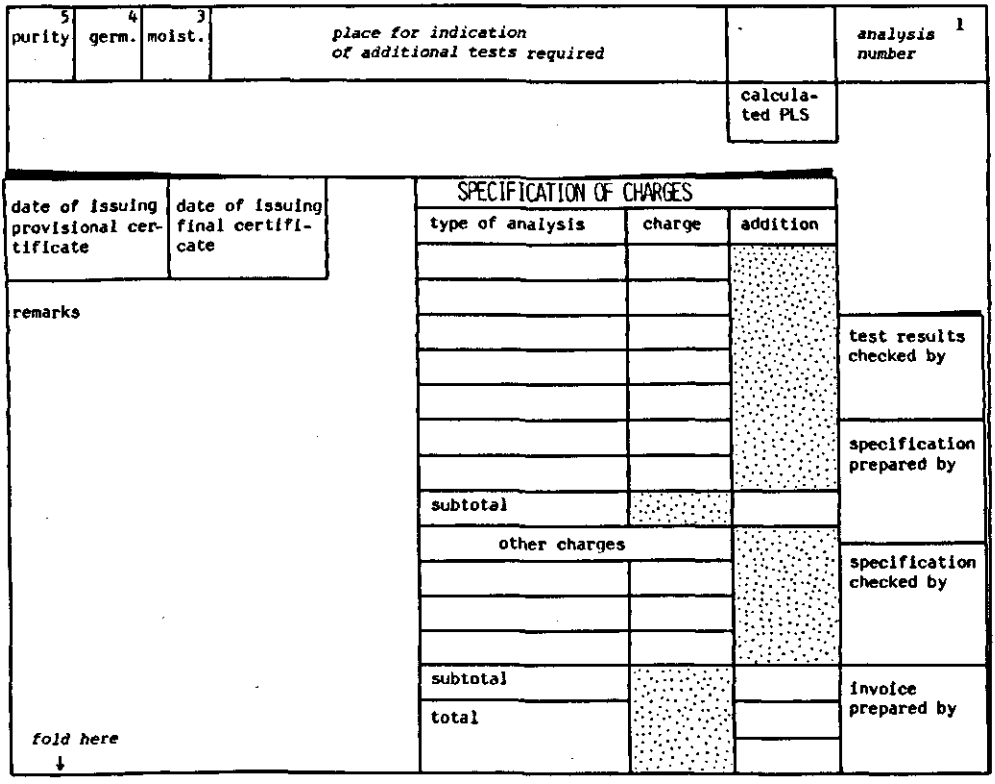



Figure 42. Form 1, Cover form.
For filing the analysis forms and request form, and used for calculating the charges. For explanation see paragraph 13.3.

third one being the Blue International Seed Sample Certificate, or B.I.C.). The third area (right of the thick line) is for use by the Seed Testing Station.

The applicant can tick the kinds of analysis wanted (purity, germination, moisture, etc.) and can ask for the analysis results:
- on national or international certificates;
- in specified languages;
- within a certain time (rush analysis).

Seed legislation of many countries requires prescribed tests.

Prescriptions for using ISTA certificates require the name and address of the applicant, some data about the seed lot such as species, cultivar name and weight of the lot. It is advisable that there should be provision for the applicant to sign the form.

The official information on the ISTA Seed Lot Certificate (O.I.C. and G.I.C.) must be provided and signed by the official sampler, otherwise no ISTA Seed Lot Certificate

| species | | | | method used | | | M.C. | analysis number 3 |
|---|---|---|---|---|---|---|---|---|
| 1ST REPLICATE | | | | | 2ND REPLICATE | | | |
| 1 | 2 | 3 | 4 | | 5 | 6 | 7 | 8 |
| | | | | number of container | | | | |
| g | g | g | g | weight of container | g | g | g | g |
| g | g | g | g | weight of seed | g | g | g | g |
| g | g | g | g | weight of container plus seed before drying | g | g | g | g |
| g | g | g | g | weight of container plus seed after drying | g | g | g | g |
| g | g | g | g | loss of weight | g | g | g | g |
| % | % | % | % | moisture content | % | % | % | % |
| | | | | date and initials of analyst | | | | |

calculation in case of pre-drying according to the formula:

$$M = S_1 + S_2 - \frac{S_1 \times S_2}{100} = \quad \%$$

$S_1$ = percentage of moisture lost by pre-drying (stage 1)
$S_2$ = percentage of moisture lost by the oven method (stage 2)

average moisture content (in case of pre-drying) $\frac{A + B}{2} = \quad \%$

Figure 43. Form 3, Moisture form.
Both replicates and possible retests can be entered on this sheet. For explanation see paragraph 13.4.

may be issued. Where any ISTA Seed Lot Certificate is required, the official sampler not only samples the lot, but also will mark and seal the bags and will forward the sample and the request form to the seed testing station. After receipt the station has to fill in the date of receipt of the sample and the analysis number.

*13.3. The cover form* (Form 1, figure 42)
As soon as the request form has been received at the station it is put in a cover form together with the other forms. The same analysis number is also put on the sample label.

The cover forms holding the request forms are filed in the administration centre. The other forms are distributed to the sections where the analyses are to be carried out.

The cover form is used to indicate the analyses that must be executed according to the opinion of the person in charge of the seed testing station, based on the national and international regulations and seed laws (sometimes this is not identical with the request of the applicant).

A special place is reserved for the calculated P.L.S. (Pure Live Seed Content): percentage pure seed x percentage normal seedlings/100.

On the right side of the front flap of the cover form the specification of the invoice (specification of charges) is recorded. On the left side the dates of issuing the certificate(s) are noted, and the spare space on the left side can be used for notes concerning the analyses (e.g. retesting necessary).

The invoice is prepared and signed by one person (at the bottom, right side).

Summarising, the cover form is used exclusively by the person(s) responsible for the registration of the sample on receipt, and for the final check of the analysis results just before the preparation of the certificates, as well as for the preparation of the invoice.

*13.4. The moisture form* (Form 3, figure 43).
The analyses are to be executed in duplicate. From the moisture form the vertical columns 4 and 5 on both sides of the central column are used. The percentage moisture content is calculated as:

$$\frac{\text{loss of weight}}{\text{initial weight of seed}} \times 100$$

When pre-drying, because columns 4 and 5 of the form have already been used for the initial analyses, column 3 has to be used for the analysis of the 50.0 g sample which has to be pre-dried. Thereafter columns 2 and 6 are used for the final moisture content determination. For the second replicate of a pre-drying determination, a second moisture form is needed. Column 4 of this form can be used for the 50.0 g which is to pre-dried, whereafter columns 3 and 5 are used for the final determinations. The two moisture forms should be marked A and B. The moisture content of the pre-dried sample is the average of the results of determinations A and B.

The average moisture content should be written on the upper side of the moisture form, left of the analysis number, and be expressed as a percentage calculated to one decimal place. The method used should be reported on the upper part of the form.

*13.5. Germination form* (Form 4, figure 44)
The space above the double line is intended for initial information and final results; the space below this line is for the analysis. Before the analysis is started the leading analyst of the germination section indicates on the form the method to be used for the seed that is going to be tested.

After planting, the date of planting and the counting dates are noted. Normally, the seeds are sown in four replicates of 100 seeds each (8 × 50 or 16 × 25). The planted seeds are put in or on a germination apparatus. It is advisable to give each germinator a number and also each tray in each cabinet or each slot of a germination table. The sample can easily be found then. Tray 5 in cabinet 2 will then be indicated by: 2.5, and will be recorded on the germination form, in this way locating the test.

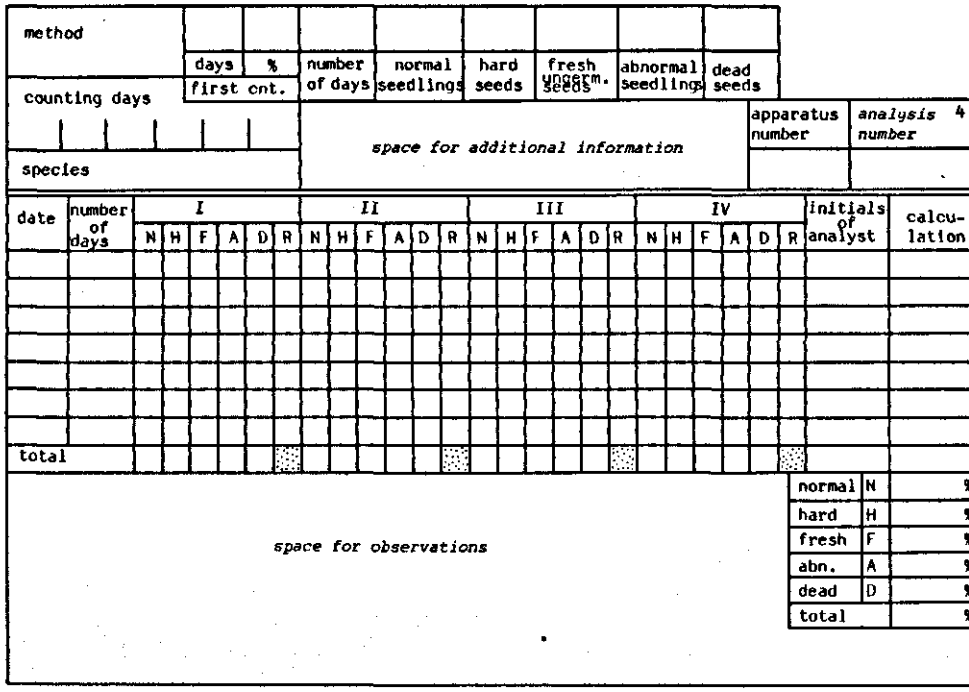



Figure 44. Form 4. Germination form.
All four replicates including the calculation of the final results can be recorded. For explanation see paragraph 13.5.

In the vertical 'date' column the date of the evaluation is written, e.g.: May 23rd, as 23/5.

In the vertical column 'number of days', the number of days since the date of planting is written. When the seed has been sown on Monday, the next Monday is the seventh full day (not the eighth). Hence, the date of planting is not included. On the horizontal lines the results of the evaluations are written. For each evaluation day one horizontal line can be used.

The categories of evaluated seeds and seedlings are:
N – normal seedlings;
H – hard seeds;
F – fresh ungerminated seeds;
A – abnormal seedlings;
D – dead seeds (e.g. rotten seeds);
R – rest; indicating the number of seeds or seedlings that cannot yet definitely be classified.

An example (see table 2): On the first counting day perhaps in one replicate 40 normal

seedlings can be removed (and recorded), but the rest (60) consists of insufficiently developed seedlings for evaluation and (or) ungerminated but not rotten seeds. These 60 are written under R. Rotten seeds and also rotten seedlings are removed and recorded (under D and A respectively) at any time during the test to prevent contamination of the healthy seeds or seedlings. For that reason, frequent inspection of the tests is advised, because removal may be necessary between counting days.

On the next counting day these 60 seeds or seedlings have to be evaluated anew. Now perhaps 30 seedlings are normal, three seedlings decayed and five seeds clearly rotten. The rest to be written under R is now 22. Apart from rotten seedlings no abnormal seedlings are removed from the test until the final count, because this facilitates checking of the type of abnormalities by the leading analyst.

On the last counting day the evaluation has to be completed. Now the 22 seeds and seedlings of column R must be subdivided as far as possible. Let us suppose that there are now:

| | |
|---|---|
| 2 normal seedlings | (N) |
| 2 hard seeds | (H) |
| 3 fresh ungerminated seeds | (F) |
| 12 abnormal seedlings | (A) |
| 3 rotten seeds | (D) |

For each replicate the results must be stated in the appropriate vertical column I, II, III or IV.

The results of the replicate of the sample are scored as follows:

Table 2.

| Date | Number of | I | | | | | |
|---|---|---|---|---|---|---|---|
| | days | N | H | F | A | D | R |
| 23/5 | 4 | 40 | – | – | – | – | 60 |
| 27/5 | 8 | 30 | – | – | 3 | 5 | 22 |
| 31/5 | 12 | 2 | 2 | 3 | 12 | 3 | – |
| | | 72 | 2 | 3 | 15 | 8 | – |
| | | | | TOTAL: 100 | | | |

The same recording is done for the other replicates. For the final calculation of the average values the right bottom side is used. As the germination test is performed in four replicates, the averages are often not whole figures; 0.25 is rounded downwards and 0.50 and 0.75 are rounded upwards. Because of the rounding off, the total percentages of all categories together will sometimes deviate from 100%, in which case a correction is necessary. This correction is never executed in the category N.

| pure seed | inert 1) matter | other 2) seeds |
|---|---|---|

*space for additional specifications concerning the seed lot*

1) *kind of inert matter*

2) *kind of other seeds*

species as stated by sender

analysis 5 *number*

species as analysed (latin) *by leading analyst*

| analysis approved *by leading analyst* | latin name of species *established by analyst* | | 1ST AND 2ND REPLICATE | | | |
|---|---|---|---|---|---|---|
| | grams | % | weighing working sample | blowing | analysis | weighing components |
| working sample | | | average % of replicates | 1) *kind of inert matter of this replicate* | | |
| pure seed | | | | | | |
| inert matter 1) | | | | | | |
| other seeds 2) | | | | | | |
| total | | | 2) *kind of other seeds of this replicate* | | | |

Figure 45. Form 5, Purity form.
One replicate is recorded on one sheet; if tests are carried out in duplicate two forms are needed. For explanation see paragraph 13.6.

As an example, when te average of four replicates is:

| | after rounding off: | the correction then will be: |
|---|---|---|
| N = 70.50% | N = 71% | N = 71% |
| H = 12.50% | H = 13% | H = 12% |
| F = 2.50% | F = 3% | F = 3% |
| A = 6.25% | A = 6% | A = 6% |
| D = 8.25% | D = 8% | D = 8% |
| Total = 100.00% | Total = 101% | Total = 100% |

The form can also, with some improvising, be used for the recording of tetrazolium results.

*13.6. Purity form* (Form 5, figure 45)
Like the other forms the purity form has two main sections separated by a double line. The lower section is for the analysis, the upper section is for the final conclusions and additional information.

A purity test should be executed in duplicate, two subsamples (half working samples) being independently drawn and tested. The two tests are referred to as the '1st replicate'

and the '2nd replicate'. Two purity forms should therefore be prepared, one to be filled with the results of the '2nd replicate', the other to be filled with the results of the '1st replicate' together with the average of the two. In that way the results obtained by one analyst cannot bias the other, the two sets of results being compared after the percentages have been calculated. The leading analyst of the purity section, when receiving a submitted sample with its duplicate purity analysis forms, designates two analysts to carry out the analyses and to draw the duplicate half working samples.
Each analyst:
- checks whether the analysis number on the label of the submitted sample agrees with the number on the form;
- writes the analysis number, species name, '1st' or '2nd' on the working sample container, and signs it;
- takes a working sample (using the procedure as outlined in paragraph 8.1), determines its weight, writes the outcome at 'working sample' at the top of the 'grams' column of the form, and writes the date and signs at 'weighing working sample';
- (if the sample requires fractionation by blowing, either as an aid or in pursuance of a prescription) subjects the working sample to blowing and encloses the fractions in separate containers;
- writes the blowing speed (= manometer reading, or valve opening reading) on each container;
- writes the blowing speed, the date and signs at 'blowing' on the form;
- takes the fractions and the form to a working table.

At the working table, the same or another analyst:
- subjects the subsample (whether or not fractionated) to a purity test;
- writes the kind of inert matter behind 1) and the scientific (Latin) names of the other seeds behind 2);
- writes the scientific name of the pure seed at 'Latin name of species (established by analyst)';
- notes the date and signs in the 'analysis' space;
- has data checked by the leading analyst;
- weighs the components in the weighing unit.

The leading analyst is responsible for any results that are submitted for reporting and signs and dates the form checking the name of the pure seed species by writing it under 'species as analysed' before transferring the form to the office if the calculation is performed by the administration centre.
In the office the clerk:
- calculates the component percentages (to two decimal places);
- compares the two series of percentages that result from the two replicates, and compares the difference with the prescribed table of tolerances;
- (if the difference between comparable percentages exceeds tolerance) takes two blank purity analysis forms, writes the analysis number in its upper right corner and orders an additional purity test;

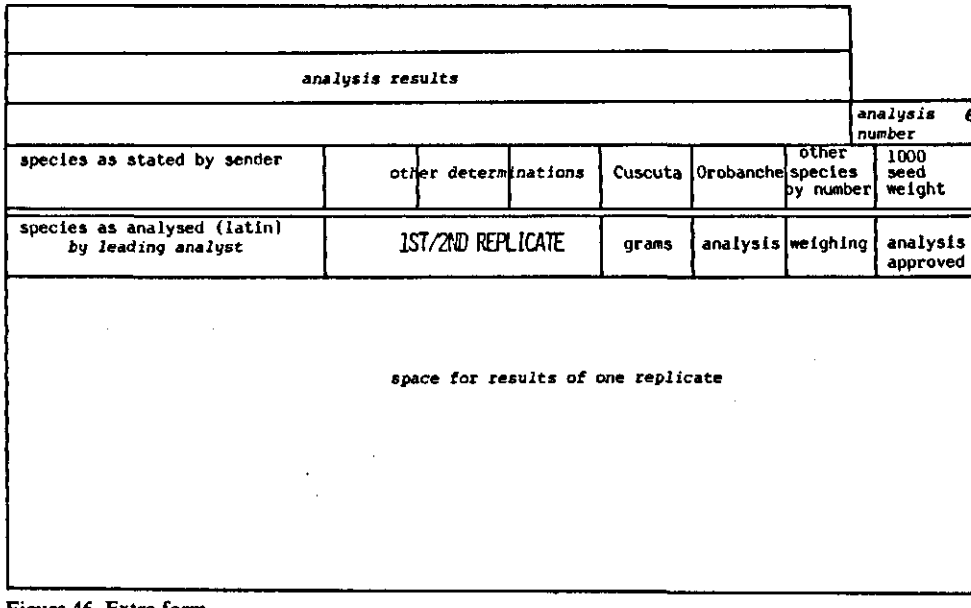



Figure 46, Extra form.
For recording special tests. For explanation see paragraph 13.7.

- (if the difference between comparable percentages is within tolerance) writes the average percentage of the test in column 'average of replicates' of one of the two forms;
- rounds off these percentages to one decimal place;
- corrects the percentages such that their total is 100.0%, by correcting the inert matter fraction only (impurities that occur to an extent of less than 0.05% are reported as 'trace', and are not included in this calculation);
- copies the corrected percentages at the head of the first form;
- copies (in the middle part of the head of the form), at '1' and '2' the data from the '1st replicate' and the '2nd replicate' of 'kind of inert matter' and 'kind of other seeds', by adding them up. Then, the data on the form are ready to be reported on a report form (certificate).

*13.7. Extra form* (Form 6, figure 46)
This form serves all kinds of special determinations that normally will be reported under the heading 'Other Determinations' of an ISTA certificate. Such tests include: other seeds by number (number count test), 1000 seeds weight, hectolitre or bushel weight, verification of species or cultivar, seed health tests, percentage of sprouted grain tests, percentage of cracked seeds tests, etc., etc.

## 14. Equipment

### *14.1. Recommended furnishing and equipment**

The equipment is arranged according to the chapters and paragraphs used in this paper. For total number per item needed (both for the 2000 and the 5000 laboratory), refer to paragraph 14.2.

*5. Sample reception*

- Rack for blank forms: 4 shelves, 30 × 220 cm, fixed to the wall at height of 60 cm, 90 cm, 120 cm and 150 cm.
- Table: top 75 × 400 cm, at 95 cm above floor level; two additional shelves underneath, 65 × 400 cm, at 20 cm and 50 cm above floor level; heavy duty.
- Numbering machine, dating stamp, stock of forms.
- Chairs in the hall to accommodate visitors.

*6. Administration centre (office)*

- Filing cabinet; a standard type, 65 cm deep, 54 cm wide, 138 cm high, with six drawers, each with a partition to hold two rows of forms produced in a single season.
- Desk (for the clerk/typist) with one set of drawers.
- Desk (for the superintendent) with two sets of drawers.
- Open tray, for temporarily filing analysis forms: inside dimension 22 × 45 × depth 10 cm, with adjustable inclined partitions that prevent the forms from slipping.
- Simple electronic calculating machine, for percentages and other calculations.
- Typewriter, telephone, stationery, stock of report forms (or certificates).
- Chairs.

*7. Moisture unit*

7.2. Oven method (figure 5)

- Aluminium containers, 6 cm diameter, 3 cm high with loose fitting lids. Supplier 1: Centraal Magazijn, Industrieweg 5, 3433 NL Nieuwegein, Netherlands; Supplier 2: H. B. Selby & Co., 48 North Terrace, Adelaide, South Australia 5000, Australia.
- Drying oven. Recommended oven T 5042 E + additional thermometer insertible in top. Supplier: Heraeus GmbH, P.O. Box 169, 645 Hanau, Germany F.R.
- Balance. Recommended type of balance is: 'precision balance 2' (see below under 8.2.).
- Grinding mill. Recommended Cemotec. Supplier: Tecator AB, P.O. Box 70, S-263 01 Höganäs, Sweden.
- Sieves. 0.5, 1.0 and 2.0 mm mesh + receptacle. Supplier: Duintjer & Zn., P.O. Box 1, 9640 AA Veendam, Netherlands.
- Desiccator ± 20 cm diameter, with thick metal or porcelain plate and blue silicagel. Readily available through any dealer of laboratory supplies.
- Pair of tongs. Model 1309 B3. Supplier: Tamson, P.O. Box 208, 2700 AE Zoetermeer, Netherlands.

7.3. Quick methods

7.3.1. Recommended: Ultra X 70 (figure 6). Supplier: A. Gronert, P.O. Box 167, D 4937 Lage (Kachtenhausen), Germany F.R. Alternative 1: Cenco moisture balance 26680–817. Supplier: Breda Scientific, P.O. Box 3336, 4800 DH Breda, Netherlands. Alternative 2: Mettler LP 15 combined with balance PC 440 and application input unit GC 301. Supplier: Mettler Instruments, CH-8606 Greifensee, Switzerland.

* The recommendation of particular models and listing of their suppliers by the authors does not necessarily indicate approval by the International Seed Testing Association.

7.3.2. Recommended: Dickey-John DjGMT Grain Moisture Tester (Figure 7). Portable, battery operated, digital read-out. Supplier: Dickey-John, P.O. Box 10, Auburn, IL 62615, USA. Working on the same principle, portable, battery operated, direct read-out: Cera-Tester. Supplier: A/S N. Foss Electric, 69 Slangerupgade, DK- 3400 Hillerød, Denmark.

*8. Purity section*

8.1. Subsampling unit

- Divider (figure 8). Recommended: riffle type divider (soil divider), Model INRA. Supplier: Tripette & Renaud, 39 Rue J.-J. Rousseau, 75038 Paris Cedex 01, France.
- Aluminium containers (figure 17). Supplier: Centraal Magazijn, Industrieweg 5, 3433 NL Nieuwegein, Netherlands. Alternative: paper bags, various sizes.
- Shallow tray, straight-edged spoons and spatula (figure 9), various sizes. These items are to be made locally.

8.2. Weighing unit

- Balances. Although mechanical balances have our preference, mainly because they are cheap, we advise electronic balances. Good mechanical balances are no longer available, or will not be available in the near future. Because the after-sales service is very important, in many countries the choice of make will be limited to two main producers of laboratory balances: Mettler and Sartorius. Every year new improved models are developed and therefore advice from our side must be limited to the type of balance (see table 1 of paragraph 8.2 in main text). Both laboratories need an analytical balance (figure 10) and a precision balance 1, but if possible also a precision balance 2 ('intermediate' type) could be purchased and will prove to be very useful. For addresses of local agents write to: Supplier 1: Mettler Instrumente AG, CH-8606 Greifensee, Switzerland; Supplier 2: Sartorius GmbH, P.O. Box 19, D-3400 Göttingen, Germany F.R.
- Weighing table (figure 11). Ready-made tables can be purchased but very often one will have to make one oneself, which is not difficult. The table consists of a heavy (metal or brick) base and a thick stone slab ($\pm$ 8 cm thick). The size of the table depends on the number of balances available and the place where they are needed. Per balance one will need a table surface of $\pm$ 75 $\times$ 75 cm. The analytical balance should have its own table. The slab should not be laid directly onto the base but on rubber blocks. In this way the heavy table top is more or less independent from the vibrations that still might occur in the base. To limit these vibrations in the base it is essential that it stands on a solid concrete floor (if available) or that it is mounted to a solid stone or concrete wall (if available). If neither of these are available accurate weighing will be impossible.

8.3. Blowing unit

- Seed blower (figure 12). Recommended: Micro Blower type 35. Supplier: Contab Instrumentation, Klisätravägen 25 A, S-130 12 Älta (Stockholm), Sweden. Alternative 1 ( for fine grasses): Ottawa blower. Supplier: Astell Hearson, 172 Brown Hill Road, Catford, London SE6 2DL, United Kingdom. Alternative 2 (for coarse separations of the larger grasses): South Dakota Blower. Supplier: Seedburo, 1022 West Jackson Blvd., Chicago IL 60607, USA.

8.4. Purity unit

- Working tables (figure 13). Although any desk that is standing firmly on the ground is suitable for a purity test, it is advantageous to have a special table constructed. This table is specifically designed for the purpose. A major advantage is that the elbows have a good support; this is very important because the analyst is usually sitting at the table for several hours every day. A good support of the elbow prevents tiredness and in the long term serious physical complaints. A diaphanoscope can be made in the table by cutting out a 4.7 $\times$ 4.7 cm rectangle, covering it by a glass plate that fits flush into the table, and mounting a small 20 W (28 V) microscope lamp underneath. In that case also an additional funnel fitted into the table is recommended. Freestanding diaphanoscopes are available, that can be used on any table: Illuminant Magnifying Glass with polarizing filters. Supplier: Tripette & Renaud, 39 Rue J.-J. Rousseau, 75038 Paris Cedex 01, France.

- Incident light. Recommended: Luxo FL-101A. Supplier: write for local agent to: Jacobson, P.O. Box 60, Oslo-6, Norway.
- Working board (figure 16). The board (30 × 50 cm) may consist of hardboard, hardboard sandwiched between dull hard plastic of a dark colour, or of sanded (dull) black glass. In any case it should be of a hard, smooth, non-static material.
- Binocular microscope (figure 16). Most binocular microscopes have a range of magnifications. If variable ('zoom') then a combination of lenses should be chosen to get a range from about 6–25 ×. If two single magnifications are possible, then 6 × and 16 × are the best combination. If only one magnification is available then 16 × is the best choice. In that case an additional (screw-on) lens of 0.5 × is often available, which will give the possibility of 8 × magnification too. Attention should be given to the stand (see main text).
- Magnifier. 3, 4 or 5 × magnification on stand. Recommended (4 × magnification): type 15900. Supplier: Breukhoven, P.O. Box 6044, 3002 AA Rotterdam, Netherlands.
- Metal spatula, tweezers and scalpel needle (figure 14). Can easily be made locally. Tweezers of the recommended type are also: 1068 B 10. Supplier: Tamson, P.O. Box 208, 2700 AE Zoetermeer, Netherlands.
- Seed collection (figures 15 and 16). A small quick-reference seed collection (figure 16) can be made by using small glass test tubes inserted in a wooden block that is provided with holes. All analysts should prepare such a collection for their own use. For the main seed collection more provisions are needed: recommended (figure 15) are metal units with 48 plastic drawers. In each drawer up to eight glass test tubes (maximum length 12 cm) can be accommodated per unit resulting in a collection of up to 384 samples. Recommended type: 48A. Supplier: write for local agent to: Raaco A.S.E., 4800 Nykøbing/ Falster, Denmark.
- Half funnel. Will have to be home-made.
- Watch glasses. Available through any dealer in laboratory supplies.

*9. Germination section*

9.2. Germination rooms

- Germination rooms. Prefabricated with well insulated panels with varying thickness of the insulation material and air-conditioning. Supplier: Zephyr, P.O. Box 507, 2700 AM Zoetermeer, Netherlands.

9.3. Germination cabinets

Depending on the circumstances, the kinds of seed to be tested and the financial possibilities, four types can be recommended:

- Inventum 'wet' cabinet – with light (DK-10, figure 21). The cabinet serves all purposes but is especially suitable for carrying out tests with the various paper substrates. No covering of the filter paper is needed, since there is no danger of drying out. Although not strictly necessary, the tests are usually placed on a moist filter paper underlay. Water flowing along the inner sidewalls provides a humidity of 100% and also regulates the temperature: constant and alternating temperatures. Supplier: Inventum, P.O. Box 4, 3720 AA Bilthoven, Netherlands.
- Zephyr 'wet' cabinet – without light. With constant and alternating temperature conditioning. The humidity and temperature are regulated by water delivered through a spray from a distributor pipe in the space between the stainless steel linings which wets both inner and outer lining. Tests do not dry out. The cabinet can be fully compared with the Inventum cabinet but is without the light facility, which limits its possibilities. Supplier: Zephyr, P.O. Box 507, 2700 AM Zoetermeer, Netherlands.
- Zephyr 'dry' cabinet – with and without light (ZGM-E, figure 22). With constant and alternating temperature conditioning. Tests have to be carried out in containers with covering. Supplier: Zephyr, for address see above.
- Navep 'dry' cabinet. Several types: with and without light, with only constant temperature conditioning or also with alternating temperature conditioning. Light is supplied by four loose cold fluorescent tubes of 8 W which can be placed at any position in the cabinet. As with the Zephyr cabinet, homogeneous temperature distribution is achieved by a ventilator for forced air circulation. Supplier: Navep, P.O. Box 256, 9700 AG Groningen, Netherlands.

9.4. Germination tables

Two alternatives are recommended:

- Inventum table (figure 23). The germination plate of stainless steel on which the germination beds are placed consists of eight parallel, rectangular tubes of stainless steel through which temperature controlled water is pumped to achieve the required temperature. The tubes are separated by a 7 mm gap. The level of the water in the tank underneath the germination plate is automatically controlled and can be changed (cleaned) by pressing a button. The whole plate can be jacked up to clean the tank thoroughly. The plate can hold 144 or 207 round beds of 10 cm and 8 cm diameter respectively. Supplier: Inventum, P.O. Box 4, 3720 AA Bilthoven, Netherlands.
- Zephyr table. The table has a removable plate of stainless steel. Underneath a copper pipe system is fitted through which the temperature regulated water is pumped to achieve the required germination temperature. The plate has as standard 176 slots cut into it on which beds of 8 cm diameter are placed and through which the paper wicks hang down into the stainless steel water bath underneath. Supplier: Zephyr, P.O. Box 507, 2700 AM Zoetermeer, Netherlands.

Note: All germination equipment is normally delivered for 220 V–50 Hz power supply. However, the power supply of all apparatus can be adapted upon request.
Light (if supplied) and temperature are automatically operated with electronic temperature regulators and timers.

9.5. Counting devices

9.5.1. Vacuum counting heads (figure 25)

For requirements see main text. Supplier: Ames Powercount Co. & E1 Drechson Co., Brookings, SD 57006, USA. The counting heads should be used in combination with a central vacuum circuit or a powerfull vacuum cleaner that is not susceptible to over-heating. Recommended: Nilfisk GS 80. Supplier: write for local agent to Fisker & Nielsen A/S, Peter Bangsvej 30, DK-2000 Copenhagen F, Denmark.

9.5.2. Counting boards (figures 26 and 30)

The board that is depicted in figure 26 is especially designed for the Bema germination container for sand tests (see below). It may be possible to have it locally made. Technical plans can be provided by the authors.

9.8. Further needs

- Rakes and scrapers. Can be made using figures 27 and 14.c respectively. The scraper fits into the box shown in figure 39.
- Containers with transparent cover for sand tests (figure 39). Recommended: Bema kiembakje. Supplier: Bema, Deltastraat 14, 4301 RC Zierikzee, Netherlands.
- Containers for paper tests (figure 40). Recommended: box 21 × 15 × 3 cm. Supplier: D.B.P. Plastics, Terbekehof 25–29, Wilrijk, Belgium.
- Bell jars (figure 24). 8 cm diameter. Supplier: Tamson, P.O. Box 208, 2700 AE Zoetermeer, Netherlands. 10 cm diameter. Supplier: Leithen Valley Plastics, Leithen Road, Innerleithen, Peebleshire, Scotland.
- Filter paper. Circles of 8 cm or 10 cm diameter and 0.6 mm thickness (code T 300), and also the rectangular sheets (code ZH 1220, ZH 1224) are supplied by: Schut & Zn., P.O. Box 1, 6866 ZG Heelsum, Netherlands. Pleated filter paper (code 3014) and the coverslip that is also needed (code 0858) are supplied by: Schleicher & Schüll, P.O. Box 4, D-3354 Dassel, Germany F.R.
- Pre-drying oven. The oven of the moisture department may be used. If an additional oven is needed, refer to 7.2 for supplier.
- Mixer. e.g. Lescha VM 130 N. Supplier: Gehabouw, P.O. Box 246, 3900 AE Veenendaal, Netherlands.
- Washing apparatus for beet seed. Supplier: J. Volkers Jr., ARVO, Bovenweg 41, 1834 CB Sint Pancras, Netherlands.

## *14.2. Survey of total equipment needed*

| | Recommended quantity | |
|---|---|---|
| | 2000 laboratory | 5000 laboratory |
| *Moisture* | | |
| aluminium container | 30 | 50 |
| oven | 1 | 1 |
| grinding mill | 1 | 1 |
| set of sieves | 1 | 1 |
| desiccator | 2 | 2 |
| pair of tongs | 1 | 1 |
| laboratory chair | 1 | 1 |
| quick moisture tester | optional | optional |
| *Purity* | | |
| divider | 1 | 1 |
| analytical balance | 1 | 1 |
| precision balance (capacity 1000 g) | 1 | 1 |
| weighing table | 2 | 2 |
| seed blower[1] | 1 | 1 |
| working table | 2 | 4 |
| built-in diaphanoscope[1] | 2 | 4 |
| or: freestanding table model | 1 | 2 |
| incident light source | 3 | 5 |
| binocular microscope | 1 | 2 |
| magnifier | 3 | 5 |
| cupboard for seed collection | 1 | 1 |
| small aluminium container[2] | 1000 | 2000 |
| large aluminium container[2] | 500 | 1000 |
| large paper bag (12 × 24 cm)[3] | 2000 | 5000 |
| laboratory chairs | 2 | 4 |

In addition small tools are needed: shallow trays, straight-edged spoons and spatulas, tweezers, scalpel needles, working boards, half funnels, watch glasses, test tubes for seed collection (see main text).

[1] Only where grasses have to be tested.

[2] Paper bags can be used instead (suggested size: 6 × 9 cm), but have the disadvantage that they can not be re-used because of danger of contamination of one sample with the other.

[3] To accommodate the working sample and the pure seed in cases where the amount of seed to be examined does not fit into the aluminium containers (e.g. with pulses, cereals or in the case of number count tests).

| | Recommended quantity | |
|---|---|---|
| | 2000 laboratory | 5000 laboratory |
| *Germination* | | |
| germination room | 1 | 2 |
| germination cabinet with light | 1 | 1 |
| germination table | 1 | 1 |
| vacuum counting device with two heads (for cereals and brassica – sized seeds) | optional | 1 |
| counting board | 4 | 4 |
| containers with transparent cover | | |
| for sand tests[1] | 600 | 1500 |
| containers for paper tests[1] | 50 | 250 |
| bell jars[2] | 200 | 200 |
| pre-drying oven | optional | optional |
| mixer | optional | optional |
| washing apparatus[3] | optional | optional |
| domestic refrigerator | 1 | 1 |
| hot water boiler | 1 | 1 |
| *Viability* | | |
| binocular microscope | – | 1 |
| small utensils: magnifier, small glass containers, tweezers, lancet knives, needles. | | |
| *Administration + sample reception* | | |
| rack for blank forms | 1 | 1 |
| table + shelves | 1 | 1 |
| numbering machine | – | 1 |
| dating stamp | 1 | 1 |
| chairs (visitors) | 4 | 2 |
| filing cabinet | 1 | 1 |
| desk | 2 | 2 |
| open tray for forms | 1 | 2 |
| calculator | 1 | 1 |
| typewriter | 1 | 2 |
| chairs | 3 | 4 |

In addition small tools are needed: rakes, scrapers, tweezers, spatulas, magnifiers, watering bottles, sieves (to separate seeds from sand). Refer to main text.

[1] Numbers are approximate and will depend on the species to be tested.
[2] Size depends on the size of the filter paper circles used (8 cm or 10 cm diameter).
[3] Only when many beet seeds have to be tested.

## 15. List of books and journals

A basis for a good seed testing library.

**General**

*International rules for seed testing (Rules + Annexes)*
Seed Science and Technology 4 (1), 1976.
German edition: Seed Science and Technology 4 (3), 1976.
French edition: Seed Science and Technology 4 (4), 1976.
ISTA Secretariat*.

*Amendments to international rules for seed testing 1976 – composite version of 1977 and 1980 admendments*
S. R. Cooper, ISTA Secretariat*, 1981.

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