Scottish Agricultural Science Agency

An Agency of the Scottish Executive Environment and Rural Affairs Department

To: Registered Merchants, Processors & Packers in Scotland, Licensed Seed Testing Stations in Scotland, Scottish Licensed Seed Samplers, Scottish Licensed Crop Inspectors
E & W Applicants in Scotland, NFU (Scotland), SSTA, SSTGA, AIC (Scotland), BSPB, DARDNI, DEFRA, NAW, NIAB, SEERAD, Interested Organisations

Our ref: SEED Cert EC 117

17 June 2005

SEED CERTIFICATION INFORMATION

CHANGES TO THE SEEDS MARKETING REGULATIONS FOR SCOTLAND Number 2

Further to our Seed certification Information letter of 13 June 2005 (EC116), please find enclosed details of procedures and changes to procedures introduced from the 1 July 2005 as a result of the introduction of new Scottish legislation. These will be discussed at the SASA open days on Thursday 23rd and Friday 24th June. Those who are unable to attend can direct queries to the contacts listed on each of the inclusions.

Update guidance booklets (eg the Cereals “blue book”) are in preparation, and will be circulated in due course.

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CONTROL PLOTS

The Oil and Fibre Plant Seed (Scotland) Regulations 2004, the Cereal Seed (Scotland) Regulations 2005 and the Fodder Plant Seed (Scotland) Regulations 2005 (collectively referred to as “the Regulations”) come into force on 1 July 2005. The Regulations change the requirements relating to control plots.

For a licensed inspection to be used for seed certification an official control plot will be required for each seed lot, and the plants produced in the control plot will have to pass an official examination for the minimum EC standard (**not** HVS) for which the crop is entered. In the absence of a control plot, any inspection will have to be official. Scottish Ministers will recognise control plots sown in other areas of the United Kingdom, provided confirmation of this fact and the result is given to Scottish Ministers. If you are uncertain that a control plot has been sown in other areas of the United Kingdom, you may wish to send a plot sample of each seed lot you intend to enter for crop production in Scotland to SASA.

Additionally, if a seed lot fails the minimum EC standard (not HVS) for varietal purity in post control, licensed inspections will not be allowed on any crops sown with such a seed lot, and official inspections will have to take place. For example, a licensed inspection will be allowed to take place on C1H seed lot that fails the C1H standards in post control, but if this seed lot were also to fail C1L in post control, a licensed inspection will not be allowed, and an official inspection will be required.

For Information contact:

Cereals:

Gerry Hall - Telephone: 0131 244 8856; E-Mail Gerry.Hall@sasa.gsi.gov.uk
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Non Cereals:

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SAMPLING FOR MOISTURE CONTENT DETERMINATION

The Cereal Seed (Scotland) Regulations 2005, which come into force on 1 July 2005, require moisture content to be carried out on seed being certified and marketed.

Primary samples for moisture tests should be taken in such a way as to minimise exposure to the atmosphere. The sample submitted to a licensed seeds laboratory or the OSTS must be in a moisture proof container, of a type approved by the Certifying Authority. A sample of at least 100 gms is required.

The following are examples of containers that can be used for submitting moisture samples:

Resealable plastic bags
Foil & polythene bags that can be heat-sealed
Glass or polythene bottles with an airtight top

The following method is used to obtain a submitted sample from a composite sample:

The composite sample is passed through a Riffle Divider once.

A moisture proof container is drawn/pulled randomly through the seed obtained in one of the two collecting vessels.

The container is then sealed, after excluding as much air as possible, placed in the sample bag and submitted to the seeds laboratory as soon as possible.

Alternatively, the whole composite sample may be submitted in a moisture proof container.

For further information please contact:

Ken Pearson, Tel: 0131 244 8885, e-mail: Ken.Pearson@sasa.gsi.gov.uk or
Russell Thomson, Tel: 0131 244 8899, e-mail: Russell.Thomson@sasa.gsi.gov.uk

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Registration of Fodder Mixtures

The Fodder Plant Seed (Scotland) Regulations 2005, which come into force on 1 July 2005, require certain information to be provided to the Certifying Authority (SASA) when the details of a mixture are not contained on a label.

If a mixture is always labelled with labels which contain the percentage by weight of each of the constituents by species and, where appropriate, by variety, there is no need to register the mixture with the Certifying Authority.

When a brand name only is used on the mixture label, a company must provide SASA with the name of the mixture, details of all the constituents and their proportions by weight. There is currently no fee for registration, which is valid for one year from the date of approval. Once a mixture has been registered, SASA must be informed of any changes to the mixture name or the constituents.

Packages over 10kg (Agricultural or Amenity Mixtures)

Once a mixture has been registered, details of species/variety/percentage by weight can be omitted from the official label. The customer **must**, however, be provided, at or before the sale, with a despatch note or invoice, which includes the details omitted from the official label.

Small EC "A" Packages (up to and including 2kg - Amenity Mixtures)

Small EC "B" Packages (above 2kg up to and including 10kg - Agricultural or Amenity Mixtures)

Even after a mixture has been registered, only the details of the percentage by weight of the constituents can be omitted from the label. The label **must** continue to include the species and variety names of all the constituents. The customer **must** also be informed, at or before the sale, that information relating to the percentage by weight of each of the constituents in the mixture will be provided by the supplier **on request**.

The registration of a mixture only permits the information detailed above to be omitted from the label. It does not affect any of the other packaging or labelling requirements set out in The Fodder Plant Seed (Scotland) Regulations 2005.

Details of mixtures should be sent to Ken Pearson at SASA or e-mailed to Ken.Pearson@sasa.gsi.gov.uk.

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MOISTURE CONTENT TESTING

The Cereal Seed (Scotland) Regulations 2005 ("the Regulations") come into force on 1 July 2005. The Regulations set 17% as the maximum moisture content of cereals and will require moisture content tests to be carried out on all cereal seed lots entered for Certification. Moisture Content tests will be carried out by either the Official Seed Testing Station (OSTS) or Licensed Seed Testing Stations (LSTSs).

Seed treatments applied as aqueous suspensions have a significant affect on seed moisture content and processors should ensure that seed meets the moisture content standard after the application of such treatments and prior to processing.

When sampling a seed lot for certification purposes, the sampler should draw the moisture sample in such a way as to minimise exposure of the sample to the atmosphere. There are two options available for submitting the moisture content sample.

Option 1

The seed lot is sampled in the usual way and the complete composite sample is submitted to the seed testing station in an airtight container.

For samples tested at LSTSs the weight of the composite sample must be at least 2.25kg; for samples to be tested at the OSTS the weight of the composite sample must be at least 1.25kg.

Option 2

The composite sample is passed through a riffle/soil divider once and a moisture proof container is filled by drawing it through the seed contained in one of the tins. The container is then sealed and the remainder of the composite sample mixed and divided as detailed in the Regulations to give a submitted sample of at least 2kg, for LSTSs, or 1kg, for the OSTS. The moisture sample is placed inside the sample bag with the certification sample. In this way, the seed for moisture content tests are separate from the rest of the seed in the sample bag on which the other certification tests have to be carried out. **Those using the OSTS to test certification samples will be provided with moisture proof containers that can be used to submit moisture samples in this way.**

Using both Options 1 and 2 the samples must be submitted for testing as soon as possible. Seed testing stations must complete moisture content tests within 5 days of the seed lot being sampled or the test will be considered invalid and another moisture content sample will be required.

There are two methods of determining the moisture content of cereals included in the International Seed Testing Association (ISTA) Rules. These are:

the high¬ constant temperature oven method; and
the moisture¬ meter method.

For both methods the equipment used must be approved by the OSTS before Moisture Content Testing is added to the license of a LSTS. For those using the meter method, the meter must be calibrated for each species tested. New calibrations are required each time a total of 100 samples are tested. The maximum time allowed between calibrations is 12 months.

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Monitoring of this test will be carried out by the OSTS during audit visits to LSTSs. It will include:

duplicate tests¬ on the day of the audit visit; and
proficiency¬ tests on the day of the audit using samples of known moisture content.

For LSTSs using the moisture meter method there will be additional checks on calibration test data and of the logbook containing details of number of tests carried out.

If you have any questions please contact:

For Information on Procedures for obtaining Moisture Content samples:

Ken Pearson - Telephone: 0131 244 8885; E-Mail Ken.Pearson@sasa.gsi.gov.uk

For information on Moisture Content testing and the provision of moisture proof containers for submission of samples to the OSTS for testing:

Caroline Cadger-Telephone: 0131 244 8908; E-Mail Caroline.Cadger@sasa.gsi.gov.uk
or
Jean Hall – Telephone: 0131 244 8803; E-Mail Jean.Hall@sasa.gsi.gov.uk

For information on calibration of Moisture Meters:

Ronald Don – Telephone 0131 244 8891; E-Mail Ronald.Don@sasa.gsi.gov.uk
or
Gillian McLaren-Telephone: 0131 244 8851; E-Mail Gillian.McLaren@sasa.gsi.gov.uk

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TETRAZOLIUM TESTING

The Cereal Seed (Scotland) Regulations 2005 ("the Regulations") come into force on 1 July 2005. The Regulations will allow the use of a tetrazolium test on all categories of certified cereals as an alternative to the full germination test. This will enable seed to be marketed rapidly. Tetrazolium tests will be carried out by either the Official Seed Testing Station (OSTS) or Licensed Seed Testing Stations (LSTSs) that have been approved to carry out such tests.

The relationship between tetrazolium and germination is very good; however, in some circumstances the tetrazolium test will give an over-estimation. For example, the tetrazolium test does not detect the effects on germination of:

- chemicals; or
- seed-borne diseases.

In addition, in seed lots where drying damage is present the germination of such lots is invariably lower than that indicated by the test.

For these reasons certification **will not** be permitted by tetrazolium testing if:

- heat damage is present; or
- the seed crop was treated with glyphosate.

Furthermore, tetrazolium testing is only available for seed lots of wheat and oats that are to be sold untreated when a disease test demonstrates that the combined levels of *Microdochium* and/or *Septoria* are less than 10%.

The result of a tetrazolium test will be reported as a percentage of viable seeds and the number of seed tested must also be reported. This is important because based on statistical probabilities there are different pass percentages depending on the number of seeds tested. For example:

- **100** seeds tested: **93% and above viable = pass**, otherwise fail.
- **200** seeds tested: **90% and above viable = pass**, otherwise fail.
- **400** seeds tested: **85% viable and above = pass**, otherwise fail.

It will be the responsibility of the applicant to ensure seed submitted for tetrazolium meets the conditions for this test. Seed tested by tetrazolium that is subsequently found not to have met testing criteria is deemed not to have been certified. A germination test must be carried out if there is any doubt about whether a tetrazolium test is applicable.

The results of a germination test will always take precedence over those of a tetrazolium test and enforcement and certification control will be based on germination.

If you have any questions please contact:

For Information on Certification procedures using Tetrazolium testing

Gerry Hall - Telephone: 0131 244 8856; E-Mail Gerry.Hall@sasa.gsi.gov.uk or
Bob Sunter - Telephone: 0131 244 8815; E-Mail Bob.Sunter@sasa.gsi.gov.uk

For information on Tetrazolium testing:

Caroline Cadger - Telephone: 0131 244 8908; E-Mail Caroline.Cadger@sasa.gsi.gov.uk
or
Gillian McLaren - Telephone: 0131 244 8851; E-Mail Gillian.McLaren@sasa.gsi.gov.uk

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For information on *Microdochium* and *Septoria* disease testing:

Valerie Cockerell-Telephone: 0131 244 8900; E-Mail Valerie.Cockerell@sasa.gsi.gov.uk or

Margaret Jacks - Telephone: 0131 244 8803; E-Mail Margaret.Jacks@sasa.gsi.gov.uk

EARLY MOVEMENT PROVISION

The Oil and Fibre Plant Seed (Scotland) Regulations 2004, the Cereal Seed (Scotland) Regulations 2005 and the Fodder Plant Seed (Scotland) Regulations 2005 (collectively referred to as "the Regulations") come into force on 1 July 2005. The Regulations change the requirements for the procedure known as early movement. Marketing of a seed lot to the first buyer by way of trade before the completion of the official germination test will be permitted, subject to the following conditions:

- the seed test report (SDG10) must show that all the other appropriate seed standards (i.e. content of seed of other plant species, analytical purity, ergot (cereals only) and moisture content (cereals only)) have been met and the report must have been presented to SASA;

- a provisional analytical report must show that the seed meets at least the minimum germination standard, and must have been presented to SASA;

- SASA must be notified in writing of the name and address of the first buyer by way of trade as soon as practicable after delivery and in any event not later than seven days after delivery;

- the first buyer by way of trade must be provided either before or at the time of delivery with;

  - a written statement that the seed lot has been marketed before completion of the official germination test; and

  - the provisional germination result;

- the person marketing the seed must guarantee a minimum percentage of germination, which must be the percentage germination of the seed ascertained in the provisional germination report;

- a separate additional "supplier's label" must be applied to each package or container of seed from the seed lot stating:

  - the supplier's name and address;
  - the seed lot reference number;
  - that the seed is being sold before the official examination of the germination of that seed lot has been concluded; and
  - the minimum percentage germination guaranteed by the person marketing the seed.

Records of all early movement transactions must be kept for at least two years after the date of the delivery of the seed to the first buyer by way of trade and must include-

- the date of delivery;
- reference number of the seed lot or part of the seed lot delivered;

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- name and address of the first buyer by way of trade;
- the date on which the preliminary test of germination was carried out and of the date of the provisional germination report;
- the result of the preliminary test of germination; and
- if appropriate, the date on which the first buyer by way of trade was informed of the result of the completed official germination test.

The "Early Movement" provision only applies to seed sold to the first buyer by way of trade. The first buyer by way of trade must be another seed merchant. Further marketing beyond the first buyer by way of trade before the completion of the official germination test is prohibited.

The requirements apply to all seed marketed in Scotland, including seed originating from England.

In the event of the official germination test showing the failure of the seed to comply with the relevant germination standard, the supplier must notify the buyer of the specific percentage of germination ascertained, in writing. That notification must be made as soon as practicable and in any event no later than 7 days after the supplier was informed of the result.

In order to make seed covered by the Cereal Seed (Scotland) Regulations 2005 more rapidly available to farmers, a Statutory Tetrazolium Test (STZ Test) has been introduced for all species and for each generation. A germination test will not be necessary where a seed lot is tested using the STZ test. Seed lots that are to be marketed using the STZ test will not be eligible for early movement, but once the test is completed and the result is satisfactory, the seed lot can be marketed to any buyer. There are some restrictions to the use of this test. Certification **will not** be permitted by tetrazolium testing if:

- heat damage is present; or
- the seed crop was treated with glyphosate.

In addition, tetrazolium testing is only available for seed lots of wheat and oats that are to be sold untreated, when a disease test demonstrates that the combined levels of *Microdochium* and/or[KJ1] *Septoria* are less than 10%.

A germination test **must** be carried out if there is any doubt about whether a tetrazolium test is applicable.

Suppliers should familiarise themselves with the separate written guidance available from SASA in respect of Statutory Tetrazolium Testing.

For Information on Certification procedures:

Cereals:

Gerry Hall - Telephone: 0131 244 8856; E-Mail Gerry.Hall@sasa.gsi.gov.uk
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DETAILED PROCEDURES FOR THE DETERMINATION OF MOISTURE CONTENT OF CEREAL SEED

1. ISTA HIGH CONSTANT TEMPERATURE OVEN METHOD

APPARATUS REQUIRED

Oven

An oven capable of maintaining a temperature of 130±2°C. The temperature variation between the various locations within the oven must be within ±2°C. The heating capacity of the oven must be such that, after preheating to the required temperature followed by opening and loading with containers, the oven will again reach the required temperature within 15 minutes.

Thermometer/Thermocouple

The instrument used to monitor temperature must be accurate to ±0.5°C.

Containers

These must be of non-corrosive metal or glass of approximately 0.5mm thickness and have lids. They should be between 6cm and 8cm diameter and have a depth of 1.5cm.

Grinding Mill

The mill used must grind evenly and in a manner that does not cause heating. At least 50% of the ground material must pass through a wire sieve of 0.50mm and not more than 10% remain on a wire sieve of 1.00mm.

Balance

An analytical balance capable of weighing accurately to 0.001g.

Desiccator

This should be fitted with a thick metal plate to promote rapid cooling of containers. A suitable desiccant should used.

HEALTH and SAFETY

Appropriate measures must be taken to ensure compliance with regulations in respect to dust, chemical, seed treatments, electrical equipment and high temperatures.

METHOD

1. The oven is switched on prior to starting tests to allow it to reach the required temperature of 130± 2°C.

2. Before drawing working samples the submitted sample must be thoroughly mixed by one of the following methods that minimises exposure of the sample to the air:

    - Stir the sample in its container with a spoon/scoop; or

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- Place the opening of the original container against the opening of a similar container and pour the seeds back and forth between the two containers.

3. Two independently drawn replicates are used to determine the moisture content.

   Each replicate must be drawn in such a manner that the sample is not exposed to the air for more than 30 seconds.

   Each replicate is obtained by grinding a representative sample of the thoroughly mixed submitted sample.

   - **If the complete certification sample has been submitted in a moisture proof container:**

A spoon/scoop is placed in the container and small portions removed from different positions and ground. This is repeated until about 4 – 5g of ground material is obtained. The ground portion is weighed to 3 decimal places in a pre-weighed container whose lid is placed under the container for weighing. The sample is then immediately covered with the lid.

- **If the sample is in a moisture bottle:**

Small amounts of seed are removed and ground until the required amount of ground seed is obtained. This is repeated until about 4 – 5g of ground material is obtained. The ground portion is weighed to 3 decimal places in a pre-weighed container whose lid is placed under the container for weighing. The sample is then immediately covered with the lid.

In both cases two independently drawn replicates are required.

4. The containers are placed in the oven (with their lids removed and underneath) as quickly as possible to reduce exposure to the air. The time is noted.

5. Fifteen minutes after placing containers in the oven the temperature is noted. This must be 130± 2^{o}C.

6. The samples remain in the oven for a further 2 hours.

7. The containers are removed from the oven and placed in a desiccator, with their lids in place, to cool for 30 – 45 minutes.

8. The containers and dried contents are weighed and the moisture content is calculated.

<u>CALCULATION OF RESULTS</u>

The moisture content is expressed as a percentage by fresh weight. It is calculated to one

decimal place using the following formula:

$$\frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

where:

M_1 = the weight in grams of the container and its cover.
M_2 = the weight in grams of the container, its cover and its contents before drying.
M_3 = the weight in grams of the container, its cover and its contents after drying.

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The moisture content of each replicate is calculated and recorded. If the difference between the two replicates does not exceed 0.2%, the result reported is the arithmetic mean. This is expressed to one decimal place. If the difference is greater than 0.2% the replicate results are out of tolerance and the determination must be repeated.

PRE-DRYING

If a moisture content result of more than 17% is obtained, a retest is obligatory involving a pre-drying stage prior to grinding the seed for the ISTA high constant temperature oven method.

The pre-drying stage involves:

1. Two independently drawn replicates are used to determine the moisture content.

   Each replicate must be drawn in such a manner that the sample is not exposed to the air for more than 30 seconds.

   - **If the complete certification sample has been submitted in a moisture proof container:**

A spoon/scoop is placed in the container and small portions removed from different positions. This is repeated until 25±1g of seed is obtained. The seed is weighed to 1 decimal place in a pre-weighed container.

   - **If the sample is in a moisture bottle:**

Small amounts of seed are removed until 25±1g of seed is obtained. The seed is weighed to 1 decimal place in a pre-weighed container.

In both cases two independently drawn replicates are required.

2. The seed in their containers is placed in an oven at 130±2°C for 5-10 minutes. The partly dried seed is then removed from the oven and allowed to cool in the laboratory for 2 hours.

3. After the 2 hour cooling and equilibration period the seed is re-weighed in the containers.

4. Immediately thereafter the seed is subject to the ISTA high constant temperature oven method, as described earlier.

CALCULATION OF RESULT

The moisture content is expressed as a percentage by fresh weight. It is calculated to one

decimal place using the following equations:

$$S_1 = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100 \quad \text{----------equation 1}$$

where:

S_1 = moisture content lost during pre-drying
W_1 = weight of container
W_2 = weight of the container + seed
W_3 = weight of the container + pre-dried seed

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(Note that W_2 – weight of the ground seed must be 25±1g)

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$$S_2 = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100 \quad \text{----------equation 2}$$

where:

S_2 = moisture content of the pre-dried seed
M_1 = weight of container
M_2 = weight of the container + ground pre-dried seed
M_3 = weight of the container + dried ground pre-dried seed

(Note that M_2 – weight of the ground seed must be 4 to 5g)

The moisture content is now calculated:

Moisture content = $(S_1 + S_2) - ((S_1 \times S_2)/100)$----------equation 3

where:

S_1 = is the moisture lost during pre-drying
S_2 = is the moisture content of the pre-dried seed

The moisture content of each replicate is recorded and if the difference between the two replicates does not exceed 0.2%, the result reported is the arithmetic mean. This is expressed to one decimal place. If the difference is greater than 0.2% the replicate results are out of tolerance and the determination must be repeated.

An example calculation is included in the appendix.

ISTA METHOD FOR MOISTURE METERS

APPARATUS

Moisture Meter

A moisture meter capable of being calibrated according to procedures in International Seeding Testing Association Rules. The method of operation of the moisture meter must be in accordance with manufacturer's instructions.

HEALTH and SAFETY

Appropriate measures must be taken to ensure compliance with regulations in respect of dust, chemical, seed treatments, electrical equipment and high temperatures.

METHOD

1. Before drawing working samples the submitted sample must be thoroughly mixed by one of the following methods that minimises exposure of the sample to the air:
   - Stir the sample in its container with a spoon/scoop; or
   - Place the opening of the original container against the opening of a similar container and pour the seeds back and forth between the two containers.

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2. Two independently drawn replicates, each of the weight/volume required for the specified meter, according to manufacturer's instructions, are used to determine the moisture content.

   Each replicate must be drawn in such a manner that the sample is not exposed to the air for more than 30 seconds.

   - **If the complete certification sample has been submitted in a moisture proof container:**

     The spoon method should be used to obtain small portions at random until the required amount is obtained. LSTSs will obtain instruction in the spoon method from the OSTS.

   - **If the sample is in a moisture bottle**:

     Small portions are taken until the required amount is obtained.

   In both cases two independently drawn replicates are required.

3. The moisture content of each replicate is determined by testing according to the manufacturer's instructions.

4. If the moisture meter method involves weighing the level of accuracy required for the result depends on the weight of seed required for the specific meter according to the following table:

Weight required by meter	Number of decimal places
0 – 9g	3
10 – 99g	2
100g and above	1

5. The moisture content is expressed as a percentage by fresh weight.

   The moisture content of each replicate is recorded and if the difference between the two replicates does not exceed 0.2%, the result reported is the arithmetic mean. This is expressed to one decimal place.

   If the difference between the two replicate moisture content results is greater than 0.2% the results are out of tolerance and the determination must be repeated.

CALIBRATION OF MOISTURE METERS

Calibration of moisture meters can be carried out in-house or using an agency provided the method prescribed in the International Seed Testing Association Rules is used. A new calibration is required for every 100 determinations, in total, that are carried out and the maximum time allowed between calibrations is 12 months..

APPARATUS REQUIRED

Moisture Meter

The meter requiring calibration.

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Moisture proof containers

For storing calibration samples.

Refrigerated Storage

For storing calibration samples at 5±2^{o}C for up to 10 days.

Apparatus as detailed for ISTA high constant temperature oven method "the reference method".

Calibration Samples

Five calibration samples are required. These must come from a minimum of two varieties of the species for which the moisture meter is being calibrated. The samples should have a range of moisture contents evenly covering the required measurement range for which the moisture meter is being checked. The range covered must include moisture contents up to 18%, if the meter is to be used for certification tests.

METHOD

1. The calibration of moisture meters may be affected by many variables, including species, variety, ripeness, humidity, temperature and level of impurities. To minimise these effects:

    - Calibration samples should have an analytical purity of at least 98%;
    - During determinations, exposure of samples to the atmosphere must be reduced to the absolute minimum;
    - A minimum of 2 varieties of each of the species for which moisture meter is being calibrated must be used;
    - Calibration samples are stored at 5±2^{o}C;
    - Calibration samples must be used within 10 days of preparation;
    - Prior to use, calibration samples must be equilibrated to the same temperature as the moisture meter;
    - Calibration samples should be free of mustiness, fermentation and sprouted seed;
    - The calibration samples must be thoroughly mixed, before working samples are drawn from them using one of the following methods:

        - Stir the sample in its container with a spoon/scoop; or
        - Place the opening of the original container against the opening of a similar container and pour the seeds back and forth between the two containers.

2. Each working sample must be drawn in such a manner that it is not exposed to the air for more than 30 seconds.

3. The moisture content of the calibration samples is determined using the reference method.

4. Three successive measurements are made on each calibration sample using the moisture meter according to the manufacturer's instructions.

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5. After drawing each sample, for either oven or meter determination, the calibration sample must be thoroughly mixed prior to drawing the next working sample.

6. After the completion of moisture meter tests the moisture content of the calibration samples are re-checked using the reference method.

CALCULATION OF RESULTS

Reference oven method

For each calibration sample there are two results; x_1 obtained before measuring the moisture content with the moisture meter, and x_2 obtained after measuring the moisture content with the moisture meter. The average of x_1 and x_2 is the true value of the moisture content provided the difference between x_1 and x_2 is no greater than 0.3%. **If the difference is greater than 0.3% the calibration must be repeated.**

Moisture meter

For each calibration sample there are three results and these should be designated as y_1, y_2 and y_3.

Calculate the average of the three values as y_x ($y_x = (y_1 + y_2 + y_3)/3$). Subtract y_x from the true value of the moisture content and designate the result as z_i.

A moisture meter is considered to be within calibration when z_i is lower than the following maximum permissible errors.

True value of the moisture content (reference method)	Maximal permissible error
Less than 10%	±0.4%
10% or more	±0.04 x true value moisture content

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EXAMPLE OF CALCULATION WHEN PRE-DRYING IS REQUIRED FOR THE MOISTURE CONTENT TEST PROCEDURE

MOISTURE CONTENT TEST

Pre -Drying Required

Sample No.: 123

MOISTURE QA CHECKS/DATE	MOISTURE TEST RESULT
Test 1 RD 20/2/05	20.5%
Test 2	

Test date: 20/2/03 Analyst GM Checked: RD Test date: Analyst: Checked:

TEST 1 Pre -Drying

BEFORE DRYING	TIN (d)	TIN (f)
	Replicate 1	Replicate 2
2. Weight of Tin + Seed	154.2	155.0
1. Weight of Empty Tin	128.4	129.3
3. Weight of Seed = 2 - 1	25.8	25.7
AFTER DRYING		
2. Weight of Tin + Seed	154.2	155.0
4. Weight of Tin + Dried Seed	151.0	151.8
5. Weight Loss = 2 -4	3.2	3.2
6. % Pre-Moisture = 5/3 x 100	12.4	12.5

TEST 2 Pre-Drying

BEFORE DRYING	TIN ( )	TIN ( )
	Replicate 1	Replicate 2
2. Weight of Tin + Seed		
1. Weight of Empty Tin		
3. Weight of Seed = 2 - 1		
AFTER DRYING		
2. Weight of Tin + Seed		
4. Weight of Tin + Dried Seed		
5. Weight Loss = 2 - 4		
6. % Pre-Moisture = 5/3 x 100		

TEST 1 Drying

BEFORE DRYING	TIN (1)	TIN (2)
	Replicate 1	Replicate 2
2. Weight of Tin + pre-dried Seed	27.038	27.884
1. Weight of Empty Tin	23.035	23.877
3. Weight of pre-dried Seed = 2 - 1	4.003	4.007
AFTER DRYING		
2. Weight of Tin + pre-dried Seed	27.038	27.884
4. Weight of Tin + Dried pre-dried Seed	26.666	27.523
5. Weight Loss = 2 - 4	0.372	0.361
7. % Dry-Moisture = 5/3 x 100	9.3	9.0
6+7 - (6x7/100) = % Moisture	20.5	20.4
Average of Rep 1 and 2 =	20.5	% Moisture

TEST 2 Drying

BEFORE DRYING	TIN ( )	TIN ( )
	Replicate 1	Replicate 2
2. Weight of Tin + pre-dried Seed		
1. Weight of Empty Tin		
3. Weight of pre-dried Seed = 2 - 1		
AFTER DRYING		
2. Weight of Tin + pre-dried Seed		
4. Weight of Tin + Dried pre-dried Seed		
5. Weight Loss = 2 - 4		
7. % Dry-Moisture = 5/3 x 100		
6+7 - (6x7/100) = % Moisture		
Average of Rep 1 and 2 =		% Moisture

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DETAILS OF TETRAZOLIUM TESTING PROCEDURES FOR CERTIFICATION OF CEREALS

1 BACKGROUND INFORMATION ON TETRAZOLIUM TESTING

The tetrazolium test is a biochemical viability test that provides a rapid means of determining the potential germination of a seed lot. The relationship between tetrazolium and germination is very good; however, in some circumstances the tetrazolium test will give an over-estimation of germination. For example, the tetrazolium test does not detect the effects on germination of:

- Chemicals; or
- Seed-borne diseases.

In addition, in seed lots where drying damage is present the germination of such lots is invariably lower than the tetrazolium.

Chemicals

Excessive application of seed treatments or their application at too high a moisture content can lead to a reduction of germination through phytotoxicity – this adverse effect is not apparent in the tetrazolium test.

The use of desiccants such as glyphosate is not recommended on seed crops and the manufacturers issue specific warnings against their use on such crops. The germination of seed from crops treated with glyphosate is invariably lower than the tetrazolium viability.

Seed-Borne Diseases

Seed-borne diseases such as *Microdochium* (*Fusarium*) and *Septoria* have an adverse effect on the germination and field emergence of wheat and oats. Fungicidal seed treatments counteract the effects of these diseases. For many years merchants in Scotland have used the tetrazolium test to estimate the germination that will be obtained when seed lots are treated. If wheat or oat seed is to be sold untreated and has high levels of *Microdochium* or *Septoria* the tetrazolium test is not suitable.

Tetrazolium can only be used for the certification of oat and wheat seed lots that are to be sold untreated when the combined levels of *Microdochium* or *Septoria* are no more than 10%.

2 LEGISLATION AND TETRAZOLIUM TESTING

The Cereal Seeds (Scotland) Regulations 2005 ("the Regulations") allow the use of a tetrazolium test on all categories of certified cereals as an alternative to the full germination test.

Previously only OSTSs could carry out a statutory tetrazolium test, which was restricted to C1 seed of winter cereals. The Regulations now allow both Official Seed Testing Stations (OSTSs) and Licensed Seed Testing Stations (LSTSs) to carry out tetrazolium tests for certification purposes on all categories and levels of cereals. However, LSTSs may only carry out the test if they have been approved to do so.

A germination test must be carried out if there is any doubt about whether a tetrazolium test is applicable. **The results of a germination test will always take precedence over those of a tetrazolium test and, whilst the reserved portions of seed lots tested by tetrazolium will be**

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checked using tetrazolium, enforcement and certification control will be based on germination.

All cereal species covered by the Regulations are eligible for tetrazolium testing.

3 WHO CAN CARRY OUT TETRAZOLIUM TESTS FOR CERTIFICATION?

Tetrazolium tests for certification purposes can be carried out by LSTSs who have had tetrazolium testing added to their schedule of seed testing. To have tetrazolium testing added, the Analyst in Charge of a LSTS must have successfully completed a tetrazolium assessment at either the OSTS Edinburgh or Cambridge.

In the past there was the option of a separate sub-sample for statutory tetrazolium testing which could only be carried out by an OSTS. This is still possible for tetrazolium testing under the new Regulations.

An applicant has two options when using an OSTS either:

- the certification sample is submitted to the OSTS for all tests;
  or
- a LSTS carries out analytical purity and other seed determination tests and submits a sub-sample for tetrazolium testing to the OSTS.

4 TETRAZOLIUM TEST PROCEDURES FOR CEREALS (BARLEY, OATS AND WHEAT)

There are two methods of tetrazolium testing for cereals within the International Seed Testing Association Rules. These are the longitudinal cut method, favoured by the OSTS for Scotland, and the excised embryo method, favoured by the OSTS for England and Wales. Both methods are valid and are described below.

5 LONGITUDINAL CUT METHOD

Treatment before staining

Replicates of seed should be placed in beakers and covered with tap water. They must be left for 15-22 hours at a temperature of 12-25°C.

Preparation for staining

Decant the water. For oats, the lemma and palea are carefully removed from the replicates of seed, ensuring that the embryos are not damaged in the process, prior to cutting.

Thereafter for all species, using a very sharp scalpel blade No. 10 (Swann Morton) cut through the middle of the embryonic axis and approximately two thirds of the length of the seed. Ensure that the seeds remain moist until preparation is complete. As soon as the seeds are cut return them to the beaker and cover with sufficient 0.5% tetrazolium solution to submerge them.

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Staining time

Incubate: 1 hour to 1.5 hours for wheat and oats; and
2 hours to 2.5 hours for barley,
in total darkness at 30 ± 10°C.

Preparation for evaluation

Decant the solution and thoroughly wash the seed before examination. Protective gloves may be worn as an additional precaution. Place the embryos on a glass slab, white tile or other appropriate surface and examine using between x10 and x16 magnification.

The seeds must be kept moist throughout preparation and evaluation; any drying out will invalidate the test.

Evaluation

For species other than barley, the top surface of the embryo is examined before the seed is opened to expose both cut surfaces. The critical areas on both cut surfaces are checked (i.e. the plumule, root initials and internal tissue).

The back surface of scutellum on one side of the embryo is also examined by detaching the embryo from the endosperm. If there is any doubt about the viability of the seed due to the staining pattern, the back of the scutellum of the other half must be examined.

Maximum area of unstained, flaccid or necrotic tissue permitted:

- At least one root initial must be stained normally. The rest of the root area can consist of unstained, flaccid or necrotic tissue
- 1/3 of extremities of scutelum can consist of unstained, flaccid or necrotic tissue

Remarks

Unstained tissue at centre of scutellum is indicative of heat damage and the seed is classed as non-viable. Any unstained tissue in the plumule area renders the seed non-viable

Note and record the viable embryos, that have the ability to germinate normally, and non-viable embryos, that do not have the ability to germinate normally.

7 EXCISED EMBRYO METHOD

Treatment before staining

Replicates of seed should be placed in beakers and covered with tap water. They must then be placed in an incubator at 40 ± 2°C for 2-4 hours.

For oats, the lemma and palea, and for barley the lemma must be carefully removed from the replicates of seed, ensuring that the embryos are not damaged in the process.

Preparation for staining

Decant the water and with a very sharp scalpel blade No. 11 (Swann Morton) held at an angle, cut through the endosperm removing a thin slice with the scutellum and the embryo attached. Ensure that the embryos remain moistened with water until preparation is complete. As soon as the embryos

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are dissected they are returned to the beaker and covered with sufficient 1% tetrazolium solution to submerge the seed.

Staining time Incubate overnight (16-20 hours) in total darkness at 30 ± 2°C.

Preparation for evaluation Decant the solution and thoroughly wash the embryos before handling. Protective gloves may also be worn as an additional precaution. Place the embryos on a glass slab and examine using x10 magnification.

The embryos must be kept moist throughout preparation and evaluation; any drying out will invalidate the test.

Evaluation Expose and closely examine the plumule, root initials and internal tissue if necessary to assess the viability of the tissues. Expose and closely examine the scutellum. If the thin layer of endosperm protecting the scutellum is firm, it may be removed by cutting. Otherwise it must be scraped away with the blunt side of a scalpel blade.

Maximum area of unstained, flaccid or necrotic tissue permitted:

- At least one root initial must be stained normally. The rest of the root area can consist of unstained, flaccid or necrotic tissue
- 1/3 of extremities of scutelum can consist of unstained, flaccid or necrotic tissue

Remarks Unstained tissue at centre of scutellum is indicative of heat damage and the seed is classed as non-viable.

Note and record the viable embryos, that have the ability to germinate normally, and non-viable embryos, that do not have the ability to germinate normally.

8 NUMBER OF SEED TESTED

The number of seed tested in a tetrazolium test is 100, 200, or 400 depending on the results at each of the three possible steps described below. The seed must be taken from either, the pure seed fraction of a purity test, or be pure seed taken from the certification sample. Seeds must be counted out at random in replicates of 2 x 50 and 3 x 100 to give sufficient seed to carry out all three steps, if necessary.

Details of the test procedure

STEP 1

Test 2 x 50 seeds

If the 2 x 50 replicates are in tolerance (tolerance tables are attached in an appendix), the average result can be reported.

If the results are in tolerance and the average is 93% viable or above, then the seed lot meets the certification standard. No further seeds need be examined.

For samples where the 2 x 50 replicates are out of tolerance or the viability is below 93%, another 100 seeds must be examined. The reason for this is that if the 2 x 50 tetrazolium

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result is between 78% and 92% there is a significant probability that the true viability of the seed lot is 85% or more and will give a pass result when more seed are examined.

STEP 2

Test one of the 1 x 100 replicates.

2 x 100 seeds have now been examined in total. If the results for the 2 x 100 tests are in tolerance, the average can be reported.

If the results are in tolerance and the average is 90% viable or above the seed lot meets the certification standard. No further seeds need be examined.

If the 2 x 100 replicates are out of tolerance or the viability is below 90%, another 2 x100 seeds must be examined. The reason for this is that if the 2 x 100 tetrazolium result is between 80 and 89% there is a significant probability that the true viability of the seed lot is 85% or more and will give a pass result when more seed are examined.

STEP 3

Test the final 2 x 100 seeds replicates.

4 x 100 seeds have now been examined in total. If the 4 x 100 results are in tolerance, the average can be reported.

If the 4 x 100 results are in tolerance and the average is 85% or above the seed lot meets the certification standard. No further seeds need be examined.

Retest Procedure

A retest can be carried out if the result is within limits of the standard. For example, with a germination standard of 85%, a retest tetrazolium test can be carried out if a result of 80% - 84%, inclusive, is obtained in an initial 400 seed tetrazolium test.

9 RESTRICTIONS ON THE APPLICATION OF TETRAZOLIUM TESTS FOR CERTIFICATION PURPOSES

Certification **will not** be permitted by tetrazolium testing if:

- heat damage is present;
  or
- if the seed crop was treated with glyphosate.

In addition, tetrazolium testing is only available for seed lots of wheat and oats that are to be sold untreated when a disease test demonstrates that the combined levels of *Microdochium* and *Septoria* are less than 10%.

It is the responsibility of the applicant to ensure seed submitted for tetrazolium meets the conditions for such testing.

Seed tested by tetrazolium that is subsequently found not to have met testing criteria is deemed not to have been certified.

10 REPORTING RESULTS

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The result of a tetrazolium test will be reported as a percentage of viable seeds and the number of seed tested must also be reported. This is important because there are different pass percentages depending on the number of seeds tested.

For example:

100 seeds tested: 93% and above viable = pass, otherwise fail.
200 seeds tested: 90% and above viable = pass, otherwise fail.
400 seeds tested: 85% viable and above = pass, otherwise fail.

Evidence of heat damage will mean that the seed lot can not be certified using tetrazolium irrespective of the result of the tetrazolium test. If heat damage is found a germination test must be initiated.

11 TRAINING AND COMPETENCE

There is a joint approach between the OSTSs for seed analyst training, with UK recognised qualifications for Seed Analysis and Laboratory Management. Tetrazolium testing will now be added to the portfolio of Seed Analysis qualifications and is recognised by all the UK Certifying Authorities.

Tetrazolium training and examinations will take the form of a 2 day training course followed by a one day assessment of competence. This course covers both the theoretical and practical aspects of tetrazolium testing. It introduces candidates to the assessment of different types of non-viable seeds – root damage, shoot damage (including split coleoptiles), root and shoot damage, sprouting, mechanical damage, heat damage, etc.

A collection of samples is used to ensure that candidates can identify different types of non-viable seed and can practice assessment of all these types of non-viable seeds. An integral part of the course is the comparison of germination and tetrazolium results. Normally the OSTSs would expect there to be a training period between completing the two day training and the one day assessment.

The one day assessment will take the form of a refresher of the “does and don’ts” of tetrazolium testing, the candidate demonstrating their cutting technique, a short theory test followed by the assessment of up to 6 samples.

Examples of theory questions are:

- When should a tetrazolium test **not** be used?
- With a tetrazolium result of 91% based on 100 seeds, what would your next course of action be?

The course is agreed and marked by both the OSTS for Scotland and the OSTS for England and Wales who will be running similar courses. Course fees will be the same at both OSTSs.

The OSTS for Scotland will only assess and teach the longitudinal cut method. The OSTS for England and Wales will assess candidates on either of the longitudinal cut or excised embryo methods.

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TOLERANCE RANGES TO BE USED TO COMPARE RESULTS OF TETRAZOLIUM TESTS

Seed Tested.	COLUMN 1 2x50 Average Viable Seed in Sample			COLUMN 2 2X100 Average Viable Seed in Sample			COLUMN 3 4X100 Average Viable Seed in Sample		
	A	B	Tolerance	A	B	Tolerance	A	B	Tolerance
	50	1	2	99	2	3	99	2	4
	49	2	3	98	3	4	98	3	5
	48	3	4	97	4	5	97	4	6
	47	4	5	96	5	5	96	5	7
	46	5	5	95	6	6	95	6	7
	45	6	6	94	7	7	94	7	8
	44	7	6	93	8	7	93	8	8
	43	8	7	92	9	7	92	9	9
	42	9	7	91	10	8	91	10	9
	41	10	7	90	11	8	90	11	10
	40	11	8	89	12	9	89	12	10
	39	12	8	88	13	9	88	13	11
	38	13	8	87	14	9	87	14	11
	37	14	8	86	15	9	86	15	11
	36	15	9	85	16	10	85	16	12
	35	16	9	84	17	10	84	17	12
	34	17	9	83	18	10	83	18	12
	33	18	9	82	19	10	82	19	13
	32	19	9	81	20	11	81	20	13
	31	20	9	80	21	11	80	21	13
	30	21	9	79	22	11	79	22	13
	29	22	9	78	23	11	78	23	14
	28	23	9	77	24	11	77	24	14
	27	24	9	76	25	12	76	25	14
	26	25	9	75	26	12	75	26	14
				74	27	12	74	27	14
				73	28	12	73	28	14
				72	29	12	72	29	15
				71	30	12	71	30	15
				70	31	12	70	31	15
				69	32	13	69	32	15
				68	33	13	68	33	15
				67	34	13	67	34	15
				66	35	13	66	35	15
				65	36	13	65	36	16
				64	37	13	64	37	16
				63	38	13	63	38	16
				62	39	13	62	39	16
				61	40	13	61	40	16
				60	41	13	60	41	16
				59	42	13	59	42	16
				58	43	13	58	43	16
				57	44	13	57	44	16
				56	45	13	56	45	16
				55	46	13	55	46	16
				54	47	14	54	47	16
				53	48	14	53	48	16
				52	49	14	52	49	16
				51	50	14	51	50	16

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