

# INTERNATIONAL STANDARD

ISO  
6639-4

First edition  
1987-02-01



INTERNATIONAL ORGANIZATION FOR STANDARDIZATION  
ORGANISATION INTERNATIONALE DE NORMALISATION  
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

## **Cereals and pulses — Determination of hidden insect infestation —**

### **Part 4: Rapid methods**

*Céréales et légumineuses — Détermination de l'infestation cachée par les insectes —*

*Partie 4: Méthodes rapides*

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Reference number  
ISO 6639-4: 1987 (E)

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6639-4 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Section five cancels and replaces ISO 1162-1975.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

# Cereals and pulses — Determination of hidden insect infestation —

## Part 4 : Rapid methods

### 0 Introduction

This International Standard describes methods of determining hidden insect infestation in cereals and pulses. It consists of the following parts:

*Part 1: General principles.*

*Part 2: Sampling.*

*Part 3: Reference method.*

*Part 4: Rapid methods.*

### 1 Scope and field of application

This part of ISO 6639 specifies five rapid methods for estimating the degree of, or detecting the presence of, hidden insect infestation in a sample of a cereal or pulse.

NOTE — The characteristics leading to the choice of rapid method are summarized in the table in ISO 6639/1.

#### Section one: Method by determination of carbon dioxide production (clauses 3 to 9)

The method is primarily intended for testing whole grains. It is not applicable for testing

- a) finely ground grain products, as there is a risk that particles of material will be sucked up with air samples; or
- b) grain products with moisture contents greater than 15 % (*m/m*), because of the risk of carbon dioxide produced by the products themselves and by micro-organisms interfering with the results.

In addition, the method is not applicable to the rapid testing of grain products on to which carbon dioxide has already been adsorbed in large quantities, for example grain stored in a confined atmosphere or when there are clear external indications of heavy infestation.

The method can be used for coarsely milled or kibbled grain products, provided that they have been sieved before testing to remove fine particles and loose insects.

The method does not permit the presence of dead adults, pupae, larvae or eggs to be detected.

#### Section two: Ninhydrin method (clauses 10 to 16)

The method is applicable to any dry grain prone to internal insect infestation, particularly wheat, sorghum, rice and similar sized grains. Large grains, such as maize, have to be partially broken (kibbled) before they can be tested. This treatment of large grains can cause some insects to be lost or fragmented, thus rendering the interpretation of results unreliable. Numbers of eggs and young larvae may be underestimated, but, in this respect, the method is no less efficient than any other.

#### Section three: Whole grain flotation method (clauses 17 to 24)

The method is suitable for detecting hidden infestation in most cereals and pulses but only on a qualitative basis.

#### Section four: Acoustic method (clauses 25 to 31)

The method is suitable for detecting living insect adults and larvae feeding inside grains. It does not permit dead adults and larvae or living eggs and pupae (non-feeding stages) to be detected.

#### Section five: X-ray method (clauses 32 to 38)

The method is suitable for detecting living and dead larvae and adults within grains. Insects which have been recently killed (for example by fumigation) may be difficult to distinguish from those still living.

## 2 References

ISO 520, *Cereals and pulses — Determination of the mass of 1 000 grains.*

ISO 565, *Test sieves — Woven metal wire cloth, perforated plate and electroformed sheet — Nominal sizes of openings.*

ISO 712, *Cereals and cereal products — Determination of moisture content (Routine reference method).*

ISO 950, *Cereals — Sampling (as grain).*

ISO 951, *Pulses in bags — Sampling.*

## Section one : Method by determination of carbon dioxide production

### 3 Principle

Incubation of a test portion of the material at a standard temperature, and estimation, by a gasometric method or an infra-red method, of the amount of carbon dioxide generated during a standard period as a measure of the total metabolism of the material.

NOTE — This method is based on work in which it was shown that respiration could be used to detect insects in produce and that the volume of airspace is approximately constant in bulk grain packed tight. The metabolic rate of dry grain, or a grain product, is very low. That of insects is so much higher that the generation of carbon dioxide in dry grain or grain product can be regarded as a sign of infestation, provided that care has been taken to avoid contamination with this gas and to ensure that adsorption by the grain is minimized.

### 4 Apparatus

**4.1 Sieve**, of suitable aperture size such that fine particles and insects can pass but the material under test is retained (see ISO 565).

**4.2 Balance**, accurate to 0,1 g.

**4.3 Apparatus for gasometric analysis** (see figure 1).

**4.3.1 Airtight sample containers**, of capacity not exceeding 750 ml. Each container shall be closed with a rubber septum.

**4.3.2 Syringes and needles**, for withdrawing samples of interstitial air. The syringes shall be completely airtight and shall be of sufficient capacity for the analysis. All-glass syringes of capacity 20 ml are suitable.

**4.3.3 Incubator or climatic chamber**, capable of being maintained at  $25 \pm 1$  °C (see 4.4.1).

**4.3.4 Gas analysis apparatus**, suitable for measuring carbon dioxide concentrations to within  $\pm 0,2$  % (V/V).

**4.4 Apparatus for infra-red gas analysis** (see figure 2).

**4.4.1 Controlled climate room**.

The analytical apparatus should be housed in a room having controlled temperature and relative humidity, preferably at  $25 \pm 1$  °C and a relative humidity of  $70 \pm 5$  %.

**4.4.2 Infra-red gas analyser**, with two interchangeable measurement ranges for carbon dioxide (0 to 50  $\mu\text{l/l}$  and 0 to 500  $\mu\text{l/l}$ ), capable of operating with dry air as the carrier gas supplied by a compressed air cylinder, an air pressure line or a leakproof diaphragm pump at a flow rate of 2 000 ml/min.

**4.4.3 Airtight sample containers**, of capacity not exceeding 750 ml. These containers comprise a cylinder made of gasproof material, approximately 100 mm in diameter, sealed at the bottom and accommodating a removable lid with an airtight closure at the top (see 4.3.1), having two orifices with nozzles permitting air to be introduced into the lower part of the cylinder after connection to the purified air line (see figure 2) and to be expelled at the top.

**4.4.4 Supply of compressed dry air** (air pressure line, compressed air cylinder or diaphragm pump) with a pressure-reducing valve. A flow-regulating valve and a flowmeter are necessary in the circuit.

**4.4.5 Three-way valves**, manually or electrically controlled.

**4.4.6 Air washing and drying tubes**, installed in the circuit before the sample container. The washer comprises a flask to allow the air to be bubbled through 10 % (m/m) sodium hydroxide solution. The desiccator contains desiccant, for example anhydrous calcium chloride.

**4.4.7 Moisture indicator**, placed between the sample container and the analyser (silica gel with saturation indicator).

### 5 Sampling

Use samples obtained as described in ISO 6639/2.

### 6 Procedure

#### 6.1 Preparation of test sample

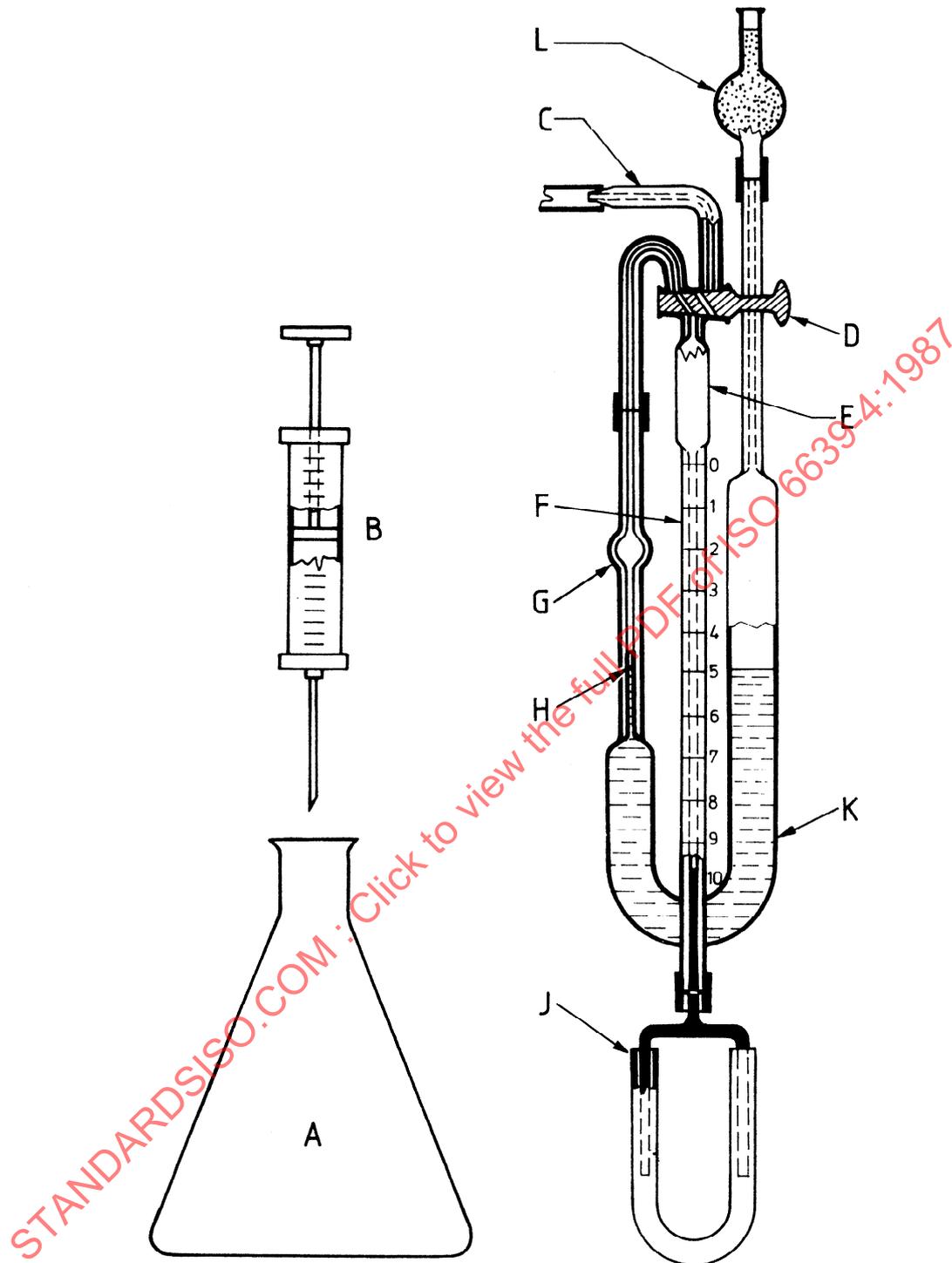
Use the sieve (4.1) to remove any fine particles and insects from the sample. If required, the insects may be identified and the number of adults, pupae and larvae recorded separately for each species.

In order to bring the sample to a suitable condition for testing, keep it for 24 h in the incubator (4.3.3), controlled at 25 °C, or in the controlled climate room (4.4.1) in a close-woven cloth bag, or a wide-mouthed jar, tray or open tin, suitably covered to prevent the entry or escape of free-living insects, while allowing exchange of air (see ISO 6639/3, subclause 5.4).

Before preparing the airtight sample container (6.2), re-sieve the sample to remove any insects which may have emerged during the preparatory period.

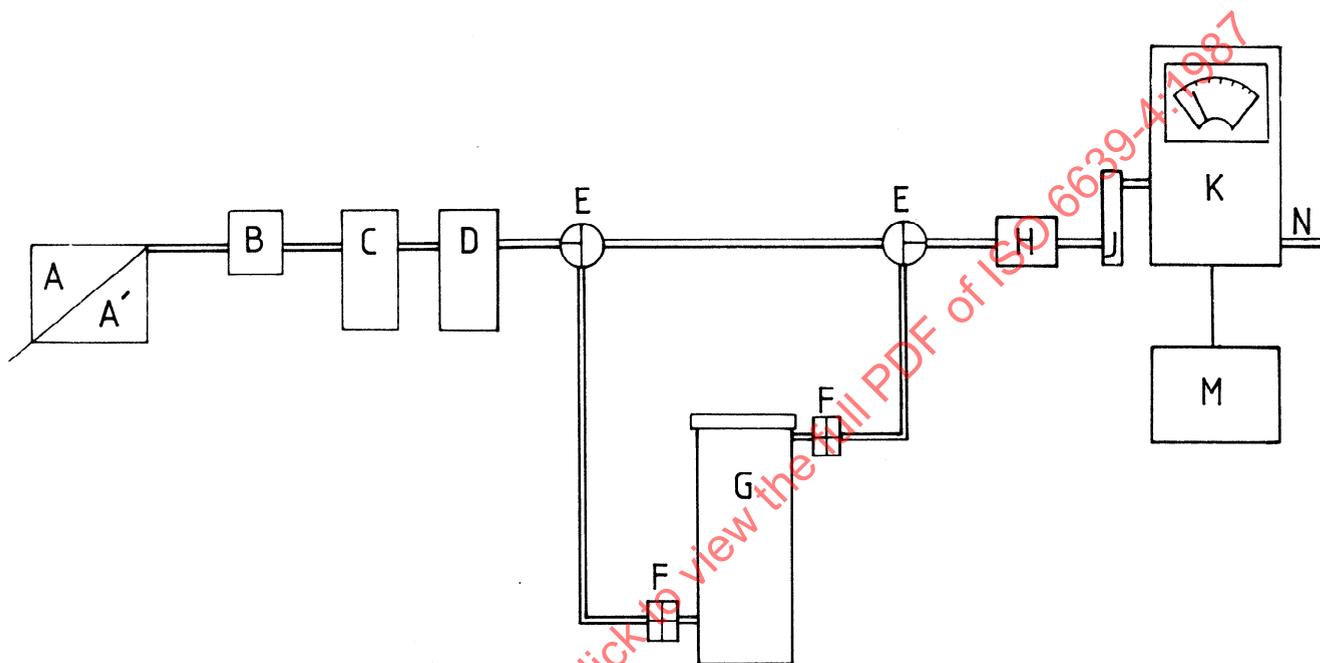
Spread the sample thinly on a tray or other suitable flat surface, and leave exposed to air for 15 to 30 min (to permit adsorbed carbon dioxide to escape). Airing is less important for infra-red analysis, but, if this is not done, the test report (clause 9) shall mention the fact.

Immediately before filling the airtight sample container, determine the moisture content of the sample by the method described in ISO 712, using test portions obtained in accordance with ISO 950 or ISO 951.



- A Airtight container for the test portion
- B Hypodermic syringe
- C Air intake
- D Three-way stopcock
- E 4 ml bulb (volume to "0" graduation on tube F)
- F Narrow-bore tube graduated in divisions of 0,01 ml from 0 to 1,00 ml
- G 1,5 ml bulb
- H Graduation mark
- J Mercury reservoir (provided with means for adjusting level in tube F)
- K U-tube containing potassium hydroxide solution
- L Soda-lime tube, to protect contents of tube K from atmospheric carbon dioxide

Figure 1 — Apparatus for gasometric analysis



- A Dry air supply (cylinder or air pressure line)
- A' Leakproof diaphragm air pump
- B Pressure regulator
- C Air washer (bubbler with 10 % (m/m) sodium hydroxide (NaOH) solution)
- D Air desiccator [anhydrous calcium chloride (CaCl<sub>2</sub>)]
- E Three-way valves (manual or electric)
- F Airtight connections
- G Sample container
- H Moisture indicator (silica gel with saturation indicator)
- J Flowmeter with flow-regulating needle-valve
- K Infra-red analyser
- M Potentiometric recorder (optional)
- N Air outlet

Figure 2 — Diagram of apparatus for infra-red gas analysis with operating accessories

## 6.2 Preparation of container and of test portion

Leave the sample container (4.3.1 or 4.4.3) open to allow water and/or carbon dioxide to escape and then weigh it to the nearest 0,1 g.

Pour approximately 300 g of the test sample into the container. Tap the container to shake the sample down, and add more of the test sample until the container is completely full.

Weigh the container containing the test portion to the nearest 0,1 g and deduce the mass of the test portion.

NOTE — Constancy of filling and packing of the sample container is not essential if the infra-red method is used.

Seal the container hermetically by means of its airtight closure (see 4.3.1 or 4.4.3).

Return the prepared sample container to the incubator or climatic chamber (4.3.3) and leave for 24 h if the carbon dioxide is to be measured by the gasometric method. If the infra-red method is to be used, the prepared sample container may be connected to the gas analyser immediately.

## 6.3 Determination by the gasometric method

Expel all air from the syringe (4.3.2), insert the needle through the rubber septum on the sample container and move the piston of the syringe backwards and forwards several times so as to mix the air in the needle thoroughly with the atmosphere in the container. Draw about 10 ml of the atmosphere in the container into the syringe and withdraw the needle from the septum.

Promptly transfer a suitable quantity of the gas sample from the syringe to the gas analysis apparatus (4.3.4). (If the gas sample cannot be transferred promptly, insert the needle into a rubber bung.) Determine the concentration of carbon dioxide in the gas sample, expressing it as a percentage by volume. Repeat the analysis on the same test portion.

## 6.4 Determination by the infra-red method

Position the valves (4.4.5) so as to isolate the circuit near the container containing the test portion. After 5 min of scanning with purified air at a rate of 1 l/min, set the analyser to zero and to the most sensitive scale (measuring range 0 to 50 µl/l).

Connect the sample container nozzles to the air inlet pipe and to the analyser (see figure 2).

Direct the flow of air through the sample by operating the three-way valves, with the analyser now set on the least sensitive scale (measuring range 0 to 500 µl/l). Circulate the purified air at a rate of 1 l/min through the sample for 15 min. Then switch the analyser to the most sensitive scale (measuring range 0 to 50 µl/l). Take the reading, in microlitres per litre per minute, of the emission of carbon dioxide in the sample directly from the analyser screen or from the recorder.

NOTE — The automatic operation of the valves and sensitivity scales may be performed by an electronic programmer and electric control valves. The measurement may also be carried out cyclically, but an

integration system is required to measure the area of the successive peaks and for accurately determining the production of carbon dioxide in the sample.

With analysers having a non-linear scale, the value obtained should be converted into microlitres per litre using the analyser calibration curve.

## 6.5 Number of determinations

Carry out two determinations on the same test portion.

## 7 Expression of results

### 7.1 Gasometric method

#### 7.1.1 Calculation and formula

The concentration, expressed as a percentage by volume, of carbon dioxide in the intergranular air of 1 kg of grain after 24 h incubation at 25 °C is given by the formula

$$\frac{C_1 + C_2}{2} \times \frac{1\ 000}{m_0}$$

where

$C_1$  and  $C_2$  are the results of the two measurements of the carbon dioxide concentration, as percentages by volume, measured on each test portion;

$m_0$  is the mass, in grams, of the test portion.

Take as the result the arithmetic mean of the values obtained in the two determinations, if the repeatability conditions (see 7.1.2) are met.

#### 7.1.2 Repeatability

The difference between the results of two determinations carried out one after the other by the same analyst should not exceed 0,2 % (V/V).

### 7.2 Infra-red method

#### 7.2.1 Calculation and formula

The concentration, expressed in microlitres per litre, of carbon dioxide produced in 1 min in the intergranular air in 1 kg of grain is given by the formula

$$C \times \frac{1\ 000}{m_0}$$

where

$C$  is the concentration, in microlitres per litre, of carbon dioxide produced in 1 min in the intergranular air of the test portion;

$m_0$  is the mass, in grams, of the test portion.

Take as the result the arithmetic mean of the values obtained in the two determinations, if the repeatability conditions (see 7.2.2) are met.

**7.2.2 Repeatability**

The difference between the results of two determinations, carried out one after the other by the same analyst, should not exceed 2 µl/(l·min).

**8 Interpretation of results**

**8.1 Gasometric method**

For wheat, peas, split peas, haricot beans, butter beans, polished rice, small yellow maize, and similar small huskless hard grains, tested by the gasometric method, the interpretation given in table 1 applies.

NOTE — For other grains, it is necessary to make a correction for the characteristic volume of interstitial air and the observed carbon dioxide concentration should be multiplied by the correction factor. Some correction factors are :

- linseed : 0,89
- large white maize : 1,18
- barley : 1,25
- oats : 1,39

**8.2 Infra-red method**

The interpretation given in table 2 applies.

**9 Test report**

The test report shall show the method used, the number of determinations carried out, and the results obtained. It shall also mention any operating details not specified in this part of ISO 6639, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

**Table 1 — Interpretation of results obtained by the gasometric method**

Production of carbon dioxide, % CO <sub>2</sub> (V/V), for 1 kg after 24 h incubation	Interpretation
less than 0,2	Probably no infestation present. Repeat test on another sample to confirm.
0,2	Possible light infestation. Repeat test on another sample to confirm.
0,3 to 0,5	Light to moderate infestation. Grain unsuitable for storage longer than 2 months without treatment.
0,6 to 0,9	Moderate to heavy infestation. Grain should be fumigated immediately.
1,0 and higher	Heavy infestation. Grain in dangerous condition and highly unsuitable for storage.

**Table 2 — Interpretation of results obtained by the infra-red method**

Rate of carbon dioxide production, µl/(l·min), for 1 kg of grain	Interpretation
less than 1,0	Probably no infestation present. Persistent small peaks could indicate a very light infestation. Repeat test on another sample to confirm.
1,0 to 1,9	Possible light infestation. Repeat test on another sample to confirm.
2,0 to 3,9	Light to moderate infestation. Grain unsuitable for storage longer than 2 months without treatment.
4,0 to 5,9	Moderate to heavy infestation. Grain should be fumigated immediately.
6,0 and higher	Heavy infestation. Grain in dangerous condition and highly unsuitable for storage.

## Section two : Ninhydrin method

### 10 Principle

Crushing a test portion, from which any visible living insects have been removed, against white paper impregnated with ninhydrin.

When an infested dry grain is crushed, the amino acids in the body fluid of insects react with the ninhydrin in the paper to give a purple spot, but the amino acids of the grain are not released and do not react.

NOTE — Grain with a high moisture content may cause a reaction itself after 2 or 3 days.

Counting of the purple spots on the paper. The number of spots is taken to indicate the level of hidden infestation in the sample.

### 11 Apparatus

**11.1 Sieve** (see 4.1).

**11.2 Kibbling device**, if required, to partially break large grains.

**11.3 Grain sample divider** (see ISO 950).

**11.4 Infestation detector**, manually or electrically operated, which consists essentially of two rough surfaced steel rolls 0,75 mm apart, between which passes a continuous strip of ninhydrin-treated paper (see figure 3).

NOTE — The Ashman Simon apparatus is suitable.

#### 11.5 Ninhydrin-treated paper

Use a roll of white paper 57 mm wide and 50 m long, already impregnated with ninhydrin, or prepare as follows.

Pass the untreated paper through a 10 g/l solution of ninhydrin in industrial denaturated alcohol. Roll the paper up and leave it to dry at 20 to 25 °C and 40 to 60 % relative humidity, in a dark place for at least 3 days. Wrap the dry treated roll in metal foil and store away from light, if possible at 20 to 25 °C and 40 to 60 % relative humidity. Under these conditions, the ninhydrin-treated paper will remain stable for 2 to 3 years.

**11.6 Balance**, accurate to 0,1 g.

### 12 Sampling

Use samples obtained as described in ISO 6639/2.

### 13 Procedure

#### 13.1 Preparation of test sample and test portion

Use the sieve (11.1) to remove all foreign matter and free insects from the sample. If required, the free insects may be identified and counted according to species and stage.

Weigh the sifted sample and divide it, using the grain sample divider (11.3), to obtain the test portions required (see 13.3 and clause 15). Each test portion should contain at least 1 000 grains (see ISO 520). Test portions of large grains should be kibbled and resifted before testing.

Weigh a test portion and/or count the number of grains in it. Prepare the infestation detector (11.4) and pass the test portion through it in accordance with the manufacturer's instructions.

#### 13.2 Determination

Remove the paper strip corresponding to the test from the detector, taking care to handle only the ends of the strip as amino acids on the skin of the fingers also react with ninhydrin to give purple stains (this may be obviated by wearing of surgical gloves or using tweezers), and allow time for purple spots to develop. At 20 °C and at higher ambient temperatures, purple spots develop within 1 h, although they can take up to 24 h to reach maximum intensity. At lower temperatures, or if more rapid development is required, the paper can be heated in an oven maintained at 50 °C, or it can be passed cautiously (to prevent burning) over a spirit-lamp flame or electric light bulb.

When purple spots have developed, mark the boundary of each with a pencil line, taking care to distinguish spots which may be so close as almost to merge.

Ignore any spots on the paper which are not purple in colour.

Count the number of marked spots.

#### 13.3 Number of determinations

Carry out two determinations on the same test sample. (See also clause 15.)

### 14 Expression of results

Express the infestation as the number of hidden insects per kilogram or per 100 grains, and take as the result the arithmetic mean of the two determinations.

### 15 Interpretation of results

If no insects are detected in the first pair of test portions, the test should be repeated with up to a total of 10 test portions before it can be reasonably concluded that the grain is free

from infestation. Even then, it should be remembered that eggs and small larvae can escape detection by the method. Therefore, if it is desired and practical, apparently infestation-free grain should be tested again after 2 to 4 weeks.

The efficiency of the method also varies according to the species of insect and size and type of grain under test. It is doubtful whether a correction coefficient valid for different grain types and different insect species can, or should, be recommended or whether it is necessary in commercial practice.

In general, a positive result should be taken to indicate that the grain is unsafe for storage. One purple spot represents one insect in the test portion, and relatively few purple spots occurring irregularly on the paper, if followed by similar results from several test portions, indicate a light to moderate infestation. Many purple spots indicate a heavy infestation requiring

immediate treatment. However, before taking any action, it should be determined whether the grain has already been effectively treated, and how recently. This is because dead insects continue to give positive results by the method until their body fluids have dried up. A large dead insect can take several weeks to dry out.

## 16 Test report

The test report shall show the method used, the number of determinations carried out, and the results obtained. It shall also mention any operating details not specified in this part of ISO 6639, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

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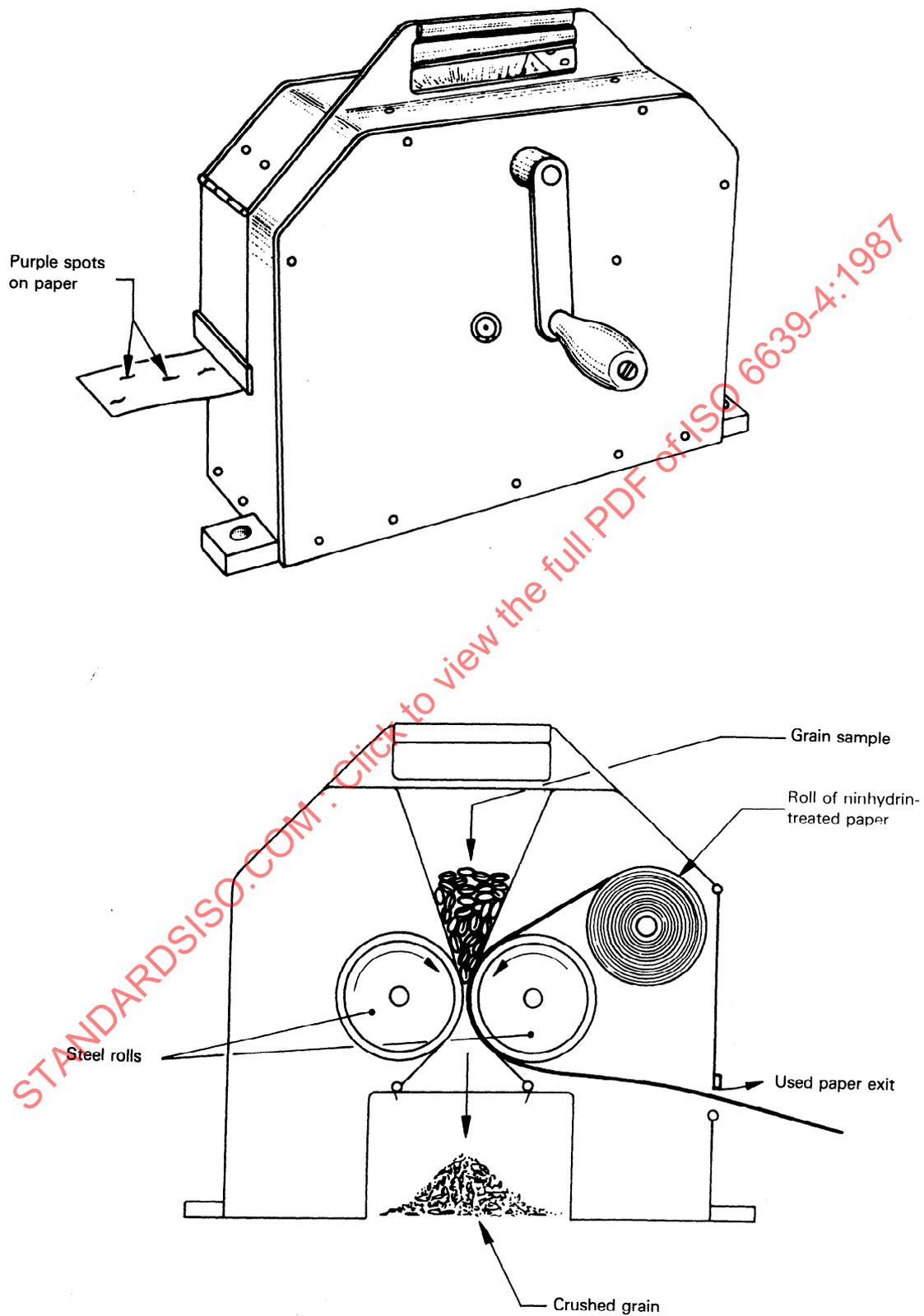


Figure 3 – Apparatus for ninhydrin detection of hidden insect infestation

## Section three : Whole grain flotation method

### 17 Principle

Hidden insect infestation reduces the mass of grain. When a mixture of sound and infested grains is immersed in a test solution in which sound grains just sink, infested grains float to the surface. The separation is usually imperfect because grains containing early instar larvae tend to sink, and un-infested grains with air pockets under the testa or some other defect may float. Floating grains are dissected, to confirm the presence or absence of insects.

### 18 Apparatus

**18.1 Hydrometer floats**, to measure relative densities in the range 1,100 to 1,300.

**18.2 Measuring cylinder**, of capacity 500 ml.

**18.3 Sieve** (see 4.1).

**18.4 Balance**, accurate to 0,01 g.

**18.5 Grain sample divider** (see ISO 950).

**18.6 Beaker**, of capacity 1 000 ml.

**18.7 Skimmer**, for removing floating grains.

### 19 Sampling

Use samples obtained as described in ISO 6639/2.

### 20 Test solution

A suitable test solution can be prepared by dissolving sodium silicate, ammonium nitrate or glycerol in water. The quantity of solute required to make 1 000 ml of test solution of approximately the correct relative density can be calculated by reference to figure 4. Check the relative density of the solution by using a suitable hydrometer float (18.1) and the measuring cylinder (18.2). If necessary, add small amounts of solute or water until the relative density is within  $\pm 0,005$  of that required.

NOTE — As grain densities vary according to type, variety and other factors, the required relative densities of test solutions also vary. Where practical, the required relative density should be determined by experiment. The following values for relative densities of test solutions are intended only as a guide :

- wheat : 1,15
- maize and sorghum : 1,19
- milled rice : 1,27
- peas : 1,27

### 21 Procedure

#### 21.1 Preparation of test sample and test portion

Use the sieve (18.3) to remove all foreign matter from the sample. Weigh the sifted sample and divide it, using the grain sample divider (18.5), into test portions, each containing about 500 grains. Count the grains in a test portion.

#### 21.2 Determination

Place the test portion in the beaker (18.6) containing the test solution. Mix thoroughly and allow to stand for 10 min, stirring briefly at 1 min intervals to release air bubbles trapped by the grains. When the grains have settled for the last time, use the skimmer (18.7) to remove all floating grains. Sort out and count all grains bearing visible evidence of insect infestation ("windows" in the testa or tunnels visible through it). Cut open the remaining grains with a suitable instrument, and count those found to contain insect larvae, pupae or adults.

#### 21.3 Number of determinations

Carry out two determinations on the same test sample.

### 22 Expression of results

#### 22.1 Calculation

Express the infestation as a percentage of grains which are infested and take as the result the arithmetic mean of the two determinations.

#### 22.2 Repeatability

The difference between the result of either determination and the mean shall not exceed the limit indicated in figure 5. If this limit of repeatability is exceeded, repeat the determination on other test portions until the requirement is satisfied.

### 23 Interpretation of results

In view of the limitations referred to in clause 17, the method is most likely to produce an underestimate of the level of infestation present. Therefore, results are of qualitative, rather than quantitative, value. One of the more accurate methods should be used if quantitative results are important.

### 24 Test report

The test report shall show the method used, the number of determinations carried out, and the results obtained. It shall also mention any operating details not specified in this part of ISO 6639, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

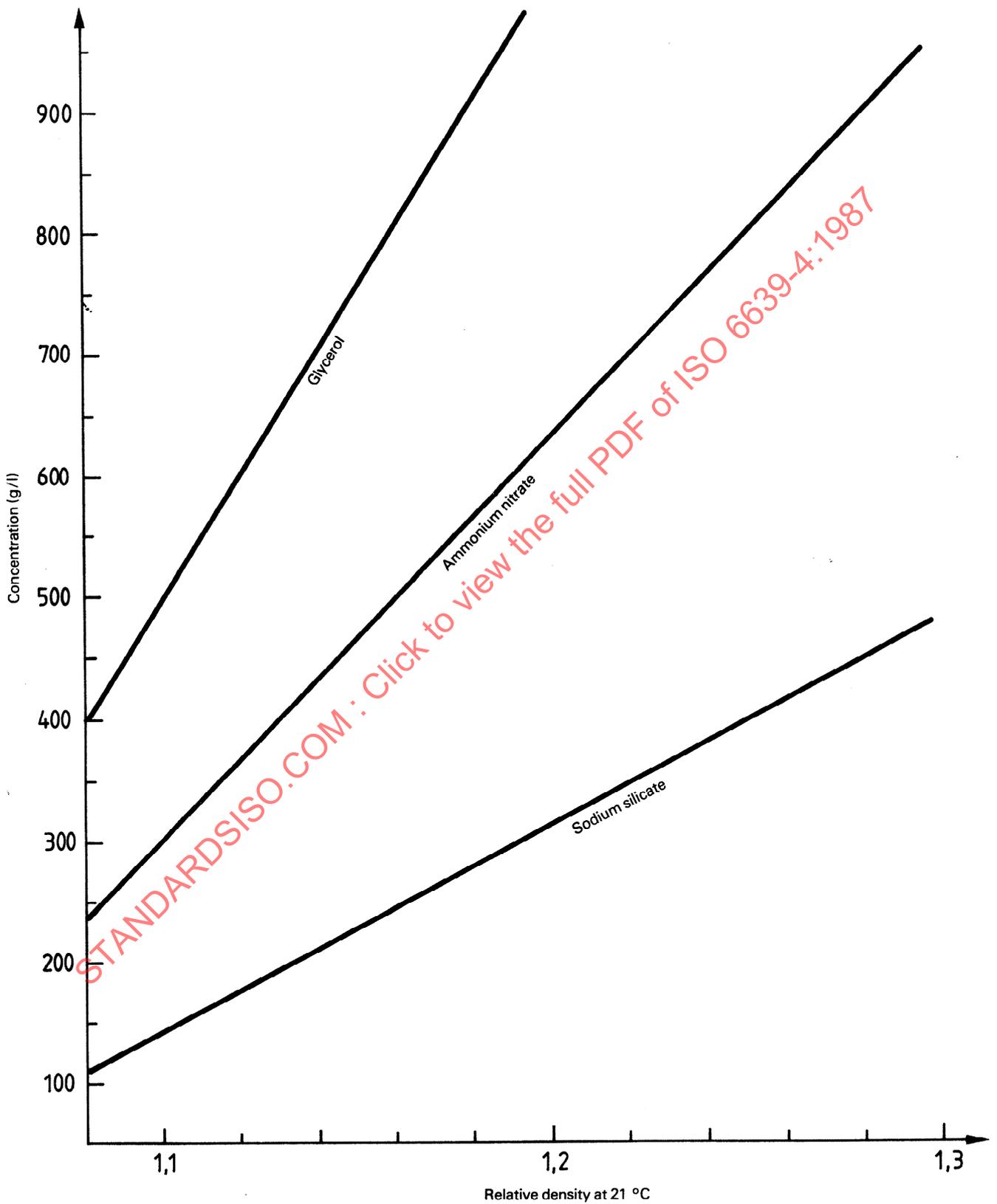
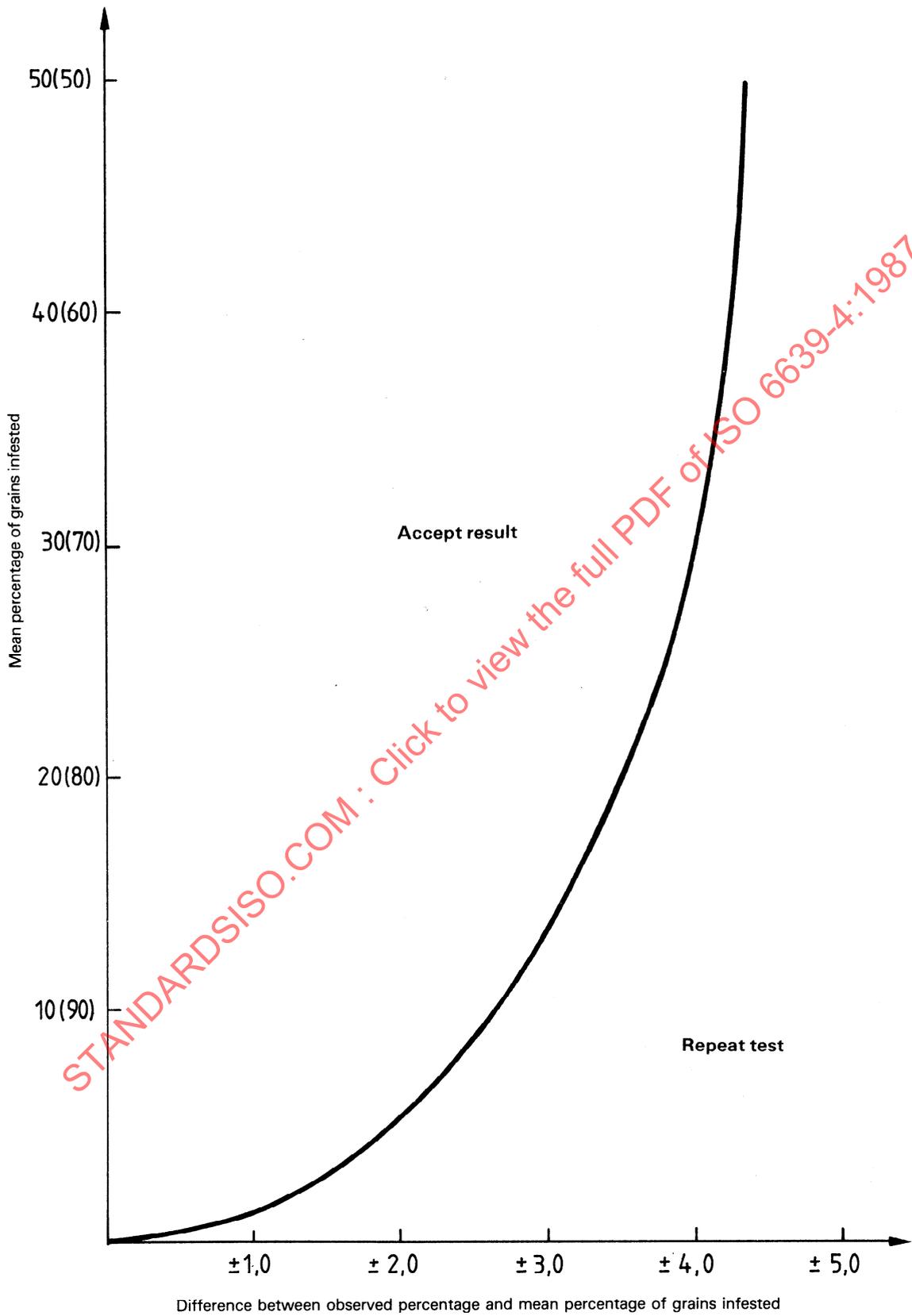


Figure 4 — Guide to the preparation of test solutions of given relative densities



**Figure 5 – Limits of repeatability for the grain flotation method**

(the curve represents the 95 % confidence limits of repeatability for representative samples of about 500 grains)

## Section four : Acoustic method

### 25 Principle

Placing a test portion in a sample container inside a well sound-proofed box. An acoustic vibration sensor, fitted inside the sample container and connected to an amplification system, transmits noise from the feeding activity of hidden insects for direct listening or for recording. Estimation of the approximate degree of hidden infestation from the noise level transmitted.

### 26 Apparatus

**26.1 Acoustic detection equipment**, comprising the following elements (see figure 6).

**26.1.1 Box**, sound-proofed by an internal lining of high-performance sound-insulating material (for example rock wool or high-density polyester foam with lead), the opening of which has a seal impervious to external noise, and within which is placed a removable sample container in the form of a thick plastic cylinder (for example high-density PVC) fitted with a hermetic closure. A vibration sensor is placed in the centre of the sample container and connected by a flexible or expanding cable to a socket outlet placed on the outer face of the box.

The box is mounted on an elastic suspension system (for example rubber pads).

#### NOTES

- 1 The arrangement of the various components of the sound-proofed box may vary while achieving the same insulation result for airborne and mechanical vibrations.
- 2 The volume of the sample container should not exceed 1 litre.

**26.1.2 Electronic amplification system**, composed of a pre-amplifier giving an amplification of 50 to 100 dB, according to the equipment, compatible with the characteristics of the

vibration sensor, with a pass band of 600 to 4 000 Hz and a signal to background noise ratio of at least  $-120$  dB/V.

NOTE — In order to improve this ratio, a filter may be added to reduce the width of the pass band (the central tuning frequency for the rice weevil is approximately 2 kHz). A filter is recommended if use is made of a counting device.

#### 26.1.3 Headphones and counting and recording system :

- a) headphones and possibly loudspeaker connected to the amplifier for direct listening to the noise produced by the hidden insects;
- b) voltage or current threshold detector and recording system for electrical impulses exceeding the adjustable threshold;
- c) low-impedance outlet connection for oscillographic control or magnetic tape recording.

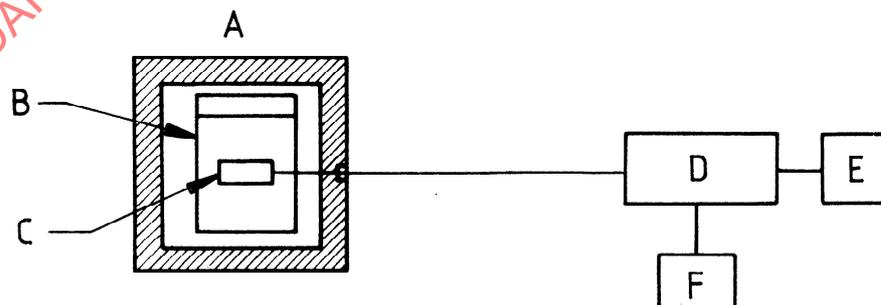
**26.1.4 Mat of high-density insulating material**, placed between the box and the horizontal support to limit the transmission of mechanical vibrations. (This precaution is optional with well-insulated boxes.)

**26.2 Sieve** (see 4.1).

**26.3 Grain sample divider** (see ISO 950).

### 27 Sampling

Use samples obtained as described in ISO 6639/2.



- A Sound-proofed and vibration-insulated box
- B Sample container for grain
- C Vibration sensor
- D Control box and signal amplifier
- E Direct listening system (headphones or loudspeaker)
- F Recording system

Figure 6 — Acoustic detection equipment

## 28 Procedure

### NOTES

- 1 It is essential to carry out the test on samples having a temperature greater than or equal to 20 °C.
- 2 Some equipment incorporates a system for heating the sample in order to increase insect activity ; this preliminary heating requires about 20 min before each determination.

### 28.1 Preparation of the test sample and test portion

Use the sieve (26.2) to remove all free insects. If required, the insects may be identified and counted according to species and stage. Divide the sample, using the grain sample divider (26.3), into the required number of test portions (see 28.3). Each test portion shall be equal to or slightly in excess of the quantity required to fill the cylinder of the box (26.1.1), and shall not be less than 500 g of grain.

### 28.2 Preparation of the apparatus

Place the box on the insulating mat (26.1.4) if necessary. Close the empty sample container, place it in the box and close the box. Connect the amplifier and listening or recording system and adjust the gain until a low continuous background noise is obtained. Switch off the now pre-set apparatus.

NOTE — With a recording system, the threshold setting should be checked periodically (for example, against a test recording on magnetic tape).

### 28.3 Determination

Fill the sample container with the test portion. Tamp the grain down gently by vibration, seal the container, place it in the box and seal the box. Wait for 5 min for the grain to stabilize, then switch on the detection system. Listen for the characteristic noises of insect activity in five listening periods of 1 min or by recording for a period of 5 min.

NOTE — When recording, the direct listening device can be used for checking any defects in recording and adjusting the setting of the detection threshold.

Once the apparatus is switched off, remove the test portion from the cylinder and weigh it to the nearest 1 g.

### 28.4 Number of determinations

Carry out two determinations on the same test sample.

## 29 Expression of results

### 29.1 Direct listening

The result of each 1 min listening period shall be noted separately, with an indication of the existence or absence of activity of hidden insects. The relative intensity of the insect activity may be assessed in order to classify the extent of infestation.

### 29.2 Counting by recording

The number of impulses recorded over 5 min shall be converted to the mean number per minute.

## 30 Interpretation of results

With less than one noise recorded per minute, the sample shall be deemed to be free from infestation.

With one noise per minute, the sample is probably infested.

With two noises per minute, the sample is lightly infested and shall be carefully observed.

After a threshold of five noises per minute, the sample is highly infested and shall be disinfested.

In the event of continuous noise, the infestation is very extensive or there are some adult insects left in the sample (re-sieve before making a new measurement).

## 31 Test report

The test report shall show the method used, the number of determinations carried out, and the results obtained. It shall also mention any operating conditions not specified in this part of ISO 6639, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.