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COUNCIL DIRECTIVE

of 21 December 1976

on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine

(77/96/EEC)

(OJ L 26, 31.1.1977, p. 67)

Amended by:

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►**M1**	Council Directive 81/476/EEC of 24 June 1981	L 186	20	8.7.1981
►**M2**	Council Directive 83/91/EEC of 7 February 1983	L 59	34	5.3.1983
►**M3**	Commission Directive 84/319/EEC of 7 June 1984	L 167	34	27.6.1984
►**M4**	Council Regulation (EEC) No 3768/85 of 20 December 1985	L 362	8	31.12.1985
►**M5**	Commission Directive 89/321/EEC of 27 April 1989	L 133	33	17.5.1989

Amended by:

►**A1**	Act of Accession of Greece	L 291	17	19.11.1979

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COUNCIL DIRECTIVE

of 21 December 1976

on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine

(77/96/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 72/462/EEC of 12 December 1972 on health and veterinary inspection problems upon importation of bovine animals and swine and fresh meat from third countries [1], as last amended by Directive 75/379/EEC [2], and in particular Article 21 thereof,

Having regard to the proposal from the Commission,

Whereas in Directive 72/462/EEC the Council provided in Article 21 for the laying down of the method and procedures required for detecting the presence of trichinae in fresh pigmeat;

Whereas the application of Directive 72/462/EEC will not have the desired effects as long as disparities exist between the Member States as to the guarantees required in respect of the detection of trichinae upon importation of fresh meat from third countries; whereas it is therefore necessary to lay down Community arrangements in this field;

Whereas, in order to protect consumer health, it is necessary that fresh pigmeat be systematically subjected to an examination using methods recognized as effective, in order to eliminate meat containing trichinae;

Whereas when the examination is carried out in the exporting third country, it must be carried out in slaughterhouses which comply with certain conditions and which contain, in particular, a screening laboratory provided with suitable equipment;

Whereas in order to be able to distinguish the meat samples examined from those not examined it is necessary to provide for the affixing of a special mark to meat which has been examined with a negative result;

Whereas there should be a procedure establishing close and effective cooperation between the Commission and the Member States for assessing the advisability of permitting establishments in the third countries to carry out this examination or to work on the meat examined; whereas there should also be a procedure for bringing the technical provisions relating in particular to the examination methods, the requirements concerning the screening laboratories and the procedure for marking examined meat into line with technical progress and with experience acquired;

Whereas the Member States should be allowed to admit fresh meat which has not been screened for trichinae in the exporting third country, provided that this meat, undergoes treatment by freezing; which ensures that any trichinae which may be present are rendered harmless, either in the exporting third country, or in the Member State for which the meat is intended; whereas this treatment must nevertheless be carried out according to certain well defined procedures and establishments fulfilling certain conditions,

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[1] OJ No L 302, 31. 12. 1972, p. 28.
[2] OJ No L 172, 3. 7. 1975, p. 17.

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HAS ADOPTED THIS DIRECTIVE:

Article 1

The definitions used in this Directive are those contained in Directive 72/462/EEC.

Moreover:

(a) 'fresh meat' means fresh meat of domestic swine;

(b) 'examination' refers to the examination to detect the presence of trichinae in fresh meat.

Article 2

1. In order to be admitted to intra-Community trade, fresh meat originating in third countries which contains skeletal muscles (striated muscles) shall be examined under the supervision and responsibility of an official veterinarian.

2. The examination shall be carried out in accordance with one of the methods provided for in Annex I, on the whole carcase or, failing this, on each half carcase, quarter carcase or piece to be imported into the Community.

3. The examination shall take place in a slaughterhouse approved in the exporting country in accordance with Article 4 of Directive 72/462/EEC and authorized to carry out this examination in accordance with Article 4 of this Directive.

4. The examination shall take place before the health marking provided for in Chapter X of Annex B to Directive 72/462/EEC.

5. If it is not possible to carry out the examination in the exporting country, the Member State for which the fresh meat is intended may authorize its importation provided that the examination is carried out within its territory at the time of the public health inspection provided for in Article 24 (2) of Directive 72/462/EEC, at an inspection post within the meaning of Article 27 (1) (b) of that Directive.

6. (a) If the outcome of the examination is negative, the fresh meat shall be marked immediately after the examination, in accordance with Annex III.

(b) In the case of ink stamping, use shall be made of a colorant within the meaning of Article 17 (3) of Directive 72/462/EEC.

Article 3

1. By way of derogation from Article 2, the Member State for which it is intended may authorize the exemption from examination of fresh meat from certain third countries or parts of such countries, provided it is frozen in accordance with the provisions of Annex IV.

2. This treatment shall be carried out in an establishment situated in the exporting third country, and described in Article 4 (1).

Freezing in the exporting third country must be the subject of certification by the official veterinarian on the health certificate accompanying the meat, as referred to in Article 22 (3) of Directive 72/462/EEC.

3. If the treatment has not been carried out in the exporting third country, it must be carried out at an inspection post as described in Article 2 (5).

Freezing in a Member State must be the subject of certification by the official veterinarian on the certificates accompanying the meat, as referred to in Article 25 of Directive 72/462/EEC.

Article 4

1. The authorization for a slaughterhouse to carry out the examination and of a cutting plant to cut up or bone meat which has undergone such examination, or the authorization for an establishment to carry out

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the freezing treatment referred to in Article 3, shall be decided on in accordance with the procedure laid down in Article 9. In addition to the requirements of Article 4 of Directive 72/462/EEC, account shall be taken of the guarantees given in respect of compliance with this Directive and, in the case of slaughterhouses, of:

(a) the presence of the rooms and apparatus necessary for carrying out the examination;

(b) the qualifications of the personnel responsible for carrying out the examination.

Authorization shall be granted to a slaughterhouse and cutting plant only where the competent authorities of the third country concerned have officially recognized that the slaughterhouse and cutting plant are in a position to satisfy the conditions laid down in Article 5 and in Annex III; also, in the case of a slaughterhouse, that it has a laboratory which complies with the conditions laid down in Annex II, Chapter I, and which is in a position to satisfy the requirements of the other chapters of Annex II and those of Annex I.

Authorization shall be granted to an establishment to carry out the freezing treatment only if the competent authorities of the third country concerned have officially recognized that the establishment is in a position to satisfy the conditions laid down in Annex IV.

2. On the list(s) referred to in Article 4 (4) of Directive 72/462/EEC, a special indication shall be inserted against the names of the establishments which have been granted an authorization within the meaning of paragraph 1.

Article 5

1. In slaughterhouses which have been granted an authorization in accordance with Article 4, swine the meat of which is intended for the Community must be slaughtered in different rooms or in the absence thereof at different times from swine the meat of which is not intended for the Community, unless the meat of such swine is examined in accordance with the same procedure.

2. The cutting and boning of meat which has undergone an examination with negative results and is intended for the Community must be carried out in cutting plants, in accordance with Article 4.

In these cutting plants, the cutting and boning of such meat must be carried out in different rooms or in the absence thereof, at different times from meat which is not intended for the Community, unless the meat is examined in accordance with the same procedure.

Article 6

The inspections in third countries provided for in Article 5 of Directive 72/462/EEC must also verify whether the present Directive is being applied.

Article 7

The Member States shall draw up and communicate to the Commission the list of the inspection posts referred to in Article 2 (5) at which:

— the examination,

— the freezing referred to in Article 3,

may be carried out.

They shall ensure that these posts have the equipment necessary for carrying out the operations in question.

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Article 8

Using the procedure laid down in Article 9, decisions may be taken to adapt or add to the Annexes hereto in the light of technological devel-

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opments; such decisions must ensure that health guarantees are maintained at their present standard.

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Article 9

1. Where the procedure laid down in this Article is followed, the matter shall without delay be referred by the chairman, either on his own initiative or at the request of a Member State, to the Standing Veterinary Committee (hereinafter referred to as 'the Committee') set up by the Council Decision of 15 October 1968.

2. Within the Committee the votes of the Member States shall be weighted as laid down in Article 148 (2) of the Treaty. The chairman shall not vote.

3. The representative of the Commission shall submit a draft of the measure to be adopted. The Committee shall deliver its opinion on these measures within a time limit set by the chairman having regard to the urgency of the questions under examination. Opinions shall be delivered by a majority of ►M4 fifty-four ◄ votes.

4. The Commission shall adopt the measures and shall implement them immediately, where they are in accordance with the opinion of the Committee. Where the measures envisaged are not in accordance with the opinion of the Committee, or if no opinion is delivered, the Commission shall without delay submit to the Council a proposal on the measures to be taken.

The Council shall adopt the measures by a qualified majority.

If the Council has not adopted any measures within three months of the date on which the matter is referred to it, the Commission shall adopt the proposed measures and shall implement them immediately save where the Council has decided against such measures by a simple majority.

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Article 11

The Member States shall bring into force on 1 January 1979 at the latest the laws, regulations and administrative provisions needed for compliance with this Directive. They shall forthwith inform the Commission thereof.

Article 12

This Directive is addressed to the Member States.

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ANNEX I

METHODS OF EXAMINATION FOR TRICHINAE

I. TRICHINOSCOPIC EXAMINATION

(a) **Apparatus**

An incandescent lamp trichinoscope with 50 × and 80 to 100 × magnification.

A pressure glass consisting of two glass plates — one of which is divided into equal fields — small curved scissors, small forceps, a knife for cutting specimens, small numbered containers for storing the specimens separately, a dropping pipette, a glass of acetic acid and a glass of potassium hydroxide solution for brightening any calcifications or softening dried meat.

(b) **Taking of specimens**

In the case of whole carcases, at least one specimen of the size of a hazelnut is to be taken from both diaphragm pillars at the transition of the sinewy part.

If there is only one diaphragm pillar, one specimen the size of two hazelnuts is to be taken. In the absence of both diaphragm pillars, two specimens approximately the size of a hazelnut are to be taken from the rib part or the breastbone part of the diaphragm or, as the case may be, from the lingual muscle or the jaw muscle or from the abdominal muscles.

For pieces of meat, from each piece take three samples of skeletal muscle, containing little fat, if possible the size of a hazelnut, and taken from different points, where possible near to bones or tendons.

(c) **Method**

If both diaphragm pillars are present the trichinae inspector must cut, from each of the above specimens taken from a whole carcase, seven pieces the size of an oat-kernel making 14 in all; if only one diaphragm pillar is present, 14 pieces, from different places and if possible from the transition to the sinewy part; he must then compress them between the glass plates in such a way that normal print can be clearly read through the slide preparation. If the flesh of the specimens to be examined is dry and old, the preparations must be softened in a mixture of one part potassium hydroxide solution to about two parts water for 10 to 20 minutes before pressing.

If, in the case of whole carcases, specimens have to be taken from the rib part or the breastbone part of the diaphragm, the lingual muscle or jaw muscle or the abdominal muscles, then 14 pieces the size of an oat-kernal are to be cut from each specimen, i.e. a total of 28.

From each of the samples taken from pieces of meat, the trichinae inspector must cut four pieces the size of an oat-kernal, making 12 pieces in all.

The trichinoscopic examination must be carried out in such a manner that each preparation is scanned slowly and carefully. If the trichinoscopic examination reveals suspect areas, the nature of which cannot be definitely ascertained even with the most powerful magnification of the trichinoscope, these must be checked by microscope.

The microscopic examination should be carried out in such a manner that each preparation is scanned slowly and carefully at a magnification of 30 to 40 ×.

In the case of an uncertain result, the examination must be continued on a further number of specimens and slide preparations, if necessary with the aid of higher magnifications, until the information required is obtained. The trichinoscopic examination must be carried out for at least three minutes.

The trichinoscopic examination must be carried out for at least six minutes in the case of substitute specimens taken from the rib part or breastbone part of the diaphragm pillars, the lingual muscle or the jaw muscle or the abdominal muscles.

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The minimum time fixed for the examination does not include the time necessary for sample-taking and for making the preparations.

As a general rule, the trichinoscopic examiner should not inspect more than 840 pieces a day, though by way of exception he may inspect up to 1 050.

II. THE ARTIFICIAL DIGESTION METHOD

(a) **Apparatus and material**

— knife for taking samples,

— small numbered containers, with closures, for storing samples, if necessary until repetition of the examinations,

— incubator,

— 2 to 3 litre glass funnel with stand, a connecting hose in rubber, clamps for fastening the connecting hose,

— a plastic sieve (approximately 18 cm in diameter, and approximately 1 mm mesh),

— gauze,

— a small tapering tube with a sealed end,

— a glass block dish,

— a meat mincer,

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— a stereo-microscope (magnification 15 to 40 ×) with a suitable light source,

— digestive liquid made up as follows:

10 g of pepsin (80 u/g FIP: Fédération internationale de pharmacie), 5 ml HCl (at least 37 %) made up to a litre with tap-water.

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(b) **Taking of specimens**

1. In the case of whole carcases a sample of at least 20 g to be taken from a diaphragm pillar at the transition to the sinewy part. In the absence of diaphragm pillars a specimen of at least 20 g to be taken from the rib part or the breastbone part of the diaphragm or from the lingual muscle or the jaw muscle or the abdominal muscles.

2. For pieces of meat, a sample of at least 20 g of skeletal muscle to be taken, containing little fat and where possible near to bones or tendons.

(c) **Method**

For the examination of a collective specimen from 10 pigs, a 10 g specimen shall be prepared from each individual 20 g sample. The remaining 10 g shall be kept for additional single-specimen examination should this be necessary.

10 specimens, each weighing 10 g, shall be combined into a collective specimen; it shall be minced in a meat mincer (with 2 mm perforations) and placed loosely in a sieve lined with a layer of gauze. The sieve shall then be placed in a funnel connected by a length of rubber hose to a small tapered tube with a sealed end; the funnel shall be filled from the edge with digestive liquid until the matter for analysis is completely covered. The proportion of matter for analysis to digestive liquid must be approximately 1:20 to 1:30.

After 18 to 20 hours of incubation at 37 to 39 ºC, the small tapered tube shall be disconnected and removed. After carefully drawing off the supernatant liquid, the sediment present in the tip of the tube is to be carefully rinsed in a dish. Then to be examined for the presence of trichinae with a stereo-microscope at 20 to 40 × magnification.

In the case of a positive or doubtful result from the examination of a collective specimen, the remaining single specimens to be examined individually after addition of a further 20 g from each pig, or in the case of pieces of meat, the addition of 20 g taken from each piece, according to (b) above.

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III. METHOD USING THE ARTIFICIAL DIGESTION OF COLLECTIVE SAMPLES

(a) **Apparatus and reagents**

— knife and tweezers for collecting specimens,
— meat mincer with 2 to 3 mm diameter perforations,
— a 3 litre Erlenmeyer flask with a rubber or cotton-wool plug,
— a conical separation funnel of 2 000 ml capacity,
— an ordinary A-base stand of approximately 28 cm length with an 80 cm stem,
— a ring, diameter approximately 10 to 11 cm which can be fixed to the stand,
— a clamp with a flat vice (23 × 40 mm) which can be attached to the stand by means of a double coupling,
— a sieve (mesh size 177 microns) with an external diameter of 11 cm fitted with brass or stainless steel mesh,
— a funnel with an internal diameter of not less than 12 cm,
— 100 ml glass measuring cylinders,
— a stereo-microscope (magnification 15 to 40 ×) with a suitable light source, or a trichinoscope with horizontal table for the compressor with suitable light source,
— when using the trichinoscope: a larval counting basin is used which may be described as follows:

  the larval counting basin is made from 3 mm thick acrylic plates, as follows:

  (i) the bottom of the basin to be 180 × 40 mm, marked off into squares,
  (ii) the sides to be 230 × 20 mm,
  (iii) the end to be 40 × 20 mm. The bottom and the ends should be inserted between the sides, thus forming a basin with two small handles in both ends. The upper side of the bottom should be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The parts should be fixed by using glue appropriate for the material,

— a number of 9 cm diameter Petri dishes (when using stereo-microscope) marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument,
— a number of 10 litre bins to be used when applying decontamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case of positive results,
— concentrated (37 %) hydrochloric acid,
— pepsin strength: 1: 10 000 NF (US National Formulary)

  corresponding to 1: 12 500 BP (British Pharmacopoea)

  corresponding to 2 000 FIP (Fédération internationale de pharmacie),
— a number of trays which can hold 50 samples of approximately 2 g each,
— a balance accurate to 0,1 g.

(b) **Collection of specimens**

1. In the case of whole carcases, a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part of the breastbone part of the diaphragm, from the lingual muscle or the jaw muscle or the abdominal muscles.

2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. (i) *Complete pools* (100 samples at a time)

Approximately 1 g of sample shall be taken from each of the 100 individual samples from the pigs. The pooled sample is put once through the mincer.

The minced meat shall be placed in the 3 litre Erlenmeyer flask together with 7 g of pepsin, approximately 2 litres of tap-water

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heated to about 40 to 41 ºC and 25 ml of concentrated hydrochloric acid. The mixture shall be shaken to dissolve the pepsin.

The pH of the solution will be about 1,5 to 2.

— For digestion, the Erlenmeyer flask shall be incubated at 40 to 41 ºC for approximately four hours. The flask shall be regularly shaken during the time of incubation, at least twice every hour.

— The digested solution shall be filtered through the sieve into the conical 2 litre separation funnel and left undisturbed on the stand for at least one hour.

— A total volume of approximately 45 ml should be run off into a measuring cylinder and divided between three Petri dishes, the bottoms of which should be marked off into squares, with 15 ml in each dish.

— Each Petri dish shall be minutely examined for larval trichinae under the stereo-microscope.

— Where larval counting basins are employed, the 45 ml shall be distributed between two larval counting basins and examined under the trichinoscope.

The larvae appear as identifiable organisms in the deposit and often, when the water is lukewarm, rolling and unrolling movements of the 'spiral' may be observed.

— Digests should be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

If the digests are unclear or not examined within 30 minutes of their preparation, they should be clarified as follows. The final sample of 45 ml is poured into a measuring cylinder and allowed to stand for 10 minutes. At the end of this time, 30 ml of supernatant fluid is removed by suction and the remaining 15 ml is made up to 45 ml with tap-water. After a further settling period of 10 minutes, 30 ml of supernatant fluid is removed by suction and the remaining 15 ml is poured into a Petri dish or larval counting basin for examination. The measuring cylinder should be washed with 10 ml of tap-water and these washings should be added to the sample in the Petri dish or larval counting basin for examination.

(ii) *Pools of less than 100 samples*

Up to 15 single samples could be added to a total pool of 100 samples and be examined together with these samples. If more than 15 samples and less than 100 samples are examined, the digestion fluid should be reduced proportionately.

2. In the case of a positive or doubtful result following the examination of a collective sample, a further 20 g sample should be taken from each pig in accordance with (b) above. The 20 g samples from five pigs should be pooled and examined by the method described above. In this way samples from 20 groups of five pigs will be examined. When trichinellae are detected in a pooled sample from five pigs, further 20 g samples should be collected from the individual pigs in the group and each should separately be examined using the method described above.

IV. THE MECHANICALLY ASSISTED POOLED SAMPLE DIGESTION METHOD/SEDIMENTATION TECHNIQUE

(a) **Apparatus and reagents**

— knife or scissors for cutting specimens,

— trays marked off with 50 squares each of which can hold samples of approximately 2 g of meat,

— a Stomacher Lab-blender 3 500 Thermo model,

— plastic bags suitable for the Stomacher Lab-blender,

— conical separation funnels of 2 litre capacity, preferably fitted with teflon safety plugs,

— stands, rings and clamps,

— sieves, mesh size 177 microns, external diameter 11 cm with stainless steel mesh,

— funnels with an internal diameter not less than 12 cm, to support the sieves,

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— 100 ml glass measuring cylinders,
— a 25 ml dispenser,
— beakers of 3 litre capacity,
— spoon or glass rod for stirring the digestion fluid in the beaker,
— a plastic syringe and tube for suction,
— a measuring spoon for 6 g,
— a thermometer accurate to ± 0,5 ºC within the range 1 to 100 ºC,
— a vibrator, e.g. an electric shaver with the head removed,
— a relay which will switch on and off at one minute interval,
— a trichinoscope with a horizontal table or a stereo-microscope, with a suitable light source,
— larval counting basin (when using trichinoscope): the larval counting basin is made from 3 mm thick acrylic plates as follows:
  (i) the bottom of the basin to be 180 × 40 mm, marked off into squares,
  (ii) the sides to be 230 × 20 mm,
  (iii) the end to be 40 × 20 mm. The bottom and the ends should be inserted between the sides, thus forming two small handles in both ends. The upper side of the bottom should be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The parts should be fixed by using glue appropriate for the material,
— a number of 9 cm diameter Petri dishes (when using stereo-microscope) marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument,
— 17,5 % hydrochloric acid solution,
— pepsin strength 1: 10 000 NF (US National Formulary)
  corresponding to 1: 12 500 BP (British Pharmacopoea)
  corresponding to 2 000 FIP (Fédération internationale de pharmacie),
— a number of 10 litre bins to be used when applying decontamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case. of positive results,
— a balance accurate to 0,1 g.

(b) **Collection of specimens**

1. In the case of whole carcases, a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part or the breastbone part of the diaphragm, from the jaw muscle or the abdominal muscle.

2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. *Digestion procedure*

(i) *Complete pools* (100 samples at a time)

— The Stomacher Lab-blender 3 500 should be fitted with a double plastic bag and the temperature control set at 40 to 41 ºC
— One and a half litres of water preheated to about 32 to 35 ºC is poured into the inner plastic bag and the water heated to 40 to 41 ºC.
— 25 ml of 17,5 % hydrochloric acid is then added to the water in the Stomacher.
— 100 samples of approximately 1 g each (at 25 to 30 ºC) taken from each of the individual samples, in accordance with (b), are then added.
— 6 g pepsin is finally added. This order of addition should be strictly adhered to in order to avoid decomposition of the pepsin.
— The Stomacher is then allowed to pound the content of the bag for 25 minutes.
— The pastic bag is then removed from the Stomacher and the digestion fluid is filtered through the sieve into a 3 litre beaker.

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— The plastic bag is washed with approximately 100 ml of water, which is then used to rinse the sieve and finally added to the filtrate in the beaker.

Up to 15 single samples could be added to a total pool of 100 samples and be examined together with these samples.

(ii) *Smaller pools less than 100 samples*

— The Stomacher Lab-blender 3 500 should be fitted with a double plastic bag and the temperature control set at 40 to 41 ºC.

— A digestion fluid is prepared by mixing about one and a half litres of water and 25 ml of 17,5 % hydrochloric acid. 6 g of pepsin is added and the whole mixed at a temperature of 40 to 41 ºC. This order of addition should be strictly adhered to in order to avoid decomposition of the pepsin.

— Of the digestion fluid, a volume corresponding to 15 ml per gram of sample is measured (e.g. for 30 samples the volume required is 30 × 15 ml or 450 ml) and transferred to the inner of the two plastic bags together with the meat samples of approximately 1 g (at 25 to 30 ºC) taken from each of the individual samples in accordance with (b).

— Water at a temperature of approximately 41 ºC is poured into the outer bag to a total volume in the two bags of one and a half litres.

— The Stomacher is then allowed to pound the content of the bag for 25 minutes.

— The plastic bag is then removed from the Stomacher and the digestion fluid is filtered through the sieve into a 3 litre beaker.

— The plastic bag is washed with approximately 100 ml of water, which is then used to rinse the sieve and finally added to the filtrate in the beaker.

2. *Recovery of larvae by sedimentation*

— Ice (300 to 400 g of ice flakes, scaly ice or crushed ice) is added to the digestion fluid, bringing its volume up to about 2 litres. The digestion fluid is then stirred until the ice has melted.

In the case of smaller pools (see 1 (ii)), the amount of ice should be reduced correspondingly.

— The chilled digestion fluid is transferred to a 2 litre separation funnel, equipped with a vibrator in an extra clamp.

— Sedimentation for 30 minutes, during which time the sedimentation funnel is vibrated intermittently, i.e. one minute vibration followed by one minute pause.

— After 30 minutes, a 60 ml sample of the sediment is quickly run off into a 100 ml measuring cylinder. (The funnel is rinsed with detergent solution after use).

— The 60 ml sample is allowed to stand for at least 10 minutes, after which time the supernatant should be withdrawn by suction, leaving a volume of 15 ml to be examined for the presence of larvae.

— For suction, a disposable syringe can be used, equipped with a plastic tube.

The length of the tube should be such that 15 ml will remain in the measuring cylinder when the flanges of the syringe rest on the cylinder's rim.

— The remaining 15 ml is poured into a larval counting basin or two Petri dishes and examined using a trichinoscope or stereomicroscope respectively.

— Digests should be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

If the digests are unclear, or are not examined within 30 minutes of their preparation, they should be clarified as follows. The final sample of 60 ml is poured into a measuring cylinder and allowed to stand for 10 minutes. At the end of this time, 45 ml of supernatant fluid is removed by suction and the remaining 15 ml is made up to 45 ml with tap-water. After a further settling period of 10 minutes, 30 ml of supernatant fluid is removed by suction and the remaining 15 ml is poured into a Petri dish or larval counting basin for examination. The measuring cylinder should be washed with 10 ml of tap-water

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and these washings should be added to the sample in the Petri dish or the larval counting basin for examination.

3. In the case of a positive or doubtful result, following the examination of a collective sample, a further 20 g sample should be taken from each pig in accordance with (b) above. The 20 g samples from five pigs should be pooled and examined by the method described above. In this way, samples from 20 groups of five pigs will be examined. When trichinellae are detected in a pooled sample from five pigs, further 20 g samples should be collected from the individual pigs in the group and each should separately be examined using the method described above.

V. THE MECHANICALLY ASSISTED POOLED SAMPLE DIGESTION METHOD/ 'ON FILTER ISOLATION' TECHNIQUE

(a) **Apparatus and reagents**

Those indicated in method IV (a).

Supplementary equipment to the abovementioned:

— 1 litre Gelman funnel, complete with filter holder (diameter 45 mm),

— filter discs; the filter discs consist of:

a circular stainless steel mesh with an aperture of 35 microns (the diameter of the disc should be 45 mm),

two rubber rings made of 1 mm thick rubber (the external diameter should be 45 mm and the internal diameter 38 mm),

the circular mesh is placed between the two rubber rings and bonded to them using a two-component glue suitable for the two materials,

— an Erlenmeyer flask with a capacity of 3 litres and fitted with a side tube for suction,

— a filter pump,

— plastic bags with a capacity of at least 80 ml,

— equipment for sealing the plastic bags,

— rennilase, strength 1: 150 000 soxhlet units per gram.

(b) **Collection of specimens**

See method IV (b).

(c) **Method**

1. *Digestion procedure*

(i) *Complete pools* (100 samples at a time)

See method IV (c) (1) (i).

(ii) *Smaller pools less than 100 samples*

See method IV (c) (1) (ii).

2. *Recovery of larvae by filtration*

— Ice (300 to 400 g of ice flakes, scaly ice or crushed ice) is added to the digestion fluid, bringing its volume up to about 2 litres.

In the case of smaller pools, the amount of ice should be reduced correspondingly.

— The digestion fluid is then stirred until the ice has melted. The chilled digestion fluid is then left for at least three minutes to let the larvae coil.

— The Gelman funnel, fitted with a filter holder and filter disc, is mounted on the Erlenymeyer flask connected to a filter pump.

— The digestion fluid is poured into the Gelman funnel and filtered. Towards the end of filtration, the passage of the digestion fluid through the filter can be assisted by applying suction with the filter pump. Suction should cease before the filter becomes dry, i.e. when 2 to 5 ml of fluid are left in the funnel.

— When all the digestion fluid has been filtered, the filter disc is removed and placed in an 80 ml capacity plastic bag, together with 15 to 20 ml of rennilase solution. The solution of rennilase is made by adding 2 g of rennilase to 100 ml of tap-water.

— The plastic bag is sealed twice and placed in the Stomacher between the inner and outer bag.

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— The Stomacher is allowed to pound for three minutes, e.g. while it is working on a complete or incomplete pool.

— After three minutes, the plastic bag, complete with filter disc and rennilase solution, is removed from the Stomacher and opened with scissors. The liquid contents are poured into a larval counting basin or Petri dish. The bag is washed out with 5 to 10 ml of water which is then added to the larval counting basin for examination by trichinoscope or to the Petri dish for examination by stereo-microscope.

— Digests should be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

Note

Filter discs should never be used when not completely clean. Unclean discs should never be allowed to dry out.

Filter discs can be cleaned by leaving them in rennilase solution overnight. Before use, they should be washed in fresh rennilase solution using the Stomacher.

3. In the case of a positive or doubtful result, following the examination of a collective sample, a further 20 g sample should be taken from each pig in accordance with (b) above. The 20 g samples from five pigs should be pooled and examined by the method described above. In this way, samples from 20 groups of five pigs will be examined. When trichinellae are detected in a pooled sample from five pigs, further 20 g samples should be collected from the individual pigs in the group and each should separately be examined using the method described above.

VI. THE MAGNETIC STIRRER METHOD FOR POOLED SAMPLE DIGESTION

(a) **Apparatus and reagents**

— knife and tweezers for cutting specimens,

— trays marked off into 50 squares each of which can hold samples of approximately 2 g of meat,

— a Moulinette blender,

— magnetic stirrers, with thermostatically controlled heating plate and teflon coated stirring rods, approximately 5 cm long,

— conical separation funnels of 2 litre capacity,

— stands, rings and clamps,

— sieves, mesh size 177 microns, external diameter 11 cm with stainless steel mesh,

— funnels with an internal diameter not less than 12 cm, to support the sieves,

— beaker of 3 litre capacity,

— measuring cylinders of approximately 50 ml capacity, or centrifuge tubes,

— a trichinoscope with horizontal table or a stereo-microscope, with a suitable light source,

— larval counting basin (when using trichinoscope): the larval counting basin is made from 3 mm thick acrylic plates as follows:

(i) the bottom of the basin to be 180 × 40 mm, marked off into squares,

(ii) the sides to be 230 × 20 mm,

(iii) the end to be 40 × 20 mm. The bottom and the ends should be inserted between the sides, thus forming two small handles in both ends. The upper side of the bottom should be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The parts should be fixed by using glue appropriate for the material,

— a number of 9 cm diameter Petri dishes (when using stereo-microscope) marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument,

— aluminium foil,

— 25 % hydrochloric acid,

— pepsin strength: 1: 10 000 NF (US National Formulary)
corresponding to 1: 12 500 BP (British Pharmacopoea)

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corresponding to 2 000 FIP (Fédération internationale de pharmacie),

— tap-water heated to 46 to 48 ºC,

— a number of 10 litre bins to be used when applying decontamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case of positive results,

— a balance accurate to 0,1 g.

(b) **Collection of specimens**

1. In the case of whole carcases, a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part or the breastbone part of the diaphragm, from the jaw muscle or the abdominal muscles.

2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. (i) *Complete pools* (100 samples at a time)

— 100 samples of approximately 1 g each, taken from each of the individual samples in accordance with (b), are chopped in the Moulinette blender. The blender should be operated three to four times for approximately one second each time.

— The chopped meat is transferred to a 3 litre beaker and sprinkled with 10 g of pepsin. 2 litres of tap-water, preheated to 46 to 48 ºC, is poured into the beaker, together with 16 ml of hydrochloric acid.

— The mincing insert of the Moulinette blender is immersed repeatedly in the digestion fluid in the beaker to remove any meat still adhering.

— The stirring rod is placed in the beaker and the beaker is covered with aluminium foil.

— The beaker is placed on the preheated heating plate of the magnetic stirrer and the stirring process started. Before starting the stirring process, the magnetic stirrer should be adjusted so that it maintains a constant temperature of 44 to 46 ºC throughout the period of operation. During the stirring process, the digestion fluid should rotate at a sufficiently high speed to create a deep whirl without splashing.

— The digestion fluid is stirred for 30 minutes, at the end of which the stirrer is switched off and the digestion fluid is poured through the sieve into the sedimentation funnel.

— The digestion fluid is allowed to stand in the funnel for 30 minutes.

— After 30 minutes, a 40 ml sample of digestion fluid is quickly run off into the measuring cylinder or centrifuge tube.

— The 40 ml sample is allowed to stand for 10 minutes, at the end of which time 30 ml of supernatant is withdrawn by suction, leaving a volume of 10 ml.

— The remaining 10 ml sample of sediment is poured into a larval counting basin or Petri dish.

— Then the cylinder or centrifuge tube is rinsed with about 10 ml of tap-water which has to be added to the sample in the larval counting basin or Petri dish. Subsequently, the sample is examined by trichinoscope or stereomicroscope respectively.

— Digests should be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

If the digests are not examined within 30 minutes of their preparation, they should be clarified as follows. The final sample of about 40 ml is poured into a measuring cylinder and allowed to stand for 10 minutes, after which time 30 ml of the supernatant fluid is removed leaving a volume of 10 ml. This volume is made up to 40 ml with tap-water. After a further settling period of 10 minutes, 30 ml of the supernatant fluid is withdrawn by suction leaving a volume of 10 ml for examination in a Petri dish or larval counting

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basin. The measuring cylinder should be washed with 10 ml of tap-water and these washings should be added to the sample in the Petri dish or the larval counting basin for examination.

If the sediment is found to be unclear on examination, the sample should be poured into a measuring cylinder and made up to 40 ml with tap-water and then the above procedure should be followed.

(ii) *Pools of less than 100 samples*

When needed, up to 15 samples of 1 g each may be added to a total pool of 100 samples and examined together with these samples according to (c) (1) (i). More than 15 samples should be examined as a complete pool. For pools up to 50 samples, the digestion fluid may be reduced to 1 litre.

2. In the case of a positive or doubtful result following the examination of a collective sample, a further 20 g sample should be taken from each pig in accordance with (b) above. The 20 g samples from five pigs should be pooled and examined by the method described above. In this way samples from 20 groups of five pigs will be examined. When trichinellae are detected in a pooled sample from five pigs, further 20 g samples should be collected from the individual pigs in the group and each should separately be examined using the method described above.

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VII. THE AUTOMATIC DIGESTION METHOD FOR POOLED SAMPLES OF UP TO 35 g

(a) **Apparatus and reagents**

— Knife or scissors for cutting specimens,
— trays marked off with 50 squares each of which can hold samples of approximately 2 g of meat,
— a Trichomatic 35 blender with filtration insert,
— hydrochloric acid solution 8,5 % ± 0,5 weight,
— transparent polycarbonate membrane filters with a diameter of 50 mm and a pore size of 14 microns,
— pepsin strength 1: 10 000 NF (US National Formulary)
corresponding to 1: 125 000 BP (British Pharmacopoea)
corresponding to 2 000 FIP (Federation Internationale de Pharmacie),
— a balance, accurate to 0,1 g,
— tweezers with a flat tip,
— a number of microscope slides with a side-length of at least 5 cm or a number of at least 6 cm diameter Petri dishes marked on their underside equipped into 10 × 10 mm large areas using a pointed instrument,
— a (stereo-) microscope with transmitted light (magnification 15 to 60 times) or a trichinoscope with a horizontal table,
— a bin for collection of waste liquids,
— a number of 10 litre bins to be used when applying de-contamination, such as formol treatment, to the apparatus and for the remaining digestive juice in the case of positive results.

(b) **Collection of specimens**

1. In the case of whole carcases a specimen to be taken of approximately 2 g from a pillar of the diaphragm at the transition to the sinewy part. In the absence of diaphragm pillars, a specimen of the same size to be taken from the rib part of the breastbone, part of the diaphragm, from the jaw muscle or the abdomina muscle.

2. For cuts of meat, a sample of approximately 2 g of skeletal muscle to be taken, containing little fat and, where possible, near to bones or tendons.

(c) **Method**

1. *Digestion Procedure*

— Place the blender with filtration-insert, connect the waste tube and lead the tube to the waste bin.

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— When the blender is switched on, the heat-up will start.

— Before start, the bottom valve, located below the reaction chamber, should be opened and closed.

— Up to 35 samples of approximately 1 g each (at 25 to 30º C) taken from each of the individual samples, in accordance with point b, are then added. Make sure that larger pieces of tendons are removed as this may clot the membrane filter.

— Pour water to the edge of a liquid chamber connected to the blender (approximately 400 ml).

— Pour about 30 ml hydrochloric acid (8,5 %) to the edge of the smaller, connected liquid chamber.

— Place a membrane filter under the coarse filter in the filter holder in the filter insert.

— 5 g of pepsin is added last. The order of addition should be strictly adhered to in order to avoid decomposition of the pepsin.

— Close the lids to the reaction- and liquid chambers.

— Select the period of digestion. Short digestion period (5 minutes) for pigs at normal age of slaughtering and extended digestion time (8 minutes) for other samples.

— The automatic dispensing starts when the start button on the blender is activated and digestion with following filtration will proceed automatically. After 10 to 13 minutes the process is completed and stops automatically.

— The lid to the reaction chamber is opened once it is checked that the chamber is emptied. If there is foam or remains of digestion liquid in the chamber repeat the procedure according to point 4.

2. *Recovery of larvae*

— Dismount the filter holder and transfer the membrane filter to a slide or a Petri dish.

— The membrane filter is examined by means of a microscope or a trichinoscope.

3. *Cleaning of equipment*

— In the case of a positive result, fill the reaction chamber in the blender ⅔ with boiling water. Ordinary tap-water is poured into the connecting liquid chamber until the lower level sensor is covered. The automatic cleaning programme is then carried out. De-contaminate the filter-holder together with the remaining equipment, for example by means of formal treatment.

— After the day's work fill the liquid chamber in the blender with water and carry out a standard programme.

4. *Method to be used when digestion is incomplete and filtration cannot therefore be carried out*

When the automatic process in the blender is carried out according to point 1, open the lid to the reaction chamber and check whether there is foam or liquid remaining in the chamber. It this is the case, carry out the following procedure:

— Close the bottom valve below the reaction chamber.

— Dismount the filter holder and transfer the membrane filter to a slide or a Petri dish.

— Put a new membrane filter in the filter holder and mount the filter holder.

— Fill water into the liquid chamber in the blender until the lower level-sensor is covered.

— Carry out the automatic cleaning programme.

— After the cleaning programme has been completed open the lid to the reaction chamber and check for liquid remains.

— If the chamber is empty, dismount the filter holder and transfer the membrane filter with a tweezer to a slide or a Petri dish.

— The two membrane filters are examined according to point 2. If the filters cannot be examined repeat the entire digestion process with extended digestion time according to point 1.

5. In the case of a positive or doubtful result, following the result of a collective sample, a further 20 g sample should be taken from each pig in accordance with point b above. These samples shall be investigated individually according to the abovementioned method.

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ANNEX II

CHAPTER I

CONDITIONS FOR THE AUTHORIZATION OF TRICHINAE (TRICHINELLA SPIRALIS) LABORATORIES

1. Trichinae laboratories must be situated next to the swine slaughterhouses and, if the establishment does not already possess such facilities to fulfil the other requirements of the Directive on third countries, have at least the following facilities available:

   (a) a lockable room adequately equipped for the preparation of specimens; its walls must be smooth and must be lined with a washable light-coloured covering or paint up to a height of 2 m. A preparation room must be provided for each method of examination used;

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   (b) an adequately equipped lockable examination room which can be darkened when examination is performed by trichinoscope;

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   (c) equipment providing adequate ventilation and, if necessary, air conditioning equipment which ensures that the room temperature does not exceed + 25 ºC;

   (d) adequate natural or artificial lighting which does not alter colours; direct sunlight must be avoided;

   (e) in the preparation room, adequate equipment for cleaning and disinfecting hands;

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   ►M3 (f) ◄ possibly, refrigerators in which to store meat samples;

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   (g) a washroom for cleansing and disinfecting examination equipment (e.g. containers for samples, compressors, knives and scissors), with:

   — a waterproof floor-covering which is rot-proof and easy to clean and disinfect,

   — smooth walls which, up to a height of at least 2 m, are lined by a washable and light-coloured covering or paint.

   This provision need not be applied when using the methods indicated under points II, III, IV, V, VI of Annex I provided the laboratories are equipped with a large suitably plumbed sink;

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   ►M3 (h) ◄ changing rooms, wash basins and rest rooms and flush lavatories;

   ►M3 (i) ◄ wash basins with hot and cold running water, provided with cleansing and disinfecting materials and disposable towels;

   ►M3 (j) ◄ watertight corrosion-resistant containers, with hermetically sealed lids, for collecting the samples after examination, and so designed as to prevent unauthorized removal of the contents;

   ►M3 (k) ◄ adequate supplies of hot and cold drinking water;

   ►M3 (l) ◄ equipment for removing waste water which fulfils the conditions for the authorization of slaughterhouses;

   ►M3 (m) ◄ proper equipment for protection against pests (insects, rodents, etc.).

CHAPTER II

REQUIREMENTS APPLICABLE TO STAFF, PREMISES, EQUIPMENT AND INSTRUMENTS IN TRICHINAE LABORATORIES

2. Absolute cleanliness is required at all times of laboratory staff, premises, equipment and instruments:

   (a) staff must wear clean working clothes and wash their hands several times during working hours and after each break;

   (b) no animal may enter trichinae laboratories;

   (c) equipment and instruments used must be kept clean and in good repair. They must be carefully cleansed and disinfected several times during and at the end of the day's work.

3. Drinking water must be used for all purposes.

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4. As regards health, staff taking meat samples for examination must comply with the rules in Annex B, Chapter IV (11) and (12) of Directive 72/462/EEC.

5. The meat samples required for examination must be taken immediately after slaughter and examined without delay in the trichinae laboratory of the slaughterhouse.

   These examinations are forbidden away from the slaughterhouse in which the animals were slaughtered.

6. To avoid fatigue and its consequences, inspection staff should be given short breaks during the working day.

CHAPTER III

REQUIREMENTS IN RESPECT OF TRICHINOSCOPES

The construction and design of trichinoscopes must meet the following minimum criteria:

1. Simple operation.

2. High light intensity:

   — accurate results must be obtainable even in a room which is not completely dark,
   — a projector bulb of 100 W (12 V) must be used as the light source.

3. Adequate magnification:

   — normal working magnification: 50 x,
   — 80 to 100 x magnification for more precise assessment of objects not clearly identifiable under normal working magnification.

4. Resolving power:

   — a clear sharp picture of well-defined colour must be obtainable at each magnification.

5. Switch mechanism:

   — each change of magnification must be accompanied by automatic adjustment of the brightness of the image.

6. Increase of contrast:

   — the condensor must be provided with an iris diaphragm enabling the contrast to be increased for the closer inspection of difficult cases,
   — the iris diaphragm must be easy to operate (e.g. control lever on the platform of the trichinoscope).

7. Easy focusing:

   — rapid focusing by means of an adjusting ring,
   — precise focusing by means of a control lever.

8. Voltage regulation:

   — so that brightness may be adjusted as required.

9. One-way movement of the compressor:

   — an automatic blocking mechanism must ensure that the compressor moves in only one direction, to prevent unintentional displacement.

10. Free view of the projector screen.

11. Projector screen:

   — at least 54 cm in diameter,
   — high reflecting capability,
   — durable,
   — can be dismantled,
   — easy to clean.

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ANNEX III

MARKING OF MEAT WHICH HAS BEEN EXAMINED FOR TRICHINAE

1. Marking of the meat must be carried out under the responsibility of the official veterinarian. For this purpose, he shall keep and maintain:
   - — the instruments intended for marking which he may hand over to the assistant staff only at the time of marking and for the length of time required for this purpose,
   - — the tags mentioned in paragraph 5. These tags shall be given to the assistant staff at the time when they must be used and in the required number.
2. The mark must be round with a diameter of 2·5 cm. The following information must appear on the mark in perfectly legible characters:
   - — towards the centre the capital letter 'T' with arms 1 cm long and 0·2 cm wide,
   - — under the letter 'T' one of the following sets of initials: CEE, EEG, EWG, EØF, EEC ►A1 or EOK ◄. The letters must be 0·4 cm high.
3. Carcases must be marked in ink or hot-branded on the inside of the thighs, in accordance with paragraph 2.
4. Heads must be marked in ink or hot-branded, with a mark meeting the requirements of paragraph 2.

   With the exception of cuts exempt from health marking by virtue of Annex B, Chapter X (43) of Council Directive 72/462/EEC, those taken in cutting plants from carcases marked in accordance with the rules must, where they bear no stamp, be marked in accordance with paragraph 2 before the health mark is affixed.

   The label provided for in the second subparagraph of the abovementioned paragraph 43 must comply with the conditions of paragraph 6 below.
5. Marking may also be effected by means of a round tag. This tag, to be affixed to each piece or to each carcase must not be reusable, must be made of strong materials and must meet all hygiene requirements. The following information must appear on the stamp seals in perfectly legible characters:
   - — towards the centre the capital letter 'T',
   - — under the letter 'T' one of the following sets of initials: CEE, EEG, EWG, EØF, EEC ►A1 or EOK ◄. The letters must be 0·2 cm high.
6. The label provided for in Annex B, Chapter X (44) of the Directive mentioned in paragraph 4 above must, in addition to the health mark, bear a clearly legible mark identical to that provided for in paragraph 2.

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ANNEX IV

THE FREEZING OF MEAT

1. Meat brought in already frozen must be kept in this condition.
2. The technical equipment and energy supply of the refrigerating room, must be such as to ensure that the temperature referred to under paragraph 6 is reached very rapidly and maintained in all parts of the room and of the meat.
3. Insulated packaging should be removed before freezing, except for meat which has already reached throughout the temperature referred to under paragraph 6 when it is brought into the refrigeration room.
4. Consignments in the refrigeration room must be kept separately and under lock.
5. The date and time when each consignment is brought into the refrigeration room must be recorded.
6. The temperature in the refrigeration room must be at least − 25 ºC. It should be measured with calibrated thermo-electric instruments and continuously recorded. It may not be measured directly in the cold air flow. The instruments must be kept under lock. The charts must include the relevant numbers from the meat inspection register on importation and the date and time of the commencement and completion of freezing, and must be retained for one year after compilation.
7. Meat with a diameter or thickness of up to 25 cm must be frozen for at least 240 consecutive hours, and meat with a diameter or thickness of between 25 and 50 cm must be frozen for at least 480 consecutive hours. This freezing process may not be applied to meat which has a larger diameter or is thicker. The freezing time shall be calculated from the point when the temperature referred to in paragraph 6 is reached in the freezing room.