II

*(Acts whose publication is not obligatory)*

# COMMISSION

## COMMISSION DIRECTIVE

**of 7 June 1984**

**amending the Annexes to Council Directive 77/96/EEC on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine**

(84/319/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 77/96/EEC of 21 December 1976 on the examination for trichinae (trichinella spiralis) upon importation from third countries of fresh meat derived from domestic swine (1), as last amended by Directive 83/91/EEC (2), and in particular Article 8 thereof,

Whereas recent studies have enabled certain methods to be devised for detecting trichinae in pigmeat; whereas the reliability of these methods from the point of view of health protection is equivalent to that of existing methods; whereas appropriate additions should, therefore, be made to Annex I to Directive 77/96/EEC;

Whereas, in order to facilitate the work of examination for trichinae, non-member countries and the Member States should be permitted to choose between the examination methods provided for;

Whereas certain technical adaptations must be made to the methods of examination for trichinae currently applied and as regards the conditions with which laboratories engaged in the detection of trichinae must comply;

Whereas the measures laid down by this Directive are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DIRECTIVE:

*Article 1*

Directive 77/96/EEC is hereby amended as set out in the Annex.

*Article 2*

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive not later than 1 January 1985. They shall forthwith inform the Commission thereof.

*Article 3*

This Directive is addressed to the Member States.

Done at Brussels, 7 June 1984.

*For the Commission*

Poul DALSAGER

*Member of the Commission*

(1) OJ No L 26, 31. 1. 1977, p. 67.
(2) OJ No L 59, 5. 3. 1983, p. 34.

*ANNEX*

A. Annex I is hereby amended as follows:

1. In point II (a):

— the 10th indent is replaced by the following:

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

— the last indent is replaced by the following:

'— 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.'

2. Point III is replaced by the following:

'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 heated to about 40 to 41 °C 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 °C 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.'

3. The following points IV, V and VI are added:

'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,

— 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 °C within the range 1 to 100 °C,

— 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 °C

— One and a half litres of water preheated to about 32 to 35 °C is poured into the inner plastic bag and the water heated to 40 to 41 °C.

— 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 °C) 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.

— 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 °C.

— 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 °C. 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 °C) taken from each of the individual samples in accordance with (b).

— Water at a temperature of approximately 41 °C 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 stereo-microscope 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 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.

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

— tap-water heated to 46 to 48 °C,

— 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 °C, 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 °C 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 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.'

B. Annex II, Chapter I, paragraph 1 is hereby amended as follows:

1. The text of point (b) is replaced by the following:

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

2. Point (f) is deleted; the points (g), (h), (i), (j), (k), (l), (m) and (n) become respectively (f), (g), (h), (i), (j), (k), (l) and (m).

3. The text of the new point (g) is replaced by the following :

'(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.'