ANNEX I

Detection methods

CHAPTER I

REFERENCE METHOD OF DETECTION
Magnetic stirrer method for pooled sample digestion

1. *Apparatus and reagents*

(a) Knife or scissors and tweezers for cutting specimens.

(b) Trays marked off into 50 squares, each of which can hold samples of approximately 2 g of meat, or other tools giving equivalent guarantees as regards the traceability of the samples.

(c) A blender with a sharp chopping blade. Where the samples are larger than 3 g, a meat mincer with openings of 2 to 4 mm or scissors must be used. In the case of frozen meat or tongue (after removal of the superficial layer, which cannot be digested), a meat mincer is necessary and the sample size will need to be increased considerably.

(d) Magnetic stirrers with thermostatically controlled heating plate and Teflon-coated stirring rods approximately 5 cm long.

(e) Conical glass separation funnels, capacity of at least 2 litres, preferably fitted with Teflon safety plugs.

(f) Stands, rings and clamps.

(g) Sieves, mesh size 180 microns, external diameter 11 cm, with stainless steel mesh.

(h) Funnels, internal diameter not less than 12 cm, to support the sieves.

(i) Glass beakers, capacity 3 litres.

(j) Glass measuring cylinders, capacity 50 to 100 ml, or centrifuge tubes.

(k) A trichinoscope with a horizontal table or a stereo-microscope, with a substage transmitted light source of adjustable intensity.

(l) A number of 9 cm diameter petri dishes (for use with a stereo-microscope), marked on their undersides into 10 × 10 mm square examination areas using a pointed instrument.

(m) A larval counting basin (for use with a trichinoscope), made of 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 must be inserted between the sides, to form two small handles at the ends. The upper side of the bottom must be raised 7 to 9 mm from the base of the frame formed by the sides and the ends. The components must be stuck together with glue suitable for the material.

(n) Aluminium foil.

(o) 25 % hydrochloric acid.

(p) Pepsin, strength: 1:10 000 NF (US National Formulary) corresponding to 1:12 500 BP (British Pharmacopoeia) and to 2 000 FIP (Fédération internationale de pharmacie), or stabilised liquid pepsin with minimum 660 European Pharmacopoeia units/ml.

(q) Tap water heated to 46 to 48 °C.

(r) A balance accurate to at least 0,1 g.

(s) Metal trays, capacity 10 to 15 litres, to collect the remaining digestive juice.

(t) Pipettes of different sizes (1, 10 and 25 ml) and pipette holders.

(u) A thermometer accurate to 0,5 °C within the range 1 to 100 °C.

(v) Siphon for tap water.

2. *Collecting of specimens and quantity to be digested*

(a) In the case of whole carcasses of domestic swine, a specimen weighing at least 1 g is to be taken from a pillar of the diaphragm at the transition to the sinewy part. Special trichinae forceps can be used provided an accuracy of between 1,00 and 1,15 g can be guaranteed.

In the case of breeding sows and boars, a larger sample weighing at least 2 g is to be taken from a pillar of the diaphragm at the transition to the sinewy part.

In the absence of diaphragm pillars, a specimen of twice the size 2 g (or 4 g in the case of breeding sows and boars) is to be taken from the rib part or the breastbone part of the diaphragm, or from the jaw muscle, tongue or abdominal muscles.

(b) For cuts of meat, a sample weighing at least 5 g of striated muscle, containing little fat is to be taken, where possible from close to bones or tendons. A sample of the same size is to be collected from meat that is not intended to be cooked thoroughly or other types of post-slaughter processing.

(c) For frozen samples, a sample weighing at least 5 g of striated muscle tissue is to be taken for analysis.

The weight of meat specimens relates to a sample of meat that is free of all fat and fascia. Special attention must be paid when collecting muscle samples from the tongue in order to avoid contamination with the superficial layer of the tongue, which is indigestible and can prevent reading of the sediment.

3. *Procedure*

I. Complete pools (100 g of samples at a time)

(a) 16 ± 0,5 ml of hydrochloric acid is added to a 3 litre beaker containing 2,0 litre of tap water, preheated to 46 to 48 °C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started.

(b) 10 ± 0,2 g of pepsin or 30 ± 0,5 ml liquid pepsin is added.

(c) 100 g of samples collected in accordance with point 2 is chopped in the blender.

(d) The chopped meat is transferred to the 3 litre beaker containing the water, pepsin and hydrochloric acid.

(e) The mincing insert of the blender is immersed repeatedly in the digestion fluid in the beaker and the blender bowl is rinsed with a small quantity of digestion fluid to remove any meat still adhering.

(f) The beaker is covered with aluminium foil.

(g) The magnetic stirrer must be adjusted so that it maintains a constant temperature of 44 to 46 °C throughout the operation. During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing.

(h) The digestion fluid is stirred until the meat particles disappear (approximately 30 minutes). The stirrer is then switched off and the digestion fluid is poured through the sieve into the sedimentation funnel. Longer digestion times may be necessary (not exceeding 60 minutes) in the processing of certain types of meat (tongue, game meat, etc.).

(i) The digestion process is considered satisfactory if not more than 5 % of the starting sample weight remains on the sieve.

(j) The digestion fluid is allowed to stand in the funnel for 30 minutes.

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

(l) The digestion fluids and other liquid waste are kept in a tray until reading of the results is completed.

(m) The 40 ml sample is allowed to stand for 10 minutes. 30 ml of supernatant is then carefully withdrawn by suction to remove the upper layers and leave a volume of not more than 10 ml.

(n) The remaining 10 ml sample of sediment is poured into a larval counting basin or petri dish.

(o) The cylinder or centrifuge tube is rinsed with not more than 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 stereo-microscope at a 15 to 20 times magnification. Visualisation using other techniques is allowed, provided examination of positive control samples has been shown to give an equal or better result than traditional visualisation methods. In all cases of suspect areas or parasite-like shapes, higher magnifications of 60 to 100 times must be used.

(p) Digests are to be examined as soon as they are ready. Under no circumstances should examination be postponed until the following day.

Where the digests are not examined within 30 minutes of preparation, they must be clarified as follows. The final sample of about 40 ml is poured into a measuring cylinder and allowed to stand for 10 minutes. 30 ml of the supernatant fluid is then 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 no more than 10 ml for examination in a petri dish or larval counting basin. The measuring cylinder is washed with no more than 10 ml of tap water and these washings are 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 is poured into a measuring cylinder and made up to 40 ml with tap water and then the procedure

described in this Section is followed. The procedure can be repeated 2 to 4 times until the fluid is clear enough for a reliable reading.

II. Pools of less than 100 g

Where needed, up to 15 g can be added to a total pool of 100 g and examined together with these samples in accordance with Section I. More than 15 g must be examined as a complete pool. For pools of up to 50 g, the digestion fluid and the ingredients may be reduced to 1 litre of water, 8 ml of hydrochloric acid and 5 g of pepsin.

III. Positive or doubtful results

Where examination of a collective sample produces a positive or uncertain result, a further 20 g sample is taken from each pig in accordance with point 2(a). The 20 g samples from five pigs are pooled and examined using the method described in this Chapter. In this way samples from 20 groups of five pigs will be examined.

When *Trichinella* is detected in a pooled sample from five pigs, further 20 g samples are collected from the individual pigs in the group and each is examined separately using the method described in this Chapter.

Parasite samples are to be kept in 90 % ethyl alcohol for conservation and identification at species level at the EU or national reference laboratory.

After parasite collection, positive fluids (digestive juice, supernatant fluid, washings, etc.) are to be decontaminated by heating to at least 60 °C.

IV. Cleaning and decontamination procedure after a positive or doubtful result

When the examination of a collective or individual sample produces a positive or doubtful result, all material in contact with meat (blender bowl and blade, beaker, stirring rod, temperature sensor, conical filtration funnel, sieve and forceps) must be carefully decontaminated by washing in warm water (65 to 90 °C). It is recommended to rinse each piece thoroughly to remove the detergent if a detergent is used during washing.

CHAPTER II

EQUIVALENT METHODS

A. Mechanically assisted pooled sample digestion method/sedimentation technique

1. *Apparatus and reagents*

(a) Knife or scissors for cutting specimens.

(b) Trays marked off with 50 squares, each of which can hold samples of approximately 2 g of meat, or other tools giving equivalent guarantees as regards the traceability of the samples.

(c) Meat mincer or electrical blender.

(d) A Stomacher lab-blender 3 500 thermo model.

(e) Plastic bags suitable for the Stomacher lab-blender.

(f) Conical separation funnels, capacity 2 litres, preferably fitted with Teflon safety plugs.

(g) Stands, rings and clamps.

(h) Sieves, mesh size 180 microns, external diameter 11 cm, with stainless steel or brash mesh.

(i) Funnels, internal diameter not less than 12 cm, to support the sieves.

(j) 100 ml glass measuring cylinders.

(k) A thermometer accurate to 0,5 °C within the range 1 to 100 °C.

(l) A vibrator, e.g. an electric shaver with the head removed.

(m) A relay which will switch on and off at one-minute intervals.

(n) A trichinoscope with a horizontal table or a stereo-microscope, with a sub-stage transmitted light source of adjustable intensity.

(o) A larval counting basin and a number of 9 cm diameter petri dishes as in Chapter I(1), points (l) and (m).

(p) 17,5 % hydrochloric acid.

(q) Pepsin, strength: 1:10 000 NF (US National Formulary) corresponding to 1:12 500 BP (British Pharmacopoeia) and to 2 000 FIP (Fédération internationale de pharmacie), or stabilised liquid pepsin with minimum 660 European Pharmacopoeia units/ml.

(r) A number of 10 litre bins to be used for decontamination of apparatus, e.g. with formol, and for digestive juice remaining where specimens test positive.

(s) A balance accurate to 0,1 g.

2. *Collecting of specimens and quantity to be digested*

As stipulated in Chapter I(2).

3. *Procedure*

I. Grinding

Grinding the meat samples in a meat mincer beforehand will improve the digestion quality. If an electrical blender is used, the blender must be operated three to four times for approximately one second each time.

II. Digestion procedure

This procedure may involve complete pools (100 g of samples at a time) or pools of less than 100 g.

(a) Complete pools (100 samples at a time):

(i) The Stomacher lab-blender 3 500 is fitted with a double plastic bag and the temperature control set at 40 to 41 °C.

(ii) One and a half litres of water preheated to 40 to 41 °C is poured into the inner plastic bag.

(iii) 25 ml of 17,5 % hydrochloric acid is added to the water in the Stomacher.

(iv) 100 samples weighing approximately 1 g each (at 25 to 30 °C) taken from each individual sample in accordance with point 2 are added.

(v) Lastly, 6 g pepsin or 18 ml liquid pepsin is added. This order must be followed strictly to avoid decomposition of the pepsin.

(vi) The Stomacher is then allowed to pound the content of the bag for 25 minutes.

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

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

(ix) Up to 15 individual samples can be added to a total pool of 100 samples and examined together with these samples.

(b) Smaller pools (less than 100 samples):

(i) The Stomacher lab-blender 3 500 is fitted with a double plastic bag and the temperature control set at 40 to 41 °C.

(ii) 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 must be followed strictly to avoid decomposition of the pepsin.

(iii) 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 = 450 ml) and transferred to the inner of the two plastic bags, together with the meat samples weighing approximately 1 g (at 25 to 30 °C) taken from each individual sample in accordance with point 2.

(iv) Water at a temperature of approximately 41 °C is poured into the outer bag to make up 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.

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

(vi) The plastic bag is washed with approximately 100 ml of water (at 25 to 30 °C), which is then used to rinse the sieve and lastly added to the filtrate in the beaker.

III. Recovery of larvae by sedimentation

— Ice (300 to 400 g of ice flakes, scaly ice or crushed ice) is added to the digestion fluid to bring 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 Section II(b)), the amount of ice must be reduced correspondingly.

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

— Sedimentation is allowed to proceed for 30 minutes, during which time the sedimentation funnel is vibrated intermittently, i.e. one minute vibration followed by a 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 is withdrawn by suction to leave a volume of 15 ml, to be examined for presence of larvae.
— For suction, a disposable syringe, equipped with a plastic tube, can be used. The length of the tube must be such that 15 ml remains 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.
— The measuring cylinder is washed with 5 to 10 ml of tap water and the washings are added to the sample.
— Digests are to be examined as soon as they are ready. Under no circumstances is examination to be postponed until the following day.

Where the digests are unclear or they are not examined within 30 minutes of their preparation, they must be clarified as follows:

— the final sample of 60 ml is poured into a measuring cylinder and allowed to stand for 10 minutes; 45 ml of supernatant fluid is then 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 is washed with 10 ml of tap water and these washings are added to the sample in the petri dish or the larval counting basin for examination.

IV. Positive or doubtful results

Where the result is positive or uncertain, the provisions laid down in Chapter I(3)(III) shall apply.

B. **Mechanically assisted pooled sample digestion method/'on filter isolation' technique**

1. *Apparatus and reagents*

As stipulated in Section A(1).

Additional equipment:

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

(b) filter discs, consisting of a circular stainless steel mesh with an aperture of 35 microns (disc diameter: 45 mm), two rubber rings 1 mm thick (external diameter: 45 mm; internal diameter: 38 mm), the circular mesh being placed between the two rubber rings and bonded to them using a two-component glue suitable for the two materials;

(c) an Erlenmeyer flask, capacity 3 litres, fitted with a side tube for suction;

(d) a filter pump;

(e) plastic bags, capacity at least 80 ml;

(f) equipment for sealing the plastic bags;

(g) rennilase, strength 1:150 000 Soxhlet units per gram.

2. *Collecting of specimens*

As stipulated in Chapter I(2).

3. *Procedure*

I. Grinding

Grinding the meat samples in a meat mincer beforehand will improve the digestion quality. If an electrical blender is used, the blender must be operated three to four times for approximately one second each time.

II. Digestion procedure

This procedure may involve complete pools (100 g of samples at a time) or pools of less than 100 g.

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

See Section A(3)(II)(a).

(b) Smaller pools (less than 100 samples)

See Section A(3)(II)(b).

III. Recovery of larvae by filtration

(a) Ice (300 to 400 g of ice flakes, scaly ice or crushed ice) is added to the digestion fluid to bring its volume up to about 2 litres. In the case of smaller pools, the amount of ice must be reduced correspondingly.

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

(c) The Gelman funnel, fitted with a filter holder and filter disc, is mounted on an Erlenmeyer flask connected to a filter pump.

(d) The digestion fluid is poured into the Gelman funnel and filtered. Towards the end of filtration, the digestion fluid can be helped to pass through the filter by applying suction with the filter pump. Suction must cease before the filter becomes dry, i.e. when 2 to 5 ml of fluid is left in the funnel.

(e) Once 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 rennilase solution is made by adding 2 g of rennilase to 100 ml of tap water.

(f) The plastic bag is sealed twice and placed between the inner and outer bags in the Stomacher.

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

(h) 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.

(i) Digests must be examined as soon as they are ready. Under no circumstances is examination to be postponed until the following day.

Note: Filter discs must never be used when not completely clean. Unclean discs must never be allowed to dry out. Filter discs can be cleaned by leaving them in rennilase solution overnight. Before use, they must be washed in fresh rennilase solution using the Stomacher.

IV. Positive or doubtful results

Where the result is positive or uncertain, the provisions laid down in Chapter I(3)(III) shall apply.

C. **Automatic digestion method for pooled samples of up to 35 g**

1. *Apparatus and reagents*

(a) Knife or scissors for cutting specimens.

(b) Trays marked off with 50 squares, each of which can hold samples of approximately 2 g of meat, or other tools giving equivalent guarantees as regards the traceability of the samples.

(c) A Trichomatic 35® blender with filtration insert.

(d) Hydrochloric acid 8,5 ± 0,5 % weight.

(e) Transparent polycarbonate membrane filters with a diameter of 50 mm and a pore size of 14 microns.

(f) Pepsin, strength 1:10 000 NF (US National Formulary) corresponding to 1:12 500 BP (British Pharmacopoeia) and to 2 000 FIP (Fédération internationale de pharmacie), or stabilised liquid pepsin with minimum 660 European Pharmacopoeia units/ml.

(g) A balance accurate to 0,1 g.

(h) Tweezers with a flat tip.

(i) A number of microscope slides with a side-length of at least 5 cm or a number of petri dishes at least 6 cm in diameter, marked on their undersides into 10 × 10 mm square areas using a pointed instrument.

(j) A (stereo-)microscope with transmitted light (magnification 15 to 60 times) or a trichinoscope with a horizontal table.

(k) A bin for collection of waste liquids.

(l) A number of 10 litre bins to be used for decontamination of apparatus, e.g. with formol, and for digestive juice remaining where specimens test positive.

(m) A thermometer accurate to 0,5 °C within the range 1 to 100 °C.

2. *Collecting of specimens*

As stipulated in Chapter I(2).

3. *Procedure*

I. Digestion procedure

(a) Place the blender with the filtration insert, connect the waste tube and place the tube so it drains into the waste bin.

(b) When the blender is switched on, heating will start.

(c) Before this is done, the bottom valve located below the reaction chamber must be opened and closed.

(d) Up to 35 samples weighing approximately 1 g each (at 25 to 30 °C) taken from each individual sample in accordance with point 2 are then added. Ensure that larger pieces of tendons are removed as they may clot the membrane filter.

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

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

(g) Place a membrane filter under the coarse filter in the filter holder in the filter insert.

(h) Lastly, add 7 g of pepsin or 21 ml liquid pepsin. This order must be followed strictly to avoid decomposition of the pepsin.

(i) Close the lids of the reaction and liquid chambers.

(j) Select the period of digestion. A short digestion period (5 minutes) must be set for pigs at the normal slaughter age and a longer time (8 minutes) for other samples.

(k) When the start button on the blender is turned on, the process of dispensing and digestion starts automatically, followed by filtration. After 10 to 13 minutes the process is completed and stops automatically.

(l) Open the lid of the reaction chamber after checking that the chamber is empty. If there is foam or any digestion liquid remaining in the chamber, repeat the procedure in accordance with Section V.

II. Recovery of larvae

(a) Remove the filter holder and transfer the membrane filter to a slide or petri dish.

(b) Examine the membrane filter using a (stereo-)microscope or a trichinoscope.

III. Cleaning equipment

(a) Where the result is positive, fill the blender reaction chamber with boiling water until it is two-thirds full. Ordinary tap water is poured into the connecting liquid chamber until it covers the lower sensor. Automatic cleaning then takes place. Decontaminate the filter-holder and any other equipment, e.g. using formol.

(b) After work is completed for the day, fill the blender liquid chamber with water and put it through a standard cycle.

IV. Use of membrane filters

Each polycarbonate membrane filter may be used no more than five times. The filter is to be turned between each use. In addition, the filter must be checked after each use for any damage which would make it unsuitable for further use.

V. Method to be applied when digestion is incomplete and filtration cannot be carried out

Once the blender has been put through an automatic cycle in accordance with Section I, open the lid of the reaction chamber and check whether there is foam or any liquid remaining in the chamber. If this is the case, proceed as follows:

(a) close the bottom valve below the reaction chamber;

(b) remove the filter holder and transfer the membrane filter to a slide or petri dish;

(c) put a new membrane filter in the filter holder and attach the filter holder;

(d) fill the blender liquid chamber with water until the lower sensor is covered;

(e) carry out the automatic cleaning cycle;

(f) after the cleaning cycle has ended, open the lid of the reaction chamber and check whether any liquid remains;

(g) if the chamber is empty, remove the filter holder and transfer the membrane filter to a slide or petri dish with tweezers;

(h) examine the two membrane filters in accordance with Section II. If the filters cannot be examined, repeat the entire digestion process with a longer digestion time in accordance with Section I.

VI. Positive or doubtful results

Where the result is positive or uncertain, the provisions laid down in Chapter I(3)(III) shall apply.

D. **Magnetic stirrer method for pooled sample digestion/'on filter isolation' and larva detection by a latex agglutination test**

This method is only considered equivalent for the testing of meat of domestic swine.

1. *Apparatus and reagents*

(a) Knife or scissors and tweezers for cutting specimens.

(b) Trays marked off into 50 squares, each of which can hold samples of approximately 2 g of meat, or other tools giving equivalent guarantees as regards the traceability of the samples.

(c) A blender with a sharp chopping blade. Where the samples are larger than 3 g, a meat mincer with openings of 2 to 4 mm or scissors must be used. In the case of frozen meat or tongue (after removal of the superficial layer, which cannot be digested), a meat mincer is necessary and the sample size will need to be increased considerably.

(d) Magnetic stirrers with thermostatically controlled heating plate and Teflon-coated stirring rods approximately 5 cm long.

(e) Glass beakers, capacity 3 litres.

(f) Sieves, mesh size 180 microns, external diameter 11 cm, with stainless steel mesh.

(g) Steel filtration apparatus for 20 μm mesh filters with a steel funnel.

(h) Vacuum pump.

(i) Metal or plastic tanks, capacity 10 to 15 litres, to collect the digestive juice.

(j) A 3D gyratory rocker.

(k) Aluminium foil.

(l) 25 % hydrochloric acid.

(m) Pepsin, strength: 1:10 000 NF (US National Formulary) corresponding to 1:12 500 BP (British Pharmacopoeia) and to 2 000 FIP (Fédération internationale de pharmacie), or stabilised liquid pepsin with minimum 660 European Pharmacopoeia units/ml.

(n) Tap water heated to 46 to 48 °C.

(o) A balance accurate to 0,1 g.

(p) Pipettes of different sizes (1, 10 and 25 ml), micropipettes according to the latex agglutination manufacturer's instructions and pipette holders.

(q) 20 microns nylon mesh filters of a diameter that fits with the filtration system.

(r) Plastic or steel forceps of 10 to 15 cm.

(s) Conical vials of 15 ml.

(t) A pestle with a Teflon or steel conical tip to fit in the conical vials.

(u) A thermometer accurate to 0,5 °C within the range 1 to 100 °C.

(v) Latex agglutination cards of the Trichin-L antigen test kit validated under the code No EURLP_D_001/2011.

(w) Buffer solution with preservative (sample diluent) of the Trichin-L antigen test kit validated under the code No EURLP_D_001/2011.

(x) Buffer supplemented with preservative (negative control) of the Trichin-L antigen test kit validated under the code No EURLP_D_001/2011.

(y) Buffer supplemented with *Trichinella spiralis* antigens and preservative (positive control) of the Trichin-L antigen test kit validated under the code No EURLP_D_001/2011.

(z) Buffer with polystyrene particles coated with antibodies supplemented with preservative (latex beads) of the Trichin-L antigen test kit validated under the code No EURLP_D_001/2011.

(aa) Disposable sticks.

2. *Collecting of specimens*

As stipulated in Chapter I(2).

3. *Procedure*

I. For complete pools (100 g of samples at a time)

(a) 16 ± 0,5 ml of 25 % hydrochloric acid (0,2 % final) is added to a 3 litre beaker containing 2,0 litres ± 200 ml of tap water, preheated to 46 to 48 °C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started.

(b) 10 ± 1 g of powder pepsin (or 30 ± 3 ml of liquid pepsin) is added.

(c) 100-115 g of samples collected in accordance with point 2 are chopped in the blender, with 150 ± 15 ml of preheated digestion buffer.

(d) The chopped meat is transferred to the 3 litre beaker containing the water, pepsin and hydrochloric acid.

(e) The mincing insert of the blender is immersed repeatedly in the digestion fluid in the beaker and the blender bowl is rinsed with a small quantity of digestion fluid to remove any meat still adhering.

(f) The beaker is covered with aluminium foil.

(g) The magnetic stirrer must be adjusted so that it maintains a constant temperature of 44 to 46 °C throughout the operation. During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing.

(h) The digestion fluid is stirred until the meat particles disappear (approximately 30 minutes). The stirrer is then switched off and the digestion fluid is poured through the sieve into the sedimentation funnel. Longer digestion times may be necessary (not exceeding 60 minutes) in the processing of certain types of meat (tongue, game meat, etc.).

(i) The digestion process is considered satisfactory if not more than 5 % of the starting sample weight remains on the sieve.

(j) The 20 microns nylon mesh filter is placed on the filtration support. The conical filtration steel funnel is fixed to the support with the block system and the steel sieve of 180 microns mesh size is placed on the funnel. The vacuum pump is connected with the filtration support and with the metal or plastic tank, to collect the digestive fluid.

(k) Stirring is stopped and the digestion fluid is poured into the filtration funnel through the sieve. The beaker is rinsed with approximately 250 ml of warm water. The rinsing liquid is poured into the filtration ramp after the digested fluid has been successfully filtrated.

(l) The filtration membrane is taken with the forceps, holding it by an edge. The filtration membrane is folded (minimal) in four and put in the 15 ml conical tube. The choice of conical tube must be adapted to the pestle.

(m) The filtration membrane is pushed at the bottom of the 15 ml conical tube with the help of the pestle and strongly pressed by doing approximately 20 successive back and forth movements with the pestle which should be positioned inside the filtration membrane folding according to the manufacturer's instructions.

(n) 0,5 ± 0,01 ml of sample diluents is added into the 15 ml conical tube by pipette and the filtration membrane is homogenised with the pestle by doing successive low amplitude back and forth movements for approximately 30 seconds, avoiding abrupt movements to limit liquid splashes according to the manufacturer's instructions.

(o) Each sample, the negative control, and the positive control, are dispensed into different fields of the agglutination card by pipettes, according to the manufacturer's instructions.

(p) The latex beads are added into each field of the agglutination card by a pipette, according to the manufacturer's instructions, without making them come into contact with the sample/s and controls. In each field, the latex beads are then gently mixed with a disposable stick until the homogeneous liquid covers the entire field.

(q) The agglutination card is put on the 3D rocker and is rocked for 10 ± 1 minutes according to the manufacturer's instructions.

(r) After the time established by the manufacturer's instructions, the rocking is stopped and the agglutination card is put on a plane surface and the reaction results are read

immediately, according to the manufacturer's instructions. In the case of a positive sample, the beads aggregates must appear. In the case of a negative sample, the suspension remains homogeneous without beads aggregates.

II. Pools of less than 100 g as set out in Chapter I(3)(II)

For pools of less than 100 g, the procedure set out in Chapter I(3)(II) must be followed.

III. Positive or doubtful results

Where examination of a collective sample produces a positive or uncertain latex agglutination result, a further 20 g sample is taken from each swine in accordance with Chapter I(2)(a). The 20 g samples from five swine are pooled and examined using the method described in Section I. In this way samples from 20 groups of five swine must be examined.

When a positive latex agglutination is obtained from a group of five swine, further 20 g samples are collected from the individuals in the group and each is examined separately using the method described in Section I.

When a positive or uncertain latex agglutination result is obtained, at least 20 g of swine muscle must be sent to the national reference laboratory for confirmation using one of the methods described in Chapter I.

Parasite samples must be kept in 90 % ethyl alcohol for conservation and identification at species level at the EU or national reference laboratory.

After parasite collection, positive fluids must be decontaminated by heating to at least 60 °C.

IV. Cleaning and decontamination procedure after a positive or doubtful result

When the examination of a collective or individual sample produces a positive or doubtful latex agglutination result, all material in contact with meat (blender bowl and blade, pestle, beaker, stirring rod, temperature sensor, conical filtration funnel, sieve and forceps) must be carefully decontaminated by soaking for few seconds in warm water (65 to 90 °C). Meat residues or inactivated larvae that could remain on their surface may be removed with a clean sponge and tap water. If required, a few drops of detergent can be added for degreasing equipment. It is then recommended to rinse each piece thoroughly to remove all traces of detergent.

E. **Artificial digestion test for *in vitro* detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® *Trichinella* AAD Kit**

This method is only considered equivalent for the testing of meat of domestic swine.

The PrioCHECK® *Trichinella* AAD Kit shall be used according to the instruction manual of the kit using separatory funnels (Lenz NS 29/32) and a glass test tube of 80 ml.

Status:
Point in time view as at 10/08/2015.

Changes to legislation:
There are currently no known outstanding effects for the Commission Implementing Regulation (EU) 2015/1375, ANNEX I.