EXPERIMENTAL INFECTION OF MICE BY *Trichinella* spp. MUSCLE STAGE LARVAE

STANDARD OPERATING PROCEDURE (SOP)

SUMMARY

1. SCOPE

This Standard Operating Procedure provides instructions to achieve the experimental infection and the maintenance of *Trichinella* spp. isolates in mice.

2. INTRODUCTION

Trichinella parasites can be maintained in laboratory only by, all attempts to obtain their life cycle in vitro failed due to the extremely complex biology of these nematodes. To maintain *Trichinella* isolates in vivo it is necessary to sacrifice the infected animals, to collect larvae from their muscle tissue and to infect new animals at least once a year for the strains which are well adapted to the mouse model or for those strains belonging to *T. spiralis* or *T. pseudospiralis*. For the other species of the genus *Trichinella*, it is necessary to allow the parasite to adapt to the new host, through serial passages every three months during the first year, every four months the second year, every six months the third year and once a year from the fourth year.
Trichinella larvae, when inoculated in mice, become infective after 30-40 days p.i., but it is better to sacrifice mice at least for 60 days p.i., since after that time, muscle larvae become more resistant to digestion.

3. REFERENCES

Reg. (CE) 2075/2005 of 05/12/2005 GU CE L338, 22/12/2005, Ann 1. cap. 1, Ann. III

Reg. (CE) 1245/2007 of 24/10/2007 GU CE L281/19, 25/10/2007

International *Trichinella* Reference Center. http://www.iss.it/site/Trichinella/index.asp

Pozio, E. 2007. Taxonomy, biology and epidemiology of *Trichinella* parasites. In, (Dupouy-Camet, J and Murrell, K.D. eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, Paris, pp. 1-35

4. DEFINITIONS

MSL Muscle Stage Larvae

5. EQUIPMENT

5.1. thermometer 1-100 °C;

5.2. analytical balance;

5.3. stereo-microscope with a substage transmitted adjustable light source (magnification 15-20X);

5.4. blender with a sharp chopping blade;

5.5. magnetic stirrer with an adjustable heating plate;

5.6. sieve made of brass or stainless steel, mesh size approx. 180-200 microns (approx. 10 cm or larger);

5.7. tubes or measuring cylinders (50 or 100 ml plastic or glass);

5.8. glass beakers;

5.9. conical glass separatory funnels;

5.10. pipettes (1, 10 and 25 ml);

5.11. micropipettes 100 µl,

5.12. syringes 1 ml and 2,5 ml;

5.13. gavage needle;

5.14. teflon-coated stir bar.

6. REAGENTS

6.1. tap water heated at 46-48°C;

6.2. hydrochloric acid (25%, molar concentration: 7.8-7.9);

6.3. pepsin (1: 10,000 NF, 1: 12,500 BP, 2,000 FIP) in powder, granular or liquid form;

7. PROCEDURE

If you start from infected muscle tissue, go to point 7.3. If you start from infected mice:

7.1. Sacrifice the infected mice by cervical dislocation or by CO_2;
7.2. Skin and eviscerate the mice;
7.3. Add in a glass beaker in sequence: an appropriate volume of tap water preheated at 46-48°C, 25% hydrochloric acid, and pepsin (according to Table 1).

Table 1. Ratio digest fluid/number of mice.

Number of mice	**Water at 46-48°C**	**HCl 25%**	**Pepsin powder (liquid)***
1	250 ml	2 ml	1,25 g (3,75 ml)
2	500 ml	4 ml	2,25 g (7,5 ml)
3-4	1 litro	8 ml	5 g (15 ml)
5-9	2 litri	16 ml	10 g (30 ml)
10-14	3 litri	24 ml	15 g (45 ml)
15-20	5 litri	40 ml	25 g (75 ml)

***1g pepsin powder = 3ml liquid pepsin**

7.4. Chop carcasses (including bones) or tissues in a blender adding a small volume of digest fluid to facilitate blending of tissues;
7.5. Pour the blended tissues in the beaker containing the digest fluid and place the stirring rod in the beaker;
7.6. Place the beaker on the magnetic stirrer and adjust the heating plate at 44-46°C;
7.7. Commence the stirring and digest for 30 min the encapsulated species (*T. spiralis, T. nativa, T. britovi, T. murreli, T. nelsoni*, *Trichinella* T6, *Trichinella* T8, *Trichinella* T9 and *Trichinella* T12), and for 20 min the non-encapsulated species (*T. pseudospiralis, T. papuae* and *T. zimbabwensis*), covering the glass beaker with an aluminium foil to keep a constant temperature and to decrease the evaporation;
7.8. Pour carefully (to avoid overflow) the digest fluid into the separatory funnel through the sieve;
7.9. Allow the larvae to sediment in the digest fluid for 30 min;
7.10. Withdraw 50 ml of the sediment and transfer it into a glass cylinder or a tube;
7.10.1. Allow the 40 ml of the sediment to stand for 10 min so that larvae can again settle;

7.11. Withdraw 40 ml of the supernatant by suction from the top of the fluid, leaving a volume of not more than 10 ml;
7.12. Add 10-40 ml of PBS at 37°C, according to the size of the sediment containing the larvae;
7.13. If the sediment is not transparent, repeat from point 7.11 to point 7.13;
7.14. Shake the tube to suspend the larvae and immediately withdraw 100 µl of the suspension by a micropipette, and count the larvae onto a gridded Petri dish with a stereomicroscope. To increase the accuracy, repeat the count on three aliquots;
7.15. Dilute or concentrate the solution containing the larvae to reach a concentration of 1,000 larvae/ml;
7.16. Keep the larvae at 37°C up to the inoculum in mice; the vitality of larvae belonging to non encapsulated species decrease very rapidly, therefore mice shall be inoculated as soon as possible, encapsulated larvae can be kept up to 3 hours before the inoculum;

The number of larvae to be inoculated per mouse depends on: a) the mouse weight; b) the mouse strain; and c) the *Trichinella* species/genotype. Mice shall be inoculated per os with a maximum of 0,5 ml of larval suspension by a gavage needle of appropriate size, on a 1-2,5 ml syringe.
Outbread mice weighting at least 20 g (e.g., Swiss CD1), shall be inoculated with 500 larvae/mouse. For Balb/c mice weighting 20g, the inoculum cannot exceed 150 larvae/mouse and serial passages shall be performed every 6 months. For other inbred or outbred mouse strains, it's suitable to test their suitability by starting with 50 larvae/mouse per inoculum. Do not use young neither male mice, since they are very aggressive and kill each other when bred for long time.
The *Trichinella* species best adapted to mice are *T. spiralis* and *T. pseudospiralis*.

8. MICE IMMUNOSUPPRESSION

Mice immunosuppression by cyclophosphamide is necessary for all those species with a low reproductive capacity index, as for instance *T. papuae, T. zimbabwensis* and *T. nativa*, and in all cases where a very high number of larvae production is requested.
Procedure: dissolve 100 mg of cyclophosphamide powder in 5 ml of distilled H_2O. Inoculate in the mouse peritoneum, 0,2 ml/mouse of the cyclophosphamide solution the same day of *Trichinella* infection.

9. SAFETY MEASURES

Laboratory staff performing the procedure, shall wear disposable gloves, mask and lab coat. The use of a liquid pepsin formulation may be advantageous as it could reduce the risk of an occupational allergic reaction in lab staff.

The cyclophosphamide solution shall be prepared under a chemical hood, due to its high toxicity.