MOUSE BIOASSAY FOR THE DETECTION OF *Toxoplasma gondii* IN MEAT
STANDARD OPERATING PROCEDURE (SOP)

SUMMARY

1. SCOPE

This Standard Operating Procedure (SOP) provides instructions to perform the mouse bioassay to detect the presence of viable *Toxoplasma gondii* cysts (bradyzoites) or tachyzoites in meat samples.

2. INTRODUCTION

One of the major transmission routes of *Toxoplasma gondii* to humans is the consumption of raw or undercooked meat harboring tissue cysts containing bradyzoites. More unfrequently, also tachyzoites can be present in meat and transmit the infection. Due to the lack of a strict correlation between the presence of anti-*Toxoplasma* antibodies in animals (e.g., pig, sheep, chicken, horse, cattle) and that of viable parasites in their tissues, the only reliable methods able to reveal infectious *T. gondii* stages in meat samples are cat and mouse bioassays. The first, which is based on the detection of *T. gondii* oocysts in the feces of kittens fed potentially contaminated meat samples, is impractical for both ethical and economic reasons. The mouse bioassay, which measures the seroconversion of mice inoculated with meat sample digests, can be more easily performed and is the subject of this SOP.

3. REFERENCES

Dubey J.P. Toxoplasmosis of Animals and Humans, 2nd Edition. CRC Press, 2010.

4. DEFINITIONS

Bradyzoites - the slowly multiplying encysted form of *Toxoplasma gondii* typical of chronic infection.

Tachyzoites - the rapidly multiplying stage of *Toxoplasma gondii* in the development of the acute phase.

5. EQUIPMENT

5.1. analytical balance;

5.2. scalpel;

5.3. measuring cylinders;

5.4. grinder or blender with a sharp chopping blade;

5.5. Erlenmeyer flasks (500 mL, 1000 mL);

5.6. magnetic stirrer;

5.7. gaze filter;

5.8. pipettes (1, 5, 10 and 25 mL);

5.9. conical funnel;

5.10. teflon coated stir bar;

5.11. refrigerated centrifuge;

5.12. micropipettes (20, 100 and 1000 uL);
5.13. syringes 1 mL;
5.14. needles 21 G;
5.15. thermostate (37 ± 2°C);
5.16. conical tubes (Falcon 50 mL; Eppendorf 1.5 mL);
5.17. refrigerator (+4 °C);
5.18. parafilm.

6. REAGENTS

6.1. 0.9% NaCl;
6.2. trypsin solution [25 mg/mL];
6.3. penicillin-streptomycin solution [10,000 U penicillin, 10 mg streptomycin/mL]
6.4. amoxicillin [0.2 g/mL] ;
6.5. ciprofloxacin [2 mg/mL] ;
6.6. cefotaxime [100 mg/mL] ;
6.7. vancomycin [2 mg/mL];
6.8. 70% denaturated ethyl alcohol;
6.9. Swiss CD1 female mice (minimum 6 weeks old).

7. PROCEDURE

7.1. Determine the meat sample weight;
7.2. Clean the sample from all fat and connective tissue with a scalpel and cut it into small pieces;
7.3. Grind the cleaned meat pieces in the grinder (or briefly in the blender) to obtain the consistency of tartar stake (hamburger);
7.4. Weigh 200 g of meat sample;
7.5. For each meat sample to be digested prepare the Digestion Solution as follows:

0.9% NaCl….270 mL

trypsin……30 mL

amoxicillin…..0.6 mL

penicillin-streptomycin solution…..6 mL

7.6. Mix in a 1L Erlenmeyer flask the grinded meat sample and 306.6 mL of Digestion Solution, close the Erlenmeyer with parafilm and add a teflon-coated stir bar;
7.7. Incubate the sample for 1.5 hours in a thermostate at 37 ± 2°C on a magnetic stirrer;
7.8. Prepare another 2 Erlenmeyers (0.5L), funnel and gauze for filtration;

7.9. Put the funnel on the Erlenmeyer and lay the double layered gauze inside the funnel;

7.10. Pour the digested sample into the funnel and gently mix until all liquid pass through the gaze filter;

7.11. Take the ends of the gaze filter and squeeze the sample to obtain additional digestion fluid;

7.12. Divide the sample in a suitable number of conical 50 ml Falcon tubes;

7.13. Centrifuge at 1800 g for 10 min at 4 °C using the centrifuge brake;

7.14. Discard the supernatant and collect the pellets;

7.15. Dilute each pellet with 15 mL of 0.9% NaCl, thoroughly resuspend the sample by vortexing and combine the resuspended pellets into one or two 50 mL Falcon tubes;

7.16. Centrifuge at 1800g for 10 minute at 4 °C using the centrifuge brake;

7.17. Discard the supernatant and resuspend the pellet in 500 µl of Solution 1 + 500 µl of Solution 2 and make up to 5 mL with 0.9% NaCl;

Solution 1

6 mL 0.9% NaCl

4 mL penicillin-streptomycin [10,000 U penicillin, 10 mg streptomycin/mL]

0.4 mL amoxicillin [0.2 g/mL]

Solution 2

4 mL NaCl 0.9%

50 µL ciprofloxacin [2 mg/mL]

15 µL cefotaxime [100 mg/mL]

20 µL vancomycin [2 mg/mL]

7.18. Aliquot the sample in 2 x 1.5 ml Eppendorf tubes for the mouse bioassay and keep the tubes a +4°C overnight before inoculation to maximize antibiotics' action;

7.19. Store the remainder of the digest at 4°C for a maximum of three days, and use it to reinoculate a mouse if mortality occurs within 3 days post-infection.

8. MICE INOCULATION

8.1. For each analyzed meat sample, isolate two mice in each cage and mark the cage;

8.2. In one mL syringe prepare the digest with the antibiotics;

8.3. Grip the mouse by the skin from the neck and back and immobilize it in the hand so that their abdomens are exposed for manipulation;

8.4. Sterilize mouse abdomen with 70% denatured ethyl alcohol;

8.5. On the right side from the linea alba, approximately in the second third of the abdomen, inoculate the tissue digest into the abdominal cavity of the mouse;

8.6. At 6 weeks post-inoculation, take a blood sample for the serologic detection of anti-*Toxoplasma* antibodies;

8.7. If mouse dye within three days from the inoculum, it is possible to re-inoculate another mouse with the sample previously stored at +4°C.

9. SAFETY MEASURES

Laboratory staff performing the procedure, shall wear disposable gloves, mask and lab coat.