

5.6.3 Immunofluorescence

Principle

Chlamydial antibodies are detected in test sera by means of ovine or caprine immunoglobulin conjugates labelled with fluorescein isothiocyanate, which react with the antibodies that are bound to the chlamydial antigens which cover the wells of teflon coated slides.

For the same reason as before, kits for the diagnosis of human infections cannot be used but slides coated with ten samples of *Chlamydia psittaci* are commercially available (*Chlamydia spot*, IF bioMerieux, France) or can be prepared in the following way:

Materials and reagents

- Fluorescent microscope.
- 10 well teflon coated slides without *Chlamydia* coating (Figure 5.10, page 95).
- Ovine or caprine immunoglobulin fluorescent conjugate.
- PBS.

Antigen preparation

1. Place *Chlamydia* cultured in eggs in the wells of the teflon coated slide. Dry for 30 minutes at 37°C and fix in acetone for 20 minutes at room temperature.

2. Dry the slides again.

3. Store at -20°C until required for use.

Procedure

1. Thaw the slides.
2. Dilute the test sera by 10-fold dilutions with PBS together with the standard positive sample.
3. Place the sera and the standard negative dilutions in the wells and incubate for 30 minutes at 37°C in a humid atmosphere.
4. Rinse the slides twice in deionised water and twice for 10 minutes in PBS.

5. Add the conjugate and incubate for 30 minutes at 37°C in a humid atmosphere.

6. Wash the slides as previously, mount and examine.

Note

- *Chlamydia* cultured in cells detach more easily from the slides than *Chlamydia* cultured in eggs.

- The ease of interpretation of the slide results is directly related to the concentration of *Chlamydia* in the samples.

- Teflon coated slides with 3 x 8 wells (L24C5T # XES 230, CML France Nemours) allow the use of multichannel pipettes.

5.6.4 Delayed hypersensitivity [9]

A delayed hypersensitivity skin test can be undertaken in goats by intradermal injection of purified *Chlamydia* cultured in eggs or in cells. The reaction is easily visible 72 hours after injection. Although this test gives fewer questionable results than complement fixation it cannot be used as an individual diagnosis. In sheep the test can be undertaken on the lower eyelid but is a lot more difficult to assess (see Figure 4.13 in the chapter on Brucellosis).

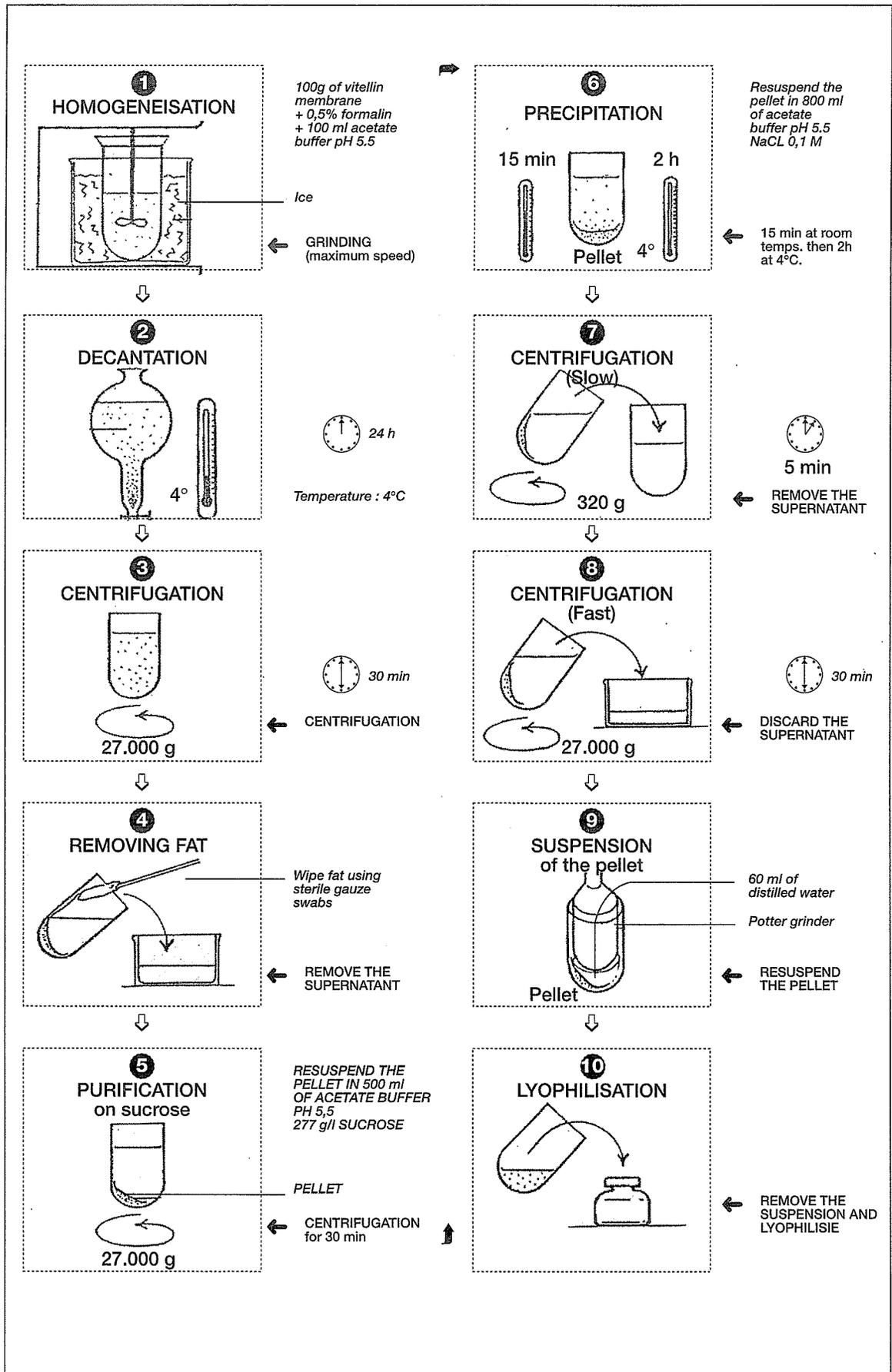
Principle

Delayed hypersensitivity detects a cell mediated response in the animal after intradermal injection of purified antigen.

5.6.4.1 Antigen preparation

Materials and reagents

- Grinder (Omni-Mixer, Sorvall).
- Potter grinder.
- Lyophiliser.
- Acetate buffer, pH 5.5.
- NaCl.
- Sucrose.



5.11 : Delayed hypersensitivity reaction
 Antigen preparation protocol

Procedure (Figure 5.11)

1. Grind 100g of infected yolk sac membrane in 0.5% formalin and 100ml of acetate buffer, pH 5.5 1M NaCl, in 250ml pots cooled in ice with the grinder (Omni-Mixer, Sorvall) at maximum speed.

2. Reduce the volume of the suspension to 100ml and allow to settle for 24 hours at 4°C.

3. Remove the gross debris and centrifuge the suspension at 27000 xg for 30 minutes.

4. Remove the supernatant. Wipe any fat from the walls of the centrifuge tubes using sterile gauze swabs and resuspend the pellet in 500ml of acetate buffer, 2M NaCl, 277g/l sucrose.

5. Centrifuge the suspension at 27000 xg for 30 minutes.

6. Resuspend the pellet in 800ml of acetate buffer, pH 5.5, 0.1M NaCl. Allow to stand for 15 minutes at room temperature to initiate precipitation, then leave for 2 hours at 4°C.

7. Centrifuge the suspension at 320 xg for 30 minutes.

8. Remove the supernatant and centrifuge at 27000 xg for 30 minutes.

9. Resuspend the pellet in 60ml of distilled water.

10. Homogenise the suspension using the Potter grinder and centrifuge at 320 xg for 5 minutes in conical bottomed tubes.

11. Remove the supernatant with a pipette and lyophilise.

12. Check the antigen by injecting 100µg of the allergen dissolved in pyrogen-free physiological saline intradermally into a guinea-pig at a previously shaven site. Read the result after about 72 hours (Figure 5.12, page 95).

Note

- Antigen can also be prepared using *Chlamydia* cultured in cells [9].

5.6.4.2 Test method**Procedure**

1. Inoculate 100µl of allergen dissolved in 0.1ml pyrogen-free physiological saline intradermally in the neck of goats or the lower eyelid of sheep.

2. Examine the reactions 72 hours after injection and assess their intensity visually and by palpation in comparison to the other eyelid of the sheep or the other side of the neck of the goat.

Note

- When the injection is given in the neck the thickness of the skin can be measured with callipers.

- The period of 72 hours from injection to assessment appears to be necessary to detect maximum positive responses. Weak reactions are never visible before 48 hours and can be non-detectable by 96 hours.

5.7 TRENDS AND FUTURE WORK

Diagnosis of abortigenic chlamydia is still usually undertaken using either the complement fixation reaction alone or in association with bacteriological examination of part of the placenta. These two techniques lack specificity and neither permit individual diagnosis, nor do they predict animals at risk from abortion.

Improved diagnostic specificity and sensitivity may be obtained by :

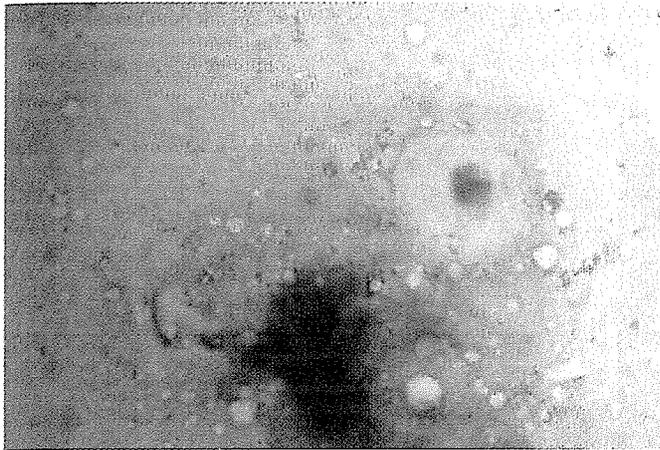
- Replacement of bacteriological assessment by detection of *Chlamydia* by immunofluorescence or better by PCR with specific emphasis on abortive strains.

- Use of an antigen specific to abortive strains of *C.psittaci* for serological diagnosis [13].

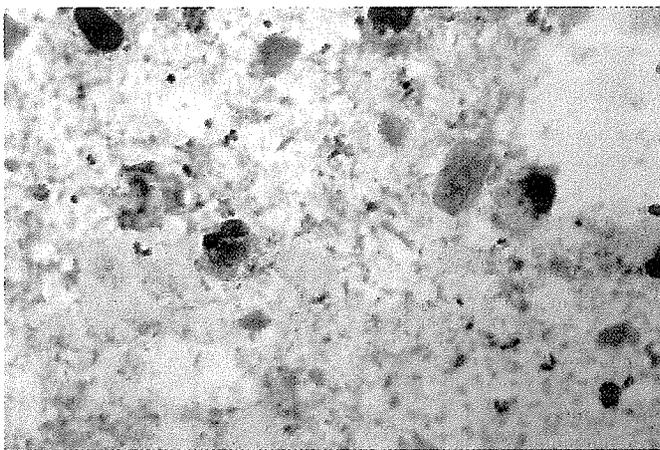
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a



b



c

Figure 3.3 :
Bacteriological examination by stamp stain of
(a) The stomach content affected by Brucellosis
(b) an impression of a cotyledon infected with *Chlamydia psittaci*
(c) or by *Coxiella burnetii*

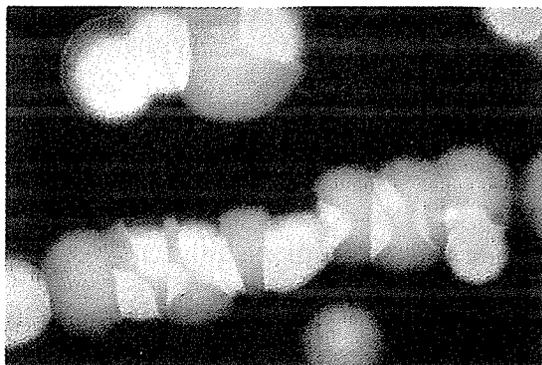


Figure 4.2 :
Smooth (blue) and rough (yellow) morphological types of *Brucella* colonies

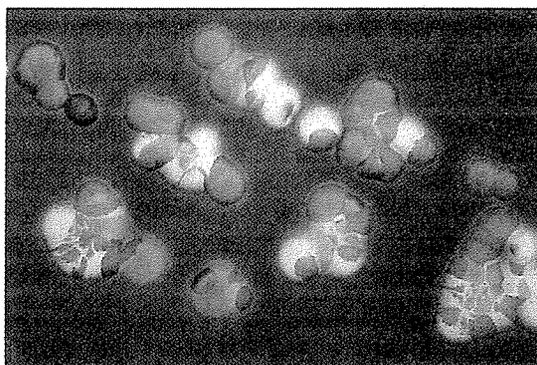


Figure 4.3 :
Brucella colonies stained by crystal violet

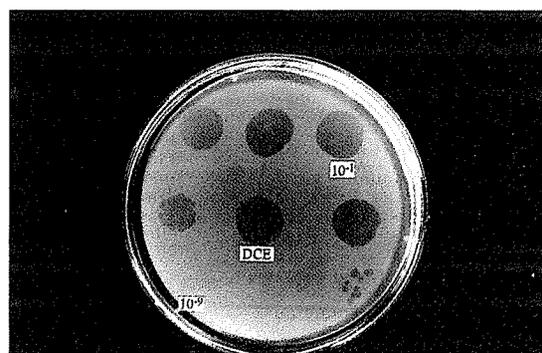
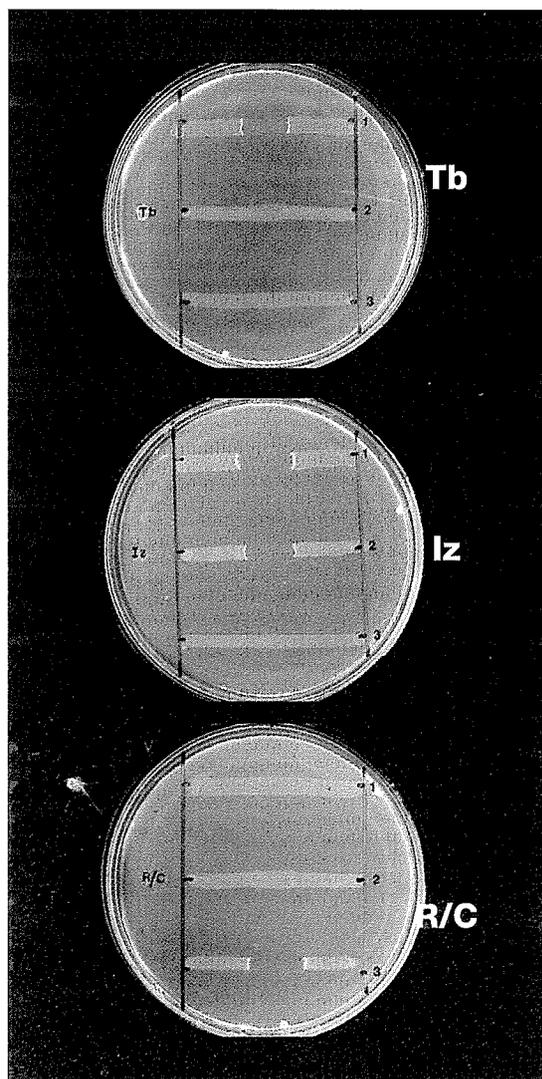


Figure 4.4 :
Determination of SWC in the example presented, the SWC corresponds to the 10⁻⁵ dilution

Figure 4.5 :
Lysotyping of *Brucella* by brucelophages Tb, Iz and R/C.

1. *B. abortus* 554.
2. *B. melitensis* 16 M.
3. *B. suis* 63/290.