STANDARD OPERATING PROCEDURE

TAYLORELLA EQUIGENITALIS IDENTIFICATION BY CULTURE METHOD

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The bacteriological diagnosis of *Taylorella equigenitalis* is based on the isolation and culture of the bacterium on solid media. Isolated suspect colonies are placed on a new culture medium to produce a pure culture on which to base the differential diagnosis after studying the bacteriological, biochemical and antigenic characters permitting identification.

The culture method is performed according to the world organisation for animal health (OIE) international standard: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 3.5.2, contagious equine metritis (current version). All National Standard Operating Procedures (SOP; e.g. NF U47-108 is the French standard for the isolation and identification of *T. equigenitalis* from genital swabs) validated and used successfully in the PT can be used for this essay.

1. COLLECTION OF SAMPLES

1.1. TISSUE MATERIAL TO BE EXAMINED

Swabbing from the urethra, urethral fossa, penile sheath, pre-ejaculatory fluid and semen are the samples of choice from the stallion.

Swabbing of the clitoral sinuses, clitoral fossa, uterus (during oestrus) and vulval/vaginal discharge (in case of metritis/endometritis) are the specimens of choice from the mare.

This method can also be applied directly on pure culture bacteria in case of second-line analysis to confirm a CEM case.

1.2. TRANSPORT OF SAMPLES

Swabs must be placed in a transport medium with activated charcoal, such as Amies medium and be kept cool during transport. They should arrive and be plated out at the laboratory no later than 48 hours after they were taken.

2. REAGENTS, PRODUCTS AND APPARATUS

2.1. CONTROL STRAINS

- At least one suitably-identified strain of *T. equigenitalis* (two strains resistant and susceptible to streptomycin if a chocolate agar with streptomycin is used),
- (in option) A suitably-identified strain of *T. asinigenitalis*.

2.2. CULTURE MEDIA (proposition; see the OIE manual)

- Chocolate agar enriched with a polyvitamin supplement (e.g. Polyvitex, Vitox) or chocolate agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B,
- Chocolat agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B (OIE recommendation: 5-15 µg/ml), 1 µg/l of trimethoprim and 1 µg/l of clindamycin,
- (in option) Chocolate agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B and 200 µg/ml of streptomycin.

Composition of culture media must be selected in accordance with the OIE Manual. Some manufacturers produce a peptone agar base that supports the growth of *T. equigenitalis*. Ready-to-use chocolate agar plates also exist with a Columbia basal agar (e.g. Oxoid, BioMérieux).

All culture media should be subjected to quality control and must support growth of a small inoculum of the suspect organism before their use on suspect samples. A control strain of *T. equigenitalis* must also be cultured in parallel.

2.3. OTHER REAGENTS

- Gram stain kit,
- Catalase (e.g. ID color catalase, BioMérieux),
- Oxidase (e.g. oxidase reagent, BioMérieux). NB: keep in the dark,
- *T. equigenitalis* specific antiserum for slide agglutination (e.g. BIONOR Monotayl, EURL polyclonal serum),
- Sterile saline solution or phosphate buffer used as diluent.

2.4. APPARATUS

Apparatus commonly found in bacteriology laboratories, and in particular:
- Class 2 biological safety cabinet or equivalent (e.g. bensen burner),
- Bacteriological incubator with 5-10% CO_2 at (37 ± 2)°C,
- Bacteriological incubator at (37 ± 2)°C,
- Microscope.

3. TECHNIQUE

3.1. INOCULATION

The control strain(s) and sample(s) are streaked directly on plates respecting the following order:
- one chocolate agar enriched with a polyvitamin supplement or one chocolate agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B,
- one chocolate agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B, 1 µg/l of trimethoprim and 1 µg/l of clindamycin,
- (in option) one chocolate agar enriched with a polyvitamin supplement plus 5 µg/ml of amphotericin B and 200 µg/ml of streptomycin,
- one blood agar may be inoculated to identify commensal flora.

3.2. INCUBATION

Incubate the plates at (37 ± 2)°C with 5-10 % of CO_2 in a damp atmosphere or by use of a candle jar.

The parameters relating to gas proportions are systematically validated at the same time as other culture and incubation conditions through observation of the growth of at least one control strain.

The control strain(s) must be initiated at each isolation test series. Only one "control" inoculation could be performed a day unless successive isolation tests require using different batches of medium, each batch having to be checked.

3.3. VIEWING PLATES

Plates should be examined for contaminants after the first 24 h incubation, after which time daily inspection is needed. If a blood agar has been inoculated, it should be interpreted after 16 to 24 h of incubation.

At least 48-72 h is normally required before colonies of *T. equigenitalis* become visible. A standard incubation time of at least seven days is advisable before certifying cultures negative for *T. equigenitalis*. Certain countries may require the prolonged incubation period as a standard procedure and should therefore ascertain the particular import requirements of those countries and/or indicate the incubation period on which their cultural findings are based.

Rarely, visual detection of colonies may take up to 14 days; the slower growth could be due to the presence of contaminating bacterial species along with the samples, but can also indicate the presence of *T. asinigenitalis*, the second species of the *Taylorella* genus.

The *T. equigenitalis* supected colonies may be up to 2-3 mm in diameter, smooth with an entire edge, glossy and greyish. They are subcultured and incubated under the same conditions as the samples for identification tests.

3.4. IDENTIFICATION TESTS

Identification tests may be peformed directly from colonies sampled from isolation plates if there are one or more suspect and correctly isolated colonies. In this case, the tests must be performed again from fresh subcultrured colonies.

3.4.1. Phenotypic and biochemical tests

- Gram stain: generally small Gram-negative coccobacillus that can have a bacillary structure. This polymorphism in size can exist even within the same colony,
- Oxidase: positive
- Catalase: positive

If the hanging drop method is used, *T. equigenitalis* is immobile and usually isolated. Its capsule cannot be revealed by the conventional India ink technique.

NB: biochemical tests can also be performed (e.g. API ZYM gallery, BioMérieux) allowing to observe positive results for acid and alkaline phosphatases.

3.4.2. CO_2 dependency test

The CO_2 dependency test is performed by inoculating two chocolate agar enriched with a polyvitamin supplement with the same saline or phosphate-buffer suspension of the colony to be identified. One chocolate agar plate is incubated in an atmosphere without CO_2 and the other in an atmosphere containing 5-10 % of CO_2 in air.

The test must also be performed from a control strain at the same time.

T. equigenitalis growth is CO_2-dependent: CO_2 requirement is revealed by the growth of bacteria on the agar plate incubated in a CO_2-rich atmosphere while the agar medium incubated in a normal atmosphere does not show any signs of growth or just a minimal culture.

3.4.3. Slide agglutination test

The agglutination test must be performed on colonies no more than 48 hours old.

If a commercial kit is used, follow the supplier's recommendations (e.g. BIONOR Monotayl).

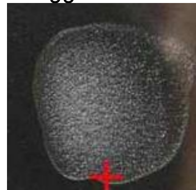
If an in-house *T. equigenitalis* specific antiserum (or if the EURL *T. equigenitalis* specific antiserum) is used, the test must be performed in parallel with:

- sterile saline solution and the *T. equigenitalis* specific antiserum,
- a control strain and the sample(s).

Agglutination must be interpreted within one minute.

No agglutination

Agglutination



NB: *T. asinigenitalis* strains are generally characterized by friable colonies (with a tendency to crumble when touched with a loop) very difficult to dissociate in saline solution. This characteristic must not be confused with autoagglutination or with a positive agglutination test using the *T. equigenitalis* specific antiserum.

4. VALIDATION AND INTERPRETATION OF RESULTS

4.1. VALIDATION OF THE ANALYTICAL PROCESS

The analytical process is validated when:
- the control strain(s) is(are) observed to grow,
- results comply with those expected for differentiation and identification tests on control strain(s).

4.2. INTERPRETATION OF RESULTS AFTER VERIFICATION OF THE COMPLIANCE OF SAMPLES TO BE TESTED

The compliance of each sample to be tested is based on observation of all chocolate agar plates. If all chocolate agar plates are:
- overgrown, the results will be uninterpretable and a set of new samples should be requested,
- sterile, "sterile culture" must be specified (outside uterus, the equine genital swabs shall not normally be sterile).

Characters indicating that the strain belongs to the *Taylorella* genus:
- slow growth at (37 ± 2)°C with 5-10% CO_2 in air => no growth visible with the naked eye in 24 to 48 h,
- colonies may be up to 2-3 mm in diameter, smooth with an entire edge, glossy and greyish,
- small Gram-negative coccobacillus (a shape polymorphism is possible),
- positive oxidase and catalase tests,
- CO_2-dependent.

Characters indicating that the strain belongs to the *T. equigenitalis* species:
- positive agglutination with a *T. equigenitalis* specific antiserum,
- detection of *T. equigenitalis* using MALDI-TOF MS.

4.3. DIFFERENTIAL DIAGNOSTIC

A result based on the conventional bacteriological tests may not be sufficient to differentiate *T. equigenitalis* from *T. asinigenitalis*.

It is strongly recommended to confirm the identification of *T. equigenitalis* using molecular testing methods such as PCR and real-time PCR.

An indirect immunofluorescence antibody test (IFAT) (e.g. Pourquier IFI *Taylorella equigenitalis* Pool of mAb) could also be used.

Furthermore, if the material is available in the laboratory, a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis could also unambiguously differentiate *T. equigenitalis* from *T. asinigenitalis*[1].

5. STORAGE CONDITIONS AND ELIMINATION OF SAMPLES

Store products and consumables in keeping with suppliers' recommendations.
Keep all control strains (e.g. in cryobeads) in a deep-freezer at ≤ - 65°C.
Keep all swab samples in a refrigerated unit at (5 ± 3)°C or a freezer at ≤ - 16°C until the results are sent.

[1] Petry S, Py JS, Wilhelm A, Duquesne F, Bäyon-Auboyer MH, Morvan H, et al. 2019. Evaluation of MALDI-TOF MS and a custom reference spectra expanded database for identification and differentiation of *Taylorella equigenitalis* and *Taylorella asinigenitalis*. Diagn Microbiol Infect Dis. 94:326-330