# OIE Procedure for Registration of Diagnostic Kits

## Abstract sheet

**Name of the diagnostic kit**: Pourquier® IIF *Taylorella equigenitalis*
**Manufacturer**: IDEXX Laboratories
**OIE Approval number**: 20160111
**Date of Registration**: May 2016

**Disease**: Contagious equine metritis

**Pathogen Agent**: *Taylorella equigenitalis*

**Type of Assay**: Indirect immunofluorescence test

**Purpose of Assay**: Certified by the OIE as fit for the detection of *T. equigenitalis* bacterial bodies from the swabs of the reproductive tract of stallions and mares for the following purposes:

1. Certify freedom from infection or agent in individual animals or products for trade or movement purposes;
2. Estimate prevalence of infection to facilitate risk analysis (surveys, herd health schemes or disease control);
3. Control of infection in stallions and mares at the start of the breeding season.

**Species and Specimen**: swabs of the reproductive tract of stallions and mares

### 1. Information on the kit

General information on the kit can be found on the IDEXX website at www.idexx.com.

### 2. Summary of validation studies

Analytical characteristics

*Repeatability*: results comparable with the culture method for special agar with and without charcoal

*Analytical specificity*: 100%

The tests are based on negative results of 18 pure cultures of bacterial strains other than T. equigenitalis which could be isolated from equine genital tract, 44 field isolates and one collection strain (CIP 107673T) of T. asinigenitalis.

*Analytical sensitivity*: 100%.

The tests are based on the detection of all 706 pure cultures of field isolates of T. equigenitalis (506 field isolates from France and 200 field isolates from Holland) and 4 T. equigenitalis reference strains (NCTC 11225, ATCC 35865, ERC 7810381 and ERC 78107419)

The limit of detection (LOD) was evaluated in comparison with two culture methods (chocolate agar and selective Agar) in a mixture of T. equigenitalis and Streptococcus zooepidemicus in two different transport media (Amies with and without charcoal). The LOD of IIF was slightly superior in transport medium without charcoal (dilution 1/130000 vs. 1/120000) and in comparison with the culture method IIF was slightly superior to chocolate agar medium (dilution 1/130000 vs. 1/100000) and slightly inferior to selective agar culture medium (dilution 1/130000 vs. 1/150000)

Diagnostic Characteristics

*Diagnostic sensitivity (DSn) and specificity (DSp) estimates*:

A. Field validation performed by OIE Reference Laboratory Netherlands (CVI Lelystad) (see section 3.3.6 A)

1) Validation based on individual horses

In the CVI validation study, from 730 horses tested, 12 horses were found positive by both culture and IDEXX IIF method.
DSn and DSp of IDEXX IIF kit on individual horses considering culture as gold standard test:

| **Test method under evaluation** | **IDEXX IIF** | **Target Species (Equine)** |
|---|---|---|
| **Diagnostic sensitivity (Isolation and identification)** | N<br>DSn<br>CI | **12**<br>**(100%)**<br>**95% confidence interval: 73.5%-100%)** |
| **Diagnostic specificity (Isolation and identification)** | N<br>DSp<br>CI | **718**<br>**(97.2%)**<br>**95% confidence interval: 95.7%-98.2%** |

2) Validation based on individual samples

A total of 2019 samples were tested by CVI and 1953 samples were found negative by both culture and IDEXX IIF test. The culture method yielded 19 positive samples and IIF method detected 65 samples as positive
DSn and DSp of IDEXX IIF kit on individual samples considering culture as gold standard test:

| **Test method under evaluation** | **IDEXX IIF** | **Target Species (Equine)** |
|---|---|---|
| **Diagnostic sensitivity (Isolation and identification)** | N<br>DSn<br>CI | **19**<br>**(94.7%)**<br>**95% confidence interval: 73.9%-99.8%** |
| **Diagnostic specificity (Isolation and identification)** | N<br>DSp<br>CI | **2000**<br>**(97.6%)**<br>**95% confidence interval: 96.8%-98.2%** |

B. Field validation performed in case of non-experienced operator (see section 3.3.6 B)

In the field validation, from 2019 samples tested, 3 samples were found positive by culture and negative by IDEXX IIF test. These 3 samples were examined a second time by the same operators and in two slides specific *Taylorella equigenitalis* elements were found. The discordant results between the first reading and the re-examination of these 2 slides are explained by an error of non-experienced operators who did not detect the specific *Taylorella equigenitalis* elements at the routine examination of slides. Because of the fact that selective re-examination of samples (discrepant or discordance analysis) induces a bias in estimates of sensitivity and specificity, we calculate the DSn and DSp of the field validation after the first reading of slides. In this particular case, because of the low number of positive animals, the diagnostic sensitivity drops from 94% to 84% for individual samples and from 100% to 83% for individual horses.

1) Validation based on individual horses

DSn and DSp of IDEXX IIF kit on individual horses considering culture as gold standard test (non-experienced operator):

| **Test method under evaluation** | **IDEXX IIF** | **Target Species (Equine)** |
|---|---|---|
| **Diagnostic sensitivity (Isolation and identification)** | N<br>DSn<br>CI | **12**<br>**(83.3%)**<br>**95% confidence interval: 51.5%-97.9)** |
| **Diagnostic specificity (Isolation and identification)** | N<br>DSp<br>CI | **718**<br>**(96.9%)**<br>**95% confidence interval: 95.4%-98.0%** |

2) Validation on individual samples

DSn and DSp of IDEXX IIF kit on individual samples considering culture as gold standard test (non-experienced operator):

| **Test method under evaluation** | **IDEXX IIF** | **Target Species (Equine)** |
|---|---|---|
| **Diagnostic sensitivity (Isolation and identification)** | N<br>DSn<br>CI | **19**<br>**(84.2%)**<br>**95% confidence interval: 60.4%-96.6%** |
| **Diagnostic specificity (Isolation and identification)** | N<br>DSp<br>CI | **2000**<br>**(97.5%)**<br>**95% confidence interval: 96.7%-98.1%** |

*Comparative performance:*

Field validation performed by OIE Reference Laboratory Netherlands (CVI Lelystad) on individual samples (see 3.3.8 A)

All samples found positive by either culture or IIF were tested by PCR. From 22 individual samples found positive by PCR, 21 were positive by IIF
DSn of IDEXX IIF on individual samples considering PCR as gold standard test

| **Test method of comparison** | | **Target Species (Equine)** |
|---|---|---|
| **Diagnostic sensitivity (real-time PCR)** | N<br>DSn<br>CI | **22**<br>**(95.4%)**<br>**95% confidence interval: 77.1%-99.8%** |
| **Diagnostic specificity** | N<br>DSp<br>CI | *ND*<br>*(DSp estimate)*<br>*(95% confidence interval)* |

Reproducibility

ANSES Dozulé (France) participates in some ring trials organized by one of the OIE laboratories for CEM (Animal Health Veterinary Laboratories Agency, GB). During the last session of June 2012 (report from the AHVLA dated July 6, 2012), the IDEXX IIF kit was used in parallel with the requested technique, bacteriology and PCR and ANSES obtained a 100% agreement with the requested technique.

The IDEXX kit was utilised for the ring trial in 2013 by 18 laboratories and in 2014 by 36 laboratories:

- In 2013 from 18 laboratories, 17 detected correctly all 6 positive swabs of the panel and one did not detect the highly diluted weak positive sample (100% correctly detected positive swabs)
- In 2014 from 36 laboratories, 34 detected correctly all 7 positive swabs of the panel and two laboratories did not detect the highly diluted weak positive sample (100% correctly detected positive swabs)

These results demonstrate a good reproducibility of the test to accurately detect the target organism even in a mixed bacterial flora which could be present in the reproductive tract of a mare and stallion

For more details see the file “Ring trial for MCE by IIF: ANSES report 2013-2014

Applications

2019 samples from 730 horses were tested with IDEXX IIF kit by CVI (Netherland) within the national screening program: export purposes (414 horses), screening (215 horses), certification artificial insemination service (91 horses) or for other reasons e.g. research/confirmation (10 horses).

In France, the IDEXX IIF kit has been used since 2013 principally for screening of stallions and mares at the start of breeding season (obligatory for “pure sang” and “trotteur français” races) and the movement of animals. All positive IIF animals should be confirmed by culture.

References

DA Gradinaru et al: Production and characterization of monoclonal antibodies against *Taylorella equigenitalis,* Vet Res (1997) 28, 65-76