OIE Procedure for Validation and Certification of Diagnostic Assays

Abstract sheet

Name of the diagnostic kit: Platelia Rabies II
Manufacturer: Bio-Rad
OIE Approval number: 20070101
Date of Registration: May 2007

Disease: Rabies

Pathogen Agent: Rabies virus

Type of Assay: The PLATELIA RABIES II kit is an *in vitro* diagnostic indirect ELISA test.

Purpose of Assay: Certified by the OIE in May 2007 as fit for purpose to determine immune status post-vaccination in individual dogs or cats (for regulation of international movement or trade) and in fox populations (for monitoring wildlife vaccination programmes).

Species and Specimen: Dog, cat and fox serum

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at rabies@bio-rad.com.

The quantitative results are expressed as ELISA Units per ml (EU/ml) which are equivalent to International Units as measured by virus neutralisation tests.

2. Summary of validation studies

STAGE 1 Validation

Calibration against the OIE international standard and determination of analytical sensitivity and specificity, repeatability (intra-assay, inter-assay and inter-lot) were completed during the Bio-Rad internal validation with the following results.

Analytical sensitivity	- lower limit of detection, 0.125 EU/ml
Analytical specificity	- not pertinent for this kit as there is no evidence from the literature of serological cross reactions to the rabies glycoprotein from closely related infections in the target species.
Intra-assay repeatability	- negative samples, 5.37 %CV - positive range of samples, 1.67-4.04 %CV
Inter-assay repeatability	- negative samples, 12.5 %CV - positive range of samples, 3.9-6.3 %CV
Inter-lot repeatability	- negative samples, 4.29-33.54 %CV (across species) - positive samples, 3.05-4.90 %CV (across species)

STAGE 2 Validation

Diagnostic specificity and sensitivity estimates were determined by both an internal Bio-Rad evaluation and an external study conducted by an OIE Reference Laboratory, both using cat, dog and fox samples. The PLATELIA RABIES II assay results were based on dog and cat samples of antibody status determined by the FAVN reference method described in the OIE *Terrestrial Manual* (5th edition) The antibody status of the fox samples was determined by a separate ELISA method used by the OIE Reference Laboratory involved in this study.

- Threshold determination

The cutoff value of the test was assigned in accordance with the recognised threshold of 0.5 IU/ml in neutralisation assays. The lower limit of detection of the PLATELIA RABIES II assay was determined to be 0.125 EU/ml.

- Diagnostic sensitivity (DSn) and specificity (DSp) estimates with 95% confidence limits (CI)

Internal evaluation study performed by Bio-Rad, France:

		DOGS	**CATS**	**FOXES**
Diagnostic sensitivity	N DSn CI	369 77.8 73.2-81.9	148 81.8 74.6-87.6	62 93.5 84.3-98.2
Diagnostic specificity	N DSp CI	197 99.5 97.2-99.9	55 98.2 90.3-99.9	145 96.5 92.1-98.9

External evaluation study performed by AFSSA Nancy, France:

		DOGS	**CATS**	**FOXES**
Diagnostic sensitivity	N DSn CI	396 88.6 85.1-91.6	98 89.8 82.0-95.0	93 88.2 79.8-93.9
Diagnostic specificity	N DSp CI	616 99.2 98.1-99.7	11 100 71.5-100	239 97.1 94.1-98.8

- Agreement between tests

Those samples showing discrepancies between the results of PLATELIA RABIES II and the reference methods are summarised as follows.

In the internal Bio-Rad study, 113/976 samples classed as having protective levels of antibody by the reference methods were below the protection threshold according to the PLATELIA RABIES II. Of these 56 were “borderline” with results in the range of 0.3-0.5 EU/ml in PLATELIA RABIES II.
Seven classed as below the protective level by the reference methods scored as protected according to the PLATELIA RABIES II, of which two were in the range 0.5-0.8 EU/ml.

In the external study at the OIE Reference Laboratory, 66/1453 samples classed as having protective levels of antibody by the reference methods were below the protection threshold according to the PLATELIA RABIES II. 27 of these were "borderline" with results in the range 0.3-0.5 EU/ml in PLATELIA RABIES II.
Twelve classed as below the protective level in the reference methods scored as protected according to the PLATELIA RABIES II, of which nine were in the range 0.5-0.8 EU/ml.

- Manufacturer's comment

The comparative studies between the PLATELIA RABIES II and the reference methods show that the majority of the discrepant results are found in "borderline" samples, with titres just above or below the cut-off value of 0.5 EU/ml.
The FAVN is based on a discontinuous method; in contrast the PLATELIA RABIES II is a continuous method. The reading of the FAVN can be difficult and the crossing from one dilution to another can lead to considerable variation in calculated titre. Furthermore, the FAVN results are subject to variation due to virus calibration, lot and experimental conditions.

STAGE 3 Validation

Interlaboratory comparisons were conducted on a panel of 20 samples at 5 different laboratories (Bio-Rad Company and 4 laboratories approved by the European Commission for performing rabies serological controls):
- 5 canine samples (3 from protected dogs + 2 from unprotected dogs)
- 5 feline samples (3 from protected cats + 2 from unprotected cats)
- 4 fox samples (3 from protected foxes + 1 from unprotected fox)
- 6 samples from a range of dilutions corresponding to 6 different dilutions of a pool of vaccinated dog sera.

These samples were titrated in three independent runs with the PLATELIA Rabies II according to the specifications of the kit. Each run included a negative control, 2 positive controls at 0.5 EU/ml and 2 ranges of dilutions.

This exercise was modelled on protocols organised annually by the EU Community Reference Laboratory – Afssa Nancy, for control of rabies serological assays performed by laboratories for international movement of pet animals.

The results demonstrated a highly reproducible performance with the PLATELIA RABIES II kit in the 5 selected laboratories. Whatever the origin of samples (dog, cat or fox), and for all categories of sample (high, medium, weak positive or negative), expected results were obtained by all participating laboratories. The linear regressions obtained for the dilution series ranging between 1.0 and 0.011 EU/ml were also reproducible.

Stage 4 Validation

This is an ongoing process. Testing laboratories should participate in proficiency testing and laboratory training programmes organized by OIE Reference Laboratories.

3. References:

1. CLIQUET F., L. SAGNE, J.L. SCHEREFFER, M.F.A. AUBERT; ELISA test for rabies antibody titration in orally vaccinated foxes sampled in the fields; Vaccine 18 (2000), 3272-3279
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of rabies virus-specific antibodies from vaccinated dogs and cats; J. of Vir. Methods 117 (2004) 1-8.

3. CLIQUET F., MÜELLER T., MUTINELLI F., GERONUTTI S., BROCHIER B., SELHORST T., SCHEREFFER J-L., KRAFFT N., BUROW J, SCHAMEITAT A., SCHLÜTER H., AUBERT M.; Standardisation and establishment of a rabies ELISA test in European laboratories for assessing the efficacy of oral fox vaccination campaigns; Vaccine 21 (2003) 2986-2993
4. FURNIER-CARUANA J., POIRIER B., HAOND G., JALLET C., FUCHS F., TORDO N, PERRIN P; Inactivated rabies vaccine control and release: use of an ELISA method; Biologicals 31 (2003); 9-16
5. ROOIJAKKERS E.J.M, UITTENBOGAARD J.P., GROEN J. AND OSTERHAUS A.D.M.E. Rabies vaccine potency control: comparison of ELISA systems for antigenicity testing; Journal of Virological Methods; 58 (1996); 111-119
6. Chapter 2.2.5., *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 2008, OIE