Bovine Brucellosis

Control of indirect ELISA kits

(Individual serum samples & pooled serum samples)

Standard Operating Procedure

SAFETY PRECAUTIONS

The laboratory shall take all precautions in order to guarantee the necessary safety, for both the operator and the environment, against the biological and chemical hazards due to the activities conducted according to this document.

1 Scope

The present document describes a standard technique aiming at controlling the fulfilment of OIE and EU requirements regarding the standardisation of indirect enzyme-linked immunosorbent assays (I-ELISA) kits for the detection of antibodies specific to smooth *Brucella* species (especially *B. abortus, B. melitensis* and *B. suis*) in bovine individual sera or pools of sera.

2 Normative references

- Bovine brucellosis, *In*: The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees), 2011, Chapter 2.4.3., OIE, Paris, *online version adopted by the World Assembly of Delegates of the OIE in May 2009*. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf
- Commission Decision of 10 December 2008 amending Annex C to Council Directive 64/432/EEC and Decision 2004/226/EC as regards diagnostic tests for bovine brucellosis (notified under document number C(2008) 7642) (Text with EEA relevance) (2008/984/EC), Official Journal of the European Union 31.12.2008, L 352/38-45.
- ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories.
- OIE Quality Standard and Guidelines for Veterinary Laboratories, 2nd Edition, 2008, *OIE, Paris.*

3 Definitions

- **Bovine brucellosis**
  Infection of bovines due to bacteria of the genus *Brucella*, naturally in the smooth (S) phase (*B. abortus*, *B. melitensis* or *B. suis*), pathogenic for most mammal species, in which this infection usually induces the production of S-*Brucella*-specific antibodies.
- **National Reference Laboratory for Brucellosis (NRL)**
  Laboratory officially designated as such by the authority of the corresponding Member State. According to the abovementioned Commission Decision, this NRL is responsible for:
  "(1) the approval of the results of the validation studies demonstrating the reliability of the test method used in the Member State;
  (2) determination of the maximum number of samples to be pooled in ELISA kits used;
  (3) calibration of working standards /.../;
  (4) quality checks of all antigens and ELISA kits batches used in the Member State;
  (5) following recommendations of, and cooperating with the EU reference laboratory for brucellosis. "

- **Official Control Laboratory (OCL)**

  Laboratory in charge of the official control of all antigens and ELISA kits batches used in the Member State (usually the Members State's NRL). This activity could be assigned to another laboratory, the NRL of another Member State, in particular.

- **International standard sera**

  International standard sera for Bovine brucellosis enzyme-linked immunosorbent assays (ELISAs) are[1]:

  - the weak positive OIE ELISA standard serum ($OIEELISA_{WP}SS$);
  - the strong positive OIE ELISA standard serum ($OIEELISA_{SP}SS$);
  - the negative OIE ELISA standard serum ($OIEELISA_{N}S$).

  The required limit of detection is the 1/16 dilution of the $OIEELISA_{SP}SS$ or the 1/2 dilution of the $OIEELISA_{WP}SS$, made up in negative serum.

  Dilutions 1/64 for the $OIEELISA_{SP}SS$ or 1/8 for the $OIEELISA_{WP}SS$ must be found negative.

- **Secondary or National standard serum**

  Secondary or national standard serum established against the abovementioned OIE standard sera and containing a defined concentration of anti-*Brucella* antibody corresponding to a defined activity in bovine brucellosis serum ELISAs.

- **Dilution**

  Initial pre-dilution of the serum made up in a dilution negative serum (7.5) before further appropriate dilution in the dilution buffer of the kit subject to control. Dilutions are made directly (no serial dilutions) and with an initial volume of neat serum of at least 10 µl.

  A minimum volume of 10 µl of the dilution is used to perform the final dilution in the dilution buffer of the kit subject to control.

4 Specific requirements before submission of a kit to control

Nil.

5 Sampling

The supplier or manufacturer sends the complete kits (microplates, reagents, final packaging, and instructions for use) to the OCL in sufficient quantity for the performance of all tests needed by the present procedure (initial control and/or control of a new batch, control of a batch during the validity period).

[1] Obtainable from the OIE Reference Laboratory for Brucellosis at AHVLA Weybridge, Addlestone, Surrey KT15 3NB, UK. Due to the limited stock left, the OIEISS should be restricted to the control of conventional antigens (RBT, CFT, SAT, MRT in particular) and must not be used for the control of ELISA kits.

6 Storage and disposal of batch samples

According to the instructions of the supplier/manufacturer during the validity period.

7 Standard sera and Reference materials

These materials are stored at a temperature < -16℃ or freeze-dried and stored at 5°C ± 3℃.

7.1 International standard sera (OIEELISA$_{WP}$SS or OIEELISA$_{SP}$SS and OIEELISA$_{N}$SS)

7.2 Secondary or national standard sera (at least the positive standard)

7.3 Reference panel of positive sera

Bovine serum samples issued from animals naturally or experimentally infected by smooth *Brucella* (*B. abortus*, *B. melitensis* or *B. suis*). These sera must be chosen in such a way that the reaction obtained is slightly above the cut-off.

7.4 Reference panel of negative sera

Bovine serum samples issued from brucellosis free animals. Some of these sera may present characteristics known as interfering with the reaction (background for instance).

7.5 Dilution Negative serum (DNS)

A pool of at least 3 negative bovine serum samples is recommended.

7.6 Reference serum for the measure of repeatability

Dilution of a bovine positive serum made up in the DNS (7.5) in such a manner that it shows a titre comparable or just above the required level of detection.

Note: materials 7.2-7.6 must be made available to any kit supplier/manufacturer on request.

8 Principle

8.1 Initial Control

This control is performed on the first batch of a new ELISA kit, submitted to the OCL for approval, once the validation dossier has been submitted by the supplier/manufacturer to the NRL and has been approved by the latter as fulfilling the OIE requirements[2].

The OCL is to check the following parameters:

[2] Chapter 1.1.4/5. Principles and methods of validation of diagnostic assays for infectious diseases. *Online version adopted in May 2009*. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.04_VALID.pdf

8.1.1 Limit of detection (LOD)

8.1.1.1 ELISA kits for individual sera:

- dilutions at 1/8, 1/16, 1/32 and 1/64 of the OIEELISA$_{SP}$SS or,

- dilutions at 1/1, 1/2, 1/4 and 1/8 of the OIEELISA$_{WP}$SS or,

- appropriate equivalent dilutions of the national or secondary bovine brucellosis standard serum (7.2),

tested twice per plate in two plates.

8.1.1.2 ELISA kits for pools of X sera:

- dilutions identical to the abovementioned dilutions, but further diluted in DNS (7.5) at 1/X and tested twice per plate in two plates.

8.1.2 Sensitivity

8.1.2.1 ELISA kits for individual sera:

- Five positive sera from the Reference panel of positive sera (7.3) tested twice per plate in two plates.

8.1.2.2 ELISA kits for pools of X sera:

- Five positive sera from the Reference panel of positive sera (7.3) diluted 1/X in DNS (7.5) and tested twice per plate in two plates.

8.1.3 Specificity

8.1.3.1 ELISA kits for individual sera:

- Five negative sera from the Reference panel of negative sera (7.4) tested twice per plate in two plates.

8.1.3.2 ELISA kits for pools of X sera:

- Five negative sera from the Reference panel of negative sera (7.4) diluted 1/X in DNS (7.5) and tested twice per plate in two plates.

8.1.4 Repeatability

Reference serum for the measure of repeatability (7.6) tested at least 20 times per plate in two plates; the same preparation of the serum dilution is distributed in each well with the same mono-channel pipette for all the wells used.

8.2 Control of a new batch

This control is performed by the OCL on samples of any new batch to be approved in the corresponding Member State and is identical to the initial control described above (8.1)

8.3 Control of a batch during the validity period

This control is performed by the OCL in the middle of the validity period of the product.

The OCL may decide not to perform this control, if the supplier/manufacturer provides the results of a corresponding internal control.

Otherwise, the OCL can decide to perform this control, if needed, especially according to information from the routine diagnostic laboratories.

The following parameters are checked:

- LOD
- Sensitivity
- Specificity
-Repeatability

9 Equipment and plastic/glass ware

Conventional serology laboratory equipment.

10 Operating procedure

The OCL must follow the instructions for use of the respective kit. Nevertheless, the positive and negative control sera of the kit may be tested in the plates more times than those prescribed in the instructions for use of the corresponding kit.

The OCL can decide to use the minimal or the maximal values of time and temperature ranges prescribed by the instructions for use.

The initial control and the control of a new batch are performed at least in two plates.

The control during the validity period is performed at least in one plate.

11 Interpretation of results

The criteria for the interpretation of results are identical for the initial control, the control of a new batch and the control during the validity period.

11.1 LOD

- a 1/2 dilution of the OIEELISA$_{WP}$SS or a 1/16 pre-dilution of the OIEELISA$_{SP}$SS made up in DNS must give a positive reaction;

- a 1/8 dilution of the OIEELISA$_{WP}$SS or a 1/64 pre-dilution of the OIEELISA$_{SP}$SS made up in DNS must give a negative reaction.

11.2 Sensitivity

All five positive sera from the Reference panel of positive sera (7.3) must give a positive reaction.

11.3 Specificity

All five negative sera from the Reference panel of negative sera (7.3) must give a negative reaction.

11.4 Repeatability

The CV of the OD values obtained for the 20 repeats of the test of the Reference serum for the measure of repeatability (7.6) must be less than (or equal to) 10 % in each plate.

12 Restitution of results

The OCL provides the supplier/manufacturer with the detailed results corresponding to the tests described in chapter 8, and, in particular, the OD values obtained and the template followed for the different plates.

13 Analysis report

The analysis report must comply with the requirements of ISO/IEC 17025.

The report clearly mentions whether the kit complies or not with the acceptability criteria.