# 1st Bovine Brucellosis Interlaboratory Proficiency Test in Asia-Pacific in 2013

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## Background

FAO-RAP, the Animal Production and Health Commission for Asia and the Pacific (APHCA) and OIE-RRAP jointly organized the "FAO-APHCA/OIE Regional Workshops on Brucellosis Diagnosis and Control – with an emphasis on Brucella melitensis" in the Asia and Pacific Region in collaboration with the Department of Livestock Development (DLD) of Thailand from 2008 to 2010 (3 workshops). It was agreed upon that there was a need to establish a regional laboratory network. This important issue has been well considered through the framework of the OIE Twinning Programme on Brucellosis between the EU/OIE/FAO Reference Laboratory for Brucellosis in France and the Department of Livestock Development – National Institute of Animal Health (DLD-NIAH) of Thailand.

## Materials and Methods

The first "2013 Asia-Pacific Bovine Brucellosis Interlaboratory Proficiency Test" was prepared (discussion and preparation of panels of 17 sera after selection of the sera, and homogeneity/stability testing) from January to July 2013 under the ANSES/NIAH OIE Twinning Programme, together with FAO-APHCA and OIE-RRAP.

Announcement and information were delivered during the 37th Session of APHCA in September 2013. Expected/ accepted results were defined according to ANSES-NIAH preliminary testing, taking into account the results of participants as well.

Qualitative, semi-quantitative and quantitative results, sensitivity, specificity and repeatability, as well as coherence between dilutions of the same serum were analysed for the assessment of the performance of each participating laboratory.

## Results and discussion

Thirty-three laboratories participated to this ILPT, including laboratories from 17 Asia-Pacific countries, 8 DLD's Regional Veterinary Research and Development Centres, 1 Government enterprise, 4 Universities and 2 Private companies in Thailand and ANSES.

The shipment of sera to laboratories was made via several means and routes, but had apparently no effect on sample quality.

Most of the results were returned in due time 28/33 labs (84.8%). Error of sample identification occurred for 0.36% (2/561) samples.

## The choice of sera

The selection of the samples was conducted taking into account the need of a variety of positive and negative samples. These dilutions were chosen owing to the results obtained:

- Four serum with positive results in every methods (RBT, CFT and I-ELISA).
- One serum with positive results in I-ELISA, but negative in RBT and CFT.
- Two negative sera from officially free animals.

**Table 1:** Preliminary results of selected sera

| Sample | Selected serum | Dilutions | No of repetition | Obtained Results: Qualitative RBT | Semi-quantitative CFT | Quantitative I-ELISA |
|---|---|---|---|---|---|---|
| Positive serum diluted in negative pooled sera | TK 44/43 | 1/10 | 2 | Strong positive | Strong positive | Strong positive |
| | TK 111/44 | 1/20 | 2 | Strong positive | Strong positive | Strong positive |
| | TK 111/44 | 1/30 | 3 | Positive | Positive | Positive |
| | TK 111/44 | 1/40 | 3 | Positive | Positive | Positive |
| | TK 111/44 | 1/200 | 3 | Negative | Negative | Positive |
| Negative serum | N 1 | - | 2 | Negative | Negative | Negative |
| | N 2 | - | 2 | Negative | Negative | Negative |

## Rose Bengal test (RBT)

Thirty-two laboratories performed RBT. The same antigens were used among all participants.
The results obtained by the laboratories were very satisfactory, 29 laboratories got the expected results with a few results not meeting the expected points (Table 2, 3, 4 and 5).

**Table 2:** Expected and accepted results in RBT

| Sample level | Expected results (no assessment of the semi-quantitative results) | Accepted results |
|---|---|---|
| 1 | Negative | Negative/Positive |
| 2 | Positive | Positive/Negative |
| 3 | Positive | Positive |
| 4 | Positive | Positive |
| 5 | Positive | Positive |
| 6 | Negative | Negative |
| 7 | Negative | Negative |

**Table 3:** Occurrence of positive and negative results obtained for RBT

| | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 | Level 6 | Level 7 |
|---|---|---|---|---|---|---|---|
| Expected results | N | P | P | P | P | N | N |
| Occurrence of (P/N) results | 2/30 | 30/2 | 31/1 | 32/0 | 32/0 | 1/31 | 2/30 |

**Table 4:** Qualitative results of participating laboratories from Asia-Pacific countries to RBT

| | | Expected results | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lab code | Antigen | Level 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 | 7 | 7 |
| 1 | Confidential | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 2 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 3 | | | Ag A | N | N | N | P | N | P | P | P | P | P | P | N | P | N | N | N | N |
| 4 | | | Ag A | N | N | N | P | N | N | P | P | P | P | P | P | P | N | N | N | N |
| 5 | | | Ag A | N | N | N | N | N | N | P | P | P | P | P | P | P | N | N | N | N |
| 6 | | | Ag A | N | N | N | N | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 7 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 8 | | | Ag A | N | N | N | N | N | P | N | N | N | P | P | P | P | N | N | N | N |
| 9 | | | Ag A | N | N | N | P | N | P | P | P | P | P | P | P | P | N | N | N | P |
| 10 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 11 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 12 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 13 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 14 | | | Ag A | N | P | P | P | P | P | P | P | P | P | P | P | P | N | N | P | P |
| 15 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 16 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 17 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 18 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 19 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 20 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 21 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 22 | | | Ag A | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 23 | | | Ag A | N | N | N | P | N | P | P | P | P | P | P | P | P | N | N | N | N |
| 24 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 25 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 26 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 27 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 28 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 29 | | | Ag A | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 30 | | | Ag A | N | N | N | N | N | N | N | P | N | P | P | P | P | N | N | N | N |
| 31 | | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 32 | | | Ag A | N | P | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |

**Table 5:** Semi-quantitative results of participating laboratories from Asia-Pacific countries to RBT

| | | Expected results | N | | | P | | | P | | | Strong Pos | | Strong Pos | | N | | N | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lab code | Antigen A | Level 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 | 7 | 7 |
| 1 | Confidential | | A | 0 | 0 | 0 | ++ | + | ++ | + | ++ | ++ | ++ | ++ | +++ | + | 0 | 0 | 0 | 0 |
| 2 | | | A | 0 | 0 | 0 | ++ | ++ | ++ | +++ | +++ | +++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 3 | | | A | 0 | 0 | 0 | + | - | + | + | ++ | ++ | +++ | +++ | - | + | 0 | 0 | 0 | 0 |
| 4 | | | A | 0 | 0 | 0 | n/a | - | - | n/a | n/a | n/a | n/a | n/a | n/a | n/a | 0 | 0 | 0 | 0 |
| 5 | | | A | 0 | 0 | 0 | - | - | - | ++ | ++ | ++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 6 | | | A | 0 | 0 | 0 | - | + | + | + | + | ++ | ++ | ++ | ++ | ++ | 0 | 0 | 0 | 0 |
| 7 | | | A | 0 | 0 | 0 | + | + | ++ | ++ | ++ | ++ | ++ | ++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 8 | | | A | 0 | 0 | 0 | - | - | n/a | - | - | - | n/a | n/a | n/a | n/a | 0 | 0 | 0 | 0 |
| 9 | | | A | 0 | 0 | 0 | + | - | + | + | + | + | + | + | + | + | 0 | 0 | 0 | + |
| 10 | | | A | 0 | 0 | 0 | + | + | + | + | + | + | + | + | + | + | 0 | 0 | 0 | 0 |
| 11 | | | A | 0 | 0 | 0 | ++ | ++ | ++ | +++ | +++ | +++ | ++++ | ++++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 12 | | | A | 0 | 0 | 0 | + | ++ | ++ | +++ | +++ | +++ | ++++ | +++ | +++ | ++++ | 0 | 0 | 0 | 0 |
| 13 | | | A | 0 | 0 | 0 | + | + | + | +++ | +++ | +++ | ++++ | +++ | ++ | ++++ | 0 | 0 | 0 | 0 |
| 14 | | | A | 0 | + | + | ++ | ++ | ++ | +++ | +++ | +++ | +++ | ++++ | ++++ | ++++ | 0 | 0 | + | + |
| 15 | | | A | 0 | 0 | 0 | + | + | + | ++ | ++ | ++ | ++ | ++ | +++ | ++ | 0 | 0 | 0 | 0 |
| 16 | | | A | 0 | 0 | 0 | + | + | + | ++ | ++ | ++ | +++ | +++ | +++ | ++ | 0 | 0 | 0 | 0 |
| 17 | | | A | 0 | 0 | 0 | + | ++ | ++ | ++ | +++ | +++ | ++++ | ++++ | +++ | ++ | 0 | 0 | 0 | 0 |
| 18 | | | A | 0 | 0 | 0 | + | + | + | ++ | ++ | ++ | +++ | +++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 19 | | | A | 0 | 0 | 0 | + | ++ | + | ++ | ++ | ++ | ++ | ++ | +++ | ++ | 0 | 0 | 0 | 0 |
| 20 | | | A | 0 | 0 | 0 | + | + | + | ++ | ++ | ++ | ++ | ++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 21 | | | A | 0 | 0 | 0 | ++ | ++ | ++ | ++ | +++ | ++ | +++ | +++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 22 | | | A | + | + | + | + | + | + | ++ | ++ | ++ | ++++ | ++++ | +++ | +++ | + | + | + | + |
| 23 | | | A | 0 | 0 | 0 | + | - | + | ++ | ++ | ++ | ++ | ++ | +++ | +++ | 0 | 0 | 0 | 0 |
| 24 | | | A | 0 | 0 | 0 | ++ | ++ | + | +++ | +++ | +++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 25 | | | A | 0 | 0 | 0 | ++ | ++ | + | +++ | +++ | +++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 26 | | | A | 0 | 0 | 0 | + | + | + | + | + | + | + | + | + | + | 0 | 0 | 0 | 0 |
| 27 | | | A | 0 | 0 | 0 | ++ | ++ | + | ++ | + | ++ | +++ | ++++ | +++ | + | 0 | 0 | 0 | 0 |
| 28 | | | A | 0 | 0 | 0 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 29 | | | A | + | + | + | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | 0 | 0 | 0 | 0 |
| 30 | | | A | 0 | 0 | 0 | - | - | - | - | + | - | ++ | ++ | + | + | 0 | 0 | 0 | 0 |
| 31 | | | A | 0 | 0 | 0 | + | + | + | ++ | ++ | ++ | +++ | +++ | ++ | ++ | 0 | 0 | 0 | 0 |
| 32 | | | A | 0 | ++ | 0 | ++ | ++ | ++ | ++++ | ++++ | ++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 |

## Complement fixation test

Fifteen laboratories participated in CFT. Five different reagents were used among all laboratories.

The expected and accepted results in the panel and occurrence of positive and negative results obtained for CFT are shown in Tables 6 and 7. The qualitative results were examined in comparison with the results obtained by the majority of participants and the preliminary tests (Table 8).

Overall results were very good. Technical failures were noticed for only 1 laboratory with unsatisfactory results.

**Table 6:** Expected and accepted results in CFT

| Sample level | Expected results Qualitative results Titre | Accepted results Qualitative results Titre |
|---|---|---|
| 1 | Negative 0-<20 ICFTU/mL | Negative with a weak titre |
| 2 and 3 | Positive ≥20 ICFTU/mL | Positive titre close to cut-off |
| 4 and 5 | Positive >20 ICFTU/mL | Positive |
| 6 and 7 | Negative 0 | Negative 0-<16.6 |

**Table 7:** Occurrence of positive and negative results obtained for CFT

| | level 1 | level 2 | level 3 | level 4 | level 5 | level 6 | level 7 |
|---|---|---|---|---|---|---|---|
| Expected results | N | P | P | P | P | N | N |
| Occurrence of (P/N) results | 0/15 | 14/1 | 14/1 | 14/1 | 14/1 | 0/15 | 0/15 |

**Table 8:** Qualitative results of participating laboratories from Asia-Pacific countries to CFT

| | | Expected results | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lab code | Antigen | Level 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 | 7 | 7 |
| 1 | Confidential | | Ag A | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 2 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 3 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 4 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 5 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 6 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 7 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 8 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 9 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 10 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 11 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 12 | | | Ag B | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 13 | | | Ag C | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 14 | | | Ag D | N | N | N | P | N | P | P | P | P | P | P | P | P | N | N | N | N |
| 15 | | | Ag E | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

## Indirect-ELISA

Nine laboratories performed the I-ELISA test. Five different reagents were used by all participants: 2 home -made kits and 3 kits from commercial suppliers.

Expected results were based on the distribution of qualitative results obtained by the ANSES-NIAH and participating Laboratories (Table 9). The discrepancies between expected and obtained qualitative results have been observed for 5 laboratories (Table 10).

**Table 9:** Expected and accepted results in I-ELISA on the panel

| Sample level | Expected Results Qualitative | Accepted Results Qualitative |
|---|---|---|
| 1 | Positive | Positive |
| 2 | Positive | Positive |
| 3 | Positive | Positive |
| 4 | Positive | Positive |
| 5 | Positive | Positive |
| 6 | Negative | Negative |
| 7 | Negative | Negative |

**Table 10:** Qualitative results of participating laboratories from Asia-Pacific countries to I-ELISA

| | | Expected results | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lab code | Kit supplier | Level 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 | 7 | 7 |
| 1 | Confidential | | Home made-1 | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 2 | | | Home made-2 | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 3 | | | Kit A | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 4 | | | Kit A | P | N | P | N | N | N | P | N | N | N | N | N | N | N | N | N | N |
| 5 | | | Kit A | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 6 | | | Kit B | P | P | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 7 | | | Kit B | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 8 | | | Kit B | P | P | P | P | P | P | P | P | P | P | P | P | P | N | N | N | N |
| 9 | | | Kit C | N | N | N | P | P | P | P | P | P | P | P | P | P | N | N | N | N |

## Conclusion

Overall results were very good for a first proficiency test. All results were presented and discussed during "The 4th FAO-APHCA/ OIE/DLD Regional Workshop on Brucellosis Diagnosis and Control in Asia-Pacific Region – Proficiency Test and Ways Forward", organized in Chiang Mai, Thailand, on 19-21 March 2014. For the laboratory that faced troubles during this ring-trial, the protocol followed and/or the quality of the antigen was certainly in cause.

Despite the overall good results obtained, there is a real need of follow-up and/or refreshing training for several Asia-Pacific laboratories and the improvement expected in the test performance should be evaluated in a next ILPT.

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