1st Proficiency Test Management and Result-analysis

ANSES & NIAH-DLD

Background

- **The 1st FAO-APHCA/OIE Regional Workshop on Brucellosis Diagnosis and Control with an Emphasis on *Brucella melitensis,* Chiang Mai, 2008**
  - **requested a follow-up workshop/training**
  - **continued for capacity building of national Veterinary Services in the Asia and Pacific region for development of a long-term control programme of the disease supported by international agencies**

- **The 2nd FAO-APHCA/OIE Regional Workshop, 2009**
  - **NIAH provided RBT antigens for stimulate activity on serological test**
  - **Next step will focus on quality assurance and strengthening of Regional collaboration on diagnosis and control of Brucellosis.**

- The 3rd FAO-APHCA/OIE Regional Workshop on Brucellosis Diagnosis

- The member countries utilized the knowledge and techniques acquired from the workshops to improve/strengthen their laboratory diagnosis capacity .

- Under the OIE Twinning Programme, the participants requested for support on Brucellosis proficiency testing to member countries in the region.

Background

- The first Asia-Pacific Bovine Brucellosis Proficiency Test 2013 was started under supervision of Dr. Bruno Garin-Bastuji
- Discussion was held during Prince Mahidol Award 28-31 January 2013

  (Dr. Bruno, Dr. Vishnu and NIAH)
- Panels of sera preparation: July 2013

  (under Twinning programme & FAO-APHCA)

Panel Preparation

Sample	Serum	Dilution	Results obtained			Samples	Replicates
			RBT	CFT	I-ELISA	Ident.	
Positive serum diluted in negative pooled sera	44/33	1/10	Strong Pos	Strong Pos	Strong Pos	5	2
	111/44	1/20	Strong Pos	Strong Pos	Strong Pos	4	2
		1/30	Pos	Pos	Pos	3	3
		1/40	Pos	Pos	Pos	2	3
		1/200	Neg	Neg	Pos	1	3
Negative	N1	-	Neg	Neg	Neg	6	2
	N2	-	Neg	Neg	Neg	7	2

- Selection of the sera: Negative and dilution of positive (ANSES & NIAH)
- Panel of 17 sera (in duplicate or triplicate to asses the repeatability)
- Homogeneity/stability testing for validation

Panel Preparation

- Homogeneity testing (July 2013, before shipment)
  - 9 samples of low titer level
  - 6 of negative and strong positive level
  - 2 technicians ANSES/ 3 technicians NIAH in repeatability conditions (in NIAH lab.)
- Stability testing
  - 3 samples / condition and per level (only 2 for N and SP), 3 NIAH technicians
  - 5 conditions : D_0, D_4 & D_8 (RT), D_8 (fridge, parcel + ice pack at RT)

→ Validation criteria : RBT (one +), CFT (one dilution), I-ELISA IDEXX (CV<10% index when OD>50%)

→ Definition of expected results

Organization

- Calendar: 1st Asia-Pacific PT Bovine Brucellosis
  - Panels preparation-July 2013
  - Announcement and information: September 2013
  - Shipment of samples: September-December 2013
    - **By hand**
    - By Courier
    - By train

    (FAO-APHCA & OIE-RRAP & DLD)
- Results: in due time (15 days after arrival) 84%
- Participants 34 labs (results 33 Labs)
  - Asia-pacific countries 18 countries

    results returned 17 labs
    - Regional VRDCs-NIAH 8 labs
    - Government enterprise 1 lab
    - Universities 4 labs
    - Private companies 2 labs
    - ANSES

Analysis

- Expected/accepted results were defined
  - According to results of participants, NIAH preliminary testing and ANSES results
- Analysis performed on qualitative and semi-quantitative and quantitative results sensitivity, specificity and repeatability, coherence between dilutions of the same serum assessed
- Tests participation

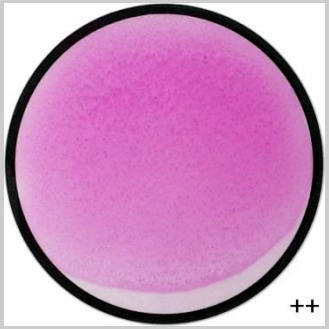
RBT	CFT	I-ELISA
32	15	9

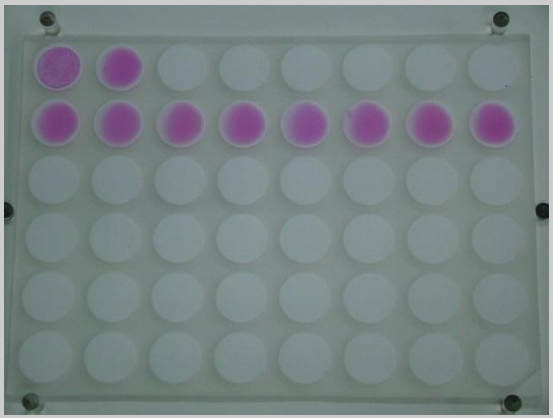
Antigens and test kits

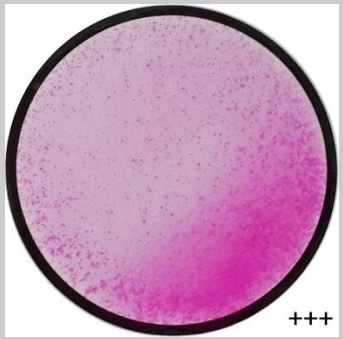
Suppliers	RBT	CFT	I-ELISA
NIAH	X		
VRI		X	
Antifix		X	
BGA Berlin		X	
IDEXX	X	X	
Biokombinat-Mongolia		X	
Kit-1 IDEXX			X
Kit-2 IDEXX			X
Syanovir			X
Home made-1			X
Home made-2			X

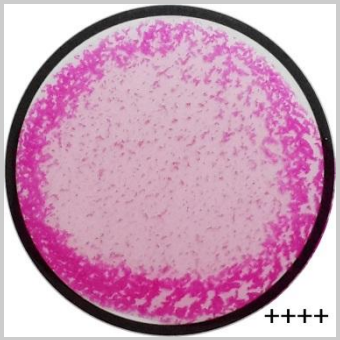
PT-Results

Rose Bengal Test









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	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	P	P	P	P	P	P	P	P	P	N	N	N	N
4	Ag A	N	N	N	P	N	P	P	P	P	P	P	N	P	N	N	N	N
5	Ag A	N	N	N	P	N	N	P	P	P	P	P	P	P	N	N	N	N
7	Ag A	N	N	N	N	N	N	P	P	P	P	P	P	P	N	N	N	N
8	Ag A	N	N	N	N	P	N	P	P	P	P	N	P	P	N	N	N	N
9	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
10	Ag A	N	N	N	N	N	P	N	N	N	P	P	P	P	N	N	N	N
11	Ag A	N	N	N	P	N	P	P	P	P	P	P	P	P	N	N	N	P
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	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
15	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
16	Ag A	N	P	P	P	P	P	P	P	P	P	P	P	P	N	N	P	P
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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
23	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
24	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	Ag A	N	N	N	P	N	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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
32	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
34	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
35	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
36	Ag A	N	N	N	N	N	N	N	P	N	P	P	P	P	N	N	N	N
37	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
38	Ag A	N	P	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N

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	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	P	P	P	P	P	P	P	P	P	N	N	N	N
4	Ag A	N	N	N	P	N	P	P	P	P	P	P	N	P	N	N	N	N
5	Ag A	N	N	N	P	N	N	P	P	P	P	P	P	P	N	N	N	N
7	Ag A	N	N	N	N	N	N	P	P	P	P	P	P	P	N	N	N	N
8	Ag A	N	N	N	N	P	N	P	P	P	P	N	P	P	N	N	N	N
9	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
10	Ag A	N	N	N	N	N	P	N	N	N	P	P	P	P	N	N	N	N
11	Ag A	N	N	N	P	N	P	P	P	P	P	P	P	P	N	N	N	P
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	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
15	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
16	Ag A	N	P	P	P	P	P	P	P	P	P	P	P	P	N	N	P	P
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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
23	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
24	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	Ag A	N	N	N	P	N	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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
32	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
34	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
35	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
36	Ag A	N	N	N	N	N	N	N	P	N	P	P	P	P	N	N	N	N
37	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
38	Ag A	N	P	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N

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	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	P	P	P	P	P	P	P	P	P	N	N	N	N
4	Ag A	N	N	N	P	N	P	P	P	P	P	P	N	P	N	N	N	N
5	Ag A	N	N	N	P	N	N	P	P	P	P	P	P	P	N	N	N	N
7	Ag A	N	N	N	N	N	N	P	P	P	P	P	P	P	N	N	N	N
8	Ag A	N	N	N	N	P	N	P	P	P	P	N	P	P	N	N	N	N
9	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
10	Ag A	N	N	N	N	N	P	N	N	N	P	P	P	P	N	N	N	N
11	Ag A	N	N	N	P	N	P	P	P	P	P	P	P	P	N	N	N	P
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	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
15	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
16	Ag A	N	P	P	P	P	P	P	P	P	P	P	P	P	N	N	P	P
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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
23	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
24	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	Ag A	N	N	N	P	N	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	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
32	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
34	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
35	Ag A	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
36	Ag A	N	N	N	N	N	N	N	P	N	P	P	P	P	N	N	N	N
37	Ag A	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
38	Ag A	N	P	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N

RBT

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	A	0	0	0	++	+	++	+	++	++	++	++	+++	+	0	0	0	0
3	A	0	0	0	++	++	++	+++	+++	+++	++++	++++	++++	++++	0	0	0	0
4	A	0	0	0	+	-	+	+	++	++	+++	+++	-	+	0	0	0	0
5	A	0	0	0	n/a	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0	0	0	0
7	A	0	0	0	-	-	-	++	++	++	++++	++++	++++	++++	0	0	0	0
8	A	0	0	0	-	+	-	+	+	++	++	-	++	++	0	0	0	0
9	A	0	0	0	+	+	++	++	++	++	++	++	+++	+++	0	0	0	0
10	A	0	0	0	-	-	n/a	-	-	-	n/a	n/a	n/a	n/a	0	0	0	0
11	A	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	+	++	++	+++	+++	+++	++++	+++	+++	++++	0	0	0	0
15	A	0	0	0	+	+	+	+++	+++	+++	++++	+++	++	++++	0	0	0	0
16	A	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	0	0	0	+	+	+	++	++	++	++	++	+++	+++	0	0	0	0
23	A	0	0	0	++	++	++	++	+++	++	+++	+++	+++	+++	0	0	0	0
24	A	+	+	+	+	+	+	++	++	++	++++	++++	+++	+++	+	+	+	+
25	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	0	0	0	+	+	+	+	+	+	+	+	+	+	0	0	0	0
32	A	0	0	0	++	++	+	++	+	++	+++	++++	+++	+	0	0	0	0
34	A	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	0	0	0	0
35	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
36	A	0	0	0	-	-	-	-	+	-	++	++	+	+	0	0	0	0
37	A	0	0	0	+	+	+	++	++	++	+++	+++	++	++	0	0	0	0
38	A	0	++	0	++	++	++	++++	++++	++	++++	++++	++++	++++	0	0	0	0

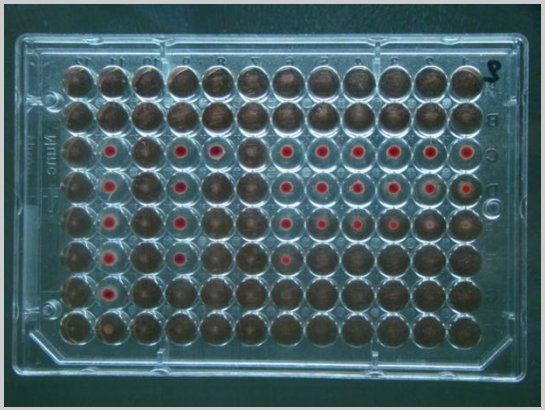
Semi-quantitative results of participating laboratories from Asia-Pacific countries to RBT

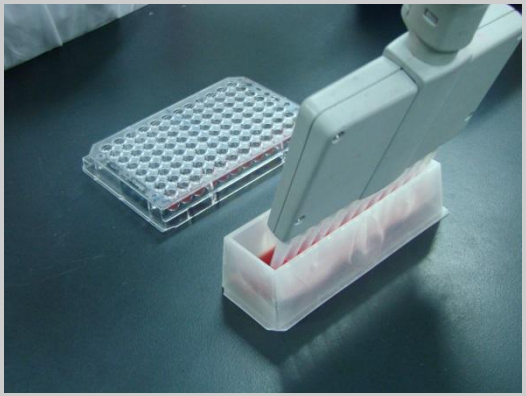
Expected results		N			P			P			Strong Pos		Strong Pos		N		N	
Lab code	Antigen A	Level 1	1	1	2	2	2	3	3	3	4	4	5	5	6	6	7	7
1	A	0	0	0	++	+	++	+	++	++	++	++	+++	+	0	0	0	0
3	A	0	0	0	++	++	++	+++	+++	+++	++++	++++	++++	++++	0	0	0	0
4	A	0	0	0	+	-	+	+	++	++	+++	+++	-	+	0	0	0	0
5	A	0	0	0	n/a	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0	0	0	0
7	A	0	0	0	-	-	-	++	++	++	++++	++++	++++	++++	0	0	0	0
8	A	0	0	0	-	+	-	+	+	++	++	-	++	++	0	0	0	0
9	A	0	0	0	+	+	++	++	++	++	++	++	+++	+++	0	0	0	0
10	A	0	0	0	-	-	n/a	-	-	-	n/a	n/a	n/a	n/a	0	0	0	0
11	A	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	+	++	++	+++	+++	+++	++++	+++	+++	++++	0	0	0	0
15	A	0	0	0	+	+	+	+++	+++	+++	++++	+++	++	++++	0	0	0	0
16	A	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	0	0	0	+	+	+	++	++	++	++	++	+++	+++	0	0	0	0
23	A	0	0	0	++	++	++	++	+++	++	+++	+++	+++	+++	0	0	0	0
24	A	+	+	+	+	+	+	++	++	++	++++	++++	+++	+++	+	+	+	+
25	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	0	0	0	+	+	+	+	+	+	+	+	+	+	0	0	0	0
32	A	0	0	0	++	++	+	++	+	++	+++	++++	+++	+	0	0	0	0
34	A	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	0	0	0	0
35	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
36	A	0	0	0	-	-	-	-	+	-	++	++	+	+	0	0	0	0
37	A	0	0	0	+	+	+	++	++	++	+++	+++	++	++	0	0	0	0
38	A	0	++	0	++	++	++	++++	++++	++	++++	++++	++++	++++	0	0	0	0

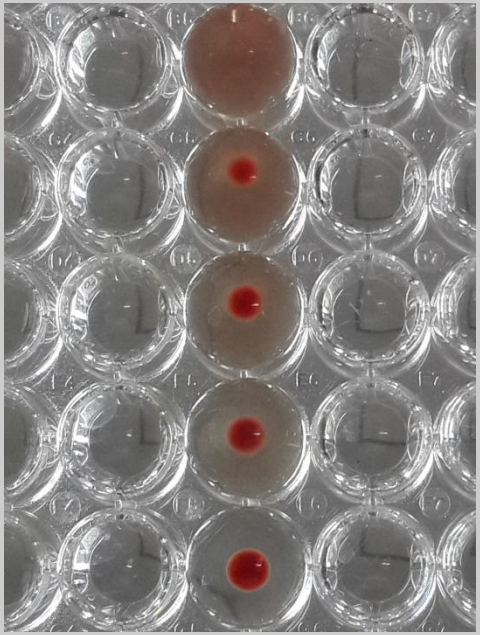
RBT

- Excess/lack of sensitivity : volume, temperature, reaction time, light, reading
- Lack of specificity : reading, protocol
- Repeatability : volume, homogenization
- Traceability
- Assessing “quantitative” agglutination

Complement fixation test







CFT

Interpretation of International complement fixation test units milliliter (ICFTU/mL)

Serum Dilution	Haemolysis inhibition			
	25% (+)	50% (++)	75% (+++)	100% (++++)
1/2	8.33	10	11.67	13.33
1/4	16.67	20*	23.33	26.67
1/8	33.33	40	46.67	53.33
1/16	66.67	80	93.33	106.67
1/32	133.33	160	187	213.33
1/64	266.67	320	373.33	426.67
1/128	533.33	640	746.67	853.33
1/256	1,066.67	1,280	1,493.33	1,706.67
1/512	2,133.34	2,560	2,986.66	3,413.34
1/1,024	4,266.68	5,120	5,973.32	6,826.68

CFT

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
8		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
9		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
12		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
13		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
14		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
15		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
17		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
18		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
19		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
20		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
22		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
23		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
25		N	N	N	P	N	P	P	P	P	P	P	P	P	N	N	N	N
36		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
37		N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N

CFT

Semi-quantitative results of participating laboratories from Asia-Pacific countries to CFT

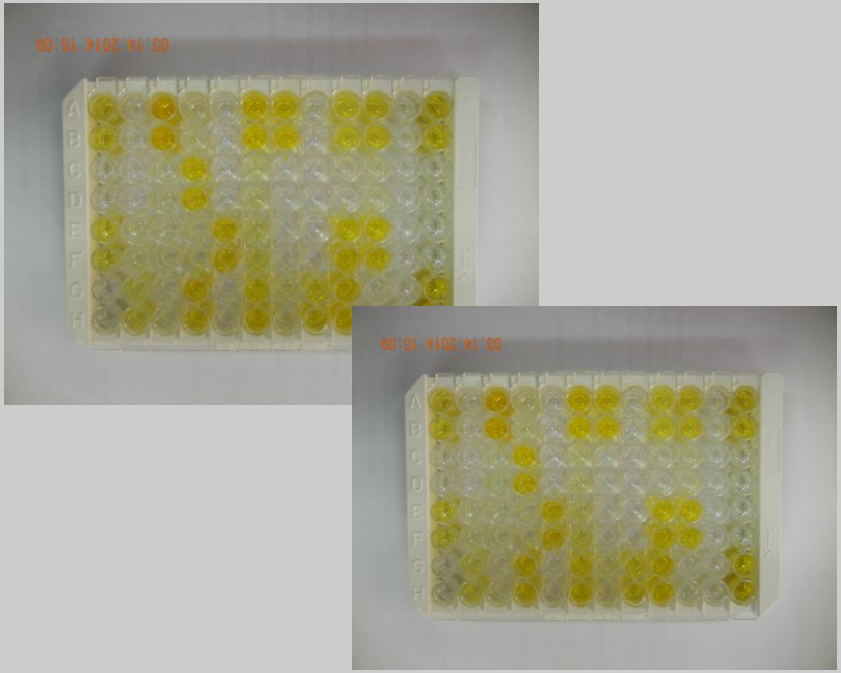
Expected Results		N			P			P			Strong Pos		Strong Pos		N		N	
Lab code	Antigen	Level 1	1	1	2	2	2	3	3	3	4	4	5	5	6	6	7	7
8	Ag A	0	0	0	23.3	23.3	23.3	33.3	33.3	33.3	46.6	46.6	93.3	80	0	0	0	0
9	Ag B	16.6	16.6	16.6	40	33.3	33.3	66.6	66.6	66.6	80	80	133	93.3	0	0	0	0
13	Ag B	0	0	0	23.3	23.3	23.3	33.3	26.6	26.6	53.3	53.3	93.3	66.6	0	0	0	0
14	Ag B	0	0	0	26.6	26.6	26.6	33.3	26.6	53.3	26.6	53.3	107	93.3	0	0	0	0
15	Ag B	<16.6	<16.6	<16.6	40	40	40	46.6	46.6	46.6	80	80	160	93.3	<16.6	<16.6	<16.6	<16.6
17	Ag B	0	0	0	26.6	26.6	26.6	46.6	46.6	46.6	53.3	53.3	107	107	0	0	0	0
18	Ag B	0	0	0	20	20	20	26.6	26.6	26.6	46.6	40	53.3	53.3	0	0	0	0
19	Ag B	0	0	0	20	20	20	26.6	26.6	26.6	46.6	53.3	80	40	0	0	0	0
20	Ag B	0	0	0	26.6	20	26.6	33.3	33.3	40	66.67	66.67	80	80	0	0	0	0
22	Ag B	0	0	0	33.3	26.6	33.3	66.6	40	33.3	53.3	66.6	107	80	0	0	0	0
23	Ag B	0	0	0	26.6	40	26.6	46.6	46.6	46.6	80	80	80	80	0	0	0	0
37	Ag B	0	0	0	33.33	26.67	33.33	46.67	46.67	53.33	80	80	106.7	106.67	0	0	0	0
12	Ag C	0	0	0	dil? 8	dil?2	dil?8	dil?8	dil?8	dil?16	dil?16	dil?16	dil?32	dil?32	0	0	0	0
25	Ag D	0	0	0	33.3	0	66.6	93.3	66.6	93.3	133	93.3	213	187	0	0	0	0
36	Ag E	0	0	0	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	0	0	0	0

CFT

- Specificity
- Sensitivity : lack for 1 lab.
- Repeatability : for one level, difference should not exceed 1 dilution (problem for 1 lab)
- Consistency between antibody levels

→ Antigen and protocol standardization

I-ELISA



I-ELISA

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
8	Home made-1	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
14	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
6	Kit A	P	N	P	N	N	N	P	N	N	N	N	N	N	N	N	N	N
38	Kit A	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
5	Kit B	P	P	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N
20	Kit B	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
37	Kit B	P	P	P	P	P	P	P	P	P	P	P	P	P	N	N	N	N
36	Kit C	N	N	N	P	P	P	P	P	P	P	P	P	P	N	N	N	N

I-ELISA

Quantitative results of participating laboratories from Asia-Pacific countries to I-ELISA

	Expected results			P				P				P				SP			SP		
Lab code	Kit supplier	Positive cut-off	Doubtful cut-off	Level 1	1	1	CV%	2	2	2	CV%	3	3	3	CV%	4	4	CV%	5	5	CV%
8	Home made-1	≥ 60%	-	43.3	39.4	40.7	4.8	69	60.8	72.1	8.7	74.7	74.4	85.8	8.3	78.7	81.4	2.4	75.6	84.7	8.0
14	Home made-2	≥ 40%	-	22.809	23.462	22.825	1.6	41.163	44.782	42.692	4.2	40.512	50.522	44.185	11.2	45.522	60.385	19.8	73.251	89.079	13.8
3	Kit A	≥ 80%	-	135.714	135.476	120.714	6.6	172.62	171.905	170.952	0.5	191.667	189.52	188.81	0.8	287.857	285.714	0.7	222.38	219.76	0.8
6	Kit A	≥ 80%	-	80	65.424	90.6215	16.1	64.633	73.785	59.209	11.2	102.938	70.508	70.170	23.2	69.266	73.220	3.9	34.689	65.650	43.6
38	Kit A	≥ 80%	-	101.11	96.5	97.86	2.4	121.38	122.08	123.94	1.1	121.36	127.59	118.72	3.7	120.46	126.65	3.5	123.11	124.65	0.9
5	Kit B	≥ 120%	110-120	145.87	145.011	75.42	33.1	184.15	173.674	129.416	17.9	185.762	178.71	197.25	5.0	185.292	185.091	0.1	187.71	182.4	2.0
20	Kit B	≥ 120%	110-120	171.577	168.076	170.682	1.1	229.82	226.628	220.717	2.0	241.18	239.43	238.6	0.5	253.754	255.179	0.4	262.54	252.49	2.8
37	Kit B	≥ 120%	110-121	147.493	161.057	147.398	5.2	274.1	268.378	252.336	4.3	311.117	266.49	274.48	8.4	300.723	274.425	6.5	287.84	269.94	4.5
36	Kit C	≥ 30%		10.9	9.8	3.4	50.4	47.9	46.8	41.5	7.5	60.4	56	59.8	4.1	67.2	70.4	3.3	84.3	85.3	0.8

Expected results		N		N	
6	6	CV%	7	7	CV%
3.8	3.7	n/a	6.8	5.1	n/a
19.278	19.215	n/a	25.683	25.408	n/a
0.714	1.190	n/a	13.333	11.905	n/a
1.808	-28.927	n/a	11.299	7.006	n/a
2.624	2.18	n/a	18.33	25.75	n/a
-1.141	-1.343	n/a	15.984	9.066	n/a
-2.603	-2.035	n/a	6.738	6.808	n/a
1.673	1.027	n/a	9.273	10.097	n/a
-4.3	-0.2	n/a	0.6	2.8	n/a

I-ELISA

- Level 1: N/P “kit” effect

→Standardization of the kit to be checked (OIEELISA$_{sp}$SS)

- Repeatability problems : check the volumes (pipettes?)
- iELISA is a very robust technique provided that standardized kit is used

Notes

- The PT-Form – not complete information
- Error of sample identification 0.38% (2/527)
  - number of some serum was fade
  - reading
  - reverse number, such as 869
  - results reporting
- Shipment of sera to labs have varied but apparently without any effect on sample quality

→ technical problems

Notes

- Very good overall results for a 1st ILPT
- Need of follow-up, training and a next PT
- RBT is the simplest test, but…not that easy to perform and to read!
- CFT is the most complicated one…but the easiest to get accurate results provided the protocol is standardized
- I-ELISA commercial, well-standardized kits usually give more reliable results than home-made kits

Thanks to all participating laboratories

Announcement

2nd Asia-Pacific Bovine Brucellosis Proficiency Test 2014

Acknowledgements

- FAO-APHCA and OIE-RRAP
- Asia-Pacific Participating Laboratories
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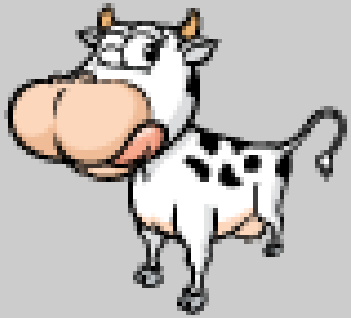
  - DLD Veterinary Expert
- Dr. Prasit Chaitaweesub
  - Director, Division of International Livestock Cooperation
- Dr. Surapong Wongkasemjit, NIAH-DLD
- Local Laboratories

Thank you for your Attention