Multiplex detection of camel antibodies to Trypanosoma evansi

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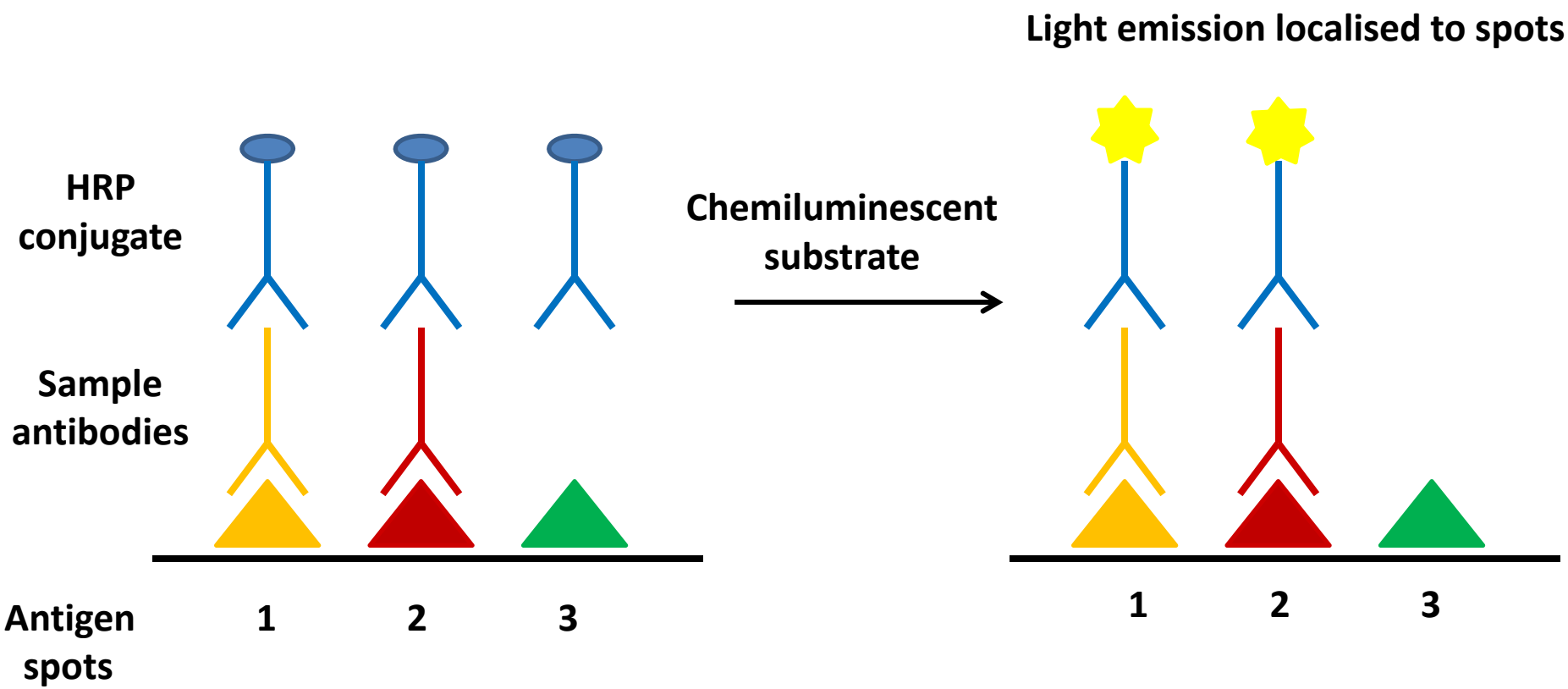
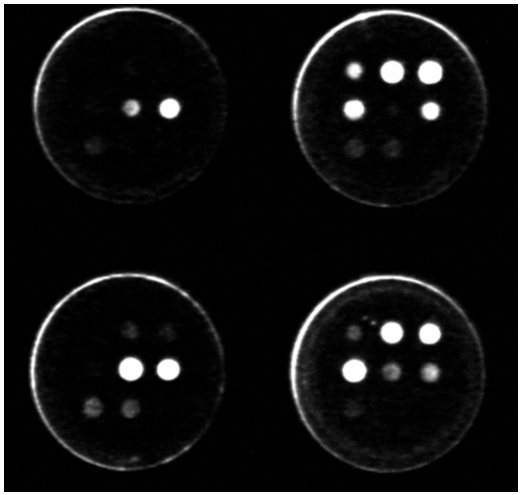
Enferplex technology



Enferplex results processing



Read-out is in

Relative Light Units (RLU)

Dynamic range 0 – 65,500

Pos/neg cutoff usually set about 1000-3000

Can be expressed as signal/noise ratio

Ag ID	RLU
Ag 1	18750
Ag 2	45600
Ag 3	51400
Ag 4	33800
Ag 5	1500
Ag 6	31350
Ag 7	2300
Ag 8	2500
Ag BLANK	300

→ **Excel Macro** → **RESULTS**

MV DIAGNOSTICS

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Enferplex test outputs

- **Positive/Negative antigen rules**

  - one, two or more antigens need to be recognised by antibody for sample to be deemed positive – depends on disease

- **Number of antigens recognised**

  - 2, 3, 4, 5, 6, 7, or 8 antigens recognised by antibody

- **Antibody load**

  - Summated signal/noise ratio of all antigens

Advantages of Enferplex technology

- **High sensitivity and specificity, controllable**
- **Quantitates antibody** with a large dynamic range
- **Results for different antigens**
  - obtained under identical assay conditions
  - provides additional biological information
  e.g. spectrum of responses, stage of disease, strain type
- **Results for different diseases** provides
  - ideal surveillance tool, high throughput
  - additional information, e.g. co-infections, interactions
- **Reasonable laboratory set up costs**
- **Multiplexing saves on reagent costs and overheads**

Trypanosoma evansi multiplex

- Developed in collaboration with ARASCO, a Saudi animal feed company
- Project to establish a camel 'health scheme' for ARASCO clients and surveillance for camel diseases, high throughput test needed
- *Trypanosoma evansi* targeted as
  - Primary pathogen causing poor condition, anaemia and abortion
  - May be a common underlying immunosuppressive infection
  - Potentially treatable through trypanocides and vector control
- Perceived deficiencies of CATT re sensitivity, specificity, reproducibility
- Incorporate other antigens into multiplex for diagnosis/surveillance
  - Paratuberculosis, Tuberculosis
  - Other agents causing abortion eg Brucella, Coxiella

- Cost effective technology with familiar ELISA-type methods

Trypanosoma evansi antigens in Multiplex

4 protein antigens identified and recombinants made in E coli:

Variable surface glycoprotein (VSG)
- RoTat1.2, major surface antigen

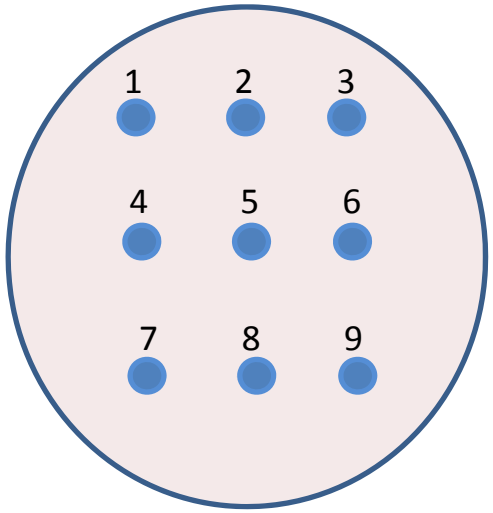
Invariant surface glycoprotein (ISG75)
- present on surface but hidden

Tandom repeat domain from calpain (GM6)
- flagellar antigen

Oligopeptidase B
- secreted enzyme, important in pathogenesis, 'propeller' domain, designed to minimise potential cross reactions with Leishmania spp.

Trypanosoma evansi Multiplex Antigen array layout

3 x 3 array
50 nl spots



1. GM6 calpain
2. ISG75
3. VSG RoTat1.2
4. Oligopeptidase B
5. Blank
6. Blank
7. Blank
8. Blank
9. Control

Trypanosoma evansi reference samples tested

Camel, positive serum

44 OIE reference lab, tested by:

- CATT
- Immune trypanolysis (ILT)
- Parasites in blood
- ELISA for antibody to native RoTat1.2
- ELISA for antibody to rISG75 produced in Pichia

Camel, negative serum

44 OIE reference lab, tested as above

100 Gran Canaria, assumed Trypanosomosis-free

MV DIAGNOSTICS

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Example Multiplex Results - OIE Positive reference sera					
Sample ID	GM6 E coli	ISG75 E coli	VSG E coli	Number of Positive Antigens	Overall Result
TE P01	0.36	7.67	0.24	1	POS
TE P02	0.21	1.67	0.45	1	POS
TE P13	0.11	0.20	3.48	1	POS
TE P16	0.43	0.53	4.94	1	POS
TE P29	0.18	0.35	2.62	1	POS
TE P06	6.39	0.80	21.81	2	POS
TE P10	2.48	0.65	9.05	2	POS
TE P12	1.55	0.41	4.51	2	POS
TE P03	20.49	7.72	2.99	3	POS
TE P04	21.82	2.00	13.24	3	POS
TE P05	21.71	1.45	5.09	3	POS
TE P07	21.74	7.62	6.26	3	POS
TE P08	16.56	3.64	3.51	3	POS
TE P09	20.65	4.63	3.14	3	POS
TE P11	21.84	5.02	6.08	3	POS
TE P14	21.44	6.39	6.38	3	POS

Example Multiplex Results - OIE Negative reference sera					
Sample ID	GM6 E coli	ISG75 E coli	VSG E coli	Number of Positive Antigens	Overall Result
TE N01	0.37	0.59	0.33	0	NEG
TE N03	0.47	0.48	0.23	0	NEG
TE N04	0.17	0.07	0.10	0	NEG
TE N05	0.19	0.45	0.14	0	NEG
TE N06	0.09	0.12	0.09	0	NEG
TE N07	0.20	0.23	0.09	0	NEG
TE N08	0.19	0.07	0.07	0	NEG
TE N09	0.65	0.75	0.22	0	NEG
TE N10	0.10	0.18	0.22	0	NEG
TE N11	0.48	0.11	0.35	0	NEG
TE N12	0.14	0.41	0.34	0	NEG
TE N13	0.26	0.10	0.22	0	NEG
TE N14	0.72	0.18	0.12	0	NEG
TE N39	0.56	1.49	0.14	1	POS

Comparison of OIE and Multiplex tests on *Trypanosoma evansi* reference samples tested by all OIE tests

		Multiplex		
		Positive	**Negative**	**Totals**
OIE	**Positive**	**43**	**1**	**44**
	Negative	**1**	**43**	**44**
	Totals	**44**	**44**	**88**

κ=0.995, SE±0.032, 95%CI 0.892-1.00, ‘very good’ agreement

Multiplex test results on samples from Gran Canaria

		Multiplex		
		Positive	Negative	Totals
Gran Canaria	Positive	0	0	0
	Negative	1	99	100
	Totals	1	99	100

Multiplex testing of serum samples from Riyadh area of Saudi Arabia

134 samples from 5 herds

29 seropositive, 105 seronegative

Herd 1	16/64	25%
Herd 2	1/7	14%
Herd 3	5/13	38%
Herd 4	6/30	20%
Herd 5	1/11	9%

Need more whole herd tests, combined with results of other tests – clinical, parasitological, immunological

In-contact animals very important when comparing tests

Camel abortion storm
Saudi Arabia, October-November 2016

2000 milking camel herd and 1000 sheep

One group of 60 camels, 44 abortions at all stages of pregnancy

T evansi known to be in the area, stable flies common

Complex aetiology? Serology positive for T evansi, Brucella, Toxoplasma, Chlamydia, Neospora – tests properly validated for camel?

Multiplex results - Camel abortion storm, KSA

Sample ID	GM6 E coli	ISG75 E coli	VSG E coli	Number of Positive Antigens	Overall Result	CATT
01	-0.00	0.07	0.25	0	NEG	-
07	0.15	0.53	0.15	0	NEG	-
09	0.04	0.58	0.76	0	NEG	-
27	0.02	0.58	0.44	0	NEG	-
28	0.91	0.64	0.15	0	NEG	-
44	0.11	0.11	0.05	0	NEG	+
51	0.12	0.42	0.42	0	NEG	++
16	0.02	9.33	0.82	1	POS	++
22	0.43	2.81	0.75	1	POS	-
40	0.12	2.74	0.41	1	POS	-
42	0.39	0.42	16.64	1	POS	+
04	2.54	0.96	1.22	2	POS	-
10	1.29	0.40	4.47	2	POS	+/-
14	1.57	0.78	3.82	2	POS	-
12	0.36	1.69	12.39	2	POS	+/-
13	-0.09	8.17	6.83	2	POS	+++
21	0.95	5.54	5.63	2	POS	+++
30	0.52	5.01	31.87	2	POS	+++
31	-0.01	6.66	1.94	2	POS	+++
33	0.00	1.13	9.70	2	POS	-
38	-0.08	9.01	3.14	2	POS	++
43	0.73	1.83	12.86	2	POS	-
15	1.28	2.93	0.59	2	POS	+++
20	14.68	9.06	0.88	2	POS	++
29	4.68	8.77	0.82	2	POS	-
47	1.56	6.21	0.21	2	POS	+++

Sample ID	GM6 E coli	ISG75 E coli	VSG E coli	Number of Positive Antigens	Overall Result	CATT
02	27.35	5.19	20.38	3	POS	-
03	31.51	3.79	26.08	3	POS	+++
05	2.73	9.26	22.08	3	POS	++
06	1.75	9.29	21.61	3	POS	+++
08	15.32	1.61	19.40	3	POS	+
11	16.13	3.39	5.43	3	POS	+++
17	4.01	1.20	1.66	3	POS	+/-
18	10.15	8.63	4.49	3	POS	++
19	1.38	8.47	3.00	3	POS	++
23	2.00	6.63	23.27	3	POS	+++
24	31.23	4.16	31.42	3	POS	+++
25	19.00	4.90	3.12	3	POS	++
26	23.41	2.75	3.38	3	POS	+++
32	8.88	8.75	2.14	3	POS	-
34	30.21	7.93	27.41	3	POS	+
35	3.45	8.10	3.02	3	POS	+
36	7.51	4.81	1.97	3	POS	-
37	3.50	9.20	4.84	3	POS	+
39	1.79	9.29	1.83	3	POS	-
41	12.98	1.28	31.24	3	POS	+++
45	30.93	4.79	16.45	3	POS	-
46	32.00	5.98	9.28	3	POS	+
48	3.92	9.28	2.74	3	POS	+
49	2.04	8.11	22.08	3	POS	+++
50	2.17	7.26	15.28	3	POS	-

Multiplex and CATT test results on samples from Camel abortion storm

		Multiplex		
		Positive	**Negative**	**Totals**
CATT	**Positive**	**31**	**2**	**33**
	Negative	**13**	**5**	**18**
	Totals	**44**	**7**	**51**

Kappa= 0.252 , SE of kappa = 0.128 , 95% CI 0.002 to 0.503
The strength of agreement is considered to be 'fair'.

Summary and Conclusions

A multiplex test comprising VSG RoTat1.2, GM6, ISG75 and Oligopeptidase B antigens has been developed for the detection of antibody to *Trypanosma evansi* types A and B.

The test shows good agreement with a panel of OIE reference tests on defined positive and negative sera,

Further validation aiming initially for well defined (clinical, parasitological, immunological) samples from

- 300 'true positives' and 1000 'true negatives',
- and 'in contact' samples from infected herds

Investigation of an abortion storm in camels in Saudi Arabia showed the utility of the test for investigation of disease outbreaks, apparently more sensitive than CATT

Further work is needed to investigate the utility of additional antigens to differentiate *T evansi* type A from other Trypanosome infections eg *T evansi* type B, *T equiperdum*, *T vivax*

The multiplex test could be adapted to other species eg cattle, buffalo, small ruminants

Questions and comments?