

Performance of Two Enzyme and One Strip Immune Assay for the Detection of IgM anti-*Treponema pallidum* Antibodies

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Background

The detection of anti-*Treponema pallidum* (Tp) IgM antibodies may be useful in the diagnosis of very early syphilis, re-infection and in the assessment of the newborn.

We evaluated 2 enzyme immunoassays and 1 line immunoassay (LIA) assay for the detection of anti-Tp IgM in blood specimens.

Conclusions

The highest sensitivity was found with the IgM recomLine line immunoassay. The sensitivity increased further when borderline results were considered positive. Cross reactivity with a specimen containing anti-malaria antibodies was found for the Euroimmun assay.

We observed that Anti-Tp IgM antibodies could be detected before RPR reactivity and that they could remain present for months even after successful treatment.

Methods

Blood specimens collected from patients presenting with a syphilitic ulcer, and specimens (N=3 for each) containing antibodies against Epstein Barr Virus, *Leptospira* sp, *Borrelia* sp, Plasmodium sp, Herpes Simplex Virus 1 and 2 and specimens from pregnant women were analyzed.

The specimens were tested using:

1. Rapid Plasma Reagin (RPR), Macrovue, Becton Dickinson
2. Treponema Pallidum Particle Agglutination (TPPA), Fujirebio
3. recomWell Treponema IgM, Mikrogen Diagnostik
4. Anti-Treponema pallidum ELISA-IgM, Euroimmun
5. recomLine Treponema IgM, Mikrogen Diagnostik

A specimen was considered positive for anti-Tp IgM when both TPPA and IgM, obtained with any of the assays, were positive.

Specimens collected from the syphilitic ulcers were tested by in-house qPCR.

Results

A total of 35 blood specimens collected from 17 patients presenting with a genital ulcer were analyzed. Tp DNA was detected in 12 ulcer specimens, 3 of the 5 negative results were not valid due to the absence of cell material.

The serology results were as follows:

Parameter	Negative	Positive	Borderline	Not done
RPR	11	24	0	1
TPPA	2	33	0	
IgM LIA	11	18	6	
IgM Euroimmun	20	15	0	
IgM recomWell	17	16	2	

Anti-Tp IgM antibodies were detected in 23 specimens.

The performance characteristics of the IgM assays are presented in the table:

Test	Concordant (%)	Sensitivity (%)	95% CI (%)	Specificity (%)	95% CI (%)
IgM LIA (A)	96,55	94,74	71,89-99,72	100	65,55-100
IgM LIA (B)	96,97	95,65	76,03-99,77	83,33	50,88-97,06
Euroimmun	77,14	65,71	42,82-82,81	100	69,87-100
recomWell(A)	84,85	76,19	52,45-90,88	100	69,87-100
recomWell(B)	85,71	78,26	55,79-91,71	100	69,87-100

Legend: CI: confidence interval; A: excluding borderline results; B: with borderline results defined as positive

Euroimmun tested positive in 1 specimen containing anti malaria antibodies

	Specimen	date	RPR	TPPA	Tp PCR	recomLine						Euroimmun		recomWell		
						Tp47	TmpA	Tp257	Tp453	Tp17	Tp15	result	result	ratio	result	U/ml
Pat A	serum	4/04/2011	n	1/320	---	±	-	-	-	-	-	n	n	0,31	n	7,54
	serum	4/08/2011	1/32	>1/20480	---	++	±	-	±	++	+	p	n	0,42	n	11,79
	ulcer	19/08/2011	---	---	p	---	---	---	---	---	---	---	---	---	---	---
	serum	14/11/2011	1/8	>1/20480	---	±	+	-	-	+	+	p	n	0,22	n	6,14
Pat B	serum	15/11/2011	1/16	1/5120	---	±	-	-	-	+	+	p	n	0,27	n	12,90
	ulcer	24/01/2012	---	---	p	---	---	---	---	---	---	---	---	---	---	---
	serum	19/07/2012	1/4	1/10240	---	-	±	-	-	+	+	p	n	0,28	n	13,52
	serum	11/03/2013	1/32	>1/20480	---	±	++	-	-	+	-	p	n	0,35	BL	21,52
Pat C	serum	11/01/2013	n	1/160	---	-	+	-	-	-	-	BL	n	0,39	p	24,90
	serum	19/07/2013	1/8	1/5120	---	±	+	-	-	-	±	BL	n	0,65	p	26,42
	ulcer	29/07/2013	---	---	p	---	---	---	---	---	---	---	---	---	---	---
	serum	11/10/2013	1/8	1/10240	---	±	+	-	±	±	-	BL	p	1,17	p	27,09
Pat D	serum	23/07/2013	n	n	---	-	-	-	-	-	-	n	n	0,07	n	3,89
	serum ulcer	26/12/2013	1/4	1/1280	p	++	++	±	-	+	±	p	p	4,41	p	45,35
Pat E	serum	2/01/2014	1/16	1/5120	---	±	±	-	-	+	±	BL	n	0,37	n	11,27
	serum	14/03/2014	1/64	>1/20480	---	±	++	-	-	++	±	p	p	1,21	p	31,77
	ulcer	24/03/2014	---	---	p	---	---	---	---	---	---	---	---	---	---	---

Example of individual test results

Legend: :
 +: reactive
 -: non reactive
 p: positive
 n: negative
 BL: borderline
 ---: not done
 Pat: patient

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