Comparison of monovalent Enzymimmunoassays (EIAs) with the gold standard 19s-FTA-ABS-IgM

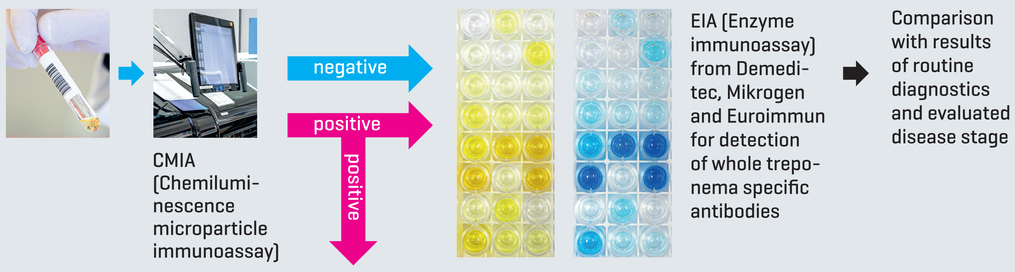
Juliane Fazio, Marie-Christin Höppner, Thomas Neiße, Kristin Meyer-Schlinkmann, Hans-Jochen Hagedorn, Dieter Münstermann
MVZ Labor Krone GbR, reference laboratory for treponema, Siemensstraße 40, 32105 Bad Salzuflen

Introduction

In 2019 more than 7,800 novel infections with treponema pallidum ssp. pallidum occurred in Germany showing an upward trend since 2010 [1]. To prevent clinical sequalae and further transmission, highly infectious patients in the early phase of syphilis have to be identified.

The current standard for the diagnosis of syphilis is a stepwise diagnostic procedure using a screening test followed by confirmatory tests. Treponema specific IgM-antibodies and non-treponema lipoid-antibodies are analyzed to estimate the activity of the infection [2,3]. Quantification of these activity markers is necessary to confirm the success of therapy in follow-up testing.

Material and Methods



A total of 1,875 samples were included: 43 early stage infections, 159 initial infections, 51 re-infections and 532 follow-ups.

Syphilis-positive, -borderline, and -negative samples were analyzed to evaluate the sensitivity and specificity of three commercially available Enzymimmunoassays from Demeditec, Euroimmun and Mikrogen for the detection of treponema-specific IgM-antibodies. In some cases, IgM-immunoblots were added to confirm specificity. All results were compared to the gold standard, 19s-FTA-ABS-IgM-test (fluorescence treponema pallidum absorption). The EIAs were performed according to the manufacturer's protocol. Spearman's Rho was calculated between EIA and FTA-ABS results for correlation.

Results

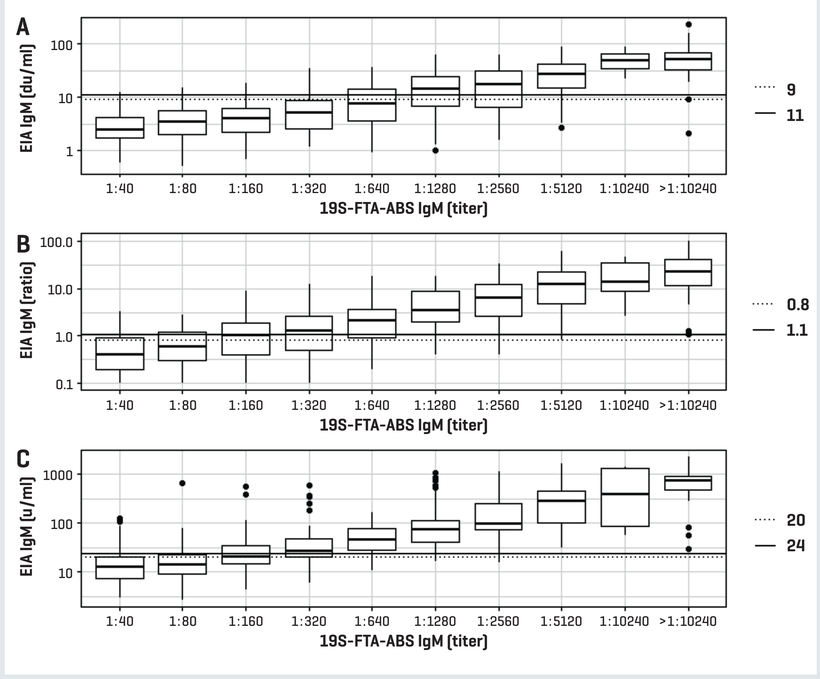


Fig. 1: Results of Demeditec (A), Euroimmun (B) and Mikrogen (C) EIA compared with titer levels of FTA-ABS-IgM (n=867). The threshold range is shown above the dotted line and the positive range is shown above the solid line according to the manufacturer's specification.

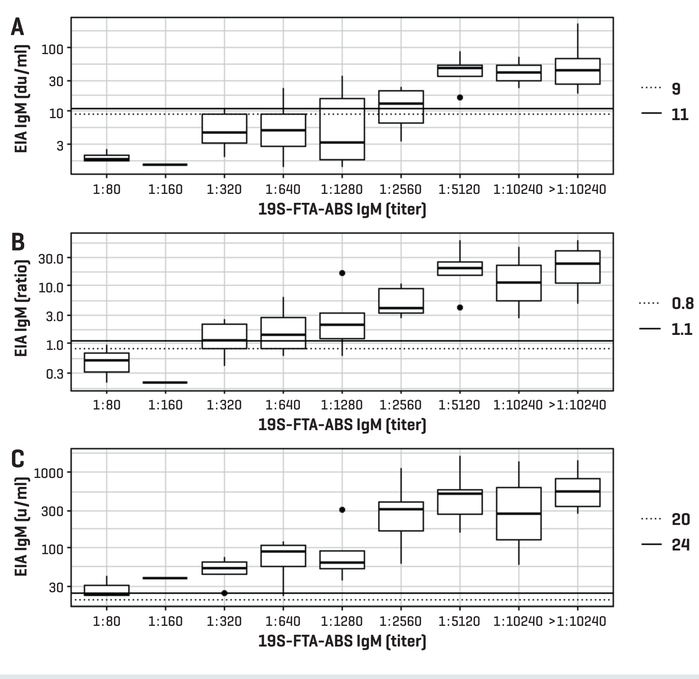


Fig. 2: Results of Demeditec (A), Euroimmun (B) and Mikrogen (C) EIA compared with titer levels of FTA-ABS-IgM (n=43) in cases of early infection. The threshold range is shown above the dotted line and the positive range is shown above the solid line according to the manufacturer's specification.

Tab. 1: Overall sensitivity, specificity and correlation of the test systems from Demeditec, Euroimmun and Mikrogen in comparison to the FTA-ABS-IgM (n=1,875).

	EIA IgM (du/ml) Demeditec	EIA IgM (ratio) Euroimmun	EIA IgM (u/ml) Mikrogen
Sensitivity (%)	28.66	62.42	60.80
Specificity (%)	99.70	99.40	98.00
Correlation to FTA-Abs-IgM (p<0.001)	0.64	0.70	0.76

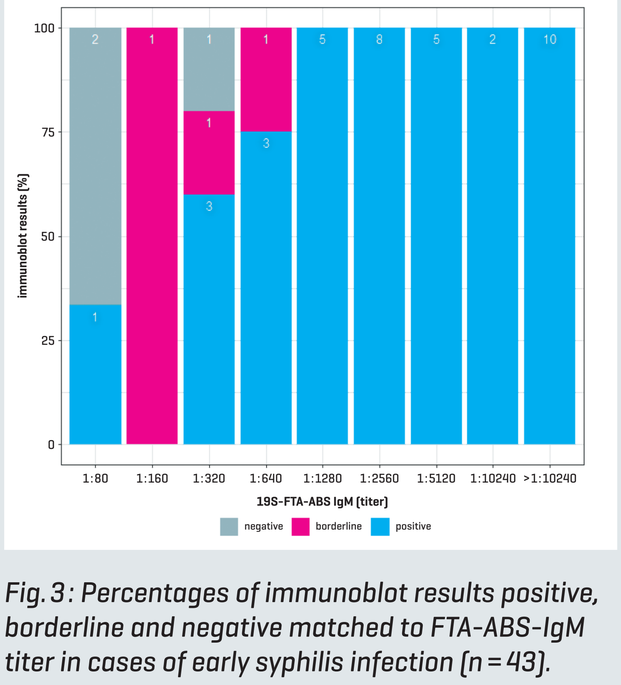


Fig. 3: Percentages of immunoblot results positive, borderline and negative matched to FTA-ABS-IgM titer in cases of early syphilis infection (n=43).

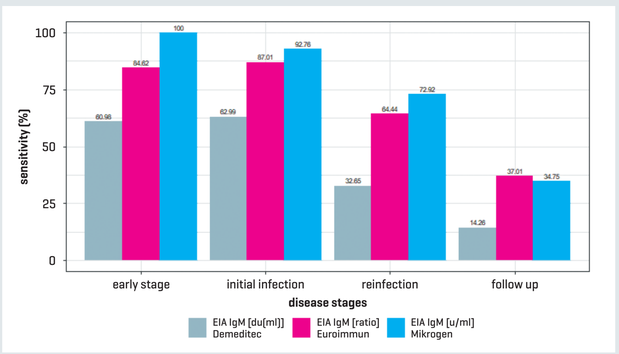


Fig. 4: Sensitivity in cases of early stages, initial infections, reinfections and in follow-up samples of Demeditec, Euroimmun and Mikrogen EIA.

In comparison to the gold standard FTA-ABS-IgM all reviewed EIAs were less sensitive in detection of samples with lower IgM titer levels (Fig. 1+2). In cases of early stages, initial infections and reinfections the Mikrogen EIA showed the highest sensitivity, in follow-up samples the Euroimmun EIA shows the highest sensitivity (Fig. 4). The overall sensitivity was best for the Euroimmun EIA (Tab. 1). Immunoblot analysis in some cases failed to detect samples with lower IgM titer in cases of early syphilis infection as well (Fig. 3).

Tab. 2: Case studies of patients with initial infections and well-defined medical history (L1 = Lues phase 1, L2 = Lues phase 2; negative, positive).

patient data and medical history				routine diagnostics					IgM EIA			IgM immunoblot									
gender	age	disease stage	assessment	CMIA	TPPA	FTA-Abs.-IgG	FTA-Abs.-IgM	VDRL	Euroimmun	Mikrogen	Demeditec	P15	P17	P445	P47	MV1	MV2	MV3	MV4	MV5	evalu-ation	VDRL-Score
M	34	L1	active, infectious	13.19	1:10000	>1:20	1:40	1:2	0.4	6.8	1.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M	29	L2	active, infectious	15.82	1:5120	>1:20	1:160	1:8	2.1	14.9	4.2	#–	#+–	#–	#+–	#++	#++	#+–	#–	#–	n	4
M	n/a	L1	active, infectious, early stage	3.33	1:160	1:10	1:320	n	0.4	63.5	1.9	#–	#–	#+	#++	#–	#–	#–	#–	#–	p	0
M	62	L2	active, infectious	18.55	1:20000	n/a	1:320	1:8	2.3	10.2	6.2	#–	#+	#–	#+–	#+	#+	#–	#–	#–	p	2
M	69	L1	active, infectious	19.58	>1:20000	>1:20	1:320	1:64	0.3	40.4	1.3	#–	#–	#–	#–	#+++	#++	#++	#++	#+	n	9
M	n/a	L1	active, infectious	5.42	1:1280	>1:20	1:640	#n	0.6	100.2	1.3	#–	#–	#+	#+++	#–	#–	#–	#–	#–	p	0
M	54	L1	active, infectious	2.08	1:2560	>1:20	1:1280	#n	0.6	35.5	1.7	#+	#–	#+–	#++	#–	#–	#–	#–	#–	p	0

Conclusion

Especially in the early phase of active infection with low levels of IgM-antibodies two of the three EIA were in many cases unable to identify highly infectious patients. One reason might be the antigen composition of the different test systems. The FTA-ABS-test works with the whole bacterial antigen in contrast to the recombinant antigens used for EIA.

Therefore, it would be necessary to recommend more follow-ups for patients with negative results and typical symptoms if these tests are used. It is currently being investigated whether a reduction of thresholds will lead to an increase in sensitivity.

References [1] World Health Organization (WHO). (2023). Syphilis. Retrieved June 16, 2023, from Fact sheets website: https://www.who.int/news-room/fact-sheets/detail/syphilis
[2] Janier et al., 2020 European guideline on the management of syphilis, JEADV 2021, 35, 574–588
[3] Deutsche STI-Gesellschaft (2021) Diagnostik und Therapie der Syphilis, Aktualisierung S2k 2021, Version 1.1 Addendum 1/21, 06/2021, URL: https://register.awmf.org/assets/guidelines/059-002l_S2k_Diagnostik_Therapie_Syphilis_2021_06.pdf, (Stand am 06.02.2023).