An assessment of the modified Bioelisa Syphilis 3.0 assay

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Background

The aim of this study was to assess the ability of a modified Bioelisa Syphilis 3.0 kit to detect anti-treponemal antibodies in 17 specimens that were previously included in an evaluation of the unmodified Bioelisa Syphilis 3.0 kit. The original version 3.0 assay was evaluated as part of a MHRA (Medicines and Healthcare products Regulatory Agency) Evaluation Report (MHRA 04109)[1].

Description of the assay

Both the previous and modified versions of the Bioelisa Syphilis 3.0 include three treponemal recombinant antigens in a sandwich configuration designed to detect anti-treponemal IgG and IgM antibodies. The assays use a 96-well microplate format coated with the recombinant antigens. The conjugate also contains the three recombinant treponemal antigens which are conjugated with peroxidase. The conjugate binds to any antibodies in the test specimen that have bound to the solid phase recombinant proteins. Tetramethylbenzidine (TMB) in the substrate reacts with the peroxidase to produce a blue colour if anti-treponemal antibodies are present in the test specimen. Any blue colour will turn to yellow on the addition of the stopping solution (1N sulphuric acid). The reactions are read photometrically at 450nm with a 630nm reference filter.

The modified Bioelisa Syphilis 3.0 assay has a slightly modified formulation which resulted from the discovery of a new function for an immunodominant protein. A patent application has been filed.

Evaluation method and specimen panel

The assays were performed according to the manufacturer's instructions using equipment available at the Evaluations Unit. The assay results were read on a Bio-Tek EL808 plate reader, which was linked to a computer with KC4 software. Lot number (L-1803) was used for the initial MHRA evaluation and lot number (K-2904) for this assessment.

To allow kit result comparison, the optical density (OD) of each specimen was divided by the cut-off (CO) value. An OD/CO greater than or equal one represents a positive result and less than one a negative result. It should be noted however that, according to the Bioelisa Syphilis 3.0 kit insert, specimens with an OD/CO between 0.9 and 1.0 are equivocal.

The sensitivity of the modified assay was re-assessed by testing it against 17 positive specimens, the status of which has been determined previously[1]. In the MHRA evaluation[1] three were repeatedly negative by Bioelisa Syphilis 3.0, two were initially negative, five were low positives (OD/CO 1 -2) and the reminding seven were a range of strong positives (OD/CO 3 – 11.6).

Results

Table 1 shows the results of the 17 specimens previously tested by Bioelisa Syphilis 3.0 and then retested by the modified version of Bioelisa Syphilis 3.0. All but one previously negative specimen were positive by the modified version. All previous low positives gave higher OD/COs. Some previous strong positives gave slightly lower OD/COs, but all were still strongly positive. The only specimen that was undetected (02S0040) was from a patient with primary untreated syphilis which was positive by darkground microscopy only. No other serological assays have generated a positive result from this specimen.

Table 1. OD/CO results for 17 specimens tested on Bioelisa Syphilis 3.0 and on the modified version of Bioelisa Syphilis 3.0

	Previous version (lot L-1803)			Modified version (lot K-2904)
MiDAS No	OD/CO	OD/CO2	OD/CO3	OD/CO
02S0010	1.71			4.25
02S0014	11.18			9.35
02S0018	11.60			9.71
02S0030	10.71			9.47
02S0040	0.06	0.03	0.03	0.08
02S0064	1.91			2.49
02S0070	5.82			4.33
02S0078	1.94			4.99
02S0079	1.75			4.28
02S0082	1.21			5.69
02S0088	3.01			7.76
02S0110	0.98	1.04	1.02	3.87
02S0115	0.84	0.93	0.88	2.23
02S0121	1.00	1.07	1.01	2.87
02S0188	3.60			6.40
03S0263	8.33			9.05
03S0287	0.72	0.62	0.74	2.04

Conclusions

The modifications made to the Bioelisa Syphilis 3.0 assay (lot number K-2904) show an increased sensitivity against previously unreactive and low positive specimens.

An extended evaluation is recommended, to confirm the increased sensitivity and to determine if specificity is affected.

Reference:

1. Cole M, Perry KR.(2004): Evaluation of ten syphilis EIAs
MHRA report 04109