

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([see an example](#)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Comparison of the Analytical level of agreement of Nine Treponemal Assays for Syphilis and Possible Implications for Screening Algorithms
AUTHORS	Castro, Arnold; Jost, Heather; Cox, David; Fakile, Yetunde; Kikkert, Susan; Tun, Ye; Zaidi, Akbar; Park, Mahin

VERSION 1 - REVIEW

REVIEWER	Elitza S. Theel, PhD Director, Infectious Diseases Serology Laboratory Mayo Clinic Rochester, Minnesota, USA I have no conflict of interest.
REVIEW RETURNED	25-Jun-2013

THE STUDY	<p>1. Page 5, line 55: Can the authors please explain why the sera in their study had such a high lipid content that they had to be pre-treated? Most laboratories do not routinely pre-treat samples. Do the authors think this may have affected their results?</p> <p>2. Page 6, line 65: Can the authors please clarify why they chose to reconstitute plasma into serum to use as a diluent for their sensitivity studies instead of using neat sera which tested negative by all 9 assays? Is there any concern that this may have introduced unnecessary variability in their evaluation?</p> <p>3. Did the authors consider including the syphilis IgG assay offered by BioRad? Many laboratories are beginning to transition to this automated platform and the evaluation of this assay would be very beneficial.</p>
RESULTS & CONCLUSIONS	<p>1. Table 1 is confusing as some values have percentages (which are not defined) and some values do not. Would the authors consider just using absolute values and adding the percentages in the text?</p> <p>2. Abstract (lines 12-14) and Results Section (lines 84-86) – Use of dashes between the percentages suggests a range, whereas I believe the authors are indicating individual percent values for reactive and nonreactive samples. Consider using ‘and’ instead of ‘-’. Also, consider adding “respectively” to the end the sentence.</p> <p>3. Consider reorganizing the results section by moving the paragraph between lines 94 – 102 to follow the end of line 88 on page 6. This would allow for better flow and clarity.</p>

REVIEWER	Belinda Yen-Lieberman, Ph.D.,
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	Professor of Pathology Lerner College of Medicine Medical Director-Clinical Microbiology, Serology & Cellular Immunology Cleveland Clinic Cleveland, Ohio, USA
REVIEW RETURNED	17-Jul-2013

GENERAL COMMENTS	This is a well written manuscript addressing the important aspects of selecting diagnostic Syphilis Serology test in clinical laboratories. Especially when many laboratories are implementing the " Reverse Test Algorithm" for Syphilis serology. The authors presented excellent data comparing the different analytical sensitivities of nine commercially available Trepanema pallidum serology tests. The authors recommended on selecting more sensitive test as the confirmatory tests.
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VERSION 1 – AUTHOR RESPONSE

Response to Reviewers

Page 5, line 55: Can the authors please explain why the sera in their study had such a high lipid content that they had to be pre-treated? Most laboratories do not routinely pre-treat samples. Do the authors think this may have affected their results?

Response

We obtain clinical sera from the Georgia Department of Health Laboratories and quite a number of samples are high in lipid content. Upon freezing and thawing the lipids forms a layer at the top of the micro centrifuge tubes that need to be physically removed to avoid interfering with the test. It is convenient to us to treat all of the samples with a lipid removal product to avoid this condition. However, if the samples undergo a minor decrease in titer there is no significant detrimental effect resulting from this procedure since we are comparing all the assays simultaneously with the same samples, (see reference (7))

2. Page 6, line 65: Can the authors please clarify why they chose to reconstitute plasma into serum to use as a diluent for their sensitivity studies instead of using neat sera which tested negative by all 9 assays? Is there any concern that this may have introduced unnecessary variability in their evaluation?

Response

There is no variability in the evaluation of the 9 assays when using plasma converted to serum as diluent. Our laboratory at CDC is the collaborating center for the WHO proficiency testing program for syphilis serology. We purchased commercially normal or nonreactive sera for the preparation of samples. It is becoming increasing more difficult to purchase bulk sera because of their availability and price per ml. However, there is an abundance of readily available nonreactive plasma that can be converted to serum according to our method, (see reference (8))

3. Did the authors consider including the syphilis IgG assay offered by BioRad? Many laboratories are beginning to transition to this automated platform and the evaluation of this assay would be very beneficial.

Response

The Bio-Rad IgG assay was not included in the study because the instrument was not delivered on time to our facilities. However, on an ongoing clinical study comparing seven treponemal assays the Bio-Rad IgG assays is included.

1. Table 1 is confusing as some values have percentages (which are not defined) and some values do not. Would the authors consider just using absolute values and adding the percentages in the text?

Response

Table 1 was modified as to include percentages values.

2. Abstract (lines 12-14) and Results Section (lines 84-86) Use of dashes between the percentages suggests a range, whereas I believe the authors are indicating individual percent values for reactive and nonreactive samples. Consider using and instead of - . Also, consider adding respectively to the end the sentence.

Response

Abstract (lines 12-14) and (lines 84-86) the dashes between the percentages were replaced by "and" and "respectively" was added at the end of the sentence.

3. Consider reorganizing the results section by moving the paragraph between lines 94 102 to follow the end of line 88 on page 6. This would allow for better flow and clarity.

Response

The paragraph between lines 94 - 102 was moved to the end of line 88.

Reviewer: Belinda Yen-Lieberman, Ph.D.,

Professor of Pathology

Lerner College of Medicine

Medical Director-Clinical Microbiology, Serology & Cellular Immunology

Cleveland Clinic Cleveland, Ohio, USA

Professor Yen-Lieberman. The authors appreciate your kind comments in reference to the manuscript and hope that the information in the study will be useful to future researchers.

This is a well written manuscript addressing the important aspects of Selecting diagnostic Syphilis Serology test in clinical laboratories.

Especially when many laboratories are implementing the " Reverse Test Algorithm" for Syphilis serology.

The authors presented excellent data comparing the different analytical Sensitivities of nine commercially available Trepanema pallidum serology tests. The authors recommended on selecting more sensitive test as the confirmatory tests.

VERSION 2 – REVIEW

REVIEWER	Elitza S. Theel Director, Infectious Diseases Serology Laboratory Mayo Clinic United States I have not conflict of interest
REVIEW RETURNED	30-Jul-2013

- The reviewer completed the checklist but made no further comments.

Correction

Castro A, Jost H, Cox D, *et al.* A comparison of the analytical level of agreement of nine treponemal assays for syphilis and possible implications for screening algorithms. *BMJ Open* 2013;3:e003347. The order of the authors was published incorrectly in this paper. The correct order is: Heather Jost, Arnold Castro, David Cox, Yetunde Fakile, Susan Kikkert, Ye Tun, Akbar Zaidi, Mahin Park.

BMJ Open 2013;3:e003347. doi:10.1136/bmjopen-2013-003347corr1