

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing
<b>AUTHORS</b>	Lee, Jong-Han; Lim, Chae Seung; Lee, Min-Geol; Kim, Hyon-Suk

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Recep Tekin Department of Infectious Diseases Dicle University School of Medicine
<b>REVIEW RETURNED</b>	20-May-2014

<b>GENERAL COMMENTS</b>	<p>It was a pleasure to review to Assessment of Comparison of the automated rapid plasma reagin (RPR) test versus the conventional RPR card test in syphilis testing. Authors reported the compared tests. . The manuscript is well written, and the findings are very interesting. The minor revisions are indicated below.</p> <ol style="list-style-type: none"><li>1. There were numerous grammatical errors</li><li>2. discussion section, give information should be other studies (for example: HBsAg, anti- Dayan S, Tekin A, Tekin R, Dal T, Hoşoğlu S, Yazgan UC, Bekçibaşı M, Gül K. HCV, anti-HIV 1/2 and syphilis seroprevalence in healthy volunteer blood donors in southeastern Anatolia. J Infect Dev Ctries. 2013 Sep 16;7(9):665-9.</li><li>3. In this study, the main highlight the desired state, must be explained</li></ol>
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<b>REVIEWER</b>	Vanessa G. Allen Public Health Ontario, Canada
<b>REVIEW RETURNED</b>	26-Jul-2014

<b>GENERAL COMMENTS</b>	<p>Overall-- this is an small but interesting study presenting the differential performance characteristics of an automated RPR syphilis testing platform compared to standard manual RPR and TPPA.</p> <p>The study was reasonably designed, with the exception of the late inclusion of the discussion of the more rapid seroconversion of the automated RPR. I am not sure that this has been demonstrated in table 5-- it would be important to see the pre-treatment RPR results, nor is there a description or what the 0.5+ to 4+ values of the manual RPR are to understand the comparison (in my lab this is a quantitative dilution e.g. 1:4). Furthermore, I am finding it difficult to understand why early seroconversion of an RPR because of low</p>
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	<p>sensitivity would be a useful clinical tool.</p> <p>Secondly, as we do not have any automated RPRs available in Canada-- I was wondering if you could specify if this is the only kit available, and if not, why this one was chosen.</p> <p>There are a few parts of the paper that could be edited for clarity, including</p> <p>p4 line 50.. what is meant by good analytic performance...is that not what the study is proposing to look at?</p> <p>p5 line 17 I think that this should be analysis (and not analyses)</p> <p>p5 line 19, we are seeing a resurgence of syphilis in many parts of the world and would be able to obtain clinically defined samples. I would suggest specifying that the infrequency is in your population</p> <p>p5 line 41-51-- some of this is redundant and could be shortened</p> <p>p5 line 53 would suggest removing the words "each step"</p> <p>p6 line 10-17 this sentence is slightly awkward-- would suggest removing "were reacted within 10 seconds" as that is in the next line and rewrite how the reagents were added.</p> <p>p6 line 32-44 I find this very hard to follow-- I am not sure that you need to describe the physical locations of the wells but rather what conditions and controls were included.</p> <p>p7 line 37-41 I find this sentence difficult to understand would you consider something like: "When both RPR tests were positive, there was a 96% concordance with the TPPA..</p> <p>p7 line 44 suggest removing "kinds of"</p> <p>p7 line 50 suggest a new sentence to describe the 2 false positive HBI tests.</p> <p>p8 line 19 I am not sure that this is tied to the hypotheses... and if so it would be good to understand whether this happens with higher and lower RPR values or both .</p> <p>p8 line 35 non treponemal tests cannot really distinguish between treated vs non treated either.. I would suggest that this paragraph be more about the advantage of automated vs manual RPR as it is the main point of the study.</p> <p>p8 line 46 I would suggest removing the word "guaranteed"</p> <p>p8 line 53 I am not sure why the FTA is being discussed at all in the discussion</p> <p>p9 line 44 as outlined above-- I am not sure why it might be beneficial to have a early seroconverting RPR from a clinical perspective unless you do not report quantitation</p> <p>p10 line 10 would suggest removing the word "some"</p> <p>table 2 would add the word syphilis in the title, would define the RPR</p>
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	<p>card values, c and h are in reverse order in Bechet and do you mean genital Herpes simplex instead of herpes penis?</p> <p>table 3 would italicize <i>Treponema palladium</i></p> <p>table 4 would refer to syphilis and add performance characteristics in title</p> <p>table 5 as above would suggest adding pre treatment RPR values</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer Name Recep Tekin

Institution and Country Department of Infectious Diseases

Dicle University School of Medicine

Please state any competing interests or state 'None declared': none declared

#### Comments to the Author

It was a pleasure to review to Assessment of Comparison of the automated rapid plasma reagin (RPR) test versus the conventional RPR card test in syphilis testing. Authors reported the compared tests. The manuscript is well written, and the findings are very interesting. The minor revisions are indicated below.

1. There were numerous grammatical errors

>We revised manuscript with native English professionals for better grammatical structure.

2. Discussion section, give information should be other studies

>We tried to give information by related references in discussion section

(for example: HBsAg, anti- Dayan S, Tekin A, Tekin R, Dal T, Hoşoğlu S, Yazgan UC, Bekçibaşı M, Gül K. HCV, anti-HIV 1/2 and syphilis seroprevalence in healthy volunteer blood donors in southeastern Anatolia. J Infect Dev Ctries. 2013 Sep 16;7(9):665-9.

3. In this study, the main highlight the desired state, must be explained

>The main highlight of this study was that the automated RPR test showed more early response of syphilis treatment compared to manual card RPR test. The automated RPR seems to be useful in reverse algorithm such as initial screening by treponemal test, then follow-up treatment response by automated RPR could be helpful in clinical settings.

Reviewer Name Vanessa G. Allen

Institution and Country Public Health Ontario, Canada

Please state any competing interests or state 'None declared': None declared

>None declared was described.

Overall-- this is an small but interesting study presenting the differential performance characteristics of an automated RPR syphilis testing platform compared to standard manual RPR and TPPA.

The study was reasonably designed, with the exception of the late inclusion of the discussion of the more rapid seroconversion of the automated RPR. I am not sure that this has been demonstrated in table 5-- it would be important to see the pre-treatment RPR results, nor is there a description or what the 0.5+ to 4+ values of the manual RPR are to understand the comparison (in my lab this is a

quantitative dilution e.g. 1:4). Furthermore, I am finding it difficult to understand why early seroconversion of an RPR because of low sensitivity would be a useful clinical tool.

> The 0.5+ to 4+ values of the manual RPR were grade of positive response as you expected. However, this grade system can be somewhat variable by person to person. The exact mechanism of early seroconversion of automated RPR test was not elucidated. We think that it is an important characteristics of automated RPR compared with manual RPR.

Secondly, as we do not have any automated RPRs available in Canada-- I was wondering if you could specify if this is the only kit available, and if not, why this one was chosen.

>This kit has been used our laboratory and compared with another automated RPR test such as Mediace RPR (Sekisui Chemical Co., Ltd, Japan) in Kim et al. report [Korean J Lab Med 2009;29:331-7]. They suggested agreement between manual RPR and two automated RPR assay kits, Mediace RPR assay and HBi Auto RPR assay, were 83.8% and 83.2%, respectively.

There are a few parts of the paper that could be edited for clarity, including

p4 line 50.. what is meant by good analytic performance...is that not what the study is proposing to look at?

>Today, automated RPR test was improved to be able to show reasonable report in clinical settings, but it could show different results from manual RPR, therefore related study should be needed.

p5 line 17 I think that this should be analysis (and not analyses)

>We changed it

p5 line 19, we are seeing a resurgence of syphilis in many parts of the world and would be able to obtain clinically defined samples. I would suggest specifying that the infrequency is in your population  
>In Korea, there was relatively low prevalence rate of sexually transmitted disease (STD) such as HIV infection including syphilis. We described in INTRODUCTION such as "Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have reached a plateau." As your recommend, we additionally described the recent case reports of Korean syphilis in the METHODS such as "There were scarce samples of true syphilis patients for its low prevalence. There were 1,424 case reports from Korean Centers for Control and Prevention (KCDC) in 2007."

p5 line 41-51-- some of this is redundant and could be shortened

>We briefly changed.

p5 line 53 would suggest removing the words "each step"

>We removed.

p6 line 10-17 this sentence is slightly awkward-- would suggest removing "were reacted within 10 seconds" as that is in the next line and rewrite how the reagents were added.

>We rewrite as your recommend.

p6 line 32-44 I find this very hard to follow-- I am not sure that you need to describe the physical locations of the wells but rather what conditions and controls were included.

>We described more briefly.

p7 line 37-41 I find this sentence difficult to understand would you consider something like: "When both RPR tests were positive, there was a 96% concordance with the TPPA..

>Yes, both RPR (automated and manual) positive specimens showed 96.9% concordance with the

TPPA positive results.

p7 line 44 suggest removing "kinds of"  
>We removed.

p7 line 50 suggest a new sentence to describe the 2 false positive HBI tests.  
>We added a new sentence.

p8 line 19 I am not sure that this is tied to the hypotheses... and if so it would be good to understand whether this happens with higher and lower RPR values or both .  
>In this study, we cannot sure that the hypotheses was related with the level of RPR. Our results (Table 5) showed the levels of manual and automated RPR and TPPA, but definite relations was not proved according to the level of test results. Further study can be designed to elucidate the correlation between the level of RPR results.

p8 line 35 non treponemal tests cannot really distinguish between treated vs non treated either.. I would suggest that this paragraph be more about the advantage of automated vs manual RPR as it is the main point of the study.  
>We rewrite as your recommendation.  
We described "Manual RPR test has been used and issued its automation. Automated RPR test was launched and has been used because of its convenient clinical settings. However, automated RPR should be fully inspected and compared with other syphilis diagnostic approaches.

p8 line 46 I would suggest removing the word "guaranteed"  
>We removed.

p8 line 53 I am not sure why the FTA is being discussed at all in the discussion  
>Traditionally, FTA-ABS test showed high specificity of syphilis diagnosis. We did not mainly include FTA-ABS test, but referenced previous study related with comparison between FTA-ABS and TPPA.

p9 line 44 as outlined above-- I am not sure why it might be beneficial to have a early seroconverting RPR from a clinical perspective unless you do not report quantitation  
>We also think early seroconversion could be meaningful with semiquantitative results.

p10 line 10 would suggest removing the word "some"  
>We removed.

table 2 would add the word syphilis in the title, would define the RPR card values, c and h are in reverse order in Bechet and do you mean genital Herpes simplex instead of herpes penis?  
>We changed as your recommend. Behcet's disease was identified.  
It was a medical charting records.

table 3 would italicize *Treponema palladium*  
>We corrected.

table 4 would refer to syphilis and add performance characteristics in title  
>We changed title.

table 5 as above would suggest adding pre treatment RPR values  
>We could find out the pretreatment VDRL test titer rather than RPR test results at that time. The automated RPR results were introduced from January 2013, so there were no automated RPR results

until 2012 year. All the obtainable pretreatment VDRL values were added in Table 5. The automated RPR results showed more decreased than manual RPR card results after treatment.

We described in DISCUSSION as

"In fact, some late or latent syphilis cases were hard to interpret the results of non-treponemal test after initial treatment (Case No. 8 or 9 in Table 5)."

### VERSION 2 – REVIEW

<b>REVIEWER</b>	Vanessa Allen Public Health Ontario, Canada
<b>REVIEW RETURNED</b>	17-Sep-2014

<b>GENERAL COMMENTS</b>	<p>page 2 line 12 I do not think that "Method comparative study" is grammatically correct. Do you mean a comparative study of lab methods? also there should be a period at the end of this sentence.</p> <p>page 2 line 53, I do not think that you can say " probably due to higher seronegative conversion after treatment than the conventional RPR card test." as there may be multiple reasons why the automated RPR is less sensitive</p> <p>page 2 line 33: I am unclear why seroconversion would be more important in the reverse algorithm testing approach-- it seem so me me that it would be useful in both cal sic and reverse if it was being used to look at response to treatment</p> <p>P4, line 15-- "Even though" is not grammatically correct. Do you mean something like "despite these low rates,"</p> <p>p4 line 19 I am not sure that I understand " So, appropriate screening, confirmation, and follow-up protocols are well established." Do you mean that these protocols are critical to public health?</p> <p>p4, line 50 do you mean large volumes of samples? Scale could be that each clinical sample is large</p> <p>p4, line 53. I would suggest removing "with good analytical performances" as this is the hypothesis of your study rather than the intro of already known data. Unless you want to say that the ideal automated RPR would be...</p> <p>p5 line 15 would suggest adding "ratio" after male to female.</p> <p>p5 line 18 would add "were" after treponemal tests and before completed</p> <p>p6 line 50 suggest adding "of" before "incubation"</p> <p>p7 line 53 If I understand this section correctly, I would suggest removing either "And there were two false positive cases in HiSens Auto RPR LTIA test. " or " while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test" as they seem to be saying the same thing". I would also then say of the 22 samples that were positive by BD and negative by LTIA" instead of -- of the 22 discrepant-- because you just said above that there are 24</p>
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discrepant...

If I did not understand correctly-- I would look at this section again to make sure that it is clearer.

p8 line 46 " suggest remove "the" before past infections

p9 line 3 suggest removing "the" before syphilitic patients"

p9 line 6 I would reword this sentence as it is a bit difficult to understand as written. perhaps " we compared an automated RPR test with a conventional RPR card test in sera already tested by TPPA." or something of that ilk.

p9, line 8-- again, I do not see the value of discussing the FTA in the discussion.

p9 line 31- as in above-- I am not sure why the lower sensitivity of the automated RPR is better in the reverse algorithm testing approach. It would seem to me that if it is useful , it would be useful in both.

p9 line 37 I would suggest replacing "began to adopt: with has been adopted in many jurisdictions"

p9 line 46 would suggest removing " important" as it is redundant to "advantage" in this sentence. I would also remove "in addition to those previously described"

p9 line 51 would you consider "seroconversion" instead of "seronegative conversion" as this is a novel phrase.

p9 line 55 there are many reasons why the RPR could be negative-- and what you have discussed earlier is that it is the conversion from RPR + to RPR - that may be advantage, not RPR - on the initial screen. This is a very different conclusion and I do not think that it is supported by your data. However, based on your data the automated RPR is too insensitive to be relied upon as a screen and should instead be used as an adjunctive test-- which I can understand .

p10, line 49 i would suggest rebooking at this sentence "The automated RPR could be more useful in the treated cases though it's sensitivity is lower after treatment." as you have focused that it is useful using it as pre and post treatment. If it is treponemal screen +, and automated RPR - in isolation this does not change your management as it could be I be latent disease or treated disease which would have different clinical approaches that would be similar if the RPR was +.

page 11, line 5 again, I am not sure why this is especially helpful in the reverse syphilis screening algorithm.

This is greatly improved, and a helpful results to have available to users. However, there are still two main points that require more explanation.

First, such as on page 9 line 55-- I agree with the conclusion that the automated RPR could be used to follow treatment, but not that it could be used to determine if someone has been treated already (as these individuals with treponemal screen +, RPR - / RPR + could

	<p>also have untreated latent syphilis)</p> <p>Second, if the less sensitive RPR is used to follow treatment, it would not matter if it is the classic or reverse screening algorithm. All you would need either way is to get from a positive to a negative RPR with treatment. I do not think that the reverse algorithm is supported based on the data in this study of following treatment response. However, one could argue that the sensitivity of the automated RPR is too low to be an appropriate screen-- which is slightly different.</p>
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## VERSION 2 – AUTHOR RESPONSE

Reviewer Name Vanessa Allen

Institution and Country Public Health Ontario, Canada

Please state any competing interests or state 'None declared': None declared

▶None declared was stated

page 2 line 12 I do not think that "Method comparative study" is grammatically correct. Do you mean a comparative study of lab methods? also there should be a period at the end of this sentence.

▶We described "a comparative study of lab methods" and added a period.

page 2 line 53, I do not think that you can say " probably due to higher seronegative conversion after treatment than the conventional RPR card test." as there may be multiple reasons why the automated RPR is less sensitive

▶We changed as "Also, the automated RPR test showed higher seronegative conversion after treatment than the conventional RPR card test."

page 2 line 33: I am unclear why seroconversion would be more important in the reverse algorithm testing approach-- it seem so me me that it would be useful in both cal sic and reverse if it was being used to look at response to treatment

▶In reverse algorithm, sensitive treponemal test is used at first line. It could detect all the syphilitic patients initially. We thought that if all the patients could be detected in reverse algorithm testing, and then the automated RPR could be used effectively for therapy monitoring.

P4, line 15-- "Even though" is not grammatically correct. Do you mean something like "despite these low rates,"

▶We changed as "despite these low rates".

p4 line 19 I am not sure that I understand " So, appropriate screening, confirmation, and follow-up protocols are well established." Do you mean that these protocols are critical to public health?

▶Sure, these system could be helpful to public health

p4, line 50 do you mean large volumes of samples? Scale could be that each clinical sample is large

▶We changed as "a large number of samples".

p4, line 53. I would suggest removing "with good analytical performances" as this is the hypothesis of your study rather than the intro of already known data. Unless you want to say that the ideal automated RPR would be...

▶We removed.

p5 line 15 would suggest adding "ratio" after male to female.



►We added ratio.

p5 line 18 would add "were" after treponemal tests and before completed

►We changed as "after treponemal test were collected from November 2012 to April 2013 in a university hospital in Korea."

p6 line 50 suggest adding "of" before "incubation"

►We added.

p7 line 53 If I understand this section correctly, I would suggest removing either "And there were two false positive cases in HiSens Auto RPR LTIA

test. " or " while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test" as they seem to be saying the same thing". I would also then say of the 22 samples that were positive by BD and negative by LTIA" instead of -- of the 22 discrepant-- because you just said above that there are 24 discrepant..

If I did not understand correctly-- I would look at this section again to make sure that it is clearer.

►We removed "And there were two false positive cases in HiSens Auto RPR LTIA".

p8 line 46 " suggest remove "the" before past infections

►We removed.

p9 line 3 suggest removing "the" before syphilitic patients"

►We removed.

p9 line 6 I would reword this sentence as it is a bit difficult to understand as written. perhaps " we compared an automated RPR test with a conventional RPR card test in sera already tested by TPPA." or something of that ilk.

►We reworded as your recommend.

p9, line 8-- again, I do not see the value of discussing the FTA in the discussion.

►We removed "The TPPA test was reported to be as sensitive as the FTA-ABS test in all the stages of syphilis and as useful as the RPR test for monitoring therapy. "

p9 line 31- as in above-- I am not sure why the lower sensitivity of the automated RPR is better in the reverse algorithm testing approach. It would seem to me that if it is useful , it would be useful in both.

►The lower sensitivity can not be an advantage of automated RPR but we thought it might be useful to show therapy response earlier than traditional RPR card assay. In reverse algorithm, treponemal specific antibody identified, it could be effectively adjusted by automated RPR for confirming treatment response.

p9 line 37 I would suggest replacing "began to adopt: with has been adopted in many jurisdictions"

►We reworded as your recommend.

p9 line 46 would suggest removing " important" as it is redundant to "advantage" in this sentence. I would also remove "in addition to those previously described"

►We removed line 46 to 46 comment.

p9 line 51 would you consider "seroconversion" instead of "seronegative conversion" as this is a novel phrase.

►We corrected as seconversion (Antibody positivity changed as antibody negativity.).

p9 line 55 there are many reasons why the RPR could be negative-- and what you have discussed earlier is that it is the conversion from RPR + to RPR - that may be advantage, not RPR - on the initial screen. This is a very different conclusion and I do not think that it is supported by your data. However, based on your data the automated RPR is too insensitive to be relied upon as a screen and should instead be used as an adjunctive test-- which I can understand .

►We found automated RPR has relatively less sensitivity than classic card RPR. Therefore, we thought that automated RPR did not seem to match with initial screening of syphilis. However, the automated RPR showed earlier treatment response than those of classic card RPR. In reverse algorithm, sensitive treponemal tests were used in first line, so we thought that the automated RPR might be used to detect earlier seroconversion than classic RPR test.

p10, line 49 i would suggest rebooking at this sentence "The automated RPR could be more useful in the treated cases

though it's sensitivity is lower after treatment." as you have focused that it is useful using it as pre and post treatment. If it is treponemal screen +, and automated RPR - in isolation this does not change your management as it could be I be latent disease or treated disease which would have different clinical approaches that would be similar if the RPR was +.

►We changed as your recommend.

page 11, line 5 again, I am not sure why this is especially helpful in the reverse syphilis screening algorithm.

►We changed conclusion as your recommend. Automated RPR test is not sensitive to be an initial screening test as you indicated.

(We found automated RPR has less sensitivity than classic card RPR. Therefore, we thought that automated RPR did not seem to match with initial screening of syphilis. However, the automated RPR showed earlier treatment response than those of classic RPR. In reverse algorithm, sensitive treponemal tests were used in first line, we thought that the automated RPR might be used to detect earlier seroconversion in treated cases).

This is greatly improved, and a helpful results to have available to users. However, there are still two main points that require more explanation.

First, such as on page 9 line 55-- I agree with the conclusion that the automated RPR could be used to follow treatment, but not that it could be used to determine if someone has been treated already (as these individuals with treponemal screen +, RPR - / RPR + could also have untreated latent syphilis)

►This study has some limitations, a small scale of case number because syphilis patients are very rare. Also, each stage of syphilis was not classified. Therefore, to solve above first question, further well designed study should be performed.

Second, if the less sensitive RPR is used to follow treatment, it would not matter if it is the classic or reverse screening algorithm. All you would need either way is to get from a positive to a negative RPR with treatment. I do not think that the reverse algorithm is supported based on the data in this study of following treatment response. However, one could argue that the sensitivity of the automated RPR is too low to be an appropriate screen-- which is slightly different.

►In this study we noted that automated RPR was not sensitive than classic card RPR. Therefore, we thought that automated RPR did not seem to match with initial screening of syphilis. However, the automated RPR showed earlier treatment response than those of classic card RPR. So, in reverse algorithm, sensitive treponemal tests were used in initial screening, then the automated RPR might be

used to detect earlier seroconversion in treated cases.

Therefore we changed conclusion as “In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to be earlier seroconversion response in treated cases than those of conventional RPR card test. If reverse algorithm is applied, sensitive treponemal tests were used in first line, and then the automated RPR might be used as adjunct to detect earlier seroconversion in treated patients.

Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.”

### VERSION 3 – REVIEW

<b>REVIEWER</b>	Vanessa Allen Public Health Ontario, Canada
<b>REVIEW RETURNED</b>	20-Nov-2014

<b>GENERAL COMMENTS</b>	<p>The manuscript is greatly improved and in my view, ready for publication and is a useful study for those in the field</p> <p>A few very small details to consider prior to publication.</p> <p>p4 line 20 either remove "so" OR replace "are well established" with ""are required"</p> <p>p5 line 15 the # of samples should actually be in the results section and not the methods section. Perhaps you could use something like "All positive sera for syphilis by one or more tests from November 2012 to April 2013 from a single centre were included along with matched controls"</p> <p>p10 line 13 missing an "e" in "treponemal"</p> <p>p11 line 4 suggest replace "were" with "are" and replace "in first line" with "as a first line screening test"</p> <p>add an "an" between "as" and "adjunct"</p>
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### VERSION 3 – AUTHOR RESPONSE

Reviewer Name Vanessa Allen

Institution and Country Public Health Ontario, Canada

Please state any competing interests or state 'None declared': None declared

The manuscript is greatly improved and in my view, ready for publication and is a useful study for those in the field

A few very small details to consider prior to publication.

p4 line 20 either remove "so" OR replace "are well established" with ""are required"

→We changed as your recommend.

p5 line 15 the # of samples should actually be in the results section and not the methods section. Perhaps you could use something like "All positive sera for syphilis by one or more tests from November 2012 to April 2013 from a single centre were included along with matched controls"

→We changed as your recommend: "All positive sera for syphilis by one or more tests from November 2012 to April 2013 from a university hospital were included along with matched controls. " was described and the # of samples was described in the results.

p10 line 13 missing an "e" in "treponemal"

→We changed as your recommend.

p11 line 4 suggest replace "were" with "are" and replace "in first line" with "as a first line screening test"

→We changed as your recommend.

add an "an" between "as" and "adjunct"

→We changed as your recommend.

**We changed minor as follows:**

**P2 line 48 "manual" was changed as "conventional"**

**P3 line 23 “sensitivity” was changed as “positivity”**

**P5 line 33 We inserted “All the study processes were followed by the World Medical Association Declaration of Helsinki.”.**