Author's response to reviews

Title: Prevalence of Trichomonas Vaginalis Infection in Egyptian Women: Cross-Sectional Study

Authors:

Ahmed Mahmoud MD (ahmad.mahmoud.ali@hotmail.com)
Nadine A Sherif MD (nadine.sherif@kasralainy.edu.eg)
Rana Abdella MD (rana.abdella@yahoo.com)
Amira R El-Genedy MBBCh (amiraraafat@live.com)
Abdalla Y El Kateb MD (aelkateb@hotmail.com)
Ahmed NH Askalani MD (a_askalani@hotmail.com)

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Author's response to reviews: see over
Cover Letter of the Revised Manuscript for reviewers:

Reviewer's report 1

Title: Prevalence of Trichomonas Vaginalis Infection in Egyptian Women: Cross-Sectional Study

Version: 2 Date: 21 July 2014

Reviewer: Asghar Fazaeli

Reviewer's report:

Major Compulsory Revisions:

1) The manuscript Title, Aim and Introduction shows that the study is a cross-sectional prevalence survey; however, later in the methods, results and discussion, the story has been shifted to a comparatively analysis of the results focusing on detection methods comparison.

This is in fact a cross-sectional study to detect the prevalence of Trichomonal infection where we used various methods for diagnosis namely, the modified Diamond culture as the gold standard test, the Kalon Latex test, wet mount with light microscopy and
Giemsa stain to improve the detection rate while comparing the accuracy of these tests in detection.

2) If the modified Diamond Culture is considered as the gold standard (detecting 30 positive cases), the specificity of the Kalon Latex Test (detecting 50 cases as positive) must be much lower than what the authors showed (97.9%).

Modified Diamond culture was the gold standard test used that detected 30 positive cases for trichomonal infection out of the 1000 women studied. Kalon LATEX agglutination detected 50 cases as positive where 20 cases would be considered false positive considering the gold standard test for comparison. Hence, the specificity was calculated as follows:

Specificity = true negative / (false positive + true negative) × 100

i.e specificity of Kalon Latex test = 950/(20+950) × 100 = 97.9%

which is indeed a high specificity inspite of the 20 false positive cases, however the positive predictive value is considerably low in comparison to the
sensitivity and specificity of the test which was also calculated as follows:

Positive Predictive value (PPV) = True positive / (true positive + false positive) × 100

i.e. PPV for the Kalon Latex test = \( \frac{30}{30+20} \times 100 = 60\% \)

Minor Essential Revisions:
1) The data of the tables are mostly repeated in the text. (table 5 and table 6 were removed)
2) The photographs could be omitted or decreased to one photo. (photographs were decreased to only one photo)
3) The last paragraph before the discussion, does not make sense. (paragraph removed)
4) In the discussion part:
   a) The first paragraph should be omitted; (omitted)
b) the lines 133 to 137 should be documented with references; (reference 16 added, line 248)

c) lines 138 to 140 should be omitted. (omitted)

4) As clinical symptoms are highlighted and analysed by the authors, other possible accompanied STI agents would be recommended to be considered. (added in the Recommendations line 181)

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited (extensive editing done)

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interest.

Reviewer’s report 2
Title: Prevalence of Trichomonas Vaginalis Infection in Egyptian Women: Cross-Sectional Study

Version: 2
Date: 28 July 2014
Reviewer: Barbara Body

Reviewer's report:
Major compulsory Revisions
I am troubled that other causes of vaginitis were not examined in this population of women with a complaint of vaginitis such as bacterial vaginitis and candidiasis and would like to see a rationale for this choice. Although we often believe that each type of vaginitis has a typical presentation, I believe that it is the rule that more often than not the presentation is not perfectly typical. For this reason, I believe the authors need to comment on why they limited their examination to only T. vaginalis, especially because the percent of infected women found to be positive by culture is so low overall. This is in contrast to studies in the US and Europe where much higher rates of trichomoniasis are found in symptomatic women.
We aimed to study the prevalence of trichomonal infection per se, not knowing what to expect. We retrospectively found that the prevalence of infection was relatively low inspite of the symptomatizing women which could have been possibly suffering from other forms of vaginitis but with a similar clinical picture. The low prevalence can be possibly attributed to the social and cultural conservative nature of the Egyptian society regarding free sexual relations. Other causes of vaginitis such as candidiasis or bacterial vaginosis have been extensively studied at our centre with no emphasis or large scale study on trichomoniasis prevalence.

In their results section (line 97 as well as 152) the authors state that the Modified Diamond culture is their reference/gold standard but in consideration of the data for Table 3 through 7 they use the number of 50 cases which corresponds to the Kalon assay not the culture. I believe this is a significant issue, as neither the work presented here or the peer reviewed literature have performed sufficient discrepant
analysis to deem those specimens positive by the Kalon test to be ‘true positive’. In the package insert for the Kalon test such specimens are called ‘false positives’. The Tables 3-7 need to reflect those positive by culture verses those positive by culture and Kalon and finally those positive by Kalon only. Some discussion of this issue beyond the single sentence (line 169-171) is warranted in this manuscript. Now that there are at least two commercially available nucleic acid amplification assays for T. vaginalis these authors could be the first to suggest this as a path toward finally resolving the performance of the Kalon test. (I am not suggesting they actually perform this comparison). If two different nucleic acid amplification tests are positive and culture is negative this could be seen as strong evidence to support the possibility of improved performance compared to culture by nucleic acid amplification and if the Kalon test also was positive, it would establish whether some or all of the present false positives are true positives.
The gold standard test we used for diagnosis of trichomonal infection and to which other tests were compared was the modified Diamond culture being very accurate, however, expensive, time consuming with a late diagnosis of infection. The Kalon Latex test used was able to diagnose 50 cases as positive for Trichomonas in comparison to the Diamond culture which only gave a positive diagnosis in 30 cases. Whether the apparently false positive cases (20 cases) by the Kalon test are truly false positive or some of which are true positive can not be resolved unless confirmatory tests - that could be superior to culture are performed as you have mentioned - such as PCR being performed along hand. This could be the scope of future studies. So, until then we would have to suppose Kalon Latex test as a very sensitive test that is comparable to culture in detection of Trichomonal infection which could be implemented as a good screening test being cheap, easy to perform and with rapid results.

In other words, a positive Kalon test is in itself poor at confirming Trichomonal infection (PPV = 60%) and further investigations must be undertaken; it did, however, correctly identify 100% of all cases (the sensitivity). However as a screening test, a negative result is very good at reassuring that a patient does
not have the infection (NPV =100% ) and at this initial screen correctly identifies 97.9% of those who do not have trichomonas infection (the specificity).

TABLE 1 has been modified to make comparison between the various tests and modified Diamond culture easily readable showing the true negatives, true positives, false negatives and positives.

The Kalon test, if accurate, performed much better than culture, finding 60% more positives, but no discrepant analysis was performed to attempt to resolve this issue. I believe the specificity needs to be addressed in the discussion. I do not believe their conclusion is warranted because no experiments were performed to show that the culture negative – Kalon positive specimens were truly positive.

Hence with large numbers of false positives and few false negatives, a positive screening test is in itself poor at confirming the disorder (PPV = 60%) and further investigations must be undertaken; it did, however, correctly identify 100% of all cases (the sensitivity). However as a screening test, a negative result is very good at reassuring that a patient does not have the disorder (NPV =100% ) and at this initial
screen correctly identifies 97.9% of those who do not have trichomonas infection (the specificity) ie true negative rate. (addressed in the discussion too: line 120)

Minor Essential Revision –
There are numerous typographical errors of periods and spacing that need to be addressed (revision done)

Discretionary revisions
The statements line 49-52 do not anything to this manuscript I suggest removing them. (removed)

Please confirm that the swabs used were cotton-wool with wooden shafts (line 63) verses Dacron with plastic shafts. (wooden shafts added)

The detail of figure 2 is not very good I recommend deleting it all together. (figure 2 deleted)
Figures 2 and 3 each have unnecessary duplicates, I recommend inclusion of only one photo for each of them (if Figure 2 is to be included).

(All figures were grouped in only one figure)

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited (extensively edited)

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I have participated in clinical trials for the clearance of nucleic acid amplification tests for Trichomonas
vaginalis from both Gen-Probe (now Hologic) and Becton Dickinson. This work was contracted through LabCorp Clinical Trials; I received no direct compensation. As an officer and employee of LabCorp I own stock in the company. I have no additional competing interests.