Original Article

Poor performance of Point-of-Care (POC) kits for *Chlamydia trachomatis* antigen detection in endocervical specimens

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ABSTRACT:

**Background:** Chlamydia trachomatis is a common sexually transmitted bacterium which primarily affects women in reproductive age and is associated with various reproductive complications. This infection being an important public health concern, several Point of Care (POC) kits are available for rapid diagnosis, but with a questionable performance. Genital Chlamydia trachomatis infection among symptomatic and healthy pregnant women of Pondicherry and surrounding Tamil Nadu is our primary objective. Evaluation of the sensitivity, specificity and practical utility of SD BIOLINE Chlamydia Rapid Test for C. trachomatis antigen detection is the secondary objective.

**Material & Methods:** This prospective cohort study was carried out at Mahatma Gandhi Medical College & Research Institute, Puducherry during December 2010 - June 2012.

Endocervical swabs and serum samples were collected from 100 patients. Antigen detection from endocervical samples was done using a solid phase immunochromatographic assay. Antibody detection for IgM, IgG, and IgA from serum samples was done using an enzyme immunoassay, as per manufacturer’s guidelines.

**Results:** All 100 cervical swabs, 75 from symptomatic and 25 from non-symptomatic healthy pregnant women were negative for C. trachomatis antigen by SD Bioline Kit. However, 13 serum samples out of the 75 symptomatic women were positive for C. trachomatis antibodies: IgM/IgG/IgA. Two of them had both IgG and IgA antibodies.

**Conclusion:** SD BIOLINE Chlamydia rapid test, a Point of Care (POC) kit, has a poor sensitivity for C. trachomatis antigen detection in the endocervical swabs. Similar poor performance of other POC kits for C. trachomatis antigen detection is reviewed.

INTRODUCTION:

*Chlamydia trachomatis* (CT) is a common sexually transmitted pathogen responsible for about 89 million new cases occurring worldwide every year (1, 2). This infection is an important public health concern as it primarily affects women in reproductive age and is associated with various reproductive complications (3). A few studies from India, have documented a prevalence of CT infection ranging from 3-20% among antenatal women and 18-30% among those with sexually transmitted diseases (STD) (4-7). Majority (70-90%) of the women with CT infections are asymptomatic, while the others manifest a wide range of symptoms indicative of urethritis, cervicitis and acute salpingitis (1,8,9). The infected women are at an increased risk of serious reproductive sequelae such as pelvic inflammatory disease (PID), infertility and ectopic pregnancy (1,2,6). CT infections in pregnant women can result in adverse obstetric outcomes such as preterm delivery, low birth weight...
and premature rupture of the membranes (10, 11). Ante-partum chlamydial infection also plays an important role in amnionitis, post-partum endometritis and post-abortal salpingitis (5). *C. trachomatis* culture, though it is a gold standard, is cumbersome and has poor sensitivity and hence non-cultural test like Enzyme Immunoassay (EIA), Direct fluorescent antibody (DFA), Polymerase chain reaction (PCR) and Nucleic Acid Amplification Test (NAAT) are preferred for direct demonstration of antigen/DNA (1-25).

The purpose of the research is to search for genital *Chlamydia trachomatis* infection among symptomatic and healthy pregnant women of Puducherry and also to validate the sensitivity, specificity and practical utility of SD BIOLINE Chlamydia Rapid Test for *C. trachomatis* antigen detection is the secondary objective.

**MATERIALS AND METHODS:**

This prospective cohort study was carried out at Mahatma Gandhi Medical College & Research Institute, Puducherry, during December 2010 - June 2012. After approval by the Institutional Human Ethical Committee (IHEC), written informed consent was obtained from 100 pregnant women, 25 of them healthy controls and 75 symptomatic women with one or more of the following signs and symptoms:

1. Fever
2. Presence of vaginal discharge.
3. Dyspareunia
4. Perineal itching
5. Dysuria
6. Abdomen / pelvic pain
7. Muco purulent cervical discharge
8. Cervical erosion
9. Cervicitis
10. Adnexa tenderness
11. Abnormal menstrual history

**Specimen collection:** (Figure – 1)

Excess mucus was removed from the exocervix using a sterile swab. Another sterile swab was inserted into the endocervical canal past the squamo-columnar junction until most of the tip not visible. The swab was rotated for 15-20 secs and brought out without contamination with exocervical or vaginal cells. The swab was returned to the transport tube for storage and transport and stored in room temperature for an hour.

**Processing of specimen:** (Figure – 2)

Extraction Procedure: Preparation of extracted Sample

1. After labeling, sample collection tube was opened.
2. Reagent A was taken upto the fill line (300 µl) and transferred into the tube.
3. Patient swab was inserted into the tube containing reagent A.
4. The bottom of the tube was compressed between the thumb and forefinger and the swab was twirled 10 times.
5. After two minutes, again the bottom of the tube was compressed between the thumb and forefinger and the swab was twirled 10 times.
6. The dropper was held vertically and reagent B was drawn up to the fill line (600µl) and transferred into the tube.
7. Again the bottom of the tube was compressed between the thumb and forefinger and the swab was twirled 10 times.
8. The liquid was expressed from the swab by compressing the middle of the tube and the swab was pulled through it and discarded.

**Assay Procedure:**

1. Test device was removed from the foil pouch and placed on a clean, dry surface.
2. Dropping cap was assembled on the sample collection tube and 4-5 drops (120-150µl) of the extracted sample was added to the sample well on the device.

3. As test began to work a purple colour moved across the membrane in the centre of the test device.

4. Result was interpreted at the end of 15 minutes.

**Antigen detection**

Antigen detection from endocervical samples was done using SD BIOLINE Chlamydia Rapid Test (Seoul, South Korea), a solid phase immunochromatographic assay for rapid and qualitative detection of Chlamydia antigen.

**Principle:**

The SD BIOLINE kit contains a membrane strip which is pre-coated with mouse monoclonal anti-*C. trachomatis* antibody on test band region. The complex of sample including chlamydia antigen and mouse monoclonal anti-*C. trachomatis* -colloid gold conjugate moves along the membrane chromatographically to the test region and forms a visible line as the antibody- antigen-antibody gold particle complex forms. Therefore the formation of a visible line in the test region indicates positive result for the detection of Chlamydia antigen. When the Chlamydia antigen is absent in the sample no visible color band in the test region.

**Interpretation of the test:** (Figure-1)

**Negative results:** The presence of only one purple colour band (control band) within the result window indicates negative results and the specimen is presumptive negative for the presence of chlamydial antigen.

**Positive results:** The presence of two purple colour bands (control and test band) within the result window indicates positive results and the specimen is presumptive positive for the presence of Chlamydial antigen.

**Invalid results:** If the purple coloured band was not visible within the result window then the result was considered invalid and was tested again with another card.

**Antibody detection**

From all the 100 volunteers, 4ml blood was collected in sterile tubes without anti-coagulant, sera were separated, aliquoted and preserved at -20°C till the time of testing. These sera were examined by Chlamydia trachomatis IgM, IgG and IgA ELISA kits, (NovaTec Immunodiagnostics GmbH, Germany) strictly adhering to the kit instructions.

**Principle:**

The qualitative immunoenzymatic determination of antibody against *C. trachomatis* is based on the ELISA (Enzyme -linked immunosorbent assay) technique. Microtitre strip wells are pre-coated with *C. trachomatis* antigens to bind corresponding antibodies (IgG, IgM, IgA) of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human (IgG, IgM, IgA) conjugate is added. The conjugate binds to the captured chlamydia specific antibodies. The immune complex formed by the bound conjugate is visualized by adding TMB (Tetramethyl benzidine) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of chlamydia specific antibody in the specimen. 0.02 M Sulphuric acid is added to stop the reaction. This produces a yellow end point colour. Absorbance at 450 nm is read using an OD (optical density) readings were taken in iMark Microplate Reader, Bio-Rad, Japan.

**Interpretation of results**

• Validation criteria was run and results were calculated based upon the absorbance value of cut-off control.
• Values higher than 10% over that cut off were considered positive.
• Values 10% below the cut-off were considered negative.
• Values in between were considered as grey zone and repeated again. And those results again in the grey zone were considered negative.

STATISTICAL ANALYSIS
The Chi-square test and Fisher’s exact test were used to compare two groups. All P values < 0.05 were considered statistically significant.

RESULTS:
All 100 cervical swabs – 75 from symptomatic and 25 from non-symptomatic, healthy pregnant women were negative for C. trachomatis antigen by the SD Bioline Kit. However, 13 serum samples out of the 75 symptomatic women were positive for C. trachomatis antibodies:
Only IgM was detected in five patients, IgG in seven patients and IgA in three patients. Two patients had both IgG and IgA antibodies. All 25 healthy controls were negative for C. trachomatis antibodies – IgM/IgG/IgA.

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Figure – 1: Specimen processing for *C. trachomatis* antigen and antibody detection

**SPECIMEN COLLECTION AND PROCESSING: ALGORITHM**

1. Patient's information was collected.
2. Consent form was signed by the patient.
3. Endocervical swab and blood sample were collected from patient and sent to the lab with requisition form.

**In Lab**

- Blood sample
- Endocervical swab was processed immediately

**Blood sample**

- Serum was separated and stored at -20°C for detection of *Chlamydia trachomatis* antibodies (IgG, IgA, IgM) by ELISA

**Endocervical swab**

- Extract was tested for *Chlamydia trachomatis* antigen by ICT (Rapid Immunochromatographic assay)

**Results were interpreted**

- Positive
- Negative

- Positive
- Negative
Figure-2: Protocol for extraction and testing for *Chlamydia trachomatis* antigen in endocervical specimens
Table-1: Performance of different POC kits for *C. trachomatis* antigen detection

<table>
<thead>
<tr>
<th>S.No</th>
<th>Rapid Kit</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>References Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QuickVue Chlamydia rapid test (RT)</td>
<td>22.7%</td>
<td>37.7%</td>
<td>Forero L et al., 2016 (28)</td>
</tr>
<tr>
<td>2</td>
<td>SD BIOLINE rapid antigen test kit</td>
<td>93.1%</td>
<td>98.8%</td>
<td>SD Diagnostic kit (26)</td>
</tr>
<tr>
<td>3</td>
<td>Chlamydia Rapid Test (CRT) (Diagnostics for the Real World (Europe), Cambridge, UK)</td>
<td>41.2%</td>
<td>96.4%</td>
<td>van der Helm JJ et al. 2012 (32)</td>
</tr>
<tr>
<td>4</td>
<td>Chlamydia Rapid Test (CRT)</td>
<td>Men- 41.4% and women 89.0%</td>
<td>Men- 74.2% and women 95.7%</td>
<td>Hurly DS et al 2014 (29)</td>
</tr>
<tr>
<td>5</td>
<td>Chlamydia test card (Ultimed Products, GmbH, Germany)</td>
<td>62.96%</td>
<td>99.60%</td>
<td>Sabido´ et al. 2009 (33)</td>
</tr>
<tr>
<td>6</td>
<td>Clearview Chlamydia Test Kit:</td>
<td>49.7%</td>
<td>97.9%</td>
<td>Yin et al., 2006 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>90%</td>
<td>Vidwan et al., 2012 (17)</td>
</tr>
<tr>
<td>7</td>
<td>Handilab-C (Zonda, Dallas, USA), Biorapid CHLAMYDIA Ag test (Biokit, S.A., Barcelona, Spain) and QuickVue Chlamydia test (Quidel Corporation, San Diego, USA).</td>
<td>17%</td>
<td>88.9%</td>
<td>Dommelen et al. 2010 (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27%</td>
<td>93.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12%</td>
<td>99.7%</td>
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DISCUSSION:
Although the culture of endocervical swabs has been considered the diagnostic gold standard for detection of cervical chlamydial infection in women, it is influenced by several factors such as method of sample collection, transportation time, storage of sample, and toxicity of swab (calcium alginate and dacron-on-plastic swabs were considered toxic to the cells in culture, while cotton-on-wood swab appeared to be less inhibitory to chlamydiae) (1). Therefore, the non-cultural methods such as direct Immunofluorescence, enzyme immunoassays and PCR are being preferably used for diagnosis of CT infections (1, 9). Once diagnosed, CT infections can be treated effectively reducing the risk of long term reproductive sequelae (3). This emphasizes the importance of identifying the patients at risk, detecting the infection and treating them with anti-chlamydial drugs (5).
Detection of *Chlamydia trachomatis* antigen in the cervical smears of women is facilitated by highly sensitive and specific test like Nucleic Acid Amplification Test (NAAT) (12). Immunofluorescence Assay (IFA) is however the gold standard serological test (9,11,12). Enzyme Immunoassay (EIA) is the alternate choice. The sensitivity and specificity of EIA was found to be 86.36% and 91.66% respectively according to Mohanty et al (13). According to Lefebvre et al., the sensitivity and specificity of EIA was 78.4% and 96.8% respectively (14). According Sachdeva et al (15) sensitivity of EIA applied to endocervical swabs was only 60 per cent, against culture as the reference. Keeping the highly sensitive NAAT as a reference, Dhawan et al reported sensitivity of only 48.1% but 100% specificity for EIA. Performance reports of different rapid kits for *C. trachomatis* antigen detection are available in the world as Point of Care Test (POC) kits as reported by different researchers in the world (16,17, 26-33), with varying levels of sensitivity and specificity which is much lower than the claims of the kit manufacturers, as detailed in Table-1. The sensitivity and specificity of Handilab Chlamydia, QuickVue Chlamydia, Biorapid Chlamydia Ag test and Clearview Chlamydia were 12%, 92%; 27%, 99%; 17%, 93% and 0%, 90% respectively (15-16), but SD Bioline Chlamydia rapid kit used by us failed to detect any positivity although the manufacturers claim a sensitivity of 93.1% (17). In our experience SD Bioline Chlamydia rapid kit had very poor sensitivity of 0%, which is similar to that of Taarini et al. (27). Vidwan et al. reported 0% sensitivity of Clearview Chlamydia Test kit (17). A very poor sensitivity of another two kits has been reported, namely, QuickVue Chlamydia test (12%) and Handilab-C (17%) (16), in detecting *C. trachomatis* antigen in the endocervical specimens.

**CONCLUSION:**
Rapid kits for *C. trachomatis* antigen detection are unreliable and unsuitable for any prevalence survey/disease diagnosis.

**REFERENCES:**


26. SD BIOLINE Chlamydia, One Step Chlamydia Antigen Test.


