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PERFORMANCE OF AN AUTOMATED MULTIPLEX IMMUNOFLUORESCENCE ASSAY FOR DETECTION OF CHLAMYDIA TRACHOMATIS IMMUNOGLOBULIN G

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Chlamydia trachomatis & serological tests
ABSTRACT

*Chlamydia* serology is indicated to investigate etiology of miscarriage, infertility, PID and ectopic pregnancy. Here, we assessed the reliability of a new automated-multiplex immunofluorescence assay (InoDiag test) to detect specific anti-*C. trachomatis* IgG. Considering IF as gold standard, InoDiag tests exhibited similar sensitivities (65.5%), but better specificities (95.1%-98%) than ELISAs. InoDiag tests demonstrated similar or lower cross-reactivity rates when compared to ELISA or IF.

KEY WORDS:

*Chlamydia trachomatis*, intracellular bacteria, serology, diagnostic test
INTRODUCTION

Prevalence of *Chlamydia trachomatis* genital infections is steadily increasing over the last decade (Rekart et al., 2013). The majority of chlamydial infections remain asymptomatic and therefore undetected causing a worldwide silent epidemic (Paavonen, 2012). Left untreated, *Chlamydia trachomatis* may lead to ascending infection and tubal damage with chronic sequelae such as tubal infertility and sterility (Baud and Greub, 2011; Paavonen, 2012). Moreover, *Chlamydia trachomatis* is recognised as an agent of miscarriage (Baud et al., 2011). Although PCR represents the ideal diagnostic approach for uretritis and cervicitis, serology remains useful to detect anti-chlamydial antibodies among women with tubal infertility, ectopic pregnancy, miscarriage, pelvic inflammatory disease and chronic pelvic pain (Baud et al., 2008; Baud et al., 2011; Baud and Greub, 2011; Haggerty et al., 2010), since in such chronic conditions, the negative predictive value is very high. Microimmunofluorescence assay (IF), still considered as the “gold standard” for the serology of this pathogen, has the disadvantage to be time-consuming, subjective and reader-dependant (Gaydos C and Essig A, 2011). However, enzyme-linked immunosorbent assays (ELISA) using the major outer membrane protein (MOMP) as antigen appears to exhibit a higher sensitivity for diagnosing chlamydial infections (Baud et al., 2010).

The present study aimed to investigate the performance of a new fully automatized “multiplex fluorescence immuno-assay” (Inodiag®, Signes, France) to detect specific anti-*C. trachomatis* IgG. Two different assay formats were investigated: Inodiag-EBs based on antibody reactivity to the elementary body (EB) and Inodiag-MOMP based on the MOMP of chlamydiae. Both Inodiag tests were compared with two other commercialized MOMP ELISA tests (MOMP-Medac® and MOMP-RBiopharm®) and to IF, which was considered to be the the gold standard.
METHOD

Studied sera were obtained from women with and without miscarriages as described previously by Baud et al. (Baud et al., 2007; Baud et al., 2009; Baud et al., 2010). A total of 265 sera were tested for the presence of Chlamydia trachomatis IgG antibodies with the new automated “multiplexed serology test” (Mu.S.T) as previously described (Baud et al., 2010; Gouriet et al., 2008a; Gouriet et al., 2008b), but using a new InoDiag platform allowing simultaneous testing of up to 12 patients in a single run. Practically, 2 different Chlamydia trachomatis antigens are present in the test: elementary bodies (InoDiag-EB) and MOMP (InoDiag-MOMP). Results of EBs and MOMP antigens were analysed separately and combined (hereafter called InoDiag-Combi, see details in Table 1).

Inodiag tests were compared with 3 other serological tests: immunofluorescence (Micro IF, ANILabsystems, Vantaa, Finland) and two C. trachomatis IgG ELISA both using the MOMP as antigen: MOMP-Medac, CT-IgG-pELISA, Medac, Webel, Germany and MOMP-R, CT pELISA, R-Biopharm, Darmstadt, Germany. All these 3 commercial tests were performed according to the manufacturers’ instructions.

To evaluate cross-reactivity, serum samples collected from patients with positive serologies against Toxoplasma gondii (n=81 cases), Waddlia chondrophila (n=83), Chlamydia psittaci (n=24), Chlamydia pneumoniae (n=96) and Brucella sp (n=11) were also analysed and the risk of cross-reactivity with Chlamydia trachomatis was statistically determined. All these serological tests were performed using commercial kits previously reported (Baud et al., 2007, Baud et al., 2010) and for Waddlia chondrophila using an home made immunofluorescence as earlier described (Baud et al., 2007).

To compare all five serological tests, the sensitivities, specificities, positive predictive values (PPVs) and negative predictive values (NPVs) were calculated using Stata (StataCorp, College Station, TX, USA). Values in the grey zone (i.e. with optical densities between the values
threshold for negativity and positivity, as defined by the manufacturer of MOMP-Medac and MOMP-RB) were excluded from these analyses. To assess cross-reactivity, we compared the correlation between serology directed against two different pathogens using the Chi$^2$ test.
RESULTS

Of the 265 sera, *Chlamydia trachomatis* IgG seropositivity rate was 14%, 19%, 16%, 11%, 13% and 9% with IF, MOMP-Medac, MOMP-RB, InoDiag-EB, InoDiag-MOMP and InoDiag-Combi, respectively. Inconclusive results were respectively observed in 0%, 3%, 7%, 2%, 3% and 10% of these serological tests. The level of agreement between the assays was similar. The percentage of concordance with results of IF was 63.9% for MOMP-Medac, 61.1% for MOMP-RBiopharm, 63.9% for Inodiag-EBs, 63.9% for MOMP and 55.6% for Inodiag-Combi.

Table 1 shows performance of the different assay using IF as gold standard. A total of 33 inconclusive samples (12%) were excluded from the inter-assays comparison as they exhibited a doubtful result by at least one assay. MOMP-RB and InoDiag-Combi exhibited the best sensitivities (72.4%), whereas InoDiag-EB exhibited the best specificity (98%).

A second analysis was conducted using a modified gold standard based on the results from IF, MOMP-Medac and MOMP-RB (see footnotes of Table 1 for details). This analysis involved 197 samples including 17 *Chlamydia trachomatis*-positive and 177 *Chlamydia trachomatis*-negative samples. InoDiag-MOMP and InoDiag-Combi exhibited the best sensitivities (100%), whereas InoDiag-EB exhibited the best specificity (99.4%).

All 6 tests cross-reacted with *C. psittaci* \( (p<0.001) \) and *C. pneumoniae* \( (p<0.05 \) – 0.001) as shown in Table 2. Moreover, IF also cross-reacted significantly with *T. gondii* and *W. chondrophila* (Table 1). InoDiag-MOMP showed the lowest cross-reactivity rate, with the different investigated species.
DISCUSSION

In this study, we assessed the performance of an automated multiplex antigen microarray (InoDiag) for the detection of *Chlamydia trachomatis* IgG antibodies.

The specificity of the Inodiag assays (94.6%-98%) was higher than the two commercialized ELISA used for comparison, whereas sensitivities were similar (65.5%-72.4%). In the present study, we showed that sensitivity was not improved by MOMP alone compared to EBs. However, the combination of MOMP and EBs by Inodiag increased sensitivity without altering specificity. All the tests studied here show low PPV, indicating that confirmation is required when positive as suggested by others (Mylonas, 2012). Moreover, we would recommend to use a second serological assay in case of doubtful result (grey zone).

The “gold standard” IF exhibits significant limitations: (i) high levels of cross-reactions with other members of the *Chlamydiaceae* family, (ii) labour-intensive and (iii) subjective and operator-dependant reading. In contrast, ELISAs (Medac or R-Biopharm) represent a test characterized by the following advantages: (i) objective reading of the results, (ii) less expensive than IF (especially when considering technician time) and (iii) high throughput, useful for epidemiological studies.

In contrast to our study, Muvunyi et al. showed that peptide-based ELISA performed as well as two different IF assays (Muvunyi et al, 2012). However, the performance of the InoDiag tests presented here are consistent with previous studies that revealed good sensitivities and specificities of ELISA assays based on peptides from the MOMP (Muvunyi et al, 2012 ; Land JA et al, 2003). The new automated microarray from Inodiag® show similar performance than ELISA. It is well designed for small laboratories, since batches of only 2 to 4 samples can be tested with InoDiag. Several pathogens can be tested simultaneously, allowing pathology-driven testing instead of the common pathogen-driven testing (Gouriet et al., 2008a; Gouriet et al., 2008b; Raoult et al., 2004). Moreover, thank to the multiplex format of the Inodiag technology,
new markers of chronic *C. trachomatis* infection might be added to improve serodiagnosis (Cappello et al., 2009).

**ACKNOWLEDGEMENTS**

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Reference List


Table 1-bis: Performance of the 3 INODIAG serological tests using a new gold standard\(^{(1)}\).

The 95% confidence intervals are shown in brackets.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>INODIAG - EBs</td>
<td>89.5% [66.9-98.7]</td>
<td>99.4% [96.9-100]</td>
<td>94.4% [72.7-99.9]</td>
<td>98.9% [96-99.9]</td>
</tr>
<tr>
<td>INODIAG-MOMP</td>
<td>100% [82.4-100]</td>
<td>97.2% [93.5-99.1]</td>
<td>79.2% [57.9-92.9]</td>
<td>100% [97.9-100]</td>
</tr>
<tr>
<td>INODIAG-Combi*</td>
<td>100% [82.4-100]</td>
<td>96.6% [92.8-98.8]</td>
<td>76% [54.9-90.6]</td>
<td>100% [97.9-100]</td>
</tr>
</tbody>
</table>

*InoDiag-Combi was designed according to the results of InoDiag-EB and InoDiag-MOMP.

True positives were defined as both tests positive and true negatives as both tests negative.

Discordant results or values in the grey zone were excluded from the analysis.

\(^{(1)}\) Performance of the tests using a new gold standard based on the results of IF, MOMP-Medac and MOMP-RB. True positives were defined as all 3 tests positive and true negatives as all 3 tests negative. All discordant results or results in the grey zone were excluded from the analysis.
Table 2: Rate of *Chlamydia trachomatis* positivity of each serological assay for sera identified IgG-positive for another pathogenic agent.

<table>
<thead>
<tr>
<th>Assay</th>
<th><em>Toxoplasma gondii</em></th>
<th><em>Waddlia chondrophila</em></th>
<th><em>Chlamydia psittaci</em></th>
<th><em>Chlamydia pneumoniae</em></th>
<th><em>Brucella abortus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>n=81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>21% [12-30] (+)</td>
<td>7% [2-13] (+)</td>
<td>79% [62-97] (+++)</td>
<td>27% [18-36] (+++)</td>
<td>9% [0-29]</td>
</tr>
<tr>
<td>MOMP-Medac</td>
<td>15% [7-23]</td>
<td>16% [8-24]</td>
<td>67% [46-87] (+++)</td>
<td>29% [20-38] (+++)</td>
<td>9% [0-29]</td>
</tr>
<tr>
<td>MOMP-RBiopharm</td>
<td>14% [6-21]</td>
<td>13% [6-21]</td>
<td>71% [51-90] (+++)</td>
<td>30% [21-40] (+++)</td>
<td>9% [0-29]</td>
</tr>
<tr>
<td>INODIAG - EBs</td>
<td>12% [5-20]</td>
<td>6% [1-11]</td>
<td>79% [62-97] (+++)</td>
<td>20% [12-28] (+)</td>
<td>0% [0-0]</td>
</tr>
<tr>
<td>INODIAG-MOMP</td>
<td>11% [4-18]</td>
<td>11% [4-18]</td>
<td>67% [46-87] (+++)</td>
<td>20% [12-28] (+)</td>
<td>0% [0-0]</td>
</tr>
<tr>
<td>INODIAG-Combi</td>
<td>9% [2-15]</td>
<td>6% [1-12]</td>
<td>67% [46-87] (+++)</td>
<td>16% [8-23] (+)</td>
<td>0% [0-0]</td>
</tr>
</tbody>
</table>

p-value < 0.05 = (+)

p-value < 0.01 = (++)

p-value < 0.001 = (+++)