Influence of storing urogenital specimens at $-20^\circ$C before testing by enzyme amplified immunoassay (IDEIA) to detect *Chlamydia trachomatis* antigen

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**SUMMARY** Urogenital specimens from 445 patients, 174 women and 271 men, were tested for antigen to *Chlamydia trachomatis* by an enzyme amplified immunoassay, IDEIA. The test results for specimens stored at $-20^\circ$C for means of 9.6 weeks (from each of the first 376 patients) and eight months (from the remaining 69) were compared with results for specimens stored at $4^\circ$C and tested within five days. Of 617 specimens (one from the urethra of each patient and one from the cervices of 172 women) cultured for *C trachomatis*, 90 (15%) gave positive results. The IDEIA results for specimens stored at $-20^\circ$C were identical with those of specimens analysed without such storage in 96.4% (595/617) of all cases. No difference was seen between urethral specimens from men or women or cervical specimens or between specimens stored for 9.6 weeks compared with those stored for eight months. In 22 cases in which the IDEIA results differed, culture positive results were missed in stored as well as unstored specimens. The median absorbance value above the cut off point for a positive IDEIA result in stored specimens was no lower than in those not stored. The few differences noted probably depended on the sampling technique rather than on the way of storing the specimens.

Genital infection with *Chlamydia trachomatis* is one of the most common sexually transmitted diseases. In 1987 in Sweden 38 000 cases of genital chlamydial infection were reported compared with 2200 cases of gonorrhoea. Culture, an established method of identifying *C trachomatis* infections, is time consuming, depends on viable organisms, and requires a well equipped laboratory as it is based on cell culture.

To cope with the increasing demand for laboratory identification of chlamydial infections, methods other than culture have been developed. These methods are based on the direct detection of chlamydial antigen in specimens by enzyme immunoassay (EIA) or immunofluorescence assay (IFA) using monoclonal antibodies. Several studies have assessed the sensitivity, specificity, and predictive values of these alternative methods. Results have varied considerably depending mainly on the reference methods and the study populations used. The main concern about these alternative methods has been their sensitivity, which has been reported to be 54% to 100% for EIA kits and 33% to 91% for IFA kits, whereas their specificity has been 92% to 100%. However, in geographical areas where culture cannot be performed because of insufficient laboratory facilities, such as some developing countries, these alternative methods are indispensable.

The IDEIA Chlamydia test (Boots-Celltech, UK), an enzyme amplified immunoassay based on genus specific monoclonal antibodies directed against a glycolipid antigen, was used for a study of prevalence of genital chlamydial infection in women in Mogadishu, Somalia. The prevalence of genital chlamydial infection in that study was 19% in pregnant women and 33% in prostitutes, which indicates the necessity and importance of further studies of this kind. Working in remote areas sometimes makes it necessary to store specimens for some time before testing. According to oral instructions from the manufacturer, the specimens for IDEIA should be stored at $-70^\circ$C if they cannot be analysed within five days. A $-70^\circ$C...
Influence of storing urogenital specimens at -20°C before testing by IDEIA to detect C trachomatis antigen

freezer is often not available, however, though many laboratories have a -20°C freezer.

The aim of this study was to assess whether storing urogenital specimens at -20°C influenced the results of the IDEIA.

Patients and methods

Patients
From mid-November 1985 to March 1986 specimens for IDEIA and chlamydial culture were taken from 445 patients, 174 women and 271 men. The mean age of the women was 26.6 (range 15 to 67) years and that of the men 28.4 (15 to 65). All the men and 118 of the women were patients attending the venereal disease outpatient clinic at Huddinge Hospital. The remaining 56 women attended a general outpatient clinic for genital problems.

Specimens
One specimen for chlamydial culture and two specimens for IDEIA were obtained from the urethra and the cervix of each woman and from the urethra of each man. Every other week the specimens for culture were taken before those for IDEIA and vice versa in alternative weeks. One of the IDEIA specimens from each site was stored at 4°C until analysed within five days (IDEIA-1) and the second was stored at -20°C until tested (IDEIA-2). The IDEIA-2 specimens from the first 376 patients (153 women and 223 men) were analysed after seven to 21 (mean 9.6) weeks and those from the following 69 patients (21 women and 48 men) after 7.7 to 8.4 (mean eight) months. The cervical specimen for IDEIA was missing from two of the 153 women whose IDEIA-2 specimens were analysed after 9.6 weeks.

Specimens for culture and IDEIA were taken with ear, nose, and throat (ENT) swabs (Swedish Hospital Supply, Möndal, Sweden). The transport medium used for IDEIA was supplied by the manufacturer, whereas we used sucrose phosphate buffer containing fetal calf serum and antibiotics for culture.11

Culture for Chlamydiae
The specimens for culture were stored at 4°C. Cultures were performed five days a week using cycloheximide treated McCoy cells in 24 well microtitre plates.11 The cultures were stained with fluorescein labelled antibodies to chlamydiae (Microtrak Culture Confirmation Test, Syva, Palo Alto, USA, or Cultureset Chlamydia Identification Reagent (IFA), Ortho Diagnostic Systems, New Jersey, USA) after incubation for 43 to 44 hours.

IDEIA
IDEIA was performed according to the instructions of the manufacturer. A result was considered to be positive if the mean absorbance value was more than 0.05 absorbance units above the mean absorbance reading of three negative controls (the cut off point).

Additional investigations
All patients were asked about infected sexual partners and were examined physically. A saline mount preparation was made of vaginal material, and smears for staining with methylene blue were taken from women and men. Cultures for gonococci were performed for all patients. The specimens for smears were taken first, and those for gonococcal culture second.

Results
Of the 617 specimens, 90 (15%) were culture positive; 68 (15%) out of the 445 urethral and 22 (13%) out of the 172 cervical specimens. The prevalence of women who were culture positive was 16% (28/174) and of men was 18% (49/271). Of the 90 culture positive specimens, 59 (66%) were positive in the IDEIA-1 and 62 (69%) in the IDEIA-2; 65 (72%) were positive in the IDEIA-1 or the IDEIA-2, or both.

In specimens stored for a mean of 9.6 weeks the results were identical in the IDEIA-1 and the IDEIA-2 in 95% (145/153) of the urethral specimens from women, 97% (146/151) of the cervical specimens, and 97% (217/223) of the urethral specimens from men (table 1). Corresponding results for specimens stored for a mean of eight months were 100% (21/21), 95% (20/21), and 96% (46/48), respectively (table 2).

The IDEIA-1 and IDEIA-2 results thus differed in a total of 22 (3.6%) of the 617 specimens; 16 (3.6%) of the 445 urethral and six (3.5%) of the 172 cervical specimens (tables 1 and 2). The IDEIA-1 gave positive results in 12 of these cases and the IDEIA-2 in 10 (table 3). Of the 90 culture positive specimens, three (3%) gave positive results only in the IDEIA-1 and six (7%) only in the IDEIA-2.

The median absorbance value above the cut off

<table>
<thead>
<tr>
<th>Source of specimens</th>
<th>Results in IDEIA-1</th>
<th>No (%) in IDEIA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female urethras (n = 153)</td>
<td>Positive 12 (8)</td>
<td>Negative 3 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (3) 133 (87)</td>
</tr>
<tr>
<td>Cervices (n = 151*)</td>
<td>Positive 14 (9)</td>
<td>Negative 3 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1) 132 (87)</td>
</tr>
<tr>
<td>Male urethras (n = 223)</td>
<td>Positive 32 (14)</td>
<td>Negative 3 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>185 (83)</td>
</tr>
</tbody>
</table>

*IDEIA results missing for two women.
Table 2 Results of IDEIA performed after storage of 90 urethral and cervical specimens at −20°C for a mean of eight months (IDEIA-2) compared with up to five days at 4°C (IDEIA-1)

<table>
<thead>
<tr>
<th>Source of specimens</th>
<th>Results in IDEIA-1</th>
<th>No (%) in IDEIA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female urethras (n = 21)</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Cervices (n = 21)</td>
<td>Positive</td>
<td>1 (5)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Male urethras (n = 48)</td>
<td>Positive</td>
<td>6 (13)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Point in the IDEIA-1 for all positive urethral specimens was 0.27 (range 0.002–2) and for all positive cervical specimens 0.35 (range 0.003–2). Corresponding values in the IDEIA-2 were 0.22 (range 0.01–2) for all positive urethral specimens and 0.58 (range 0.002–2) for all positive cervical specimens.

Table 4 shows that in culture positive specimens for which results in the IDEIA-1 and IDEIA-2 differed the median absorbance value above the cut off point was lower (0.04 for IDEIA-1 and 0.06 for IDEIA-2) than in culture positive specimens for which both IDEIA gave positive results (0.5 for IDEIA-1 and 0.38 for IDEIA-2). That was true for urethral and cervical specimens.

Eight culture negative specimens (seven urethral and one cervical) were positive in the IDEIA-1 and the IDEIA-2. Four of them probably corresponded to specimens with false negative cultures (table 4), as the patients had clinical signs of urethritis or cervicitis, or both, and negative gonococcal cultures and one of them also had a sexual partner with proved chlamydial infection. The median absorbance value above the cut off point for the remaining four specimens was 0.01 (range 0.002–0.06) in the IDEIA-1 and 0.03 (range 0.02–0.07) in the IDEIA-2 (data not shown). In culture negative specimens for which the results in the IDEIA-1 and the IDEIA-2 differed, the median absorbance values in the IDEIA-1 (0.01) and the IDEIA-2 (0.05) were closer to the cut off point than in culture positive specimens for which both IDEIA results were positive.

That was true for urethral as well as cervical specimens.

Discussion

Storing urogenital specimens at −20°C for a mean of 9.6 weeks or eight months before testing did not appreciably influence the IDEIA results compared with those obtained within five days. That was true for specimens from women as well as from men and for urethral as well as for cervical specimens. These results are consistent with the fact that the glycolipid chlamydial antigen, the lipopolysaccharide, is stable under these conditions. The mean absorbance values

Table 3 Outcome of IDEIA in 22 specimens giving different results when tested within five days after collection (IDEIA-1) compared with after storage at −20°C for up to eight months (IDEIA-2)

<table>
<thead>
<tr>
<th>Source of specimens</th>
<th>IDEIA-1 (n = 16)</th>
<th>IDEIA-2 (n = 6)</th>
<th>Total (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>10 (2)</td>
<td>2 (1)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>Negative</td>
<td>6 (3)</td>
<td>4 (3)</td>
<td>10 (6)</td>
</tr>
</tbody>
</table>

*Cut off point for positive result was more than 0.05 absorbance units above the mean reading for three negative controls.
above the cut off point for IDEIAs performed after storage were also similar to those for IDEIAs performed within five days. The sampling method is known to be important for the outcome of the laboratory identification of *C. trachomatis*. The differences noted between results of tests performed before and after storage were, therefore, probably caused by the sampling method. This interpretation is supported by the fact that when the test results differed, culture positive specimens gave negative IDEIA results before and after storage. In fact, although the difference was not significant, in patients whose results differed more culture positive specimens gave negative IDEIA results when tested within five days than after storage at −20°C (six compared with three, table 3). The freezing procedure may have favoured the release of elementary bodies, resulting in somewhat better detection after storage than before because of higher antigen concentrations.

In culture positive specimens, the absorbance values for the positive IDEIA results were closer to the cut off point when only one IDEIA gave a positive result (either before or after storage) than specimens for which both IDEIAs gave positive results. The differences may have been related to the concentrations of antigen.

In culture negative patients with no clinical indications of a false negative culture and giving positive IDEIA results before or after storage of specimens, the absorbance values for the positive IDEIA were much closer to the cut off point than in culture positive patients giving positive results in both IDEIAs (table 4). That was true for IDEIAs performed after storage at −20°C as well as those performed within five days. It may have been because of the well known difficulty of assessing the level of absorbance for the cut off point for a positive EIA result. In most EIA systems there is a “grey zone” close to the cut off value, which gives false negative or false positive results. Special attention should be paid to results in the “grey zone”, and confirmatory tests should be performed.

We conclude that urogenital specimens for IDEIA may be stored at −20°C for up to eight months before analysis without any appreciable influence on the test results.

We thank Nobel Medica AB, Sweden, for supplying the IDEIA Chlamydia test kits and Miss Eileen Belardo for revising the English text.

References