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Diagnosis of conjunctivitis in primary care: comparison of two different culture procedures

E Tellegen,1 G ter Riet,1,2 J H Sloos,3 H C P M van Weert1

ABSTRACT
Background: In general practice, infectious conjunctivitis is a common and mostly (64%) self-limiting disorder. In case of an aberrant course or severe symptoms, a general practitioner may take a culture. Direct inoculation is considered the reference standard, but usually a swab is sent to a laboratory.

Objectives: To compare the diagnostic performance of the swab, transported by surface mail with direct inoculation.

Methods: 19 general practitioners took two samples of the conjunctiva from 88 patients with symptoms suggestive of infectious conjunctivitis by rolling a cotton swab across the conjunctiva of the lower fornix. One swab was used to inoculate three agar plates directly, while the other was sent in a Stuart medium to the laboratory and inoculated at the time of arrival. The numbers of positive cultures of both methods were compared.

Results: A pathogen was found in 31 of 88 samples (35% (95% CI 26 to 46%). Surprisingly, the number of positive cultures was higher for the Stuart medium (27/88) than for direct inoculation (23/88). The difference was 4.5% (90% CI 0 to 12, p = 0.388; one-sided McNemar test for paired proportions). In five of the 19 samples that were positive in both tests, the cultured pathogens were different.

Conclusions: The Stuart medium detected more bacteria than direct inoculation. The lower 90% CI, testing non-inferiority at p = 0.05, indicates that it is unlikely that the Stuart medium misses any positive cultures compared with direct inoculation.

Superficial bacterial infections like eye, skin and throat infections are common in general practice, and in case of an eye infection more than 80% are treated with antibiotics without additional diagnostic tests to identify the causative agent being performed.1–3 In case of a prolonged course or severe complaints, a general practitioner may take a culture to identify a causative agent.

If a specimen for bacteriological culture is taken, the time and storage condition between collection and inoculation of the specimen onto culture media are supposed to influence the accuracy of the culture results. Preferably, specimens should be transported to the laboratory within 2 h, and specimens for bacterial culture must not be stored or transported for more than 24 h.4 In general, direct inoculation onto appropriate culture media with a specimen of the infected site is supposed to be better than using a swab in a transportation medium. In fact, direct inoculation is considered the reference standard.5–6

Performing direct inoculation, however, is not feasible in general practice. Silletti et al showed that in in-hospital patients with wound infections, a swab in a transportation medium was as sensitive as a primary broth culture.7 Nevertheless, these culture methods have never been compared in patients in general practice, where transportation time might be longer, and storage and transportation conditions may be even less favourable. In this study, we compare the results of both techniques in adult patients, suspected of infectious conjunctivitis.

In addition, we were interested to know whether specific species of bacteria would be missed because of the use of a transportation medium.

METHODS
Participants and culture methods
From March 2006 until November 2007, 19 general practitioners (GP) included 88 adult patients with symptoms suggestive of infectious conjunctivitis.5 All GPs received personal instructions by the investigators and were trained to take samples of the conjunctiva and to inoculate these specimens on culture media and in a Stuart transportation medium. In addition, the GPs received written instruction. Eight months later (half-way through the study) these instructions and training were repeated. GPs took one sample by rolling a cotton swab (Laboratory Service Provider, Velzen-Noord, The Netherlands) across the conjunctiva of the lower fornix of the affected eye. This swab was spread onto agar plates in three separate smears; each smear on one-third of the surface of the plate. Three agar plates were used, including blood agar enriched with 5% sheep blood, chocolate agar and MacConkey agar. All media were manufactured at the laboratory with standard ingredients (Becton Dickinson, Cockeysville, Maryland). After inoculation, the agar plates were incubated in a stove for 48 h at 35°C; the chocolate agar plates were incubated for the same period and at the same temperature, but in a 7% CO2 atmosphere. A specialised courier transported the plates under standardised conditions to the laboratory. When not used, the culture plates were replaced every 6 weeks, before the expiration date of the agar plates.

GPs took a second sample of the same fornix with a second swab. This swab was placed in a Stuart medium and sent the same day by surface mail to the research laboratory. Immediately after arrival in the laboratory, the swabs were inoculated onto the agar plates as described above.

Identification of pathogens
In the laboratory, cultures were analysed daily according to the guidelines of the American Society
Table 1  Culture results of 88 patients

<table>
<thead>
<tr>
<th>Transportation medium</th>
<th>Directly inoculated agar plates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>65</td>
</tr>
</tbody>
</table>

for Microbiology. Colonies suspected of being pathogens were further analysed according to the guidelines and identified using standard biochemical procedures. For this study we used methods to detect aerobic bacteria only. Positive cases were interpreted as the cultures in which a bacterial species, identified as pathogenic according to the literature, had grown. Bacterial species which are normal inhabitants of the human skin were considered as non-pathogens (ie, skin flora like coagulase-negative Staphylococcus spp and Corynebacterium spp). For some species it is not known whether these should be considered as pathogens. For this study we considered these species as non-pathogens.

Statistical analysis

This study was a spin-off project of a larger study on rational diagnostic strategies for suspected infectious conjunctivitis. Therefore, no sample size calculation was performed for this study. We used 95% CIs for single proportions according to the Wilson and Exact McNemar test for the difference between paired proportions. Stata software (version 10.1) was used for all calculations.

We compared the proportion of positive cultures in both methods by means of a χ² test. Since, at the end of data collection, the Stuart medium showed more pathogens, we report the results as two-sided.

RESULTS

Between March 2006 and November 2007, 19 GPs included 95 patients. The samples of five of these patients were excluded because of contamination of the agar plates. The other 88 cultures were used. Of all specimens collected in the Stuart medium, 69% arrived at the laboratory the next day, 16% 2 days after collection, and 11% arrived at the third day. For three specimens, it took more than 3 days to arrive at the laboratory.

We cultured a pathogen with either method in 31/88 (35%;95% CI 28 to 43) of the patients. In directly inoculated specimens, the culture was positive in 23 out of 88 cases (26%; 95% CI 18 to 36) (table 1). In the cultures made after transportation in Stuart medium, 27 out of 88 were positive (31%; 95% CI 22 to 41). As the 95% CI for the difference of 4.5% as calculated using the exact McNemar test for paired proportions ranged from −4 to 15%, we also tested for non-inferiority The one-sided 90% CI ranged from 0 to 12 (p = 0.388).

A pathogen was detected 19 times (22%; 95% CI 14 to 31) with both methods. In 14 cases out of these 19, the same species was cultured with both methods (table 2). In the other five cases in which both methods showed a positive result, on three occasions the detected pathogens were different, and on two occasions an additional pathogen was found in the specimen collected in the Stuart medium. In four cases, the direct inoculation detected a pathogen while the Stuart medium-based culture was negative. In eight samples, the agar plates which were directly inoculated showed no growth, although out of the specimens transported in the Stuart medium, a pathogen was detected. Detection of more than one pathogen in the same specimen occurred only after transportation in the Stuart medium. As expected, the most frequently detected pathogen was S aureus. In 19 out of 88 (22%; 95% CI 14 to 31) specimens, this bacterium was cultured.

DISCUSSION

Unexpectedly, we found more positive cultures in the samples that used the transportation medium than by direct inoculation. The one-sided 90% CI (testing non-inferiority at p = 0.05) indicates that it is unlikely that the Stuart medium is as sensitive as direct inoculation.

The source of bacteria, cultured in the Stuart media, most likely is the conjunctiva, as the Stuart swabs are sterile before opening and were closed properly after inserting the swab. Furthermore, the cultured species belonged to the expected spectrum of pathogens. In addition, the percentages of positive cultures and detected pathogens in patients with symptoms of conjunctivitis are in accordance with those in other studies.

The most plausible explanation is that pathogens were missed by direct inoculation. Although we instructed the GPs several times, GPs’ techniques of inoculation might well have been imperfect.

Table 2  Detected bacteria

<table>
<thead>
<tr>
<th></th>
<th>Direct inoculation (no)</th>
<th>Transportation medium (no)</th>
<th>Both culture methods (no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Non-pathogens or doubtful pathogens*</td>
<td>39</td>
<td>42</td>
<td>17</td>
</tr>
</tbody>
</table>

*Including Actinobacter junii, Pasteurella haemolytica, Agrobacterium radiobacter, Comamonas testosteroni, unknown Gram-negative rods, coagulase-negative staphylococci, non-haemolytic streptococci and Gram-positive rods.

Take home messages

- In primary care circumstances, the frequently used method of detecting a pathogen in patients with symptoms of conjunctivitis by sending a swab in transport medium to the laboratory seems to be equivalent to more complicated techniques like direct inoculation.
- No special species are missed using a transport medium.
Direct inoculation still is used as a reference standard for bacterial culture. It has been previously demonstrated that in hospital patients with wound infections, the use of a transportation medium achieved results comparable with those of direct inoculation and may be considered as an acceptable diagnostic method.7 As far as we know, this has never been studied for transportation by surface mail, when a sample may be in a mailbox for well over 12 h.

We found that the use of a Stuart medium in general practice detected more bacteria than direct inoculation. The lower 95% CI indicates that it is unlikely that the Stuart medium misses more than about 4% of positive cultures, as compared with direct inoculation. It would be interesting to compare samples, taken under general practice circumstances and sent in a Stuart medium to a laboratory with direct inoculation of a sample, taken from the same patients in the laboratory in an adequately powered comparative study.

Until then, we consider using a swab with a Stuart medium for transport as an efficient and probably valid method in general practice to diagnose bacterial conjunctivitis, as the number of pathogens which are missed seems low, and no special species are involved.

Competing interests: None.

Patient consent: Obtained.

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REFERENCES

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