Culture and semi-quantitative PCR for urogenital *Mycoplasma* and *Ureaplasma* from eSwab™ transport medium

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**Background**

The eSwab™ collection kit, a modified liquid Amies transport medium with a regular shape FLOQSwab™ (Copan Italia Spa, Brescia, Italy) is widely used for urogenital infections but its suitability for culturing *Mycoplasma* and *Ureaplasma* species has never been thoroughly investigated. Further, culture methods – in contrast to PCR – usually do not allow discrimination of *U. urealyticum* (Uu) and *U. parvum* (Up) which may have a different clinical significance. This study was designed to: 1. compare semi-quantitative results of culture and PCR; 2. determine the relative frequencies of Uu and Up; and 3. to estimate survival of these organisms in eSwab™ collection device.

**Material & Methods**

**Clinical Specimens**

A total of 295 urogenital specimens submitted for diagnostic workup using the eSwab™ collection kit were included in the study. The time between taking the specimens and inoculation was retrieved retrospectively from the laboratory information system where available. Seven specimens were deleted from the analysis due to incomplete recording of results (therefore, numbers differ slightly from the abstract).

**Culture**

Liquid Urea-Agarine LYO 2 broth (3 ml; bioMérieux, France) was inoculated with 4x drops (approx. 100 µl) of eSwab medium and incubated aerobically at 37°C. Solid Mycoplasma/Ureaplasma Agar (Oxoid, UK) was inoculated with 3 single drops (approx. 25 µl each) of the inoculated LYO 2 broth and incubated anaerobically at 37°C. Results were recorded for 3 days with semi-quantitative information (negative, +, ++, ++++) for the solid medium.

**Semi-quantitative PCR**

Nucleic acids were extracted from 200 µl eSwab™ liquid Amies medium using the easyMAG™ extraction system with an elution volume of 50 µl. Five µl extract was then used for amplification with the Anyplex™ II STI-7 Kit (Seegene, Korea) which allows the semi-quantitative detection of 7 urogenital pathogens (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum* and *U. parvum*).

**Results**

<table>
<thead>
<tr>
<th>Results</th>
<th>Culture M. hominis</th>
<th>Culture Ureaplasma</th>
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</thead>
<tbody>
<tr>
<td>PCR</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>24h</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>48h</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>72h</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2: Comparison of semi-quantitative culture on solid and semi-quantitative PCR for *M. hominis* and *Ureaplasma urealyticum*/*parvum* for a total of 288 specimens

**Conclusions**

- *Mycoplasma*/*Ureaplasma* Agar alone is insufficient for the cultural detection of urogenital *Mycoplasma* and *Ureaplasma* species even if incubated for 3 days.
- LYO 2 broth incubated for 3 days is almost as sensitive as PCR but does not allow reliable discrimination of the species involved especially in mixed infections.
- Anyplex™ II STI-7 PCR is the most sensitive and rapid method and allows discrimination of *M. hominis*, *U. urealyticum* and *U. parvum*. Semi-quantitative results correlate well with those of culture but are generally approx. 1 category higher.
- *U. parvum* is significantly more often found than *U. urealyticum* and *M. hominis*.
- The amies liquid medium of the eSwab™ collection device supports survival of *Mycoplasma* and *Ureaplasma* species for at least 30 hours and is suitable for both culture and PCR.

**Fig 1:** Influence of time between taking the specimen and inoculation on the difference of semi-quantitative results between culture and PCR

![Fig 1: Influence of time between taking the specimen and inoculation on the difference of semi-quantitative results between culture and PCR](image)

**Table 1:** Sensitivity of culture and PCR for *M. hominis* and *Ureaplasma urealyticum*/*parvum* for a total of 288 specimens

- all five specimens confirmed as true positives by an independent PCR assay

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