The significance of specimen storage and transport for a quick microbiologic diagnosis under consideration of economic issues and the interests of patients1),2)

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Abstract

Mandatory are short periods of sample storage, including transport time. Generally, 2 to a maximum of 4 h is a prerequisite for microbiological rapid diagnostics of acceptable quality (s. all Microbiological – Infectiological Quality Standards – MIQ). This requirement is 1) to prevent overlooking potentially pathogenic microorganisms by extended storage times resulting in their death after 2 h, or overgrowth by colonisation of other bacteria or fungi, 2) to avoid economic losses, e.g., because of the combination with the German Diagnostic Related Group (G-DRG) with short times of stay in the hospital or to achieve so-called complication points by detecting resistant microorganisms in time and therefore optimising reimbursement, 3) to fulfil the demand for a specific therapy aimed at the causative infectious agent which may not be detected after a longer storage time and to avoid higher mortality rates by failure or delayed times of specific therapy because of delayed isolation of resistant bacteria.

Keywords: bacterial survival; economy; microbiology; pathogenic bacteria; patients; rapid diagnosis; samples; storage; transportation time.

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Introduction

For the first time the European Standard according to DIN ISO 15189 includes pre-analytics as obligatory in the spheres of control and responsibility of the hospital [1]. This important pre-analytic part of microbiologic and hence infectiologic diagnostics was long considered a rather neglected quantity, demonstrated by the numerous instances of external relocation of microbiologic diagnostics with overly long storage and transport times. Before they are transported to the far away laboratory, patient samples are stored for many hours and on weekends for several days with all the consequences for quality described in this paper. The effect of this has been very much underestimated. The pre-analysis component of diagnostics is outside the sphere of responsibility and control of the physician, the nursing staff and the administration and is also barely controllable by the lab. What are the consequences that threaten?

For example, if patient samples arrive in the microbiology lab markedly delayed, the infectious agents not only might die, but most particularly they might be recognized too late. Such belated recognition, especially of resistant germs, results in the delayed start of a specific therapy with increased mortality and extended hospitalisation [2–6], of which the latter is associated with higher costs and low revenues, for example, in the DRG system. This also cancels out higher compensation because of complication issues [1].

During their years at medical school all physicians learn that bacteria can die off. However, in general there is ambiguity about the dynamic or the course of death of the various pathogens.

The following pages therefore provide a summary of findings by several publications concerning the survival rate of typical infectious agents after storage of clinical patient samples and “artificially” inoculated samples. Because of reduced economic resources, costly and independent studies of this kind were rarely conducted during the last few years. But experimental studies of older vintage still have considerable validity and have been included, since the culture of potentially pathogenic microorganisms has not changed substantially in the last decades, in fact, not since Koch and Pasteur, and in spite...
of molecular biology it will yet remain the central diagnostic instrument in microbiologic-infectiologic diagnostics.

The central question is whether and how far the pathogens in the samples can or cannot survive longer storage, including storage in transport media.

**Transport media**

**Artificially inoculated specimens for investigation of the suitability of transport media**

Specimens that have been artificially inoculated “in vitro” with bacteria in high concentrations with or without transport media containing gel do not reflect the conditions of “clinical” patient specimens. Although agents in high concentrations can survive several days, patient specimens in contrast to artificially inoculated samples frequently not only contain fewer bacteria but constantly disintegrating cell components, whose enzymes the bacteria damage to a varying degree and whose survival rate they noticeably shorten [7, 8].

**Gel-containing transport media** must be critically evaluated in spite of their preservative properties: It must be noted that extreme loss occurs with sensitive germs, including all anaerobes, even in transport tubes with special transport media (Table 1) [9]. Second, there is the dilution effect caused by the gel [9, 10], which has a negative effect on low concentrations of infectious agents. Third, transport tubes containing gel are not suitable for specimens from the respiratory tracts, like sputum or bronchial mucus (with large amounts of specimen material), they are meant for smears.

However, according to the quality standards of microbiology/infectiology and in conformity with all medical associations, no smears, but rather tissue samples only, should be taken in the case of wound and bone infections [11, 12].

**“Sensitive” anaerobic and aerobic infectious agents**

“Sensitive” infectious agents, which can occur in every specimen, die off quickly, demonstrably after no more than 2–4 h of storage. In spite of high initial inoculation many are no longer detectable after 24 h (Table 1) [8, 9, 13]. These results are confirmed by newer studies [10, 14]. It is common knowledge that anaerobes are especially sensitive [9] and after only 6 h a culture can no longer be established [14].

β-hemolytic streptococci also could be found again in only 5–10% of cultured smears after just a short time, after 4 h in only 2–3%, whereby the main loss was due to absorption onto the cotton applicators [15]. The yield improves markedly after rinsing the smears in broth, a confirmation of the principle of using not only solid cultures, but to also continuing to use liquid cultures in diagnostics, particularly with non-recoverable specimens.

**“Clinical” patient specimens**

It is a generally little known fact, that clinical patient specimens taken directly from the patient show much higher losses of potentially pathogenic germs than artificially inoculated samples [10, 16].

**Patient specimens from various locations**

1. **Patient specimens in urinary tract infections** (for detailed description see MIQ-Urinary Tract Infections) [17]. Under room temperature conditions the germ count changes within 1–2 h, hence urine samples must be transported to the lab without delay and immediately refrigerated.

As demonstrated in several studies, a disadvantage of the frequently used boric acid incubation of urine is the toxic and germ count-reducing effect of boric acid, especially in native urine, which can result in misdiagnoses.

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**Table 1** Survival rate of aerobic and anaerobic germs with and without gel at a storage time of 24 h.a,b

<table>
<thead>
<tr>
<th>Germs (Suspension 10⁶ CFU/mL)</th>
<th>Survival rate in the culture tube</th>
<th>Without gel</th>
<th>With gel (Port A Cul)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonococci</td>
<td>0%</td>
<td></td>
<td>&lt;1%</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>0%</td>
<td></td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>4%</td>
<td></td>
<td>18%</td>
</tr>
<tr>
<td>Streptococcus pyogenes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0%</td>
<td></td>
<td>n.p.</td>
</tr>
<tr>
<td><strong>Anaerobic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. difficile</td>
<td>1%</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>&lt;1%</td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>30%</td>
<td></td>
<td>87%</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>&lt;1%</td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>6%</td>
<td></td>
<td>28%</td>
</tr>
<tr>
<td>Porphyromonas</td>
<td>&lt;1%</td>
<td></td>
<td>5%</td>
</tr>
</tbody>
</table>

n.p., not performed. aPerry JL [8]; bRoss PW [15]. Result Table 1: Even in transport media losses of 70%-80% occur on average (some up to 100%).
especially in regard to the low limiting concentrations between $10^2$ and $10^4$ that apply to children and adult males as well as catheter or vesicle puncture urine. Because of their numerous disadvantages slide cultures should only be used in exceptional instances [17].

2. Wound smears (see Tables 2 and 3) [16]. The comparison of clinical specimens that were prepared or transported immediately confirms the high rate of loss between 50 and 80% of potentially pathogenic Gram-positive infectious agents following transport. Also reduced is the identification of important Gram-negative infectious agents like *P. aeruginosa*, *Serratia, Enterobacter* and *Proteus*, even when tubes with transport medium are used (Table 3). The rather high losses in these clinical specimens after only 2–5 h of storage and transport were unexpected.

3. Specimens from patients with respiratory track infections (see Table 4) [7]. Only very few bacteria can be found in clinical specimens, e.g., fresh sputum, that have been stored more than 4 h. In this study these consisted of 10% pneumococci and 30% haemophilic bacteria. It is significant that important gram-negative bacteria like *Enterobacter* also are much less detectable after 4 h. This study also confirms the low valence of saliva [7]. Quantitative cultures of suitable sputum specimens show a three times higher yield over a “qualitative” direct smear without dilution, which is why purulent specimens should be spread with additional dilution [7].

When stored for 24 h, even at 4°C, a large part of the specimens not only show a high loss of pneumococci and *H. influenzae*, but *S. aureus* as well can only be detected in barely half of the specimens (Table 5) [18]. Patient specimens that have been stored for 48 h [18] or shipped in the mail [19] have obsolete effects [8, 9, 15, 18]. These same effects are being reported in more recent studies on other specimens with storage times of

### Table 2: Wound infections I – Gram-positive infection agents: Effect of delay through storage and transport on the isolation rate of Gram-positive potentially pathogenic bacteria and on colonisation germs in clinical specimens (9).

<table>
<thead>
<tr>
<th>Gram-positive cocci</th>
<th>“positive” specimens</th>
<th>Changes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediate culture</td>
<td>After 2–5 h Storage/transport time</td>
</tr>
<tr>
<td></td>
<td>(on ward)</td>
<td></td>
</tr>
<tr>
<td>Potentially pathogenic bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. aureus</em> (cotton swab)</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>β-häm. Streptococci (all specimens)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Enterococci (culturette)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>“Colonisation germs”</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. epidermidis</em> (culturette)</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Viridans Streptococci (cotton swab)</td>
<td>13</td>
<td>20</td>
</tr>
</tbody>
</table>

**“positive” specimens = Patient specimens, in which the listed Gram-positive bacteria were detected. Result Table 2: Only 2–5 h of storage and transport damage gram-positive infectious agents, → to –80%.

### Table 3: Wound infections II – Gram-negative infectious agents: Effect of delays during transport on the quality of the clinical specimens or the isolation rate (9).

<table>
<thead>
<tr>
<th>Gram-negative bacteria</th>
<th>“positive” specimens</th>
<th>Loss, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediate culture</td>
<td>After 2–5 h Storage/transport time</td>
</tr>
<tr>
<td></td>
<td>(on ward)</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td><em>Serratia</em> (all specimens)</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td><em>Enterobacter</em> (all specimens)</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td><em>Proteus</em> (Transcul)</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>

Result Table 4: Only 2–5 h of storage and transport damage even Gram-negative infectious agents → –30%–60%.

### Table 4: Respiratory track infections – Sputa – clinical specimens: Isolation rate of infectious agents from sputa after storage [19].

<table>
<thead>
<tr>
<th>Germs</th>
<th>Isolation rate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 h (n = 103)</td>
<td>4 h (n = 78)</td>
<td>Saliva (n = 20)</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>30%</td>
<td>20%</td>
<td>0%</td>
</tr>
<tr>
<td><em>Haemophilus</em> spp.</td>
<td>38%</td>
<td>8%</td>
<td>0%</td>
</tr>
<tr>
<td><em>Enterobacter</em> sp.</td>
<td>26%</td>
<td>4%</td>
<td>24%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>15%</td>
<td>5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Result Table 4: Depending on storage time and specimen – loss rate up to 100%.
24–48 h [10, 14]. On weekends therefore, daily processing must be mandatory.

4. Stool samples

At a storage time of 24 h stool samples show a total loss of *C. difficile*, even in tubes with transport media (Table 1) [9]. The bacteria density of other pathogenic intestinal germs, such as the species of *Salmonella* and even more so of *Shigella*, decreases strongly after two days. *Campylobacter jejuni*, in particular, was no longer detectable at this point [20]. This leads to the conclusion that *C. difficile* and the other germs mentioned die off after only a few hours and that stool samples must not be stored for longer periods of time, but, as with all other samples, must be prepared within the shortest possible period. The loss was less pronounced at a storage temperature of 4°C, so that stool samples should always be stored at this temperature, should immediate processing not be possible.

### Colonisation germs

**What is the significance of colonisation germs of the flora of skin and mucous membranes for diagnostics?**

*S. epidermidis*, the most frequent skin flora germ, viridans streptococci and others, multiply rapidly. In comparison to an immediate preparation, these were isolated from wound smears more frequently and in high concentrations after storage times of 2–5 h (Table 2) [16]. This applies also to other typical colonisation germs, such as *neisseria* [7, 16] and *E. coli* in respiratory specimens (Table 5) or candida after a storage time of 24 h [7, 18].

These studies confirm that colonisation germs multiply within only a few hours and can overgrow infectious agents that then are no longer detectable.

When bacteria of the normal flora are determined to be infectious agents, such findings are not only false, but because of complex testing in the laboratory they are also costly and may cause high expenditures through subsequent incorrect treatment. The clinic physician cannot, as a rule, recognise such false findings without any clue, for example, about overly long transport times.

### Conclusions

Without any doubt, microbiology-infectiology also requires a quick and good diagnosis. Most time delays come about not because of transport time, but rather the storage times before and after the transport.

1. **The death of potentially pathogenic agents**

With increasing storage and transport times known sensitive germs, such as anaerobes, pneumococci or *Haemophilus influenzae* (Tables 1, 4) and also β-haemolytic streptococci (Table 2) [21] die off, frequently in as little as 2–4 h. If, however, they are recognised, there is a possibility of a targeted and therefore, efficient therapy with reasonably low-priced non-broad-spectrum antibiotics, e.g., *Pneumococci*, with penicillin (in Germany the rate of resistance is very low), even when performing the quick antigen test.

Even more serious is the considerable loss of potentially pathogenic germs, such as *S. aureus* (Table 5) and equally resistant germs, such as MRSA or *P. aeruginosa* or *Serratia* etc. (Table 3), which we know to grow poorly even under normal conditions. Hence short storage times and quick transport are of high importance for all specimens that could contain these agents, most particularly in cases of serious infection.

2. **Multiplication of skin flora and colonisation germs**

Studies show that increasing storage times are associated with a large multiplication of skin and mucous membrane flora germs and of other colonising bacteria (Table 2), which in only a few hours overgrow the original pathogenic aerobic and anaerobic germs. In the case of long storage times of much more than 4–5 h, resistant germs are not only overlooked, but it becomes impossible to determine with any certainty whether the isolated germ is a colonisation germ or the potentially pathogenic agent. This means that, when storage times are exceeded, infections are overlooked or incorrectly diagnosed and this will lead to wrong therapy decisions on the part of the physician.
3. Storage temperature

Because of the strong overgrowth by skin flora and colonisation germs, frequently within only a short time, storage of specimens would seem more favourable at 4°C, although pathogenic agents die off even at that temperature.

4. Number of specimens

Since numerous infectious agents die off even with short storage and transport times and the yield of infectious agents is decidedly higher with more than one specimen, e.g., with infected wounds [15], at least two samples should be taken when an infection is suspected. This applies especially with only one session, e.g., samples that must be obtained intra-operatively. In the case of joint or bone infections multiple patient specimen – maybe three, ideally four to five – are routinely taken from the various areas of the infected region [12].

5. National recommendations

The new S3 guidelines of the Paul-Ehrlich-Society for Chemotherapy, the German Society for Pneumology and the German Society for Infectiology formulate as follows for microbiologic studies on epidemiology, diagnostics, antimicrobial therapy and management of adult patients with deep respiratory track infections obtained outside a hospital setting "pre-requisites are ... the guarantee of the necessary logistic pre-condition (transport and processing within 2–4 h)" [22].

6. European standards for pre-analytics and mandatory documentation

Any commitment according to the European Standard DIN ISO 15189 for monitoring pre-analytics has no value, if it is not known, what role the length of storage time before, during and after transport plays for the survival of the potentially pathogenic and most particularly the resistant infectious agents. Hence it is important that nursing staff or physicians consistently enter the time at which the specimen was obtained into the field predefined by the laboratory in the request display mask or on the request slip, without which the control of storage and transport duration and therefore, a complete evaluation of the findings is not possible as demanded in DIN ISO 15189.

7. International recommendations of professional societies

Numerous national and international recommendations by professional societies and studies [11, 23–27] point out that delays during transport and storage result in an inexcusable delay in communicating the findings and in diagnostics. Optimal microbiologic test results are possible only with short transport and storage times between the time the specimen is obtained and a microbiologic analysis is performed. Therefore, the predominant requirement is that all microbiologic specimens are processed in the laboratory immediately or within two hours after they are collected.

8. “Relocation”

Concerning the ever more frequently practiced external relocation of microbiologic laboratories for "economic" reasons and the time delay through transport of the specimen not only from the patient to the ward and on to the central collection site, but then onward to the laboratories located at great distance:

The patient does not fall ill at the time the specimen is picked up. Also, the specimen most often is not obtained at the time it is picked up from the central collection site in the clinic. Specimen transport within a clinic on average already takes up to 5 h. When using a relocated external laboratory, the specimens remain unprocessed until they are transported yet many more additional hours, on weekends between 24 and 48 h, with the corresponding detrimental effects for the growth of the agents, the therapy and the patient.

References