ZENTRAL STERILISATION 2

Key Words
- flexible endoscopes
- reprocessing
- inspection
- hygiene
- microbiology

Hygienic and Microbiological Inspection of Flexible Endoscopes after Reprocessing

*DGKH, DEGEA, DGSV, DGVS, AKI, AK RDG, Manufacturers of flexible endoscopes**

This recommendation will be featured as an annex to the "Guideline for validation of automated cleaning and disinfection processes for reprocessing heat-sensitive endoscopes", which is being currently drafted by, among others, the German Society of Hospital Hygiene (DGKH), German Society of Sterile Supply (DGSV), German Society of Endoscopy Assistant Personnel (DEGEA) and Working Group Instrument Preparation (AKI).

Here the complete list of societies and expert representatives involved in compiling the guideline:

German Society of Hospital Hygiene (DGKH)

German Society of Endoscopy Assistant Personnel (DEGEA)

German Society of Sterile Supply (DGSV)

German Society for Digestive and Metabolic Diseases (DGVS)

Working Group Instrument Preparation (AKI)

Working Group of Washer-Disinfector Manufacturers (AK RDG)

Endoscope manufacturers

1 General instructions

1.1 Introduction

At present, there are no standard methods for hygienic and microbiological inspection of endoscopes after reprocessing, and, indeed, even inappropriate procedures are used in some cases. This gives rise to a number of incorrect results. To remedy that situation, the present standard operating procedure, which takes account of the experiences gathered in recent years, proposes a detailed procedure for standardisation of inspection. It can be used for routine inspection of reprocessed endoscopes as well as during validation (performance qualification).

2 Sampling plan for routine inspections

A sampling plan must be drawn up for each endoscope, while taking account of the critical sites of each endoscope family.

2.1 Frequency

Each endoscope should be subjected to hygienic and microbiological inspection at least once per year (1).

During routine inspection of endoscopes at least one endoscope from each endoscope family used in the establishment must be inspected. If different methods of reprocessing (automated and/or manual) are used in the establishment, it must be ensured that both reprocessing methods are included.

Depending on the frequency of use, quality of reprocessing and on the endoscope family the intervals for routine inspections shall be defined during risk analysis. These could differ from those proposed in the recommendation cited above (1).

2.2 Types of samples collected

The following samples must be collected for each endoscope

- Swab samples
  - from distal end
  - from the Albarran lever recess (if present)
  - if applicable, from sites that are particularly difficult to access, e.g. in endosonic devices at the balloon fixing points
- Liquid samples
  - Rinse samples
    - from each channel that can be purged

Note: If the "sponge method" or a similar method is used additionally, it must be ensured that the sponge (sterilised!) or similar object cannot be left behind in the channel.

  - Water samples
    - from the optics cleaning bottle

2.3 Neutralisation

Disinfectant residues can persist in the endoscope channels as well as in the optics cleaning bottle if they are not properly reprocessed. This could have implications for the microbiological results obtained since it could impede reliable detection of microorganisms.

It is therefore recommended that in principle the rinse liquids and water samples be collected in a test tube containing a neutralisation solution. This would inactivate any disinfectant residues.

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Note: Information on the composition of the neutralisation solution must be obtained from the manufacturer of the respective disinfectant. When preparing the neutralisation solution concentration, one must bear in mind that this will be diluted in line with the quantity of rinse liquid used .

The neutralisation solution can be omitted when using washer-disinfectors for which the absence of any relevant disinfectant residues was demonstrated at the time of their validation.

If, instead of adding a neutralisation solution to the sample, an inhibition test is used, then a validated method must demonstrate that all relevant microorganisms can be taken into account.

2.4 Inspection methods

The inspection methods to be used are based on the following principles:

- Swab samples: The swab samples are plated out directly to nutrient media as well as transferred to enrichment broth. Both methods permit only qualitative detection of the existing microorganisms.
- Liquid samples: A specified volume of the rinse liquid is passed through a membrane filter with suitable pore sizes and then placed on a suitable nutrient medium. In parallel, the liquid samples can also be plated out directly to selective nutrient media (see. 4.1). After incubation, counting the number of colonies on the filter and making appropriate conversions, the number of colony forming units (cfus) per millilitre is calculated. This allows a quantitative detection of the microorganisms present.

Note: The pour plate method is not recommended because it impedes growth of aerobic bacteria such as e.g. Pseudomonas aeruginosa*, and could thus lead to false negative results.*

Because of the reduction of the detection limit, the use of a dilution series or direct plating out of e.g. 100 µl/plate is only indicated if a high number of colonies is expected in the sample.

Dip slides can only be used if their detection limit meets the requirements, i.e. they must be suitable to detection of 1 cfu/ml (3).

3 Sampling

To assure proper hygiene, two persons are generally needed for sampling. Sampling must be conducted under aseptic conditions. Contamination by the person taking the sample must be excluded. To that effect, the person collecting samples must wear a clean, unworn, gown or appropriate disposable clothing before each test and at each test site. Hygienic hand disinfection is also obligatory before taking each sample.

The endoscope must be freely suspended before a sample is collected. It is recommended that swab samples be first collected.

- Swab sample: The sterile swab is moistened with sterile physiological (0.9%) NaCl solution under aseptic conditions. Each critical site specified in the sampling plan is carefully swabbed with a moistened swab that is then transferred to a test tube containing transport medium and sent immediately to the laboratory.
- Rinse sample: Using a sterile disposable syringe, approx. 25 ml sterile physiological (0.9%) NaCl solution is injected carefully into the endoscope channel (from the proximal to the distal end).From the liquid flowing out at the distal end, 20 ml is collected in a sterile collecting test tube (containing, if necessary, 20 ml double concentrated neutralisation solution (if the collecting test tube contains e.g. 20 ml of a neutralisation solution, this neutralisation solution must be double concentrated). For each endoscope channel a new sterile disposable syringe and a sterile collecting test tube must be used.

Note: The collecting test tube must not be allowed to come into contact with the endoscope (not on the inside either).

To purge the channels it is often advisable to use cleaning adapters, while ensuring that the adapter has also been first cleaned and disinfected, and possibly even sterilised, so as to rule out any risk of contamination it might pose.

If a neutralisation solution is used, both liquids are mixed carefully together by swirling the sample.

- Water sample: Using a sterile disposable syringe, at least 20 ml liquid is withdrawn from the optics cleaning system via the associated connection hose and transferred to a sterile collecting test tube (if the collecting test tube contains e.g. 20 ml of a neutralisation solution, this neutralisation solution must be double concentrated).
- Transporting samples: After collection, the samples must be transported as quickly as possible to the laboratory so that further processing is assured within 24 hours of collecting the sample. The sample must be refrigerated if the transportation time until processing in the laboratory is > 4 h.

4 Processing samples

4.1 Swab samples

Each swab is streaked out to the surface of a blood agar plate by twisting it in wavy snake lines.

- Then each swab is transferred for enrichment to a test tube with nutrient medium (e.g. Brain Heart Infusion (BHI) broth), possibly containing neutralisation solution) and extracted using a vortex mixer.
- Both the blood agar plates and broth are incubated at 36 ± 1 °C under aerobic conditions.
- The first inspection is performed after 24 hours and the results recorded. The second inspection is done after 44 ± 4 hours.
- If there is growth on the blood agar plates or turbidity of the broth, microorganisms are further differentiated by plating out to selective nutrient media.

The following selective nutrient media should be used for the following microorganisms:

MacConkey agar	for Enterobacteriaceae
Cetrimide agar	for *Pseudomonas aeruginosa*
Baird-Parker agar	for staphylococci
Selective-elective agar	for streptococci
Slanetz-Bartley agar or Kanamycin-Esculin agar	for faecal streptococci
Middlebrook agar	for mycobacteria
GVPC* agar	for legionellae

**GVPC = glycine vancomycin polymyxn and cycloheximide*

The selective nutrient media are incubated in accordance with the manufacturer's specifications.

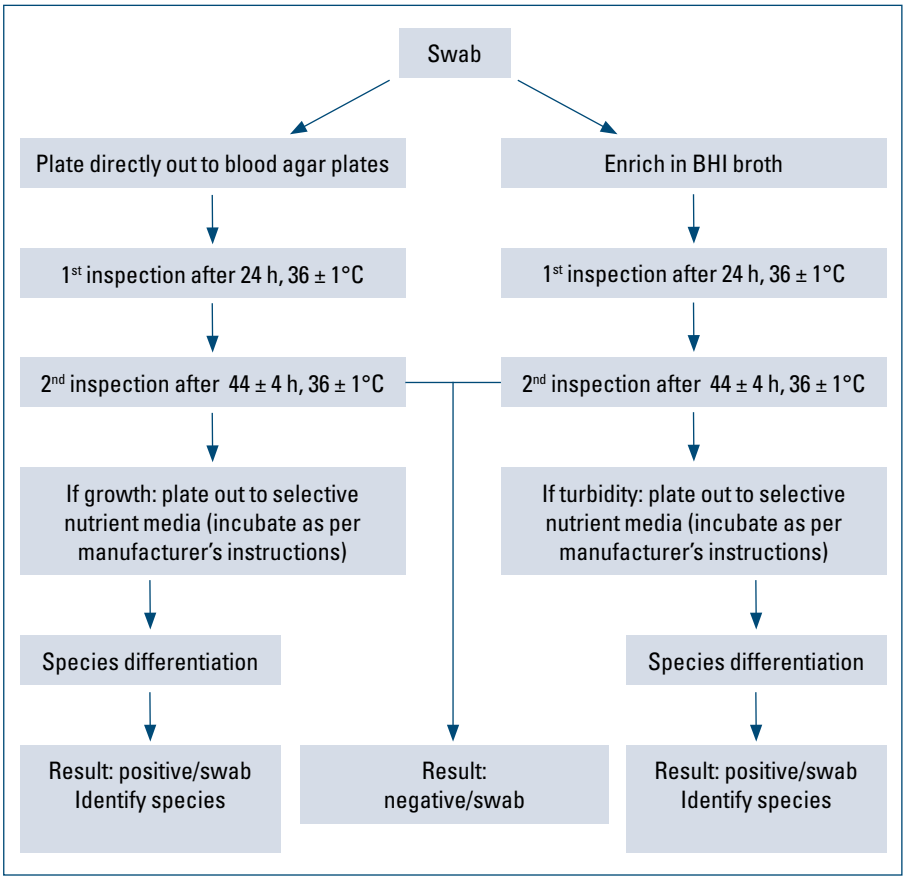

Fig. 1: Hygienic and microbiological inspection of endoscopes – investigation scheme for swabs

Note: A special culture method is needed, in each case, for detection of both mycobacteria and legionellae; these are not outlined in detail here.

4.2 Liquid samples

Aliquots of 10 ml of the liquid samples (when using a neutralisation solution 20 ml) are filtered (pore size 0.2 µm) and then the filter is placed on a blood agar plate.

In addition, 1 ml aliquots are spatulated directly onto various selective nutrient media.

- The blood agar plates are incubated at 36 ± 1 °C under aerobic conditions.
- The first inspection is performed after 24 hours and the results recorded. The second inspection is done after 44 ± 4 hours.

The following selective nutrient media should be used for the following microorganisms:

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The selective nutrient media are incubated in accordance with the manufacturer's specifications.

Note: A special culture method is needed, in each case, for detection of both mycobacteria and legionellae; these are not outlined in detail here.

5 Evaluation

5.1 Swab samples

If no growth is found in the enriched broth or on the blood agar plates, the result is given as negative for each swab.

If there is growth on the selective nutrient media, the identified species are recorded in the test report as positive per swab.

5.2 Liquid samples

The microorganisms detected on the blood agar plates (membrane filters) are counted. The results are given as cfu/ml of sample volume.

5.2.1 Rinse samples

For the rinse samples, the results are calculated as cfu/endoscope channel.

$$\frac{\text{cfu/filter} \times \text{ml sample volume/channel}}{\text{ml filtered sample}} = \text{cfu/endoscope channel}$$

Example: 5 cfu in 10 ml sample or 20 ml sample volume yields 10 cfu per endoscope channel.

CAUTION: *For 0 cfu/ml the result is < 2 cfu/endoscope channel (detection limit of example!).*

The microorganisms on the selective nutrient media are also counted. The results are given as cfu/plate (per species).

The cfu per endoscope channel are calculated as follows:

$$\text{cfu of plate} \times \text{neutralisation factor} \times \text{sample volume in ml} = \text{cfu/endoscope channel}$$

Example 1: With neutralisation medium and 20 ml sample volume: 10 cfu/plate × 2 × 20 ml = 400 cfu per endoscope channel

CAUTION: *For 0 cfu/plate the result is < 40 cfu per endoscope channel (detection limit of example!).*

Example 2: Without neutralisation medium and with 20 ml sample volume: 10 cfu/plate × 1 × 20 ml = 200 cfu per endoscope channel

CAUTION: *For 0 cfu/plate the result is < 20 cfu per endoscope channel (detection limit of example!).*

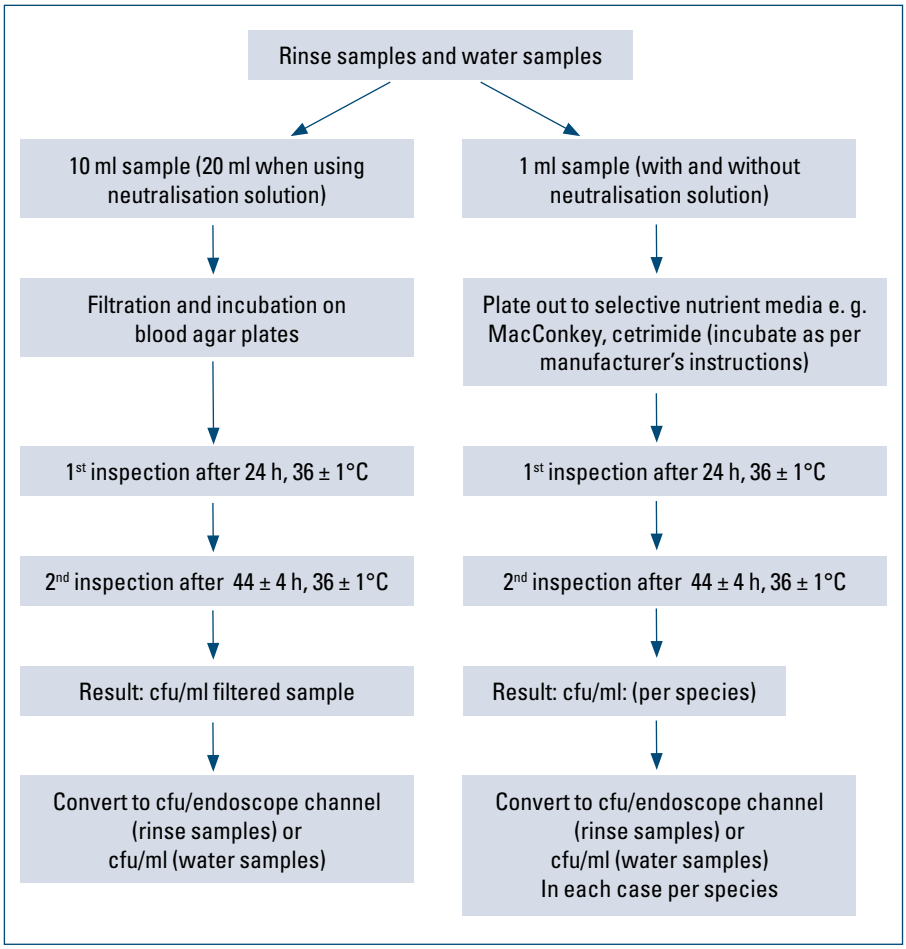

Fig. 2: Hygienic and microbiological inspection of endoscopes – investigation scheme for liquid samples

5.2.2 Water samples

For the water samples the results are calculated as cfu per ml of optics cleaning system water.

$$\frac{\text{cfu/filter}}{\text{ml filtered sample}} = \text{cfu/ml}$$

Example: 5 cfu in 10 ml sample volume yields 0.5 cfu per ml of optics cleaning system water.

CAUTION: *For 0 cfu/filter the result is < 0.1 cfu per ml of optics cleaning system water (detection limit of example!).*

The microorganisms on the selective media are also counted. The results are given as cfu/ml (per species).

The cfu/ml for the optics cleaning system water is calculated as follows:

cfu of plate × neutralisation factor = cfu/ml optics cleaning system water

Example 1: With neutralisation medium for 10 ml sample volume: 10 cfu/plate × 2 = 20 cfu per ml of optics cleaning system water

CAUTION: *For 0 cfu/plate the result is < 2 cfu per ml of optics cleaning system water (detection limit of example!).*

Example 2: Without neutralisation medium and with 10 ml sample volume: 10 cfu/plate × 1 = 10 cfu per ml of optics cleaning system water

CAUTION: *For 0 cfu/plate the result is < 1 cfu per ml of optics cleaning system water (detection limit of example!).*

6 Evaluation of results

6.1 The requirements for proper reprocessing will have been met if

- The maximal total count should be ≤ 20 cfu per channel (≤ 1 cfu/ml rinse sample for 20 ml sample volume)
- The following microorganisms must not be present:
  - *Escherichia coli*, other enterobacteriae and enterococci
  - *Pseudomonas aeruginosa* and other pseudomonads, non-fermenters
  - Nosocomial pathogens such as *Staphylococcus aureus*
  - Mycobacteria and legionellae (as per risk analysis)
  - *Streptococcus viridans*: in endoscopes used for examination of regions of the upper gastrointestinal tract or upper respiratory tract which are not (normally) microbially colonised (e.g. bronchoscope, lateral-view duodenoscope used for endoscopic retrograde cholangiopancreatography (ERCP) (as per risk analysis)

Note: *For coloscopy examination conducted within the framework of coloscopy agreements, the recommendations of the respective Associations of Statutory Health Insurance Physicians must be observed.*

6.2 Interpretation of positive results

Growth of certain microorganisms indicates various sources of potential errors (1, 2):

Reprocessing

- Detection of *Escherichia coli*, other enterobacteriae and enterococci (faecal microorganisms): indicators of inadequate cleaning and/or disinfection
- Detection of *Streptococcus viridans* (throat flora): indicators of inadequate cleaning and/or disinfection
- Detection of *Pseudomonas aeruginosa* and other pseudomonads or non-fermenters (water microorganisms): indicators of inadequate quality of the final rinse water used

Sampling

- Detection of microorganisms that are of relevance in the context of hygiene

such as *Staphylococcus aureus* or *Staphylococcus epidermidis* (skin and ambient microorganisms): indicators of e.g. endoscope contamination following reprocessing due to inappropriate storage and/or poor personnel hand hygiene

Remark: *Changes to the endoscope (e.g. wear, damage) can also give rise to detection of microorganisms during inspection, despite the process itself complying with the parameters defined at the time of validation.*

7. References

1. Recommendation by the Robert Koch Institute "Hygiene requirements for reprocessing flexible endoscopes and endoscopic accessories" on the internet at (www.rki.de) _Prevention of Infection_Hospital Hygiene_Guidelines/Recommendations by the Commission for Hospital Hygiene [RKI-Empfehlung „Anforderungen an die Hygiene bei der Aufbereitung flexibler Endoskope und endoskopischen Zusatzinstrumentariums" im Internet unter (www.rki.de) _Infektionsschutz_Krankenhaushygiene_Empfehlungen der Kommission für Krankenhaushygiene]
2. ESGE-ESGENA guideline for quality assurance in reprocessing: Microbiological surveillance testing in endoscopy. Endoscopy 2007; 39: 175–181.
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