Comparison of endoscope sampling and culturing methods

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Disclosure of Conflicts of Interest : Eurofins Biotech Germande reports having been consulted and having received financial support from medical device manufacturers to performed studies on the efficacy of medical devices.

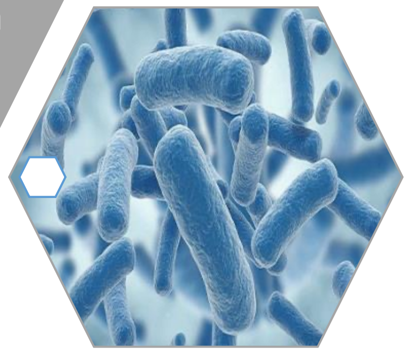
World Federation for Hospital Sterilisation Sciences

Introduction

Infectious risk?

Microbial contamination

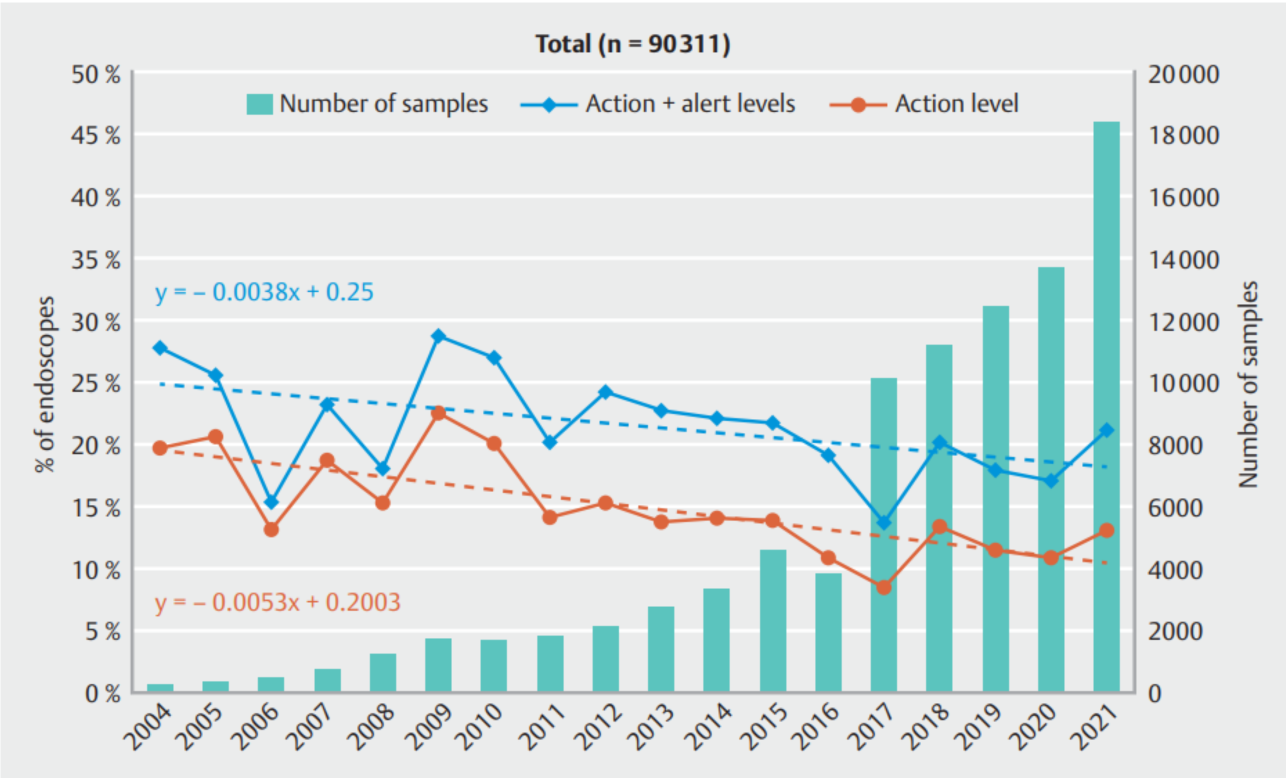
Endoscope contamination rate



What is the microbiological quality of our endoscopes?

Is there a risk for the patients?

Introduction

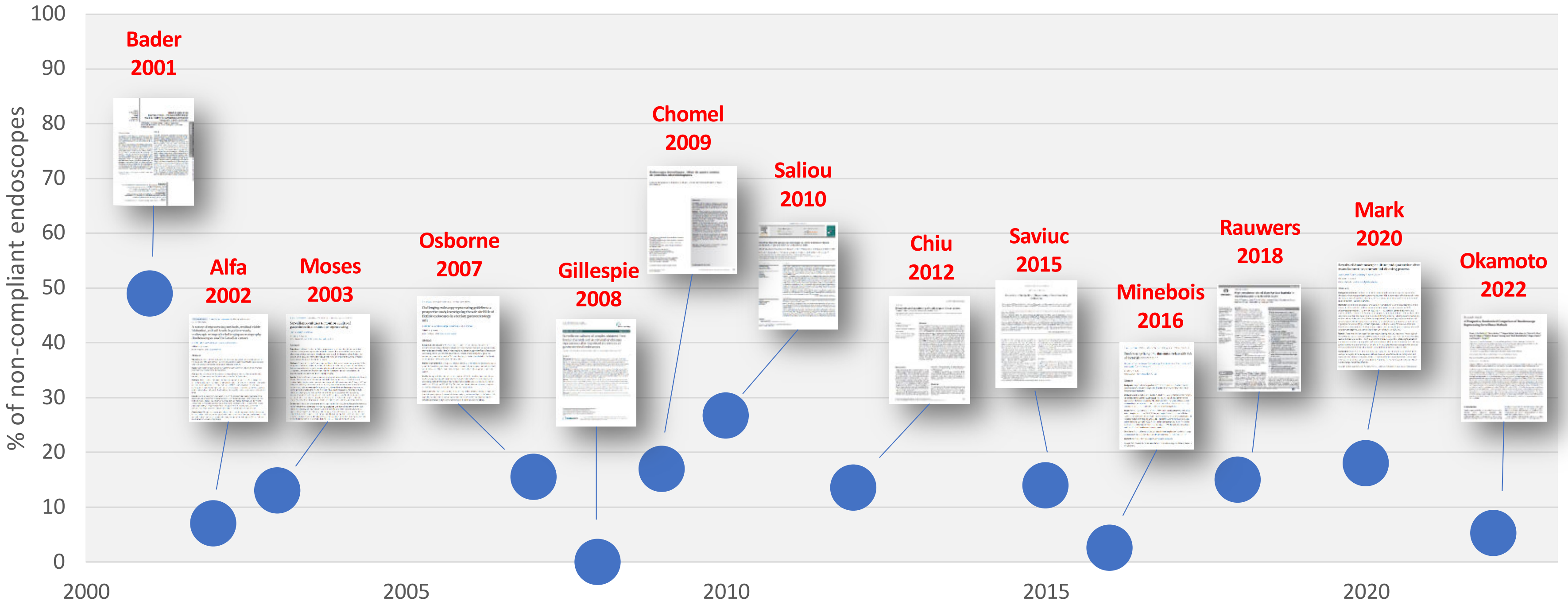


In 2021, in France, **13%** of endoscopes are **at the action level** and 8.1% are at the alert level, meaning that the contamination level of **21.1%** of endoscopes **exceeds** what has been defined as a **maximum acceptable value**.

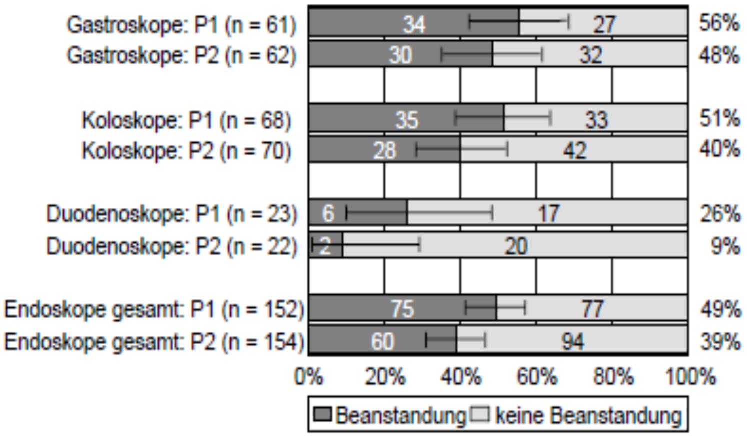
Pineau Lionel. Endoscope reprocessing: Retrospective analysis of 90,311 samples. Endosc In t Open 2023; 11: E247–E257

Introduction

Studies published in the literature indicate that the non-compliance rate of ready to use endoscopes varies from 0.4% to 49.0 %

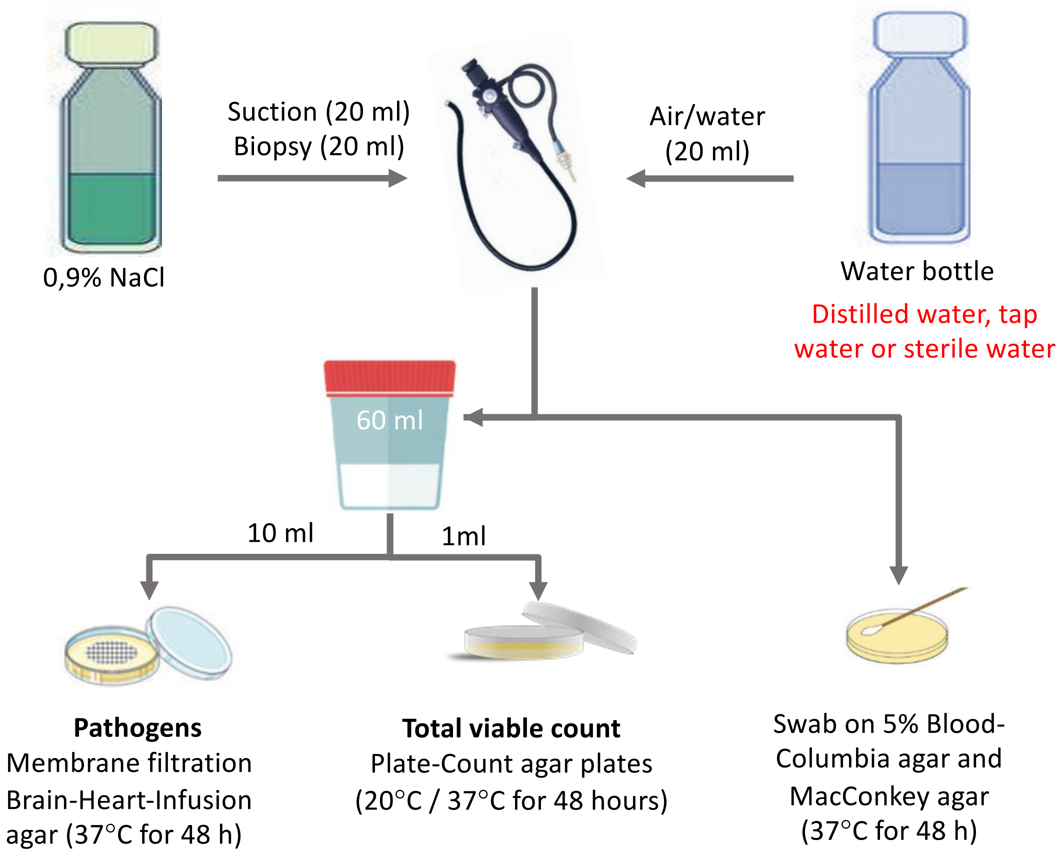


Bader L et al, 2002



« In 2 test periods, endoscopes ready for use were found contaminated at high rates:
Period 1: **49%** of 152 endoscopes;
Period 2: 39% of 154 endoscopes). »

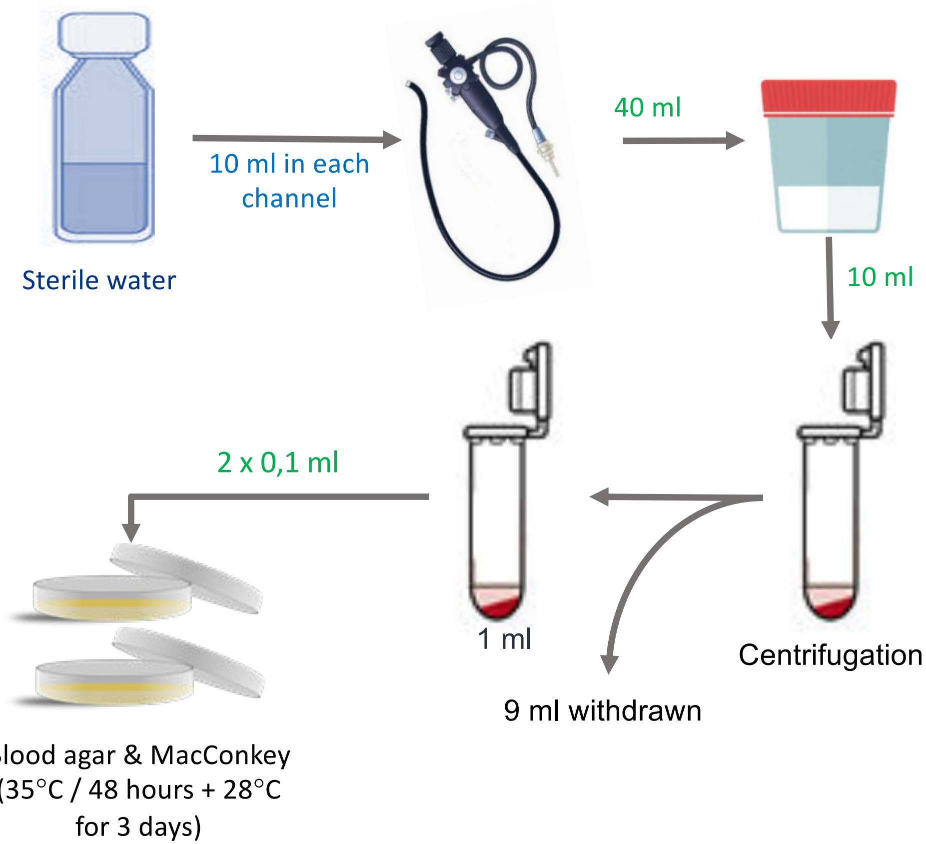
Sampling and culturing method



Gillespie et al, 2007

« There were 2374 screening tests performed during the 5-year period, including 287 AFER, 631 bronchoscopes for mycobacteria and 1456 endoscope bacterial screens.
There were no positive results of the AER or bronchscopes for mycobacteria.
Of the 1456 endoscopic bacterial samples, 6 were positive. i.e. **0,4%** »

Sampling and culturing method



Introduction


Review

Contaminated flexible endoscopes: Review of impact of channel sampling methods on culture results and recommendations for root-cause analysis

Michelle J. Alfa PhD[1] and Harminder Singh MD[2]

Abstract

Recently, infection transmission risk associated with contaminated, patient-ready flexible endoscopes has attracted attention. Outbreaks of multidrug-resistant organisms resulting in infection and/or colonization have been particularly concerning. Recent CDC and FDA recommendations focus on reducing "exogenous" infection transmission and specifically recommend that endoscopy sites have quality systems in place for endoscope reprocessing. Another key recommendation is the culture of patient-ready endoscopes to detect contamination with organisms of concern. Remaining gaps in the guidelines include ensuring that optimal endoscope-channel sample methods are used and ensuring effective root-cause analysis and remediation when contamination is detected. In this review, we summarize the critical aspects of endoscope sample collection and present a practical approach to root-cause analysis and remedial action plans.

(Received 4 October 2020; accepted 18 March 2021)

So many different methods !!.

Recent CDC and FDA recommendations focus on reducing "exogenous" infection transmission and specifically recommend the **culture of patient-ready endoscopes** to detect contamination with organisms of concern.

Remaining gaps in the guidelines include ensuring that optimal endoscope-channel sample methods are used and ensuring effective root-cause analysis and remediation when contamination is detected.

Alfa MJ, Singh H. Contaminated flexible endoscopes: Review of impact of channel sampling methods on culture results and recommendations for root-cause analysis. Infect Control Hosp Epidemiol. 2021 May 7:1-16. doi: 10.1017/ice.2021.128

Endoscope Sample Extraction Fluid	Channels Sampled	Method; FBF, FB, F	Fluid Volume	Friction for Instrument Channel	Neutralizer Added to Sample	Concentration of Sample for Culture	% Extraction Efficacy	% Culture Positive[a]	Reference
Sterile DI or RO water									
	All channels; separate	F	3–10 mL	None	No	No	Not stated	2.8	Alfa 2012[36]
	All channels; separate	FBF & F	7.5–20 mL Total: 60 mL	Bristle brush	DNP 2X	Filtration	Not stated	25.8	Pineau 2013[37]
	All channels; separate	FBF & F	20 mL	Bristle brush	No	No	Not stated	0	Ofstead 2015[38]
	All channels; pooled	F (retro- & antigrade)	20 mL	None	No	No	Not stated	31	Buss 2008[39]
Culture media[c]									
	Instrument	F	10 mL	None	No	No Broth enriched	Not stated	Outbreak	Classen 1988[55]
	Instrument	F	5–15 mL	None	No	No	Not stated	21	Moses 2003[41]
	Instrument	FBF	Not stated	Bristle brush	No	Centrifuge	Not stated	Outbreak	Epstein 2014[4]
	All channels; separate	F	20 mL	None	No	No	Not stated	0	Paula 2015[56]
	All channels; pooled	F	100 mL	None	No	Filtration	Not stated	18	Aumeran 2012[54]
DNP Neutralizer									
	All channels; pooled	F	120 mL	None	DNP used for flush	Filtration: 100 mL	Not stated	9–23	Saviuc 2015[57]
	All channels; pooled	F	300 mL	None	DNP used for flush	Filtration: 100 mL	Not stated	45	Saliou 2015[58]

Alfa MJ, Singh H. Contaminated flexible endoscopes: Review of impact of channel sampling methods on culture results and recommendations for root-cause analysis. Infect Control Hosp Epidemiol. 2021 May 7:1-16. doi: 10.1017/ice.2021.128

Introduction

Review

Contaminated flexible endoscopes: Review of impact of channel sampling methods on culture results and recommendations for root-cause analysis

Michelle J. Alfa PhD[1] and Harminder Singh MD[2]
[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada and [2]Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Abstract
Recently, infection transmission risk associated with contaminated, patient-ready flexible endoscopes has attracted attention. Outbreaks of multidrug-resistant organisms resulting in infection and/or colonization have been particularly concerning. Recent CDC and FDA recommendations focus on reducing "exogenous" infection transmission and specifically recommend that endoscopy sites have quality systems in place for endoscope reprocessing. Another key recommendation is the culture of patient-ready endoscopes to detect contamination with organisms of concern. Remaining gaps in the guidelines include ensuring that optimal endoscope-channel sample methods are used and ensuring effective root-cause analysis and remediation when contamination is detected. In this review, we summarize the critical aspects of endoscope sample collection and present a practical approach to root-cause analysis and remedial action plans.

(Received 4 October 2020; accepted 18 March 2021)

Importance of sampling solution.

In conclusion, the efficiency and therefore the value of the monitoring of endoscope reprocessing by microbiological cultures is dependent on the sampling solutions used. A **sampling solution with a tensioactive action** is more efficient than saline in detecting biofilm contamination of endoscopes.

A single flushing of internal channels with **saline solution removes only a very small number of bacteria.**

C. Aumeran, E. Thibert, F. A. Chapelle, C. Hennequin, O. Lesens and O. Traoréa. Assessment on experimental Bacterial Biofilms and in Clinical Practice of the Efficacy of Sampling Solutions for Microbiological Testing of Endoscopes. Journal of Clinical Microbiology. March 2012. Volume 50. Number 3. 938–942

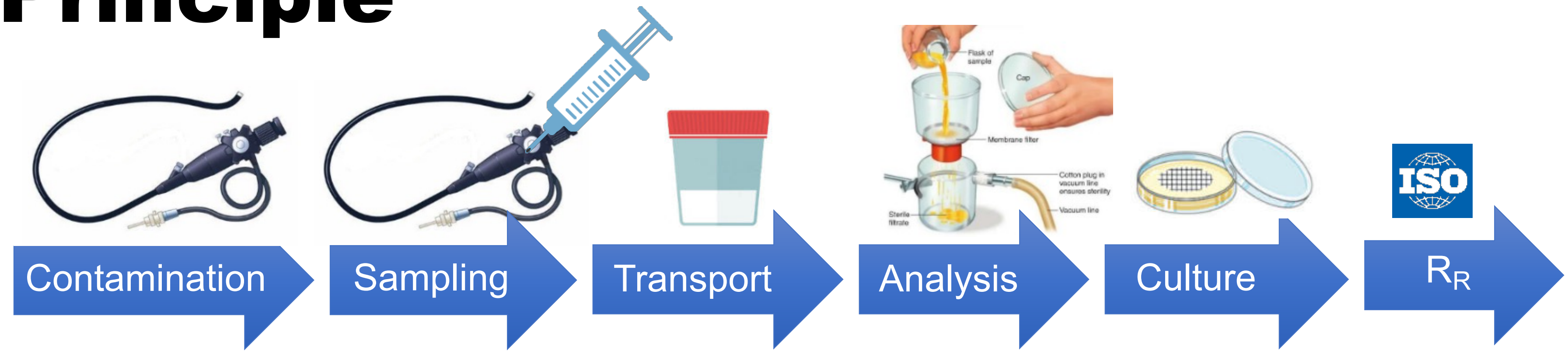
Introduction

- Since the recent outbreaks associated with duodenoscopes, the **interest of endoscope sampling** to assess regularly the adequacy of endoscope reprocessing, is well accepted.
- Studies published in the literature indicate that the **contamination level** (or non-compliance rate) of ready to use endoscopes varies **from 0.4% to 49.0 %**
- **Differences observed** between these studies regarding, the sampling method (flush vs flush-brush-flush, one channel vs all channels, …), the nature of the sampling solution (water, 0.9% NaCl, neutralizer,…), the sample culturing protocols (filtration vs centrifugation,…), the interpretation criteria and the limited number of samples analysed, **make difficult the comparison and the interpretation of these values**.
- **What is the most appropriate sampling & culturing method to be used as a quality indicator?**

Objectives

- Compare the efficacy of 5 endoscope sampling and culturing methods.
- Define the critical parameters for an endoscope sampling and culturing method.

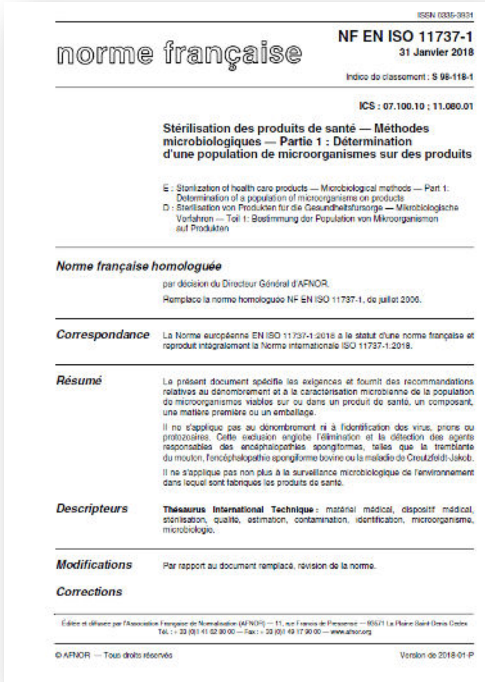
Principle



- 1 endoscope : 1 duodenoscope (TJF-Q180V),
- 3 microbial strains: *E.coli*, *S. aureus* and *P. aeruginosa*,
- 3 microbial concentrations are tested : 10 CFU/scope, 100 CFU/scope and 1000 CFU/scope,
- 5 sampling methods are compared: Germany, Netherland, France, Australia and FDA,
- 2 transportation times: 1 and 24 hours,
- RR: Recovery ratio (ISO 11737-1)
- 6 assays are performed per conditions i.e. 6 x 2 x 3 x 3 = 108 assays per sampling method

Efficacy of the sampling/culturing methods

If my endoscope contains 100 CFU, how many bacteria will I be able to collect with my sampling method?



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norme française

NF EN ISO 11737-1
31 Janvier 2018

Indice de classement : S 98-118-1

ICS : 07.100.10 ; 11.080.01

Stérilisation des produits de santé — Méthodes microbiologiques — Partie 1 : Détermination d'une population de microorganismes sur des produits

E : Sterilization of health care products — Microbiological methods — Part 1: Determination of a population of microorganisms on products
D : Sterilisation von Produkten für die Gesundheitsfürsorge — Mikrobiologische Verfahren — Teil 1: Bestimmung der Population von Mikroorganismen auf Produkten

Norme française homologuée

par décision du Directeur Général d'AFNOR.

Remplace la norme homologuée NF EN ISO 11737-1, de juillet 2006.

Correspondance La Norme européenne EN ISO 11737-1:2018 a le statut d'une norme française et reproduit intégralement la Norme internationale ISO 11737-1:2018.

Résumé Le présent document spécifie les exigences et fournit des recommandations relatives au dénombrement et à la caractérisation microbienne de la population de microorganismes viables sur ou dans un produit de santé, un composant, une matière première ou un emballage.

Il ne s'applique pas au dénombrement ni à l'identification des virus, prions ou protozoaires. Cette exclusion englobe l'élimination et la détection des agents responsables des encéphalopathies spongiformes, telles que la tremblante du mouton, l'encéphalopathie spongiforme bovine ou la maladie de Creutzfeldt-Jakob.

Il ne s'applique pas non plus à la surveillance microbiologique de l'environnement dans lequel sont fabriqués les produits de santé.

Descripteurs **Thésaurus International Technique** : matériel médical, dispositif médical, stérilisation, qualité, estimation, contamination, identification, microorganisme, microbiologie.

Modifications Par rapport au document remplacé, révision de la norme.

Corrections

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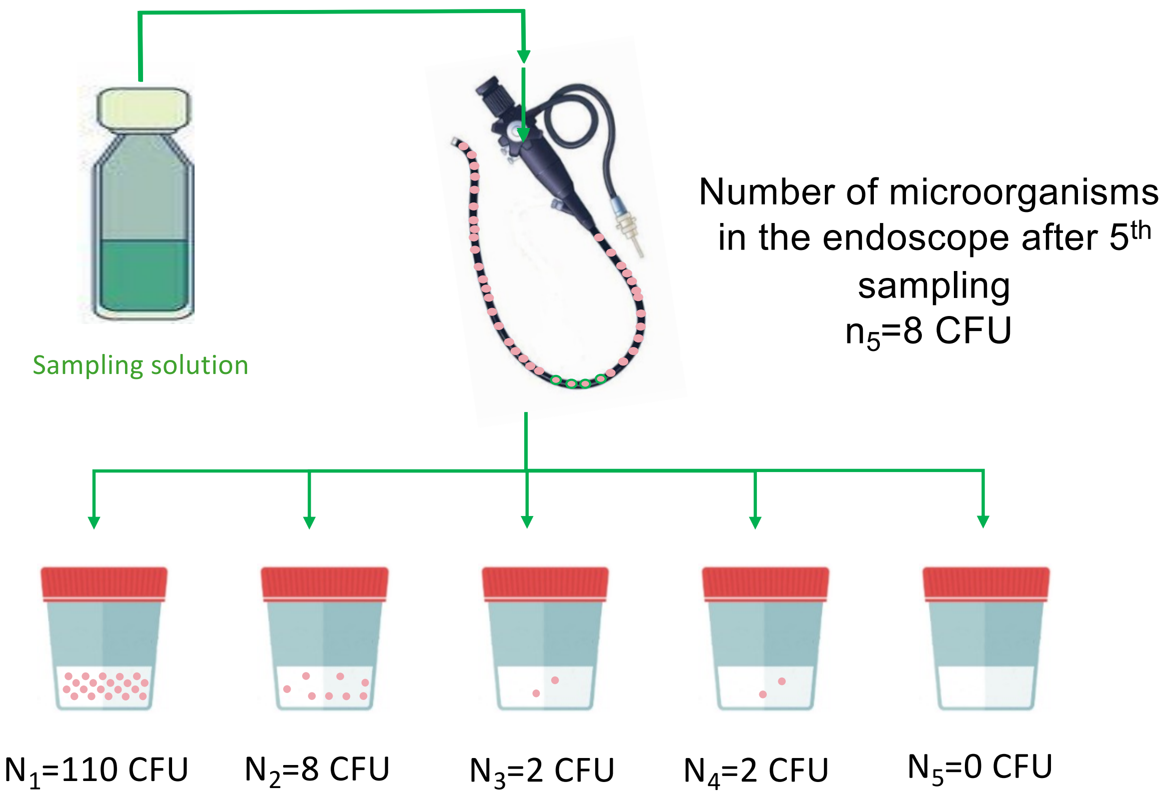
 Version de 2018-01-P

Validation by repeated sampling to establish the relationship between the number of microorganisms recovered and the actual number of microorganisms present on the product.

Recovery ratio



$$R = N1/\sum_{k=0}^{n} N_k$$
$$= 110/\ (110+8+2+2)$$
$$= 110/122 = 90\%$$

$$\sum_{k=0}^{N} N_k = n_0$$

All microorganisms initially present in the endoscope have been sampled

Sampling methods

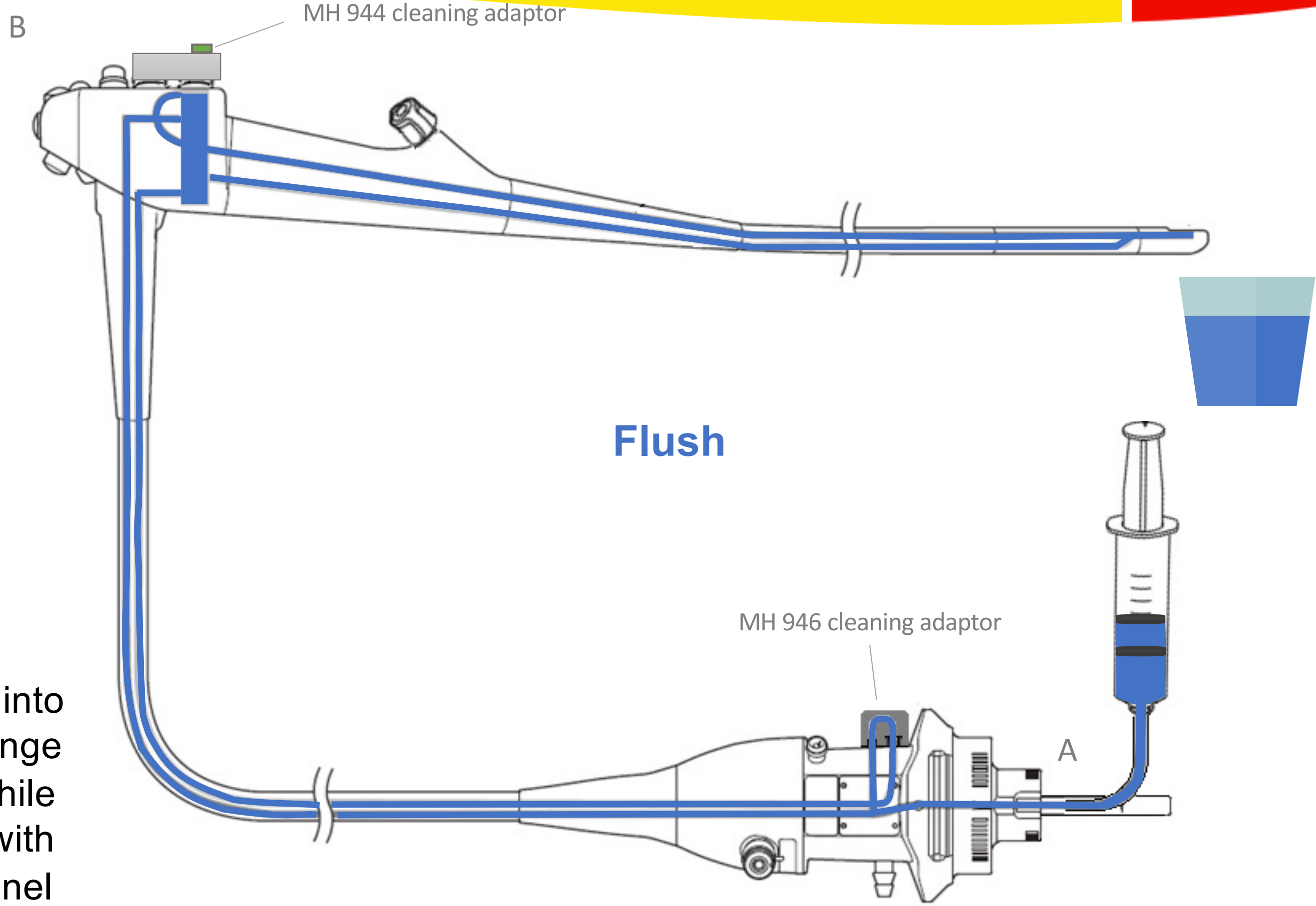
COUNTRY:		FRA	AUST	NL	USA	GER
Sample sites	Instrument channel	Y (FSF[a]) [2]	Y (FB[c]) [1]	Y (FSBF[e]) [2]	Y (FBF[b]) [2]	Y (F) [2]
	Suction/instrument channel	Y (FSF) [3]	Y (F[d]) [3]	Y (FSBF) [3]	**N**	Y (F) [3]
	Air/water channel	Y (FSF) [4]	Y (F[d]) [2]	Y (FSF) [4]	**N**	Y (F) [4]
	Elevator recess (distal end) with brush or swab	Y [1]	**N**	Y [1]	Y (1]	Y [1]
Sampling solution		NDP + thio	**Sterile water**	NaCl 0.9%	**Sterile water**	NaCl 0.9%
Addition of neutralizer to extracted sample		No (NDP + thio used for sampling)	**No**	**No**	Y (NDP + thio)	Y (NDP + thio) two time concentrated
Sample volume (sampling solution + neutralizer)		100 mL (distal end) + 130 mL (channels)	30 mL	60 mL	82 mL	3 x 50 mL
Friction for Instrument channel (bristle brush)		**N**	Y	Y	Y	**N**
Number of samples		2 (all channels pooled &distal end)	1 (all channels pooled)	2 (all channels pooled & distal end)	1 (Instrument channel & distal end pooled)	4 (All channels separately & distal end)

FRA: France, USA: United States of America, AUST: Australia, GER: Germany, NL: The Netherlands
(a) FSF: Flush-Suction-Flush, (b): FBF: Flush-Brush-Flush, (c): FB: Flush-Brush, (d) F: Flush, (e): FSBF: Flush-Suction-Brush-Flush. Y: Yes, N: No, NDP + thio: Neutralizing Pharmacopeia Diluent plus thiosulfate. [x]: figures in square brackets define the chronology in which channels/sites were sampled.

Method

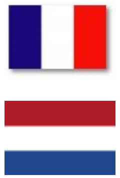
Sampling of the Air/water channels using the flush method

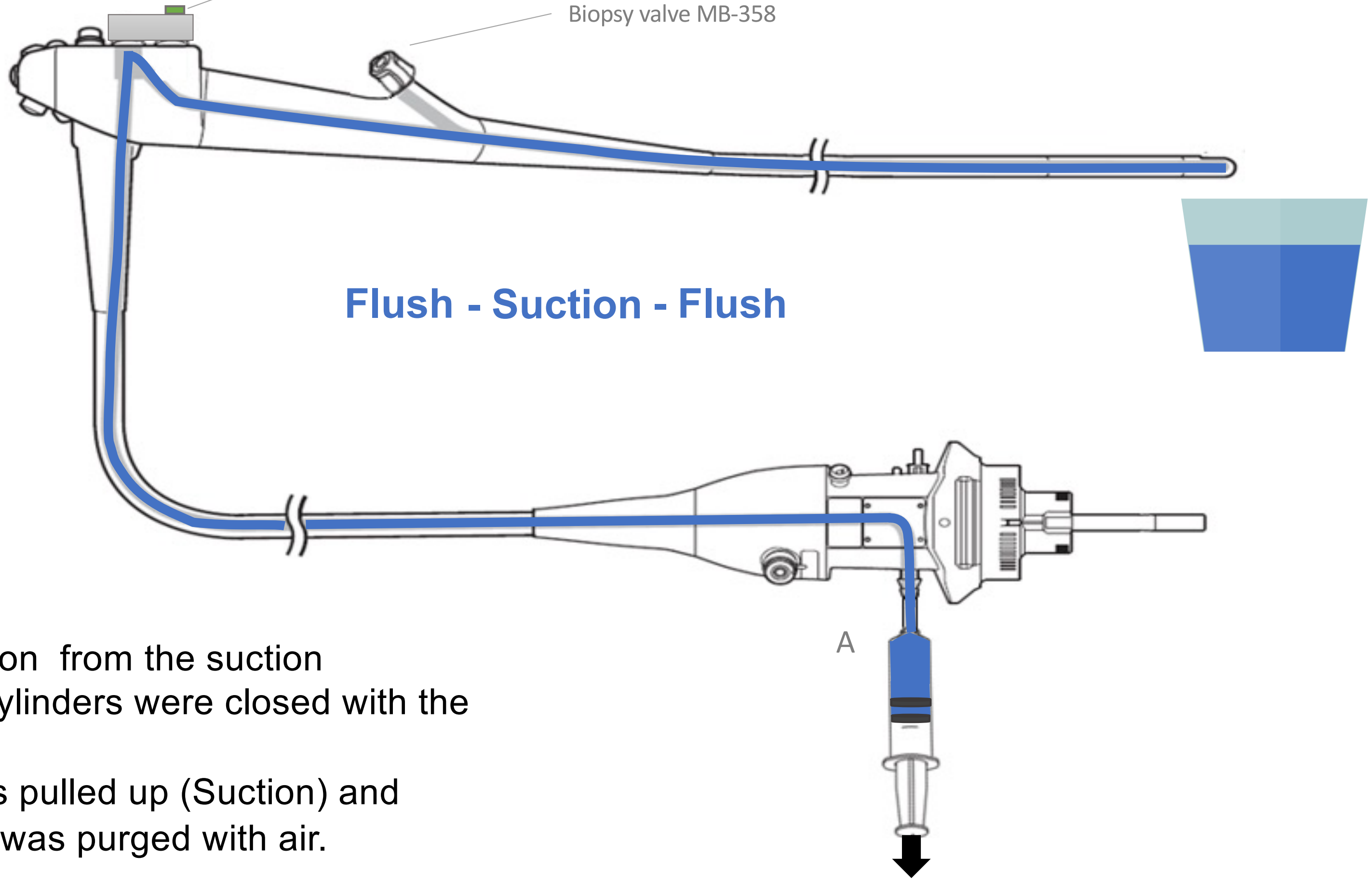
Injection of the sampling solution into the air/water channel using a syringe connected to air connector (A) while the valve cylinders were closed with the MH-944 connector (B). Channel were then purged with air.



Method

Sampling of the suction/instrument channel using the flush-suction-flush method



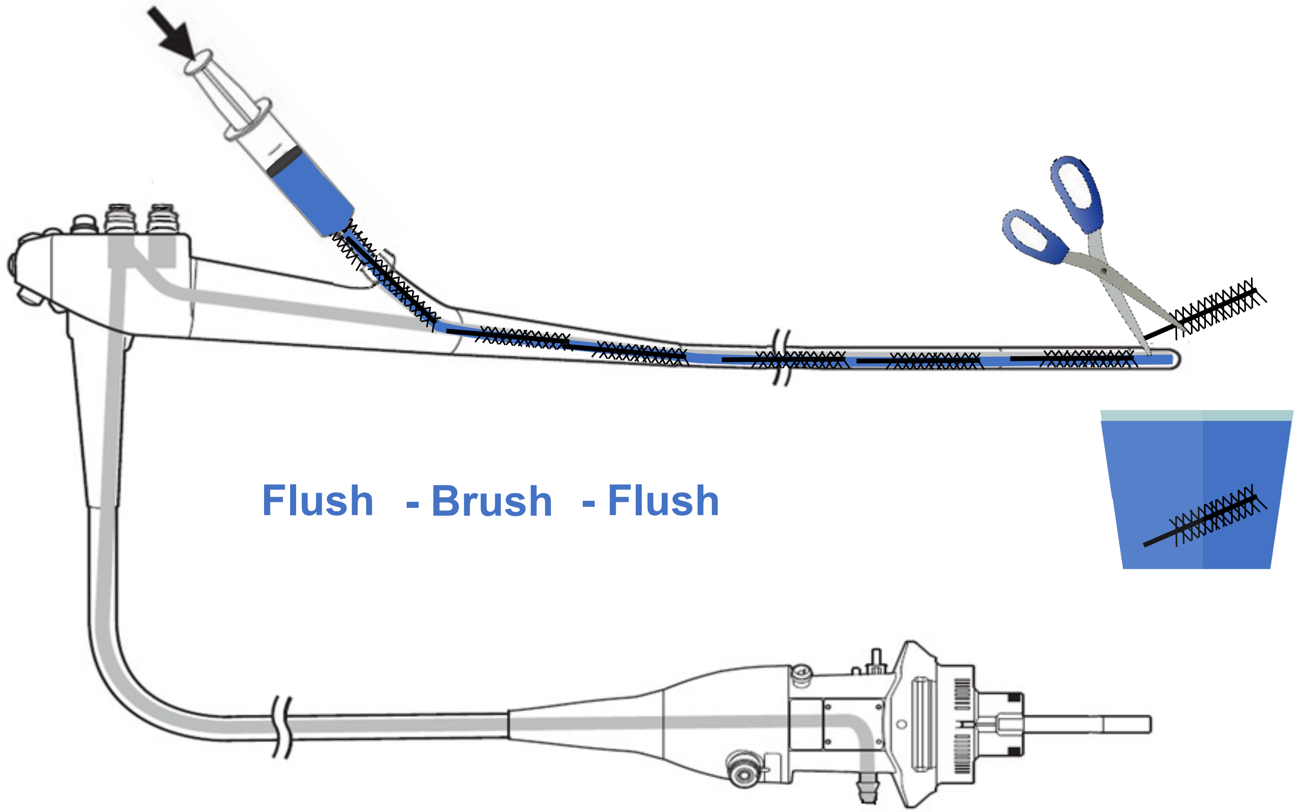


Injection of the sampling solution from the suction connector(A) while the valve cylinders were closed with the MH-944 connector (B) (Flush).
The plunger of the syringe was pulled up (Suction) and down (Flush) and the channel was purged with air.

Method

Sampling of the instrument channel using the flush-brush-flush method

- Injection of the sampling solution in the instrument channel followed by an air purge,
- Brushing of the channel,
- New injection of sampling solution and air purge.

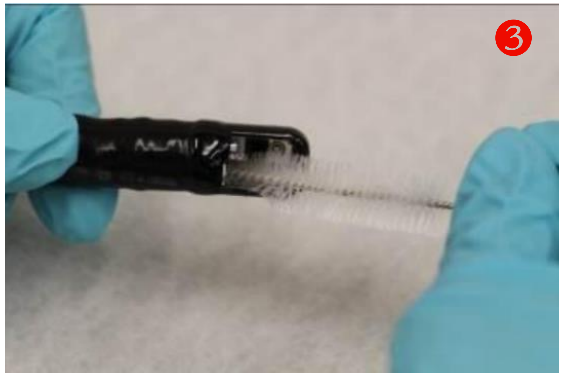


Note: for Australian method repeat all stages on suction and suction/instrument channel.

Sampling method

Sampling of the duodenoscope distal end

1. Swabbing along the seam between the distal cap and the distal end
2. Elevator recess flush – elevator down and up
3. Elevator brush (large brush)
4. Elevator brush (small brush)



Instruction n° DGOS/PF2/DGS/VSS1/2016/220 du 4 juillet 2016 relative au traitement des endoscopes souples thermosensibles à canaux au sein des lieux de soins. Available at: https://www.legifrance.gouv.fr/circulaire/id/41172 . Last accessed 11/10/2023.

GESA - Gastroenterological Society of Australia. Infection control in endoscopy 2nd Edition. 2003. Available at https://www.asp.com/sites/default/files/pdf/best-practices/GESA-guideline-gastrointestinal-endoscopey-(Australia).pdf Last accessed 11/10/2023.

Gillespie E, Despina Kotsanas D, Stuart RL. Microbiological monitoring of endoscopes: 5-year review. J Gastroenterol Hepatol 2008; 23:1069–1074

Rauwers AW, Voor In't Holt AF, Buijs JG et al. High prevalence rate of digestive tract bacteria in duodenoscopes: a nationwide study. Gut 2018; 67: 1637–1645.

FDA/CDC/ASM. Duodenoscope Surveillance Sampling and Culturing Protocols. 2018 Available at: https://www.fda.gov/media/111081/download . Last accessed 23/04/2023.

Hygiene Requirements for the Reprocessing of Medical Devices. Bundesgesundheitsbl 2012 · 55:1244–1310. Available at https://www.rki.de/DE/Content/Infekt/Krankenhaushygiene/Kommission/Downloads/Hygiene_Requirements_Medical_Devices_2012.pdf?__blob=publicationFile . Last accessed 24/05/2023.

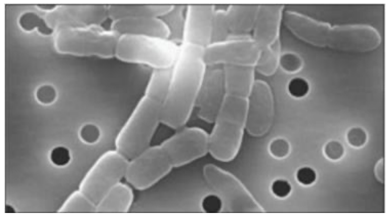
Culturing methods

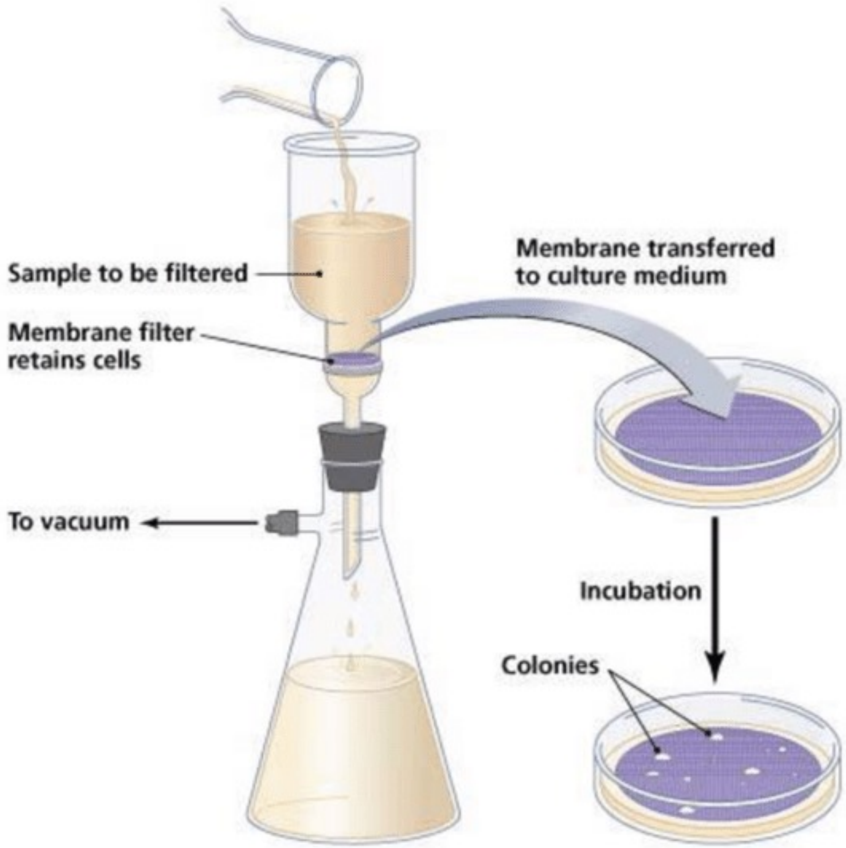
Tested Method	FRA	AUST	NL	USA		GER
Culture method	Filtration	**Centrifugation**	Filtration	Filtration	**Centrifugation**	Filtration
Total volume used for sample extraction	230 mL	30 mL	60 mL	82 mL	82 mL	3 x 50 mL
Total sample volume analyzed	230 mL	0.2 mL	60 mL	82 mL	82 mL	3 x 50 mL
% of sample volume analyzed	100%	**6.6%**	100%	100%	100%	100%
Culture medium	Trypticase soy agar (TSA)	Blood + MacConkey agar	R2A Agar	Blood agar	Blood agar	Blood agar
Incubation time	5 days	5 days	3 days	3 days	3 days	2 days
Incubation temperature	30°C	35°C then 28°C	35°C	35°C to 37°C	35°C to 37°C	36°C
Result expression according to source	CFU/ endoscope	CFU/mL	CFU/20 mL	CFU/ endoscope	CFU/ endoscope	CFU/ channel

(a) F: Filtration. (b) C: centrifugation

Membrane filtration

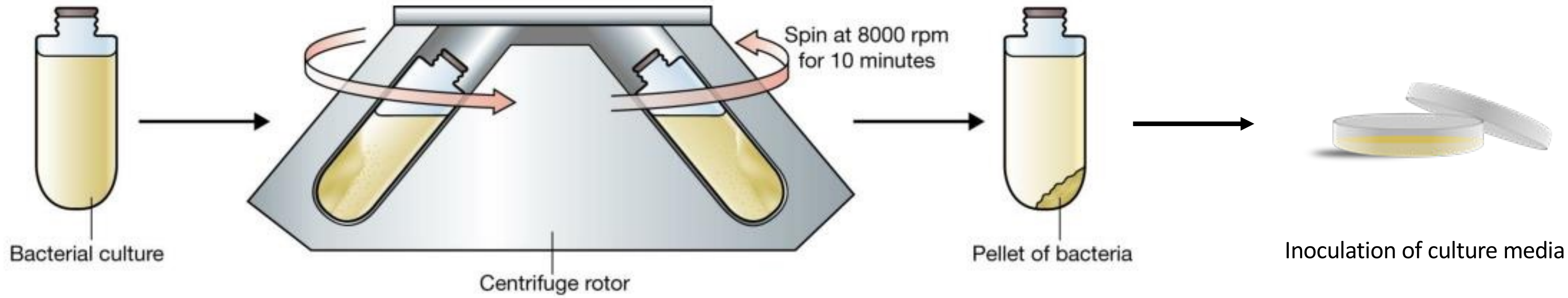
A vacuum is created in the receiving flask. The air pressure forces the liquid through the filter. The microorganisms are retained on the filter surface. This filter is then transferred to a petri dish containing a pre-poured set medium, where colonies arise from the bacteria on the surface of the filter.





https://plantlet.org/general-methods-of-microbial-isolation/

Centrifugation



The centrifuging and washing method is used to concentrate microorganisms in a small volume, but a number of questions remain unanswered: the sensitivity of the microorganisms to centrifugation, the recovery efficiency of the method…

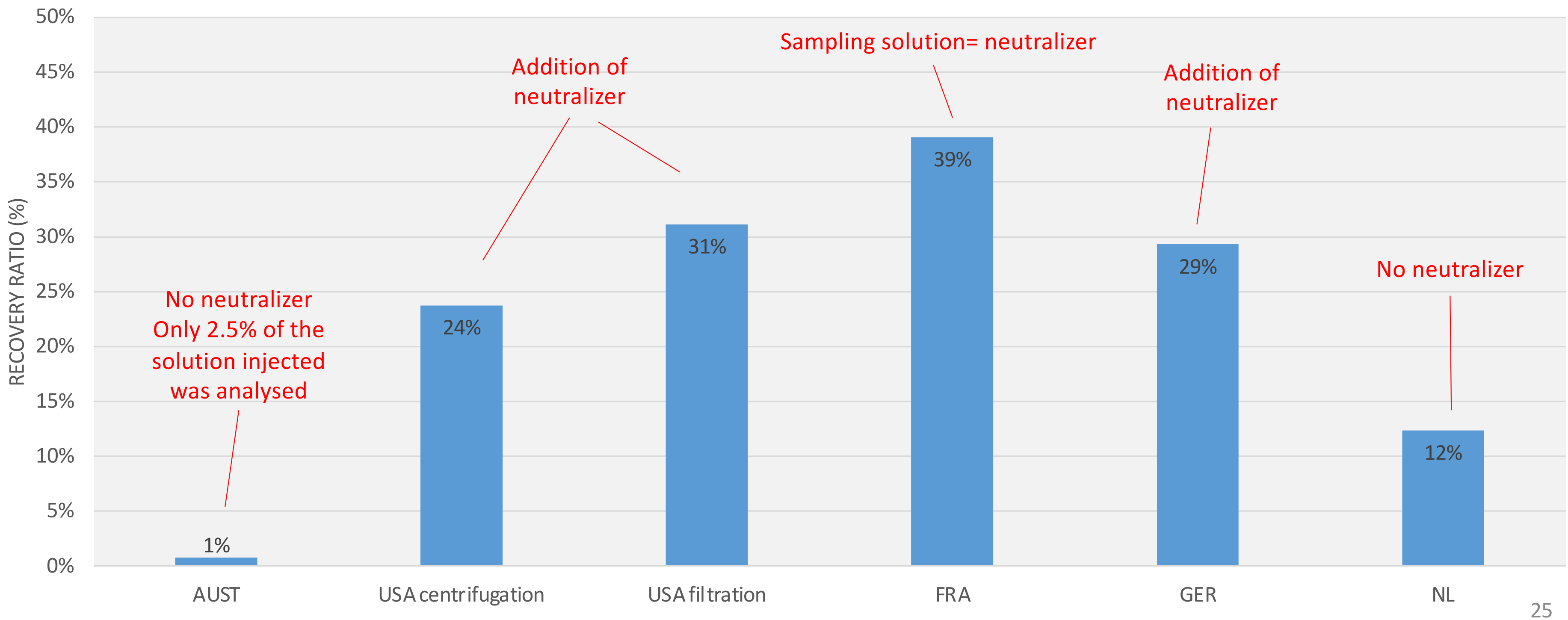
https://blog.naver.com/PostView.nhn?isHttpsRedirect=true&blogId=dwrkdehddn&logNo=220685632010

Results

- Comparison of the efficacy of 5 endoscope sampling methods.
- What are the critical parameters for an endoscope sampling and culturing method.

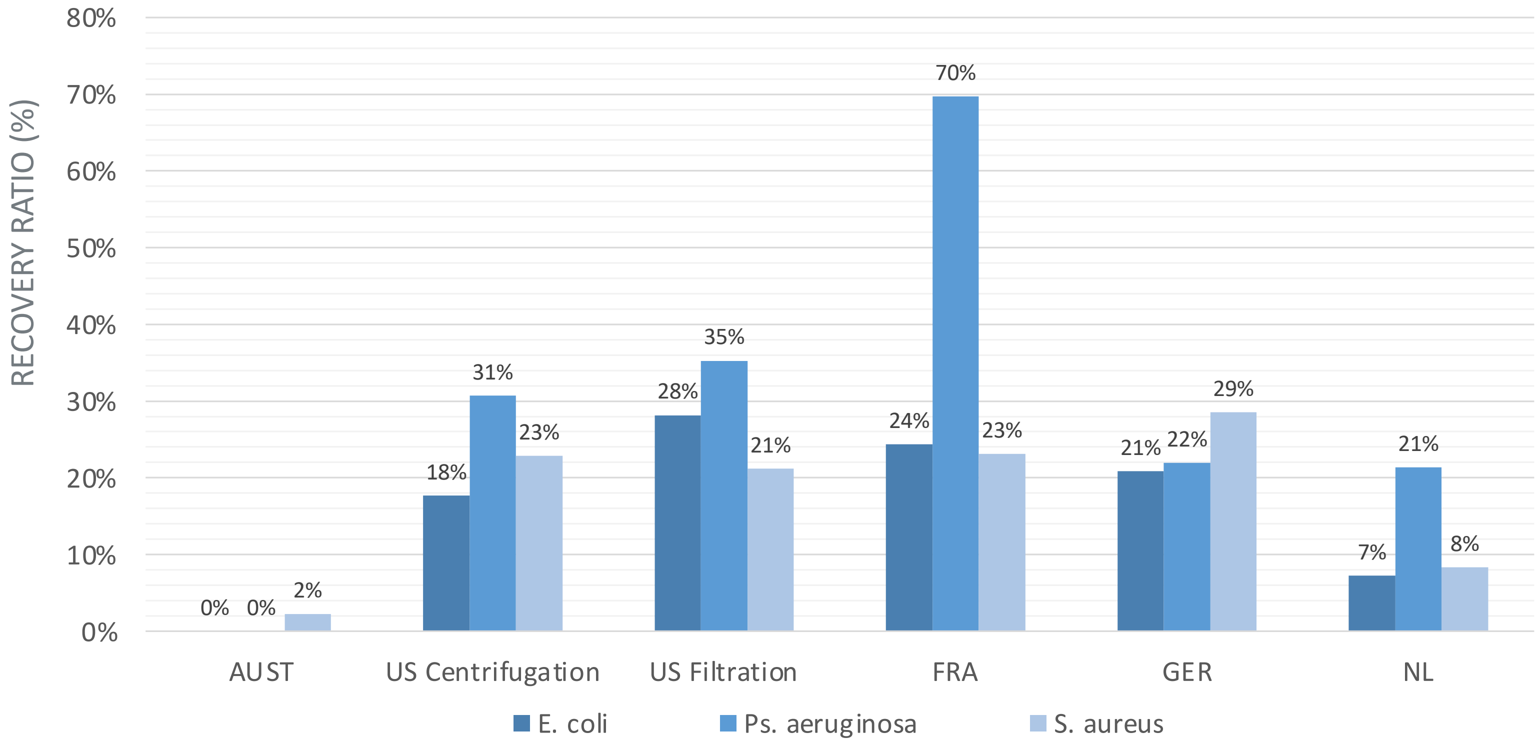
Results

Efficacy of the sampling/culturing methods



Results

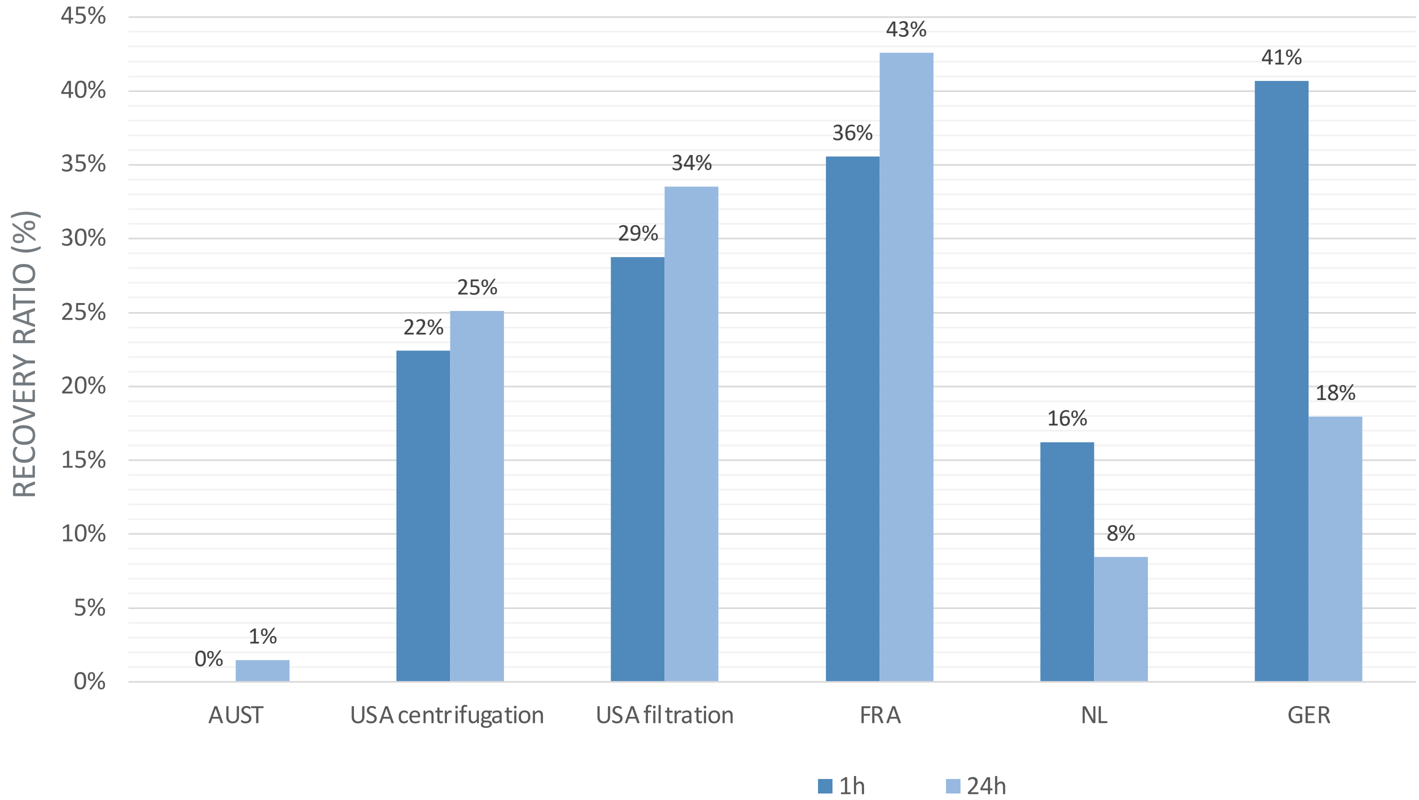
Influence of the microorganism



The efficacy of the sampling/culturing method varies according to the nature of the microorganisms present in endoscope channels.

Results

Influence of the transportation time

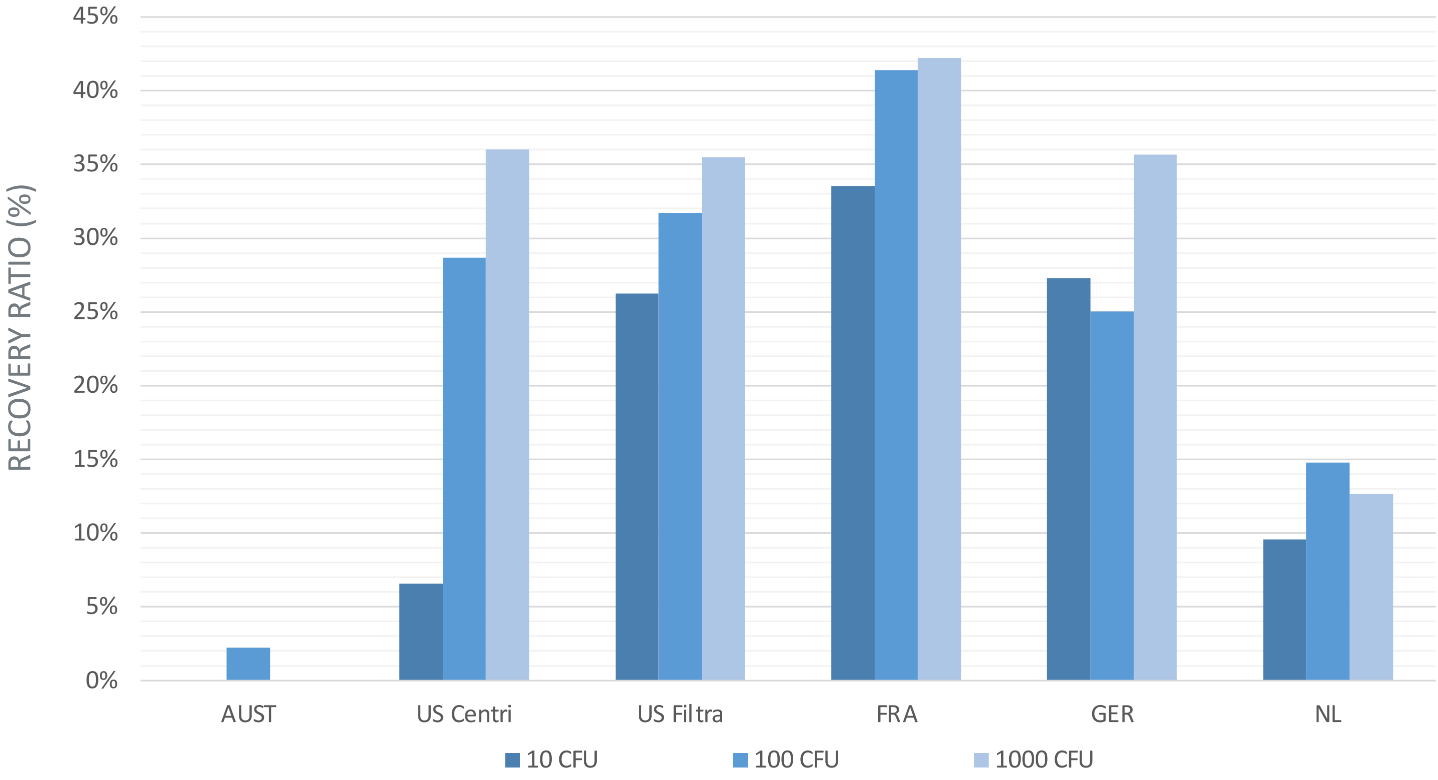


No difference between 1h and 24h transportation time for US and FRA sampling methods.

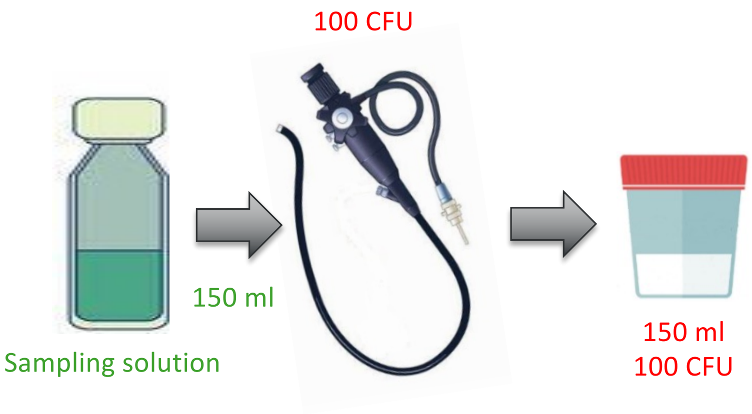
For the GER and NL method a decrease of the recovery ratio is observed if the sample is analysed 24h after sampling.

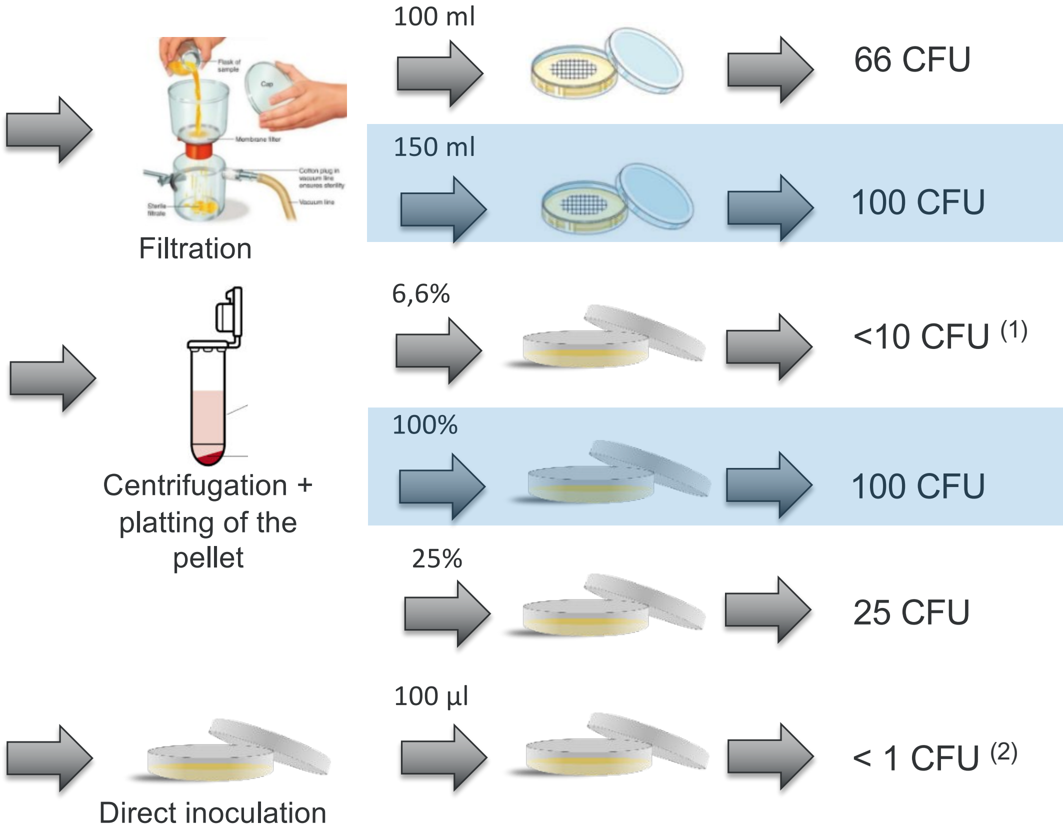
Results

Influence of the initial contamination level



The efficacy of the sampling and culturing method decreases of about 10% when the endoscope contamination level varies from 1000 CFU to 10 CFU/endoscope.





Comparison of results obtained after sampling and culturing of an endoscope containing initially 100 CFU with the different methods tested.

(1) <40 CFU/endoscope (2) $<10^3$ CFU/endoscope

Importance of brushing

MAIN ARTICLES | Flush/brush/flush method for reprocessed endoscopes

Originalia

Elution of working channels with the flush-brush-flush-method for microbiological testing of reprocessed endoscopes

Part 1: Description of the method and microbiology results of the field study

M. Wehrl[1], P. Barone[2], H. Biering[3], F.H.H. Brill[4], M. Dabrowski[5], D. Diedrich[6], J. Gebel[7], S. Gemein[7], D. Geyer[8], A Halvarsson[2], B. Hücker[5], A. Kampe[4], B. Kampf[9], K. Kruse[10], J. Lenz[11], H. Martiny[12], U. Orschel[6], M. Plevschinski[8], O. Riebe[13], K. Roth[14], V. Schilberg[15], V. Schmidt[11], L. Schnieder[14], T. Schwemmer-Cordes[16], T. Seis[2], E. Stec[6], C. Uhlig[8], P. Wehnes[10]

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Conflict of interest:
All authors confirm that there is no conflict of interest according to the guidelines of the International Committee of Medical Journal editors (ICMJE).

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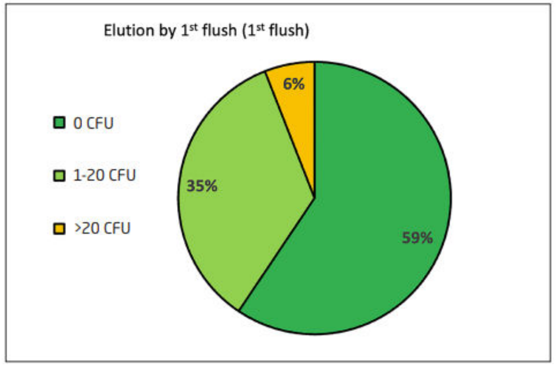
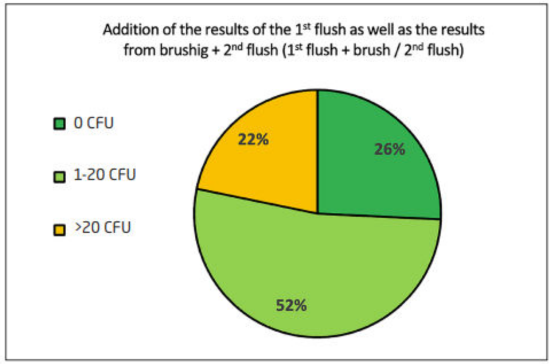
- overall process performance
- product control
- performance qualification, performance requalification

1 Introduction

In accordance with the hitherto valid Annex 10 [1] of the first edition of the *Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Heat-Sensitive Endoscopes*, compiled by the DGKH, DEGEA, DGSV, DGVS and AKI* [2], microbiological testing of reprocessed endoscopes (product control) is carried out by swab sampling of critical external areas and flush sampling of all endoscope channels. The basis for this description is Annex 8 of the KRINKO/BfArM** Recommendation *Hygiene Requirements for Reprocessing Medical Devices* [3]. For flush sampling 25 ml of sterile 0.9 % sodium chloride solution (NaCl solution) was injected into each of the channels present and 20 ml was separately collected from the distal tip. The swab and flush samples were evaluated using microbiology culture methods to obtain the number of eluted microorganisms (quantitative testing) and by using selective nutrient media for detection of hygienically relevant indicator organisms (qualitative testing). Elution of residual viable microorganisms from the channels through flush sampling followed by detection in cultures is currently the only established method in Germany for microbiological testing of endoscope channels that are not directly accessible.

When testing the microbiological condition of endoscope channels the recovery rate of the detected microorganisms is of elementary importance. The recovery rate is the quantitative measure of the proportion of detected microorganisms in relation to the number of microorganisms actually present in the test object, i.e. the respective endoscope channel. Low recovery rates result in underestimation of the actual bioburden and may lead to an unrecognised risk from undetected pathogens.



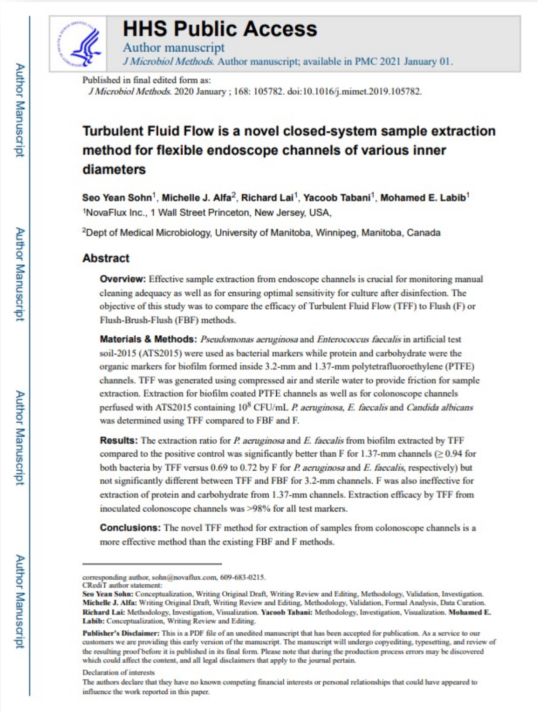


Percentage distribution of the n = 101 results obtained for the total colony count for the two test methods, divided into the categories 0 CFU, 1 – 20 CFU and > 20 CFU per working channel.

“The results obtained demonstrate that the microorganism recovery rate can be sharply increased by using an endoscope cleaning brush, followed by a 2nd flush”.

M. Wehrl et al. Elution of working channels with the flush-brush-flush-method for microbiological testing of reprocessed endoscopes2022. Zentralsterilization, Volume 30, 2772-277

Turbulent flow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Turbulent Fluid Flow is a novel closed-system sample extraction method for flexible endoscope channels of various inner diameters

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Abstract

Overview: Effective sample extraction from endoscope channels is crucial for monitoring manual cleaning adequacy as well as for ensuring optimal sensitivity for culture after disinfection. The objective of this study was to compare the efficacy of Turbulent Fluid Flow (TFF) to Flush (F) or Flush-Brush-Flush (FBF) methods.

Materials & Methods: *Pseudomonas aeruginosa* and *Enterococcus faecalis* in artificial test soil-2015 (ATS2015) were used as bacterial markers while protein and carbohydrate were the organic markers for biofilm formed inside 3.2-mm and 1.37-mm polytetrafluoroethylene (PTFE) channels. TFF was generated using compressed air and sterile water to provide friction for sample extraction. Extraction for biofilm coated PTFE channels as well as for colonoscope channels perfused with ATS2015 containing 10^8 CFU/mL *P. aeruginosa, E. faecalis* and *Candida albicans* was determined using TFF compared to FBF and F.

Results: The extraction ratio for *P. aeruginosa* and *E. faecalis* from biofilm extracted by TFF compared to the positive control was significantly better than F for 1.37-mm channels (≥ 0.94 for both bacteria by TFF versus 0.69 to 0.72 by F for *P. aeruginosa* and *E. faecalis*, respectively) but not significantly different between TFF and FBF for 3.2-mm channels. F was also ineffective for extraction of protein and carbohydrate from 1.37-mm channels. Extraction efficacy by TFF from inoculated colonoscope channels was >98% for all test markers.

Conclusions: The novel TFF method for extraction of samples from colonoscope channels is a more effective method than the existing FBF and F methods.

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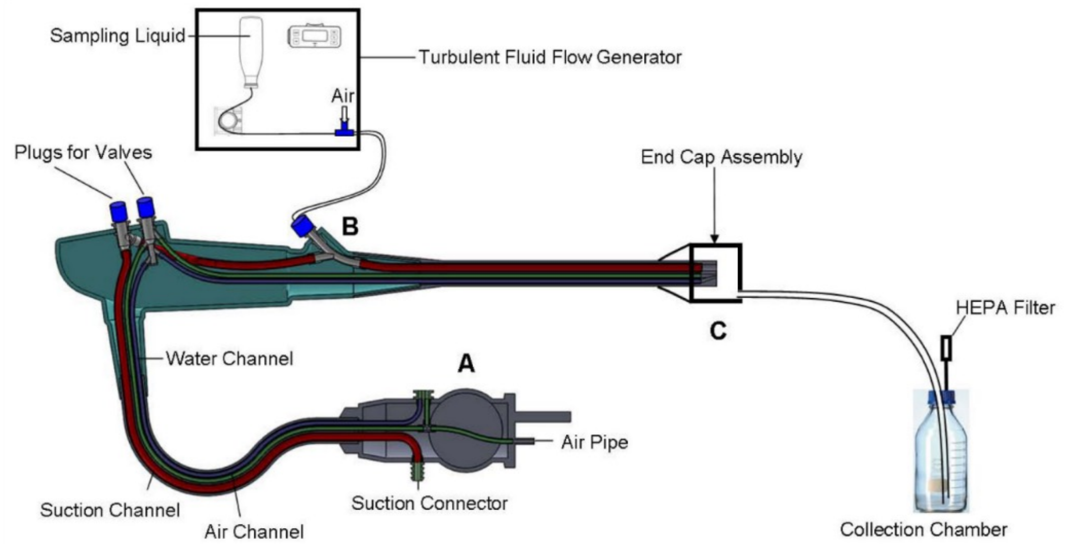
Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



"The novel Turbulent Fluid Flow (TFF) method for extraction of samples from colonoscope channels is a more effective method than the existing FBF and F methods"

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Conclusion

Endoscope sampling and culturing practices need to be harmonized

1. The sampling solution shall include **neutralizing** as well as a **tensioactive agents**.
2. **All channels** in the endoscope should be sampled.
3. Ideally use of **friction** during sample collection for all channels.
4. Ensuring that **80%** of the total sample injected into the channels is collected.
5. Ensuring that the sampling solution **maintains microbial viability** for 24 hours at refrigeration temperature (4°C).
6. The entire sample collected should be concentrated by 0.45 µm (or 0.2µm) **membrane filtration** and cultured on agar medium.
7. Harmonized interpretation criteria shall be defined.

Thank you for your attention !!!