Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab® transport system

Demuyser, Thomas; De Geyter, Deborah; Van Dorpe, Daisy; Vandoorslaer, Kristof; Wybo, Ingrid

Published in:
Journal of Microbiological Methods

DOI:
10.1016/j.mimet.2017.11.009
https://doi.org/10.1016/j.mimet.2017.11.009

Publication date:
2018

License:
Unspecified

Document Version:
Final published version

Journal of Microbiological Methods 144 (2018) 73–78

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab® transport system

Thomas Demuyser, Deborah De Geyter, Daisy Van Dorpe, Kristof Vandoorslaer, Ingrid Wybo*

Department of Microbiology, Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium

ARTICLE INFO

Keywords:
eSwab
Fastidious anaerobes
Viability recovery

ABSTRACT

Anaerobic infections are difficult to diagnose and treat, because of the often slow in vitro growth, the polymicrobial nature and the increasing antimicrobial resistance. Furthermore because of their fastidiousness, anaerobic bacteria often stay unrecognized in clinical practice. Clinical specimens potentially harboring these species require special handling to permit satisfactory recovery of these potential important pathogens. In a clinical setting, temporary storage and transportation to the laboratory are unavoidable before these specimens can be cultured. In the current study we expand the knowledge about the recovery of a wide range of clinically relevant anaerobic bacteria from an eSwab® container after different storage durations and temperatures. Our findings support the use of the eSwab® container as a relative short-term storage unit for anaerobic species. When stored at 2–4 °C immediately after inoculation, all anaerobic species (except for *Clostridium clostridioforme*) can be recovered from the liquid Amies medium until 1 day post-specimen collection. Because most samples in the clinical setting are processed in this time span, the eSwab® container is sufficiently capable of retaining viability in daily routine. However; because of inevitable centralization of clinical laboratories, adequate storage of these specimens for an extended period of time will be essential in the future. Therefore in certain cases, when viability is desired for longer periods (> 1 day), storage of the containers at 2–4 °C is certainly advisable.

1. Introduction

In the microbiology laboratory, the pre-analytical phase is especially important, because specific laboratory errors can be directly linked to therapeutic or patient management decisions. Inadequate specimen collection, handling, labelling, and transport are mistakes that can lead at worst to incorrect antibiotic treatment or treatment of the wrong patient for the wrong disease (Hawkins, 2012). Therefore some major points of attention should be taken into consideration for an adequate pre-analytical phase of microbial sample analysis. As such, organisms (e.g. viruses, anaerobic bacteria…) require certain specific transport systems to ensure adequate specimen storage and survival (Schofield, 2006).

The metabolism of obligate anaerobes is based on fermentation in which oxidation of available organic compounds to organic acids and alcohols occurs. They are characterized by the fact that they cannot resist the presence of oxygen in the environment because they are unable to metabolize the reactive oxygen derivatives that are formed in the presence of oxygen (Finegold, 1995; Hentges, 1996). Many anaerobic bacteria have an endogenous origin and are amongst the main organisms present on the skin, mucous membranes and the intestine (Brook, 2016). Because of the widespread presence of anaerobes in the human body, virtually all types of infections can occur. However there are four major sites of anaerobic infection, i.e. lung, gastro-intestinal tract, the female genital tract and at the skin and soft tissues (Finegold, 1995).

Clinical specimens potentially harboring anaerobic bacteria require special handling to permit satisfactory recovery of these important pathogens. In an ideal situation, specimens for anaerobic incubation and testing should be subjected to culture immediately after collection and subsequently incubated in a suited anaerobic environment. In a clinical setting however, temporary storage and transportation to the laboratory are unavoidable. As such, limited recovery can be due to problems in several key steps of the pre-analytical phase: at the time of sample collection, during transport to the laboratory and sometimes when the specimen has to be shipped to a reference laboratory for specialized testing (Peterson, 1997). Centralization of specialized clinical laboratory analyses is getting more and more attention, especially from an economical point-of-view. Because not all laboratories have the proper equipment to incubate and handle anaerobic specimens, transportation and extended storage will increasingly become of utmost importance.

* Corresponding author at: Department of Microbiology, Vrije Universiteit Brussel, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium.
E-mail address: Ingrid.Wybo@uzbrussel.be (I. Wybo).

https://doi.org/10.1016/j.mimet.2017.11.009
Received 13 September 2017; Received in revised form 9 November 2017; Accepted 12 November 2017
Available online 13 November 2017

Several specific anaerobic transport containers and collection media have been developed and the recovery of anaerobic bacteria from these recipients has been extensively studied (Citron et al., 2000; Fujimoto et al., 2014; Mena et al., 1978; Peterson, 1997; Stoner et al., 2008; Tyrrell et al., 2016; Van Horn et al., 2008). In general, these media consist of a semi-solid or liquid pre-reduced medium, where a fluid aspiration can be injected or a swab can be inserted.

The eSwab® (distributed by Copan Diagnostics, Brescia Italy since 2005) is not a specific medium for anaerobic organism storage, yet today it is a widely used transport system for both aerobic and anaerobic species (Van Horn et al., 2008). With its liquid modified Amies medium, it lends itself to automation, in contrast with semi-solid systems. Apart from the screw-top tube, the eSwab® consists of a nylon-flocked swab, designed to optimize specimen collection and to maximize release of the species in the liquid medium (Tyrrell et al., 2016). After adequate swabbing of the wound or infected site, the swab is inserted into the tube and the shaft of the swab is broken off. As such the cap can be fixed on the tube, locking the swab inside after it is fully closed. Alternatively, liquid samples, collected by needle aspiration, can be injected in the container and small solid tissue biopsies can be inserted.

In the present study, we aimed to expand the current knowledge about the storage and transport of anaerobic bacteria specifically in the eSwab® container. We applied different incubation periods, inoculum concentrations and storage temperatures to cover a wide range of clinically relevant conditions. Furthermore we applied these conditions on a wide range of 18 clinically relevant pathogenic anaerobic bacteria (Table 1). As such we do not only aim to evaluate the recovery of different pathological species from the eSwab® at different time points after inoculation, we also seek to verify what storage temperature is optimal for bacterial recovery at these different time points.

2. Materials & methods

The eSwab® transport system consists of a nylon-flocked swab, stored in a screw-cap tube containing 1 mL of modified liquid Amies medium (Amies, 1967). Eighteen of the most "clinically relevant" fastidious anaerobic bacteria were selected for the evaluation of anaerobic viability in an eSwab® container (Table 1). The different species were isolated, identified and stored at −80 °C before application in the current study. Identification of the primary culture was accomplished by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), with MALDI Biotyper 3.0 software and Reference Library 3.3.2.0 (Bruker Daltonik GmbH, Bremen, Germany). This recovery study was performed, based upon the approved standard Clinical & Laboratory Standards Institute (CLSI) guidelines (document M40-A) for quality control.

First a bacterial *primary plate* was prepared from the frozen organisms. After sufficient growth, a suspension from the *primary plate* was prepared in nutrient broth to achieve a turbidity of 0.5 McF (0.5 McF = 1.5×10^8 colony-forming units (CFU)/mL). This stock suspension was diluted 10 × in the eSwab® container to reach a first *inoculum* concentration of approximately 1.5×10^7 CFU/mL. In the next steps, the stock suspension was diluted 100 × and 10,000 × (to 1.5×10^6 and $1.5\ 10^4$ CFU/mL) in nutrient broth medium (LabM, Heywood, UK). This suspensions were diluted again 10 × in eSwab® containers to reach a second and third *inoculum* concentration of ~1.5×10^5 CFU/mL and ~1.5×10^3 CFU/mL.

Remark: a final theoretical inoculum of 75 CFU did not correspond to the observed number of CFU in the *Clostridium* species. Therefore the eSwab® containers, containing *Clostridium* species were diluted 100 × less. Only an initial theoretical inoculum of 1.5×10^7 and 1.5×10^5 CFU/mL were included for *Clostridium clostridioforme, Clostridium difficile* and *Clostridium ramosum*.

eSwab® containers of each final inoculum concentration were stored at 2–4 °C or at room temperature (22–24 °C) for 8 h, 24 h, 48 h, 72 h or one week. After the appropriate storage time, the eSwab® medium (1 mL liquid Amies medium containing 1.5×10^7 CFU/mL) was serially diluted 10.000 × in nutrient broth medium (to reach a content of 1.5×10^3 CFU/mL). 50 µL of each organism suspension was inoculated to duplicate in-house Brucella agar plates (brain heart infusion supplemented with 0.5% yeast extract, 5% sheep or horse blood, 5% vitamin K_1, and 1% hemin; sterilized for 15 min at 121 °C) and the suspension was spread with a sterile bent rod over the entire surface of the plate.

Secondly eSwab® medium (containing 1.5×10^5 CFU/mL) was diluted 100 × in nutrient broth medium (1.5×10^3 CFU/mL). 50 µL of this dilution is plated in duplicate to an anaerobic plate. Finally 50 µL of eSwab® medium (containing 1.5×10^3 CFU/mL) was plated directly in duplicate to anaerobic plates. As such a theoretical identical number of 75 CFU is plated in duplicate for every experimental condition.

The obtained number of CFU was determined after 72 h of plate incubation in a Whitley A45 anaerobic workstation (don Whitley Scientific, Shipley, UK). Bacterial recovery is determined as a percentage of CFU detected on the plate versus the CFU from the *reference plate* (0 h storage of eSwab® container) after appropriate storage (overview of applied conditions in Table 2).

Table 1
Anaerobic species included in the current recovery study.

GRAM negative	GRAM positive
Bacteroides fragilis	Non-spore forming
Bacteroides thetaiotaomicron	*Finegoldia magna*
Bilophila wadsworthia	*Parvimonas micra*
Fusobacterium necrophorum	*Peptostreptococcus anaerobius*
Fusobacterium nucleatum	*Eggerthella lenta*
Porphyromonas asaccharolytica	*Propionibacterium acnes*
Porphyromonas gingivalis	Spore forming
Prevotella buccae	*Clostridium clostridioforme*
Prevotella intermedia	*Clostridium difficile*
Prevotella melaninogenica	*Clostridium ramosum*

3. Results

Eighteen of the most frequent pathogenic anaerobic bacteria were included in this study. The results for all different species are depicted in Figs. 1–3 and are described in detail in Demuyser (2017) (Demuyser *submitted*).

Certain species of Gram-negative bacteria could be recovered in a reliable manner for an extended period. As depicted in Fig. 1, both *Bacteroides* species could be recovered from the eSwab® medium until 1 week of storage and *Bilophila wadsworthia* was recovered until 72 h at both storage temperatures. *Fusobacterium nucleatum* was retained viable from the eSwab® container until 1 week of storage at 2–4 °C and until 48–72 h at room temperature. Apart from these four species, other Gram-negative bacteria could not be conserved for such an extended period. *Fusobacterium necrophorum* and *Porphyromonas asaccharolytica* were recovered until 24 h at 2–4 °C and until 8 h post-inoculation at room temperature. Also *Porphyromonas gingivalis* was optimally recovered until 72 h from 2 to 4 °C stored recipients, while at room

Table 2
Applied conditions in the current recovery study.

Inoculum of the eSwab® (CFU/mL)	1.5×10^7	1.5×10^5	1.5×10^3			
Storage time in eSwab® container	0 h (ref. plate)	8 h	24 h	48 h	72 h	1 week
Storage temperature (°C)	2–4	20–24				

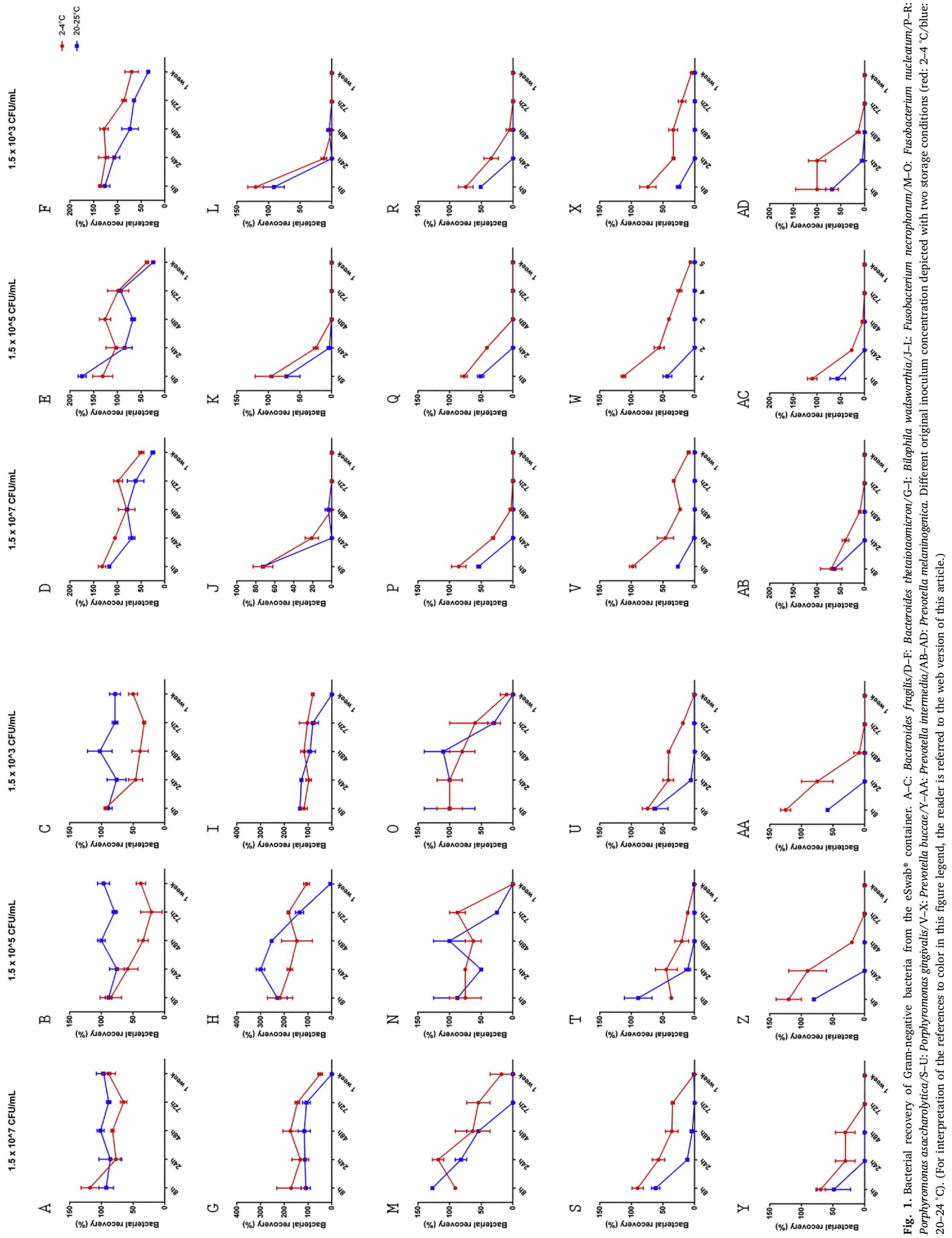

Fig. 1. Bacterial recovery of Gram-negative bacteria from the eSwab® container. A–C: *Bacteroides fragilis*/D–F: *Bacteroides thetaiotaomicron*/G–I: *Bilophila wadsworthia*/J–L: *Fusobacterium necrophorum*/M–O: *Fusobacterium nucleatum*/P–R: *Porphyromonas asaccharolytica*/S–U: *Porphyromonas gingivalis*/V–X: *Prevotella buccae*/Y–AA: *Prevotella intermedia*/AB–AD: *Prevotella melaninogenica*. Different original inoculum concentration depicted with two storage conditions (red: 2–4 °C/blue: 20–24 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

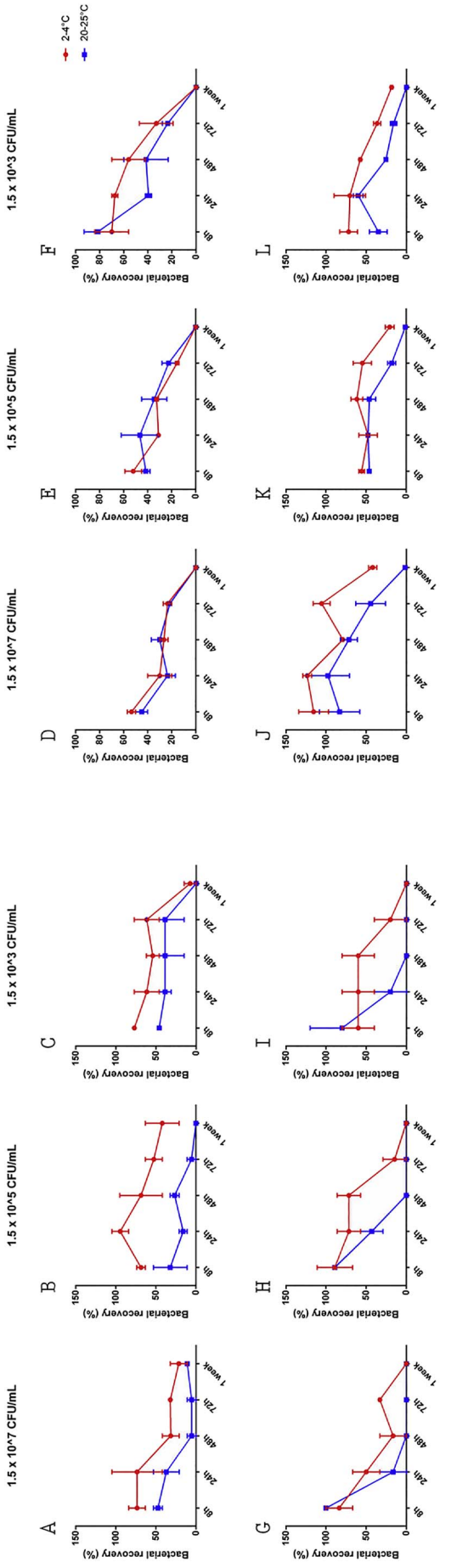

Fig. 2. Bacterial recovery of Gram-positive bacteria (non-spore forming) from the eSwab® container. A–C: *Finegoldia magna*/D–F: *Parvimonas micra*/G–I: *Peptostreptococcus anaerobius*/J–L: *Eggerthella lenta*/M–O: *Propionibacterium acnes*. Different original inoculum concentration depicted with two storage conditions (red: 2–4 °C/blue: 20–24 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

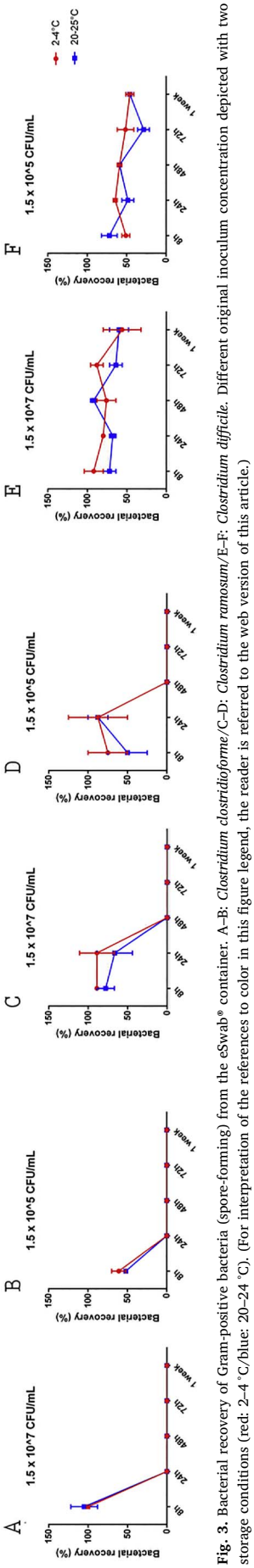

Fig. 3. Bacterial recovery of Gram-positive bacteria (spore-forming) from the eSwab® container. A–B: *Clostridium clostridioforme*/C–D: *Clostridium ramosum*/E–F: *Clostridium difficile*. Different original inoculum concentration depicted with two storage conditions (red: 2–4 °C/blue: 20–24 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temperature recovery was obtained until 24 h. Finally the 2–4 °C storage conditions also seemed optimal for recovery of *Prevotella* species. They were recovered only until 8 h at room temperature, while the viability was extended at 2–4 °C storage until 48 h (and even until 1 week for *Prevotella buccae*).

Similarly to the latter Gram-negative bacteria, the Gram-positive species included in this study were optimally recoverable from eSwab® containers stored at 2–4 °C (Fig. 2). *Finegoldia magna, Eggerthella lenta* and *Propionibacterium acnes* were retained from these recipients until 1 week of storage (until 72 h at room temperature), while *Peptostreptococcus anaerobius* was recovered until 72 h (until 24 h at room temperature). Finally *Parvimonas micra* was recovered until 72 h, independent of storage temperature. Recovery of *Clostridium* species does not seem to be affected by storage temperature (Fig. 3). On the other hand storage duration differentially affected survival of these species. As such *Clostridium clostridioforme* was only recovered until 8 h post-inoculation and *Clostridium ramosum* until 24 h. *Clostridium difficile* on the other hand was retainable until 1 week of storage.

4. Discussion

Suitable specimen transport and storage systems are essential in clinical laboratory diagnosis. In this study, we investigated the use of the Copan eSwab® container as a transport medium for clinical specimen containing anaerobic bacteria.

In the current experimental protocol, we chose to inject (pipet) the different bacterial inoculums in the eSwab® container to mimic aspiration sampling. Furthermore, this allowed us to precisely control the experimental conditions and evaluate the final theoretical number of CFU on the agar plates. As such, we were able to exactly quantify the recovery percentage of the different anaerobic species after the different storage durations (and at the different storage temperatures). Tyrrell et al. (2016) applied a different approach in their study, because they chose to inoculate the containers with accompanying swabs (Tyrrell et al., 2016). As such the clinical setting of wound or abscess swabbing was mimicked. This different approach allowed them to evaluate the survival of bacteria in the eSwab® container after swab inoculation. Although this approach is clinically relevant, the results cannot be interpreted straightforward or compared to our results, because of the additional factor of swabbing and subsequent bacterial release from the nylon flocked swab into the liquid Amies medium.

Even though different studies applied several experimental conditions (such as storage time), ideally materials for anaerobic testing should be subjected to culture within 3–6 h after prelevation (J. Infect. Chemother., 2011). Therefore the most relevant time point to evaluate bacterial recovery can be considered around 8 h post-inoculation. In daily clinical practice, most samples have reached the lab and are processed in that timespan. As such we show in our study that the eSwab® container fulfills the most important criteria, because all anaerobic species were recovered after 8 h of storage both at 2–4 °C and at room temperature. When specific analyses are needed or when samples are transported to an external laboratory for identification or susceptibility testing, recovery of the anaerobic bacteria from the eSwab® container could be desired after a longer storage duration.

To conclude we state that although the liquid Amies containers are not recognized as a specific transport medium for anaerobic culture samples in official guidelines, our data clearly support the idea that it is suitable for short-term (up to 8 h) storage of material containing anaerobic bacteria, at both room temperature and at 2–4 °C. Interestingly the inoculum concentration of bacteria in the eSwab® does not seem to affect the recovery rate from the sample. Because most samples in the clinical setting are processed in a time span of 2–4 h, the eSwab® container is sufficiently capable of retaining viability in daily routine. However because of inevitable centralization of clinical laboratories, adequate storage of these specimens for an extended period of time will be essential in the future. In these cases we strongly advise

to store the recipients at 2–4 °C for the remainder of the transportation time. As such all species (except for *Clostridium clostridioforme*) were recovered from the eSwab® medium until 24 h post-inoculation, applying inoculum levels as low as 1.5×10^3 CFU/mL. Furthermore 50% of the species included in this study were recovered until 1 week post-inoculation (i.e. *Bacteroides fragilis, Bacteroides thetaiotaomicron, Bilophila wadsworthia, Fusobacterium nucleatum, Prevotella buccae, Finegoldia magna, Eggerthella lenta, Propionibacterium acnes* and *Clostridium difficile*) and 75% until 2 days post-inoculation (additionally: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella melaninogenica, Peptostreptococcus anaerobius* and *Parvimonas micra*).

The results presented in this study do not fully represent clinical samples because pure bacterial suspensions were used to inoculate the eSwab® containers. When sample collection of infected sites is performed in hospitalized patients, mixtures of several (both aerobic and anaerobic) species are often obtained. Even though the application of different plates and incubation environments can selectively inhibit or stimulate the growth of certain species, careful assessment of the cultured specimen is always necessary. Yet, because of the introduction of MALDI-TOF MS, a reliable, fast and easy identification of a single colony is possible today.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Amies, C.R., 1967. A modified formula for the preparation of Stuart's Transport Medium. Can. J. Public Health 58, 296–300.
Brook, I., 2016. Spectrum and treatment of anaerobic infections. J. Infect. Chemother. 22, 1–13.
Citron, D.M., Warren, Y.A., Hudspeth, M.K., Goldstein, E.J., 2000. Survival of aerobic and anaerobic bacteria in purulent clinical specimens maintained in the Copan Venturi Transystem and Becton Dickinson Port-a-Cul transport systems. J. Clin. Microbiol. 38, 892–894.
Demuyser, T., 2017. Detailed data from the evaluation of anaerobic bacteria recovery from the Copan eSwab® transport system. J. Microbiol. Methods.
Finegold, S.M., 1995. Anaerobic infections in humans: an overview. Anaerobe 1, 3–9.
Fujimoto, D., Takegawa, H., Doi, A., Sakizono, K., Kotani, Y., Miki, K., Naito, T., Niki, M., Miyamoto, J., Tamai, K., Nagata, K., Nakagawa, A., Tachikawa, R., Otsuka, K., Katakami, N., Tomii, K., 2014. Comparison of two transport systems available in Japan (TERUMO kenkiporter II and BBL Port-A-Cul) for maintenance of aerobic and anaerobic bacteria. J. Infect. Chemother. 20, 26–29.
Hawkins, R., 2012. Managing the pre- and post-analytical phases of the total testing process. Ann. Lab. Med. 32, 5–16.
Hentges, D.J., 1996. In: Baron, S. (Ed.), Anaerobes: General Characteristics. Medical Microbiology, Galveston (TX).
Chapter 1–2. Anaerobic infections (general): testing anaerobic infections. J. Infect. Chemother. 17 (Suppl. 1), 13–25.
Mena, E., Thompson, F.S., Armfield, A.Y., Dowell Jr., V.R., Reinhardt, D.J., 1978. Evaluation of Port-A-Cul transport system for protection of anaerobic bacteria. J. Clin. Microbiol. 8, 28–35.
Peterson, L.R., 1997. Effect of media on transport and recovery of anaerobic bacteria. Clin. Infect. Dis. 25 (Suppl. 2), S134–136.
Schofield, C.B., 2006. Preventing errors in the microbiology lab. MLO Med. Lab. Obs. 38 (10), 12–14 (16–18; quiz 22-13).
Stoner, K.A., Rabe, L.K., Austin, M.N., Meyn, L.A., Hillier, S.L., 2008. Quantitative survival of aerobic and anaerobic microorganisms in Port-A-Cul and Copan transport systems. J. Clin. Microbiol. 46, 2739–2744.
Tyrrell, K.L., Citron, D.M., Leoncio, E.S., Goldstein, E.J., 2016. Comparison of the Copan eSwab system with an agar swab transport system for maintenance of fastidious anaerobic bacterium viability. J. Clin. Microbiol. 54, 1364–1367.
Van Horn, K.G., Audette, C.D., Tucker, K.A., Sebeck, D., 2008. Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria. Diagn. Microbiol. Infect. Dis. 62, 471–473.