REGIONE CAMPANIA
AZIENDA SANITARIA LOCALE NAPOLI 1 CENTRO
Via Comunale del Principe 13/a
80145 - NAPOLI
P. I.V.A. 06328131211

N. 438 di Repertorio del giorno 4 Luglio 2019

VERBALE III SEDUTA PUBBLICA DI GARA

Verbale di gara procedura aperta per la fornitura triennale di reagenti, accessori manuali, prodotti chimici e materiale vario di laboratorio (vetreria, plastica, ecc.) occorrente ai laboratori analisi, servizi di anatomia patologica, servizio di genetica e servizi trasfusionali della Asl Napoli 1 Centro.

L'anno 2019 il giorno 4 del mese di Luglio, alle ore 10,45 nella sede dell'UOC Acquisizione Beni e Servizi dell'ASL Napoli 1 Centro (Part. IVA – Cod. Fiscale 06328131211) – Via Comunale del Principe, 13\a, si è riunita la Commissione di gara per il prosieguo dei lavori afferenti la gara indicata in oggetto, che con delibera n. 636 del 14/06/2019 è stato nominato il seggio di gara così costituito:

Presidente	Dr. Alfredo di Lauro
Componente	Dr. Raffaele Postiglione
Segretario	Sig.ra Antonietta Meterangelis

In via preliminare si prende atto che sono presenti alla seduta odierna i rappresentanti delle Ditte i cui nominativi sono di seguito riportati:

Dr. Alfonso Calderini	Ditta: Alfamed s.r.l.
Dr. Alfonso Calderini	Ditta: Alfamed s.a.s.
Sig.ra Naomi Calderini	Ditta: Histoline s.r.l.
Dr. Pio Grimaldi	Ditta: D.I.D. s.p.a.
Dr. Giovanni Mauriello	Ditta: Kaltek s.r.l.
Sig.ra Giuseppina Favetti	Ditta: Laboindustria s.p.a.

Il Seggio di gara, riprende l'apertura della documentazione Amministrativa seguendo la cronologia con il quale sono stati acquisiti al sistema SIAPS della precedente seduta di gara:
Histo-Line Lab. S.r.l., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;
Alfamed S.r.l., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;
Biosigma s.r.l., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;

Alfamed s.a.s., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;
Carlo Erba Reagents s.r.l., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;
Aiesi Hospital s.a.s., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;
Kaltek s.r.l., la documentazione amministrativa è conforme a quanto richiesto dagli atti di gara, e pertanto la ditta viene ammessa alla fase successiva;

Il Rappresentante delegato della Ditta Diagnostic International Distribution S.p.a. Pio Grimaldi chiede di mettere agli atti una dichiarazione relativamente al lotto 11 per i Rif. 1,2,3,4 e 6 in merito a tamponi sterili floccati chiedendo che venissero escluse le Ditte Alifax, Biolife ed ogni altra che abbia partecipato ed offerto al Lotto in questione in quanto non floccati.
Il Seggio di gara allega tale dichiarazione al presente verbale, che ne forma parte integrante e sostanziale e, considerato che tale dichiarazione fa riferimento ad una valutazione tecnica, si ritiene non di competenza del seggio in quanto deputato alla sola valutazione della documentazione amministrativa.
Il Seggio di gara, approva gli atti relativi alla documentazione amministrativa e tenuto conto anche delle sedute precedenti, ammette tutte le Ditte che hanno presentato offerta alla fase successiva sul portale SIAPS nella apposita sezione.

Del che si è redatto il presente verbale letto e confermato, viene sottoscritto alle ore 14:37

Presidente Dr. Alfredo di Lauro
Componente Dr. Raffaele Postiglione
Segretario Sig.ra Antonietta Meterangelis

Per le ditte

Dr. Alfonso Calderini	Ditta: Alfamed s.r.l.	
Dr. Alfonso Calderini	Ditta: Alfamed s.a.s.	
Sig.ra Naomi Calderini	Ditta: Histoline s.r.l.	
Dr. Pio Grimaldi	Ditta: D.I.D. s.p.a.	
Dr. Giovanni Mauriello	Ditta: Kaltek s.r.l. (entra alle ore 12,30)	
Sig.ra Giuseppina Favetti	Ditta: Laboindustria s.p.a.	Abbandona la seduta alle ore 13,37

Procedura aperta per la fornitura triennale di reagenti e accessori manuali, prodotti chimici e materiale vario di laboratorio (vetreria,plastica etc.) occorrente ai laboratori analisi, servizi di anatomia patologica, servizio di genetica e servizi trasfusionali della ASL NA 1 Centro.

PREMESSO :

Che la Ditta Diagnostic International Distribution S.p.a. (D.I.D.), ha partecipato a questa procedura di gara offrendo al Lotto 11 Allegato "A" prodotti con marchio FLOQSwabs della COPAN Italia S.p.a. essendo di quest'ultima distributore autorizzato alla vendita e ai servizi ad essa connessi nel territorio italiano.

DICHIARA :

Che questa Spett.le ASL NA 1 Centro all'Allegato "A" Lotto 11 ai Rif. 1,2,3,4 e 6 richiede specificamente ed inequivocabilmente **"TAMPONI STERILI FLOCCATI".**

Che Copan Italia S.p.a. è titolare del **brevetto Europeo numero 1608268**, validato anche in Italia, avente ad oggetto la sonda floccata, commercializzata con il marchio FLOQSwabs. Tali dispositivi sono stati sviluppati e prodotti esclusivamente da COPAN.

CHIEDE :

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In fede,

Napoli, li 04-07-2019

IL DELEGATO
Pio Grimaldi

Allegati:

1) Delega a trattare
2) Dichiarazione COPAN s.p.a. Italia "Brevetto europeo FLOQSwabs
3) Studi comparativi

DIAGNOSTIC
INTERNATIONAL
DISTRIBUTION SPA

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della società ANORA S.r.l.

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Ns Offerta n. 27/2019
Pratica n. 4497/2019

SPETTABILE
Azienda Sanitaria Locale Napoli 1 Centro
VIALE COMUNALE DEL PRINCIPE 13/A
80145 NAPOLI
(Codice Cliente 002949)

Milano, 31-05-2019

Oggetto: PROCEDURA APERTA PER LA FORNITURA TRIENNALE DI REAGENTI, ACCESSORI MANUALI, PRODOTTI CHIMICI E MATERIALE VARIO DI LABORATORIO (VETRERIA, PLASTICA, ECC.) OCCORRENTE AI LABORATORI ANALISI, SERVIZI DI ANATOMIA PATOLOGICA, SERVIZIO DI GENETICA E SERVIZI TRASFUSIONALI DELLA ASL NAPOLI 1 CENTRO.

DELEGA A TRATTARE
(resa ai sensi del D.P.R n. 445 del 28-12-2000

in forma autenticata con allegato documento d'identità del sottoscrittore)

Il sottoscritto Dott. Enrico Maffioli nato a Milano il 20-05-1948 e residente in Piazza Carlo Amati, 6 - MILANO, in qualità di Presidente del Consiglio Amministrazione e Legale Rappresentante della Società D.I.D. Diagnostic International Distribution S.p.A., con sede commerciale e legale in Milano - P.zza C. Amati n. 6 - C.F. e P.IVA n. 00941660151,

DELEGA

il Dott. Pio Grimaldi nato a Roma il 18-06-1954 e residente in Corso Vittorio Emanuele 112 a Napoli, della Società Medline S.r.l. quale agenzia della sottoscritta Società per la regione della Campania affinché rappresenti con i più ampi poteri di contrattazione la Società D.I.D. Diagnostic International Distribution S.p.A. alla seduta pubblica che si terrà il giorno 12 giugno 2019 alle ore 10,30 presso gli uffici della Vostra sede.

A questa fase di gara ed eventualmente alle successive convocazioni, il Dott. Pio Grimaldi vi parteciperà con facoltà di svolgere ogni attività connessa al mandato, di definire i patti e condizioni degli stipulandi contratti, di migliorare eventuali offerte ove previsto e richiesto dalla Pubblica Amministrazione.

In fede

D.I.D. Diagnostic
International Distribution S.p.A.
Il Consigliere Delegato
Dr. Enrico Maffioli

Cognome MAFFIOLI
Nome ENRICO
nato il 20/05/1948
(atto n. 1077 1 S) R4
a MILANO ( )
Cittadinanza ITALIANA
Residenza MILANO
Via PIAZZA AMATI CARLO N. 6
Stato civile
Professione DIRIGENTE

CONNOTATI E CONTRASSEGNI SALIENTI

Statura 1,71
Capelli BRIZZOLATI
Occhi CASTANI
Segni particolari

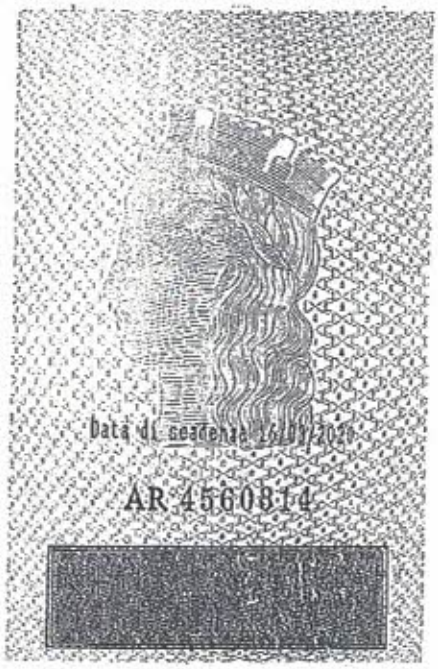
Firma del titolare

Milano 17/03/2010

IL SINDACO

Impronta del dito indice sinistro

Euro 5,42



Data di scadenza 16/07/2020

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COMVNE DI MILANO

CARTA D'IDENTITA

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MAFFIOLI

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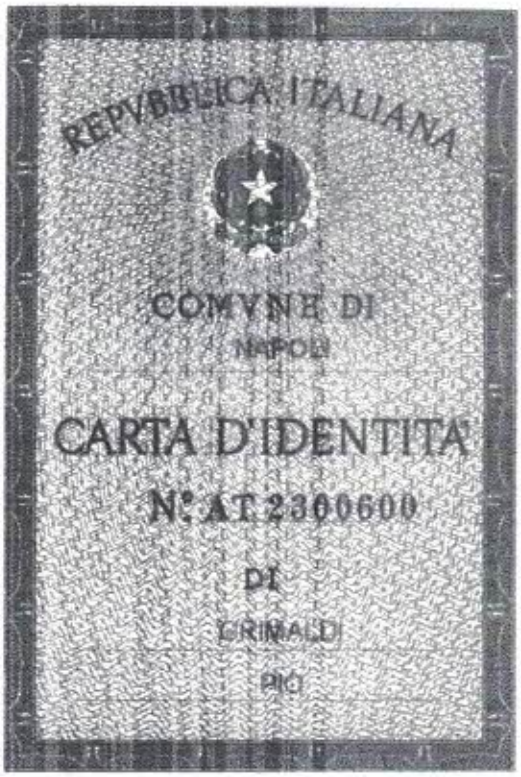
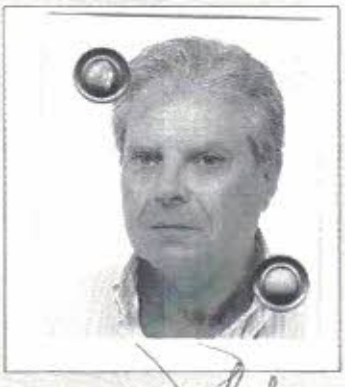


Cognome GRIMALDI
Nome PIO
nato il 18/06/1954
(atto n. 1909 P. S.)
a ROMA (RM) ( )
Cittadinanza ITALIANA
Residenza NAPOLI (NA)
Via CSO VITTORIO EMANUELE 112 Sc. A
Stato civile CONIUGATO
Professione OMESSO ART.35 DPR 30/5/89 N.223

CONNOTATI E CONTRASSEGNI SALIENTI

Statura 1,74
Capelli Brizzolati
Occhi Verdi
Segni particolari NESSUNO



Firma del titolare

NAPOLI (NA) li 08/08/2011

IL SINDACO

Impronta del dito indice sinistro

Demaio Giuseppe
Collaboratore Informatico

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Innovating Together™

Brescia, 21 settembre 2018

Con la presente, Copan Italia S.p.A. ("Copan"), con sede in via F. Perotti 10, 25125 Brescia, dichiara di essere il titolare, tra gli altri, del brevetto europeo numero 1608268, validato anche in Italia, avente ad oggetto la sonda floccata, commercializzata con il marchio FLOQSwabs®. Tali dispositivi sono stati sviluppati e prodotti esclusivamente da Copan.

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In fede,

Copan Italia S.p.A.

Stefania Triva
Legale Rappresentante

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P.IVA - ID VAT nr. IT 01740560170 Capitale Sociale: € 6.000.000 I.V.

Comparison Between the Flocked ESwab and the Foam ∑- Transwab® for the Maintenance of Fastidious Aerobic Bacteria and Candida species

Abed El-Razeq Issa, Musa Y Hindiyeh and Hiyam Marzouqa
Caritas Baby Hospital, Bethlehem, PALESTINIAN TERRITORY, OCCUPIED

Abstract

Background: It is well established that aspirates of fluids are superior to specimens collected on swabs. However, because of the ease of using swabs the clinical microbiology laboratories continue to receive patient samples on swabs. Recently, two modified Liquid Amies based swab collection devices were manufactured to enhance specimen collection and release. The ESwabs (Copan Diagnostics) are nylon flocked transport system and the ∑-Transwab® (Medical Wire) are soft polyurethane absorbent foam. **Methods:** The CLSI M40-A quantitative swab elution method was followed to compare the survival of fastidious aerobic bacteria and Candida species in these two systems at room temperature (RT) and at 4°C. The survival of the following ATCC organisms was evaluated: *S. pneumoniae* (6305), *H. influenzae* (10211), *N. gonorrhoeae* (43609), *N. meningitidis* (13090), *S. pyogenes* (19615), *C. albicans* (14053) and *C. parapsilosis* (22019). Briefly, 1:10 dilution of freshly prepared 0.5 McFarland standard of each organism was prepared and 100µl aliquots were placed in sterile eppendorf tube to inoculate the swabs. The survival of the organisms was determined at 0, 6, 24, and 48 hrs on 5% SBA or Chocolate agar after incubation at 35 °C. To meet the CLSI criteria, no more than 3 log10 decline in CFU count should be observed. **Results:** Mechanically there was a clear advantage for the ESwab since it smoothly absorbed the seeding inoculums, while the ∑-Transwab® [illegible] averaging 50µl. Both ESwabs and ∑-Transwab® met the CLSI criteria for the maintenance of the viability of *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* for 48 hours at RT and at 4°C. Only the ESwab maintained the viability of *N. gonorrhoeae* and *N. meningitidis* for 24 and 48 hrs, respectively at RT and 4°C. The ∑-Transwab® maintained the viability of both *Neisseria* species only at 4°C. Both swabs maintained the viability of *C. albicans* and *C. parapsilosis* at RT and 4°C for 48 hrs. **Conclusion:** The liquid-based multipurpose collection and transport systems allow running multiple tests on the same sample. The ESwab appears to be superior to the polyurethane foam for specimen collection. In addition, ESwab met the CLSI acceptance criteria for all the organisms tested, while the ∑-Transwab® failed to maintain the viability of both *Neisseria* species tested at RT.

Introduction

Diagnosis of bacterial infection in patient samples largely depends on specimen collection and transport to the laboratory. Failure to collect an appropriate patient sample can lead to patient misdiagnosis and inappropriate treatment. It is well established that [illegible] and aspirates of fluids are better than samples collected on swabs (1, 2). However, because of the expediency of using swabs, clinical microbiology laboratories continue to receive clinical samples on swabs. [illegible] have been extensively utilized in the preparation of swab tips. These fibers undergo extensive processing in order to allow them to effectively collect and release patient sample and to maintain the viability of the microorganisms. The swabs in combination with Amies [illegible] have been extensively evaluated. Recently, Copan Diagnostics introduced the ESwab, a new flocked swab with modified liquid Amies (1ml) transport system designed to optimize specimen collection and to [illegible] specimen [illegible]. ESwab utilizes [illegible] nylon flocked fiber technology (3, 4). The perpendicular nylon fibers act like a soft brush to allow the improved collection and release of patient sample. In this study, the CLSI M40-A quantitative swab elution method was followed to compare the survival of fastidious aerobic bacteria and Candida species in the two modified Liquid Amies based swab collection devices, the ESwab (Copan Diagnostics) which are nylon flocked transport system and the ∑-Transwab® (Medical Wire) which are soft polyurethane absorbent foam at room temperature (RT) and at 4°C.

Materials & Methods

Bacterial Stains:
S. pneumoniae (ATCC 6305), *H. influenzae* (ATCC 10211), *N. gonorrhoeae* (ATCC 43609), *N. meningitidis* (ATCC 13090), *S. pyogenes* (ATCC 19615), *C. albicans* (ATCC 14053) and *C. parapsilosis* (ATCC 22019).

Transport Swab Systems:
ESwabs (Copan Diagnostics): nylon flocked transport system.
∑-Transwab® (Medical Wire & Equipment): soft polyurethane absorbent foam.

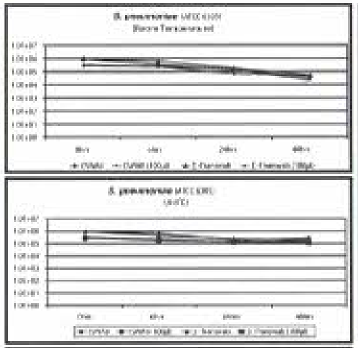
Agar Plates: 5% sheep blood agar plates (Cat# [illegible]) and Chocolate Agar (Cat# [illegible]) were purchased from Hy Labs (Park Tamar, Rehovot, Israel). Bacterial strain cultures: All bacterial strains were cultured on the appropriate media (5% Sheep blood Agar or Chocolate Agar) for 18-24 hours at 37°C. Well isolated colonies were used for sample preparation. Some fastidious organisms required 48 hours incubation before sample preparation.

Experimental Design: The CLSI document M40-A was followed to evaluate the different transport swabs. [illegible]

Data Analysis: Bacterial recovery was determined by counting the colonies recovered in each of the dilutions. [illegible]

Results

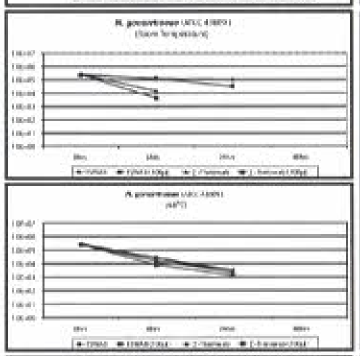
Figure 1: Recovery of bacteria isolates after 0, 6, 24 and 48 hours incubation at Room Temperature and 4-8 °C



***S. pneumoniae* ATCC 6305**

ESWAB
∑- Transwab

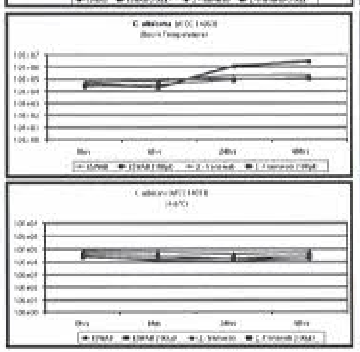
Passed Criteria @ RT & 4-8 °C



***N. gonorrhoeae* ATCC 43609**

ESWAB
Passed Criteria RT & 4-8 °C

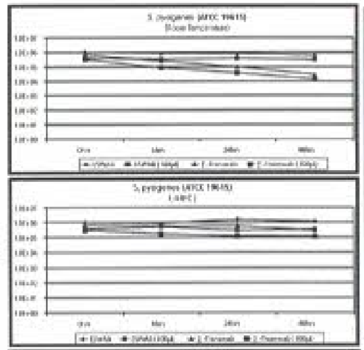
∑- Transwab
Failed Criteria @ RT
Passed @ 4-8 °C



***C. albicans* ATCC 14053**

ESWAB
∑- Transwab

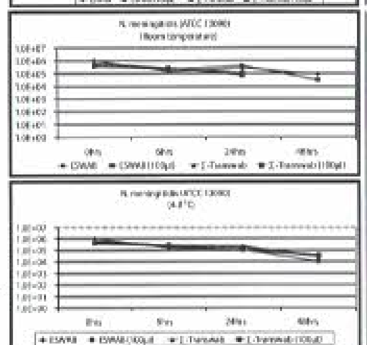
Passed Criteria @ RT & 4-8 °C



***S. pyogenes* ATCC 19615**

ESWAB
∑- Transwab

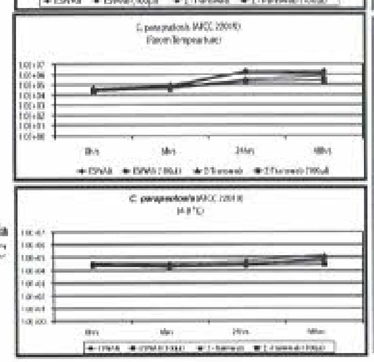
Passed Criteria @ RT & 4-8 °C



***N. meningitidis* ATCC 13090**

ESWAB
Passed Criteria RT & 4-8°C

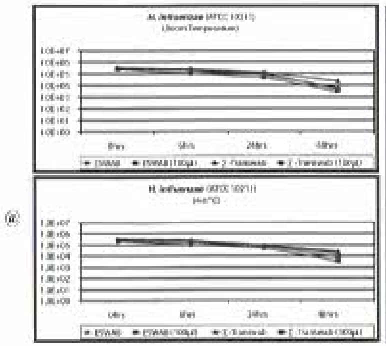
∑- Transwab
Failed Criteria @ RT
Passed @ 4-8°C



***C. parapsolosis* ATCC 22019**

ESWAB
∑- Transwab

Passed Criteria @ RT & 4-8°C



***H. influenzae* ATCC 10211**

ESWAB
∑- Transwab

Passed Criteria @ RT & 4-8 °C

Conclusions

- ESwabs smoothly absorbed the 100µl inoculums.
- ∑-Transwab absorption was less efficient averaging 50µl.
- Both liquid Amies based swabs fulfilled the CLSI guidelines at 4°C for all organisms tested.
- Only the ESwabs fulfilled the CLSI guidelines for all organisms tested at Room Temperature.
- ∑-Transwab failed to maintain both Neisseria species tested post 6 hours at Room Temperature.
- Both systems maintained the viability of both Candida species tested.
- Similar results were obtained after using the swabs or 100µl aliquot to inoculate the plates.
- Overall, the ESwabs outperformed the ∑-Transwab for the maintenance of fastidious aerobic bacteria.

Reference

1. Rishmawi N, Ghneim R, Kattan R, Ghneim R, Zoughbi M, Abu-Diab A, Turkuman S, Dauodi R, Shomali I, Issa Ael-R, Siriani I, Marzouka H, Schmid I, Hindiyeh MY. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. J Clin Microbiol. 2007 Apr;45(4):1278-83.
2. Hindiyeh M, Acevedo V, Carroll KC. Comparison of three transport systems (Starplex StarSwab II, the new Copan Vi-Pak Amies Agar Gel collection and transport swabs, and BBL Port-A-Cul) for maintenance of anaerobic and fastidious aerobic organisms. J Clin Microbiol. 2001 Jan;39(1):377-80.
3. Nys S, Vijgen S, Magerman K, Cartuyvels R. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of Escherichia coli, Streptococcus agalactiae and Candida albicans. Eur J Clin Microbiol Infect Dis. 2010 Apr;29(4):453-6.
4. Van Horn KG, Audette CD, Sebeck D, Tucker KA. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. J Clin Microbiol. 2008 May;46(5):1655-8.

C-2153

Evaluation of Fastidious Bacterial Survival in Two Swab Transport Systems

Suzane Silbert, Carly Romero, Michael Meo, Ray Widen • Esoteric Testing Lab, Tampa General Hospital • Tampa, FL

ABSTRACT:

Swabs are frequently used to collect and transport specimens to clinical laboratory. However they are often considered to be a less desirable specimen collection device. In the last years new swab systems were designed to improve the absorbance of the sample and at the same time, optimize release of microorganisms into the liquid transport medium, ensuring optimum sensitivity for subsequent test procedures. The aim of this study was to evaluate two of these new swab systems to transport and maintain fastidious bacteria: ESwab Transport System (Copan, USA) and Sigma Transwab System (MW&E, UK), both with liquid Amies medium. Methods: The following isolates were evaluated for survival after incubation at room (25°C) and refrigerator (4°C) temperatures: *Streptococcus pyogenes*: ATCC 19615, *N. gonorrhoeae*: ATCC 43069, *H. influenza*: ATCC 10211, *S. pneumonia*: ATCC 6305, *P. anaerobius*: ATCC 27337 and *F. nucleatum*: ATCC 25586. A vortex elution method (CLSI M40-A) was performed using a 0.5 McFarland suspension of each strain and a 1:10 dilution was prepared. Swabs were inoculated in triplicate with 100µL of each microorganism suspension and held at 25°C and 4°C for 0h, 24h and 48h. Bacterial survival was evaluated after 48h incubation at 35°C. Results: Cultures from all swabs dilutions were averaged. Bacterial recovery from swabs held for 0h was similar for both systems. However, after 24h and 48h incubation, bacterial recovery rates from Sigma Swab were lower compared to the ones from the ESwab. Both swab systems maintain bacterial viability up to 48h when stored at 4°C, and for both species of *Streptococcus* when stored at 25°C. None of the swabs maintained viability for *N. gonorrhoeae*, *P. anaerobius* and *F. nucleatum* after 48h at 25°C and only Copan ESwab was able to maintain viability of these species for 24h at 25°C. *H. influenzae* survived for up to 48h at 25°C in the ESwab and up to 24h at 25°C in the Sigma Swab. Conclusion: Bacteria tested showed greater survival in the Copan ESwab system compared to Sigma Swab. In summary, it was possible to conclude that Copan ESwab performed better than Sigma Swab for transport and storage of fastidious bacteria at room temperature.

INTRODUCTION:

In the last years new swab systems were designed to improve the absorbance of the sample and at the same time, optimize release of microorganisms into the liquid transport medium, ensuring optimum sensitivity for subsequent test procedures. Two of these new swab systems are: ESwab Transport System (Copan, USA) and Sigma Transwab System (MW&E, UK), both with liquid Amies medium.

The ESwab (Elution Swab) Transport System is a new liquid based transport system for bacteriology swab samples. Unlike traditional fiber wound swabs Copan's nylon flocked swabs provide superior sample absorption and release characteristics. The entire patient's sample is instantly eluted on contact with the ESwab transport medium; there is no need for the operator to rim, ring or vortex the swab.

Sigma Transwab System (Σ-Swab®) is a new specimen collection device that incorporates a screw cap tube and Liquid Transport Medium. The specimen is collected using Σ-Swab®, an open celled, polyurethane foam-tipped swab which allows complete flow through of reagents and microorganisms. After the specimen is placed into the tube of liquid medium, the microorganisms in the specimen are dispersed through the medium, producing a uniform suspension ready for use.

OBJECTIVES:

➢ **To evaluate 2 new swab systems to transport and maintain fastidious bacteria:**

ESwab Transport System (Copan, USA)

X

Σ-Swab® (Sigma) Transwab System (MW&E, UK)

METHODS:

➢ **Bacteria tested:**

- *Neisseria gonorrhoeae* ATCC 43069
- *Haemophilus influenzae* ATCC 10211
- *Streptococcus pyogenes* ATCC 19615
- *Streptococcus pneumoniae* ATCC 6305
- *Peptostreptococcus anaerobius* ATCC 27337
- *Fusobacterium nucleatum* ATCC 25586

➢ **Swab Transport Systems Brands Tested:**

- ESwab Transport System (Copan, USA)
- Sigma Transwab System (MW&E, UK)
  (Both with Liquid Amies Medium)

➢ **Temperatures:**

- RT (25°C)
- Refrigerated (4°C)

➢ **Protocols tested (M40-A, CLSI):**

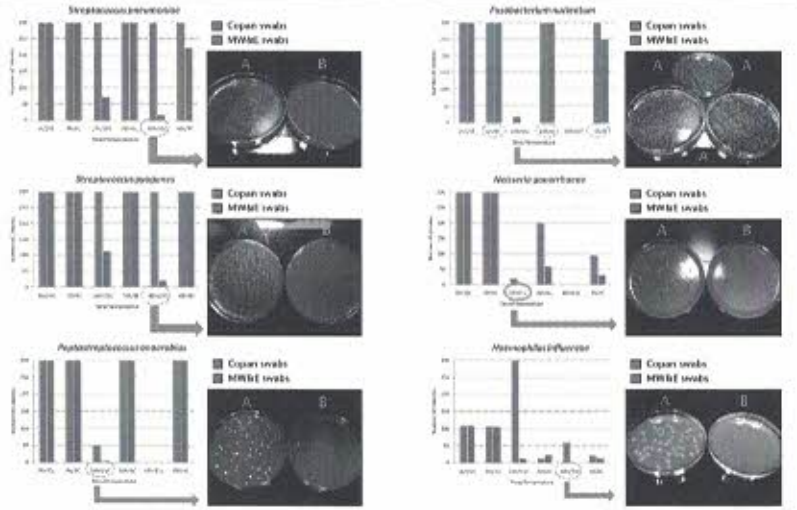
- Vortex Elution Method

➢ **Periods of time:**

- 0h, 24h, 48h

➢ **All the tests were done in triplicate**

RESULTS:



VORTEX ELUTION METHOD:

- A 0,5 McFarland suspension in saline (85%) was prepared from an 18-24hr culture of each organism. The 0,5 suspension was diluted (1:10) in saline;
- 100µl of each organism suspension was transferred into wells of a microtiter plate using a volumetric pipette;
- Tests were performed in triplicate for each swab brand, lots and time points (0, 24 and 48 hours);
- Each swab was rolled into the 100µl suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time at room temperature (25°C);
- The first swab (time point zero hour) were cultivated from within 15 minutes;
- After hold in appropriate time, all the swabs were placed in a tube with 1mL of sterile saline and vortex for 15 seconds. Serial dilutions were prepared in saline (10-1 and 10-2);
- After vortexing with tube, a 100µl of each suspension was plated in agar media specific for each species tested. Cultures were performed in duplicate and were incubated at 35°C for 48hs;
- Counts were then performed. Average counts for 24 and 48 hs were compared to the zero hour counts for the same dilution and organism.

CONCLUSIONS:

Bacterial recovery from swabs held for 0h was similar for both systems. However, after 24h and 48h incubation, bacterial recovery rates from Sigma Swab were lower compared to the ones from the ESwab.

Both swab systems maintain bacterial viability up to 48h when stored at 4°C, and for both species of *Streptococcus* when stored at 25°C. However, after 24h and 48h incubation, bacterial recovery rates from Sigma Swab were lower compared to the ones from the ESwab.

Bacteria tested showed greater survival in the Copan ESwab system compared to Sigma Swab.

In summary, it was possible to conclude that Copan ESwab performed better than Sigma Swab for transport and storage fastidious bacteria at room temperature.

C-2143

Evaluation of Two New Liquid Based Transport Swabs for Their Ability to Maintain Fastidious Bacteria

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ABSTRACT:

Microbiology work practices are undergoing significant change creating a demand for swab transport systems that have a broader multipurpose capability beyond just traditional culture. We evaluated two new liquid based transport systems ESwab (ES; Copan, Murrieta, California) incorporating a flocked swab and Sigma Swab (SS; Medical Wire & Equipment, UK) a foam swab for their ability to maintain the viability of a selection of important aerobes and anaerobes prior to adopting a system for wide scale use. Viability tests were performed using a modified *Roll-Plate Method* (CLSI M40-A) on swabs held at room temperature (RT, 20 - 25°C) and refrigerated temperature (RF) for 0, 24 & 48h time points. Test strains included *N. gonorrhoeae* (NG) ATCC 43069, *H. influenzae* (HI) ATCC 10211, *S. pyogenes* (SP) ATCC 19615 and *P. anaerobius* (PA) ATCC 27337, *P. melaninogenica* (PM) ATCC 25845. Swabs from each device were inoculated with 100µl of dilutions made from 0.5 McFarland suspension of each organism with the target of producing 30 – 300 CFUs on time zero culture plates. Swabs were inoculated in triplicate. Colony counts were compiled for each dilution & incubation time point and compared to the counts to determine percent recovery. ES maintained viability of 4/5 strains after 24h at RT and 3/5 strains at RF with higher percent recovery than SS in all cases. At 24h at RT NG recovery was 14% with ES, 0%: SS; HI 56% ES, 10% SS; SP 100% ES, 36% SS; PA 60% ES, 28% SS. At RF NG recovery was 29% with ES, 9% SS; HI 92% ES, 75% SS; SP 82% ES, 53% SS; PA 81% ES, 51% SS; PM 67% ES, 0% SS. No NG test was performed at 48h but of the remaining organisms ES recovered 4/5 at RT and 4/5 at RF whereas SS 3/5 and 3/5 respectively. ES is able to maintain viability of selected aerobes and anaerobes for at least 24h at RT and demonstrated a greater percent recovery compared to SS. The flocked applicator with ES provided a more reliable absorption and transfer device than SS foam applicator. The SS applicator had a tendency to gouge the agar and occasionally fell out of the cap onto the agar plate.

INTRODUCTION

Amies Gel and Liquid Stuart medium combined with fiber wrapped swabs have be used in routine clinical microbiology for decades. Changing trends in specimen testing and workflow challenges in microbiology have created a demand for a new type of swab that will allow a multiplicity of tests to be performed on one swab sample and enable automated specimen processing. Whereas fiber wrapped swabs limit the scope of testing and precluded any possibility of automatic processing, a new generation of transport swabs now on the market address both of these needs. We looked at two manufacturers offering swab transports that elute their entire swab sample into liquid phase thereby providing a homogenous suspension of liquid sample; Copan ESwab comprising 1ml of Liquid Amies combined with a patented Flocked Swab (Copan Diagnostics, Murrieta, CA) and Medical Wire Sigma Swab comprising 1ml of Liquid Amies and a Polyurethane Foam Swab (MW&E, Corsham, UK). We compared the ability of both manufacturers to maintain the viability of specific aerobes and anaerobes at room temperature and refrigerator temperature for up to 24 and 48hrs.

METHODS

We based our testing method on the CLSI M40-A Roll-Plate methodology. Serial ten-fold dilutions of test organisms described in the CLSI standard are designed to achieve a dilution that will result in countable colonies from each swab device when plated onto culture plates at zero time. The goal is to charge swabs with quantitative amount of colony forming units so a viable and countable change in viability can be measured over the holding time. Having performed numerous swab studies in the past we have found that it is difficult to apply general McFarland opacity rules to organisms of entirely different colonial characteristics and cell size and expect to achieve approximating 300 CFUs consistently. Oftentimes CFU counts are too low or too high. From experience we have developed one-step dilutions from primary McFarland suspension that can reliably produce CFU growth which is easily countable by eye. This also minimizes manipulations, dilution errors, exposure to oxygen and shortens the overall processing time.

All test strains are prepared from new lyophilized cultures of ATCC control bacterium according to the instructions provide by the supplier. All cultures are passed one time using appropriate non-selective medium from which primary 0.5 McFarland suspensions are prepared.

ONE-STEP DILUTIONS

From the primary 0.5 McFarland organism suspension the test suspensions used for seeding swabs are prepared by performing the following dilutions:

Strain	ATCC	One-Step Dilution of 0.5 McFarland*
Streptococcus pyogenes (SP)	19615	1:2000
Haemophilus influenza (HI)	10211	1:30000
Neisseria gonorrhoeae (NG)	43069	1:4000
Prevotella melaninogenica (PM)	25845	1:1000
Peptostreptococcus anaerobius (PA)	27337	1:500

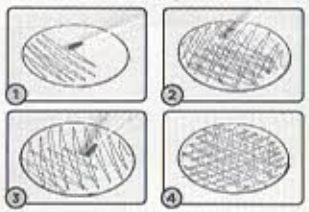
*100µl volumes of one-step dilutions used to seed each swab

To expedite the process of inoculating multiple ESwabs and Sigma Swabs 100µl aliquots of each organism test suspension are plated into sterile wells of a microtiter plate. Each test swab is then inserted into one well until it touches the bottom where it is allowed to absorb the inoculum for a minimum of ten seconds (see Fig 1). Swabs are rotated to assist the uptake of the organism suspension. Seeded swabs are removed from the incubator tray well then broken off into their respective transport medium tubes as directed by the manufacturer's instructions. Swabs are seeded and tested in triplicate for each organism and time point as indicated in the summary table below:

Test Organisms	Holding Temp	Time 0	Time 24 h	Time 48 h
SP, HI, PM and PA	20 - 25°C (RT)	3 swabs	3 swabs	3 swabs
	4 - 8°C (RF)		3 swabs	3 swabs
NG	20 - 25°C (RT)	3 swabs	3 swabs	
	4 - 8°C (RF)		3 swabs	

PLANTING SWABS:

1. Hold swabs in transport tubes for 5-15 minutes before initial (zero-time) plating.
2. Vortex the transport tubes with the swab inside for 10 – 15 seconds.
3. Remove the swab from the transport device and inoculate swab to the dried surface of appropriate culture media by streaking the swab over the entire sterile agar surface, rotating the swab between thumb and index fingers to ensure that all surfaces of the swab equally contact the surface of the culture media. Repeat by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum, as outlined in NCCLS document M2— Performance Standards for Antimicrobial Disk Susceptibility Tests. Avoid the rim of the agar to allow countable CFU (Fig 2).



4. Place in incubator at 35-37 °C in appropriate atmosphere of incubation within 15 minutes.
5. Perform the same plating procedure for seeded swabs stored for 24 and 48 hour time points.

RESULTS:

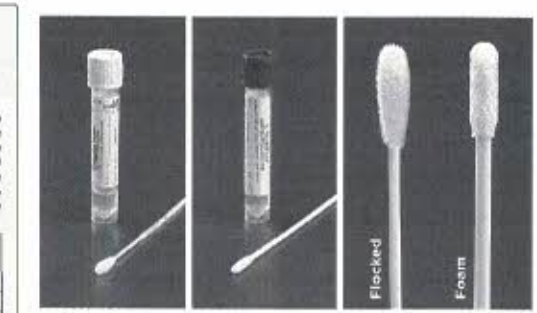
After 24hrs incubation of culture plates (48hrs for anaerobes) visible countable colonies for each swab cultured at each time point were recorded. An average CFU count was determined from each triplicate set of swab cultures and each holding time point. Product performances were compared for each swab transport with the zero time counts for each specific device by recording the base line zero time CFU counts with CFU counts at subsequent time points. Decline in viability for each organism in each device was expressed in terms of percent recovery compared to zero time. In accordance with CLSI M40-A criteria, for organism viability to be acceptable, average CFU counts must be ≥5 CFUs following the seeded holding time.

Organism	Product	Incubation	0 Time	0 Time Av. CFUs	24 hrs	48 hrs
Streptococcus pyogenes	Sigma	RT	100%	188	36%	32%
	ESwab	RT	100%	325	100%	100%
	Sigma	RF	100%	188	53%	57%
	ESwab	RF	100%	325	82%	66%
Haemophilus influenzae	Sigma	RT	100%	40	10%	0%
	ESwab	RT	100%	165	56%	18%
	Sigma	RF	100%	40	75%	68%
	ESwab	RF	100%	165	92%	88%
Neisseria gonorrhoeae	Sigma	RT	100%	66	0%	
	ESwab	RT	100%	149	14%	
	Sigma	RF	100%	88	9%	
	ESwab	RF	100%	149	29%	
Prevotella melaninogenica	Sigma	RT	100%	20	0%	0%
	ESwab	RT	100%	102	0%	0%
	Sigma	RF	100%	82	3%	0%
	ESwab	RF	100%	102	67%	24%
Peptostrep. anaerobius	Sigma	RT	100%	107	28%	0%
	ESwab	RT	100%	442	60%	12%
	Sigma	RF	100%	107	51%	28%
	ESwab	RF	100%	442	81%	76%

Percent recovery of bacteria at all time points was significantly greater with ESwab in comparison with Sigma Swab. ESwab maintained 5/5 bacteria at refrigerated temperature in accordance with the CLSI M40-A performance standard whereas Sigma Swab supported 4/5. At room temperature, ESwab supported 4/5 and Sigma Swab supported 1/5 organisms.

Some observations we noted during this comparative study:

1. When attempting to seed the Foam swab provided with Sigma Swab the tip appeared to be hydrophobic and despite rolling and squeezing attempts would not draw up the organism suspension easily. Conversely the Flocked Swab rapidly absorbed the 100ul organism suspension (Fig 3).



2. We noted that the number of CFUs at time zero with Sigma Swab was consistently lower than Copan ESwab and may reflect the poor ability to absorb sample.
3. The Foam swab had a tendency to gouge the agar during plating. The seam line on the Foam tip may have contributed to this.
4. Both Copan and MW&E transport tubes have a cap intended to capture the broken swab shaft so the operator can use the screw cap like a handle for plating and manipulating the swab. The Sigma Foam Swab often fell out of the cap.

CONCLUSIONS

Liquid based swab transport systems that elute their sample entirely into transport medium offer a convenient solution for automation and expanded testing capability. From our study the Copan format utilizing a flocked Swab appears to be the most efficient in terms of mechanics (sample absorption and release) and the ability to maintain viability of bacteria.

Fig 1*

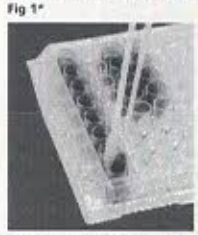
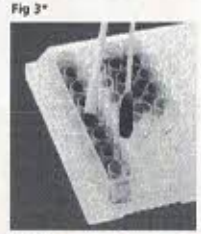


Fig 3*



*Dye used to represent presence of bacterial suspension

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Liquid based microbiological transport systems: Conformity assessment of two commercial devices

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ABSTRACT

We compared two types of liquid-based microbiology devices for microorganism viability according to standardized quantitative elution method CLSI M40-A2. The eSwab® met CLSI acceptance criteria of viability maintenance for all microorganisms tested. The Σ-Transwab® failed to meet CLSI acceptance criteria for *Peptostreptococcus anaerobius*, *Prevotella melaninogenica*, *Fusobacterium nucleatum* and *Haemophilus influenzae*.

Transport systems and devices are essential components of the process of microbiology laboratory testing. It is recognized that the early steps in the total testing processing are critical to the production of clinically relevant information. A variety of microbiological transport systems and devices exist. It is imperative that users systematically evaluate systems for performance effectiveness, ensure standards of performances, and to allow for internal validation of product effectiveness, thus selecting the best for the needs of the physician and the patient (CLSI M40-A2, 2014). There are multiple variables involved in the manufacture of a transport device, as transport medium, collection device, packaging and environment. It is fundamental that the assessment of the device be based on measurable performance characteristics for the device (CLSI M40-A2, 2014).

Recent studies have established that simulated transport performance at cold temperature yields superior results compared to that at room temperature. These data support the current CLSI recommendation that room temperature transport does not represent the optimal holding temperature for maximum preservation of microbiological samples (Nys et al., 2010; Van Horn et al., 2008a,b; Buchan et al., 2014; Stoner et at., 2008; Arbique et al., 2000). The CLSI document M40-A2 (2014) also recommends that if the conditions of the end user differ from those indicated, the actual transport condition should be tested in order to evaluate viability of microorganisms.

Recently, two modified Liquid Amies based swab collection devices were manufactured to enhance specimen collection and release: the eSwabs® (Copan Italia SpA, Brescia, Italy) incorporates a nylon flocked transport system and the Σ-Transwabs® (Medical Wire & Equipment, UK) includes a soft polyurethane foam bud.

The aim of this study was to compare the eSwab and the Σ-Transwab liquid-based microbiology (LBM™) devices for recovery and viability at controlled-room temperature (RT) of the following ATCC organisms: *Haemophilus influenzae* (10211), *Neisseria gonorrhoeae* (43069), *Streptococcus pneumoniae* (6305), *Streptococcus pyogenes* (19615), *Bacteroides fragilis* (25285), *Fusobacterium nucleatum* (25586), *Prevotella melaninogenica* (25845), *Peptostreptococcus anaerobius* (27337), and *Propionibacterium acnes* (6919). The ATCC microorganisms listed are the minimum that must be included in a test battery to evaluate a transport device (CLSI M40-A2, 2014).

The CLSI document M40-A2 (2014) was followed to evaluate the two transport swabs. Briefly, a 0.5 McFarland (about 1.5×10^8 CFU/ml) standard of each organism freshly grown at 37 °C for 18–24 h was prepared in 0.85% saline. For the flocked fiber swabs with an uptake volume of 100 μl (eSwab), the suspension was further diluted 1:10 to achieve a concentration of about 1.5×10^7 CFU/ml and the inoculum was 100 μl. For the foam swab device with an uptake volume of 50 μl (Σ-Transwab), the suspension of 0.5 McFarland was further diluted 1:5 and the inoculum was 50 μl, according to CLSI guidelines. Triplicate swabs were inoculated into their respective transport system and stored at designed controlled-room temperature 20–25 °C for 0, 24 and 48 h. After the appropriate storage time, including 0 h (tested within 15 min from inoculation) serial 1:10 dilutions of each swab system were prepared to obtain suspensions equivalent to about 10^6–10 CFU/ml. In duplicate, 100 μl samples were used to quantify the organisms in each of the dilutions on TSA with 5% sheep blood agar for aerobic and anaerobic and Chocolate agar with Vitox for fastidious organisms respectively. The organisms were spread over the agar surface with a plate spreader, and the plates were

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incubated at 37 °C in 5% CO_2 incubator, or in anaerobic atmosphere. To meet the CLSI M40-A2 criteria, no more than 3 log_{10} decline in CFU count should be observed. The initial inoculum (about 1.5×10^7 CFU/ml) was verified by serial 1:10 dilutions plated on duplicate in appropriate medium and incubated at 37 °C in appropriate atmosphere. Colony counts were obtained to confirm that the inoculum size was acceptable (about 1.5×10^7–1.5×10^9 CFU/ml) (CLSI M40-A2, 2014).

Bacterial recovery was determined by counting the colonies recovered from each dilution. The number of the organisms recovered is

Table 1
Organism recovery (log change of CFU counts) from Copan eSwab and Σ-Transwab over different incubation time (T = 0, 24, 48 h) at controlled room-temperature storage (acceptability CLSI criteria: ΔLOG no more than 3 log_{10}).

	Copan eSwab					Medical Wire I-Transwab				
	T = 0	T = 24	Δ(log) T24	T = 48	Δ(log) T48	T = 0	T = 24	Δ(log) T24	T = 48	Δ(log)T48
B. fragilis ATCC 25285	1.48E+06	2.32E+06	0,20	1,05 E+06	−0,15	8,03 E+05	4,68E+05	−0,23	7,82E+04	−1,01
F. nucleatum ATCC 25586	1,03E+06	1,20 E+06	0,07	4,32 E+05	−0,38	5,02 E+05	1,14E+04	−1,65	0,00E+00	Fail
P. acnes ATCC 6919	2,23E+06	1,93 E+06	−0,06	2,08E+06	−0,03	1,30 E+06	9,00E+05	−0,16	5,00E+05	−0,41
P. anaerobius ATCC 27377	1,71E+06	3,23 E+06	0,28	1,93 E+06	0,05	7,93E+05	3,30E+00	−5,38	0,00E+00	Fail
P. melaninogenica ATCC 25845	1,59E+06	1,02 E+05	−1,19	3,00E+03	−2,72	1,00E+05	0,00E+00	Fail	0,00E+00	Fail
H. influenzae ATCC 10211	1,22E+06	1,98 E+06	0,21	1,69 E+06	0,14	4,33E+05	3,40E+03	−2,11	7,67E+01	−3,75
S. pyogenes ATCC 19615	1,77E+06	2,72 E+06	0,19	2,81 E+06	0,20	4,40 E+05	2,62E+03	−2,23	2,08E+03	−2,33
S. pneumoniae ATCC 6305	2,48E+05	1,26 E+06	0,71	8,07 E+05	0,51	2,05E+05	5,27E+04	−0,59	1,37E+04	−1,18
N. gonorrhoeae ATCC 43069	4,60E+05	5,72E+04	−0,91			2,95E+05	8,50E+02	−2,54		

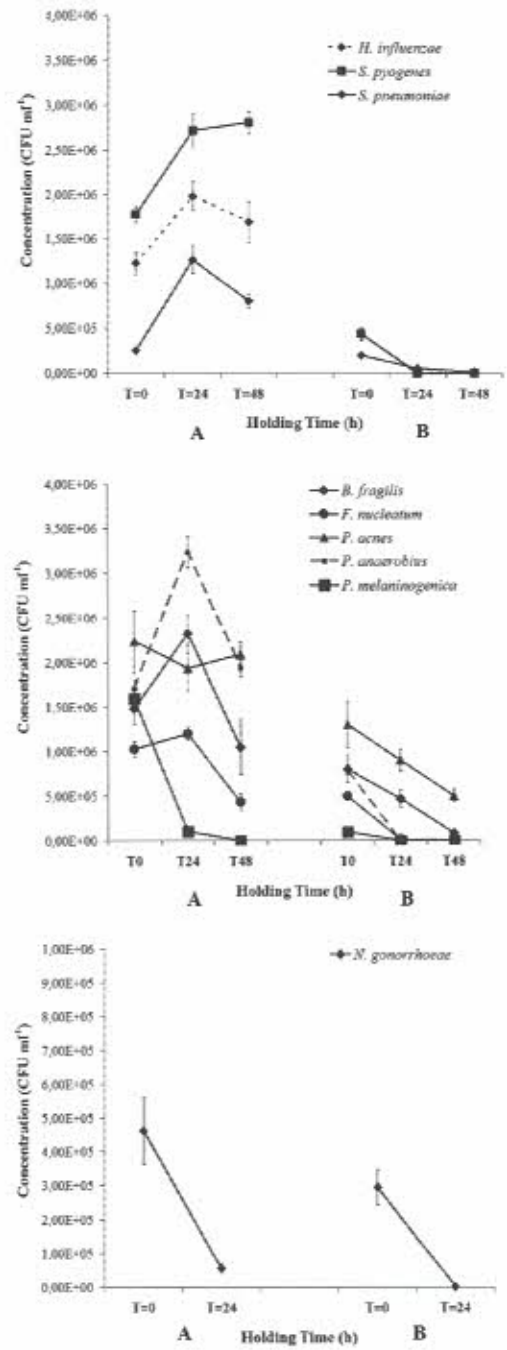

Fig. 1. Changes in CFU counts over 48 h period. Aerobes, anaerobes and fastidious organism plated after different incubation times (T = 0, 24, 48 h) in eSwab (A) and Σ-Transwab (B) devices. The mean values and the standard deviations are reported.

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expressed as an average for triplicate samples evaluated and as a percentage of the baseline counts (counts at time zero). CLSI M40-A2 criteria were used for evaluation as follows: a swab system was considered acceptable for the tested bacteria if the change in CFU from the 0 h time point declined no more than 3 $\log_{10}$ (ΔLOG). CLSI M40-A2 establishes a storage evaluation time of 24 h for *N. gonorrhoeae* and 48 h for all other organisms.

Mechanically, there was a clear advantage for the eSwab since it smoothly absorbed the loading inoculum, while the Σ-Transwab inoculum adsorption was much less efficient, averaging 50 μl. As shown in Table 1, the different CFU count values at 0 h time-point reflect the critically different absorbance capabilities of the two swabs. The eSwab fulfilled the CLSI acceptance criteria of viability maintenance for all microorganisms tested at T0, T24 and T48 timepoints. The Σ-Transwab failed to meet CLSI acceptance criteria after 24 h of storage for *P. anaerobius* and *P. melaninogenica*, and after 48 h for *F. nucleatum* and *H. influenzae* (Table 1). In contrast with Van Horn et al. (2008a,b) experiments, in our study the eSwab met CLSI acceptance criteria also for *P. melaninogenica* after 24 h and 48 h of room temperature storage. Most interestingly, as observed for *Campylobacter* spp. in FecalSwab and eSwab by Hirvonen and Kaukoranta (2014), the extension of storage up to 24 h at room temperature improved the cell viability and recovery of *S. pneumoniae* (Table 1).

In this study we aimed to evaluate the conformity assessment and performances of two commercial liquid-base microbiology devices at controlled-RT. The choice of testing only this temperature of storage was to simulate the actual transport conditions in our routine setting, in line with CLSI M40-A2 (2014) recommendation. Under the storage condition evaluated, the Copan eSwab has shown a better recovery capability than Σ-Transwab device, meeting CLSI acceptance criteria of viability maintenance for all microorganisms tested. Σ-Transwab showed less efficient inoculum adsorption and failed to meet CLSI acceptance criteria for *P. anaerobius*, *P. melaninogenica*, *F. nucleatum* and *H. influenzae*.

Conflict of interest

None of the authors has a potential conflict of interest to declare. (See Fig. 1.)

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