Viability Study of Nontuberculous Mycobacteria Commonly Causing Skin/Soft Tissue Infections Using the Copan ESwab Collection and Transport System

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Revised Abstract

Objective: The aim of this study was to evaluate the performance of Copan ESwab™ collection and transport system (Copan Diagnostics Inc.) for the viability of nontuberculous mycobacteria commonly causing skin and soft tissue infections.
Method: Viability tests were performed in triplicate using ESwabs and the roll plate method as per the CLSI M40-A2 document. ESwabs were prepared in duplicate with one set held at room temperature (RT) (20ºC to 25ºC) and the other held at refrigerator temperature (2°C to 8^{0}C) for 0, 24 and 48 hours (hrs) prior to processing. Five selected mycobacterial isolates consisting of *Mycobacterium abscessus, M. fortuitum, M. chelonae, M. marium,* and *M. haemophilum* were planted onto Columbia blood agar plates with 5% sheep blood and incubated at 30°C in O_2 for 5 to7 days. These organisms were then used to prepare three independent 0.5 McFarland suspensions. From this working suspension four 1:10 serial dilutions (10^7, 10^6, 10^5 and 10^4) were prepared in sterile saline. One hundred microliters of each organism suspension were transferred into wells of a round bottom microtiter plate. Each swab was then immersed into the organism suspension and allowed to absorb for 10 seconds before inserting into its corresponding ESwab tube. For each 0-hr set, the ESwab was removed from the transport device after 15 minutes. The swabs were discarded and the tubes vortexed again for 5 seconds. One hundred microliters of the suspension were added to Middlebrook 7H10 agar (MA), streaked and incubated at 30ºC in O_2 for 5 to 7 days. *M.haemophilum* was streaked on blood agar plates. Colonies were counted and averaged for three swabs for each time point and dilution. Average colony counts at 24 and 48 hrs were compared to the 0 hr inoculated swabs.
Results: All five organisms, were able to produce countable colonies at dilutions of 10^6, 10^5 and/or 10^4 and within ~300 to 500 CFU/mL at 24 and 48 hrs, respectively, compared to 0 hr counts.
Conclusion: Results suggest that the Copan ESwab collection and transport system is able to maintain and recover nontuberculous mycobacteria for up to 48 hrs at room and refrigeration temperatures. Routine swab collections are considered less than optimal for the recovery of mycobacterial isolates; however, this study indicates that mycobacterial organisms can, with high efficiency, be recovered from ESwabs. Further study is required to determine the utility of the ESwab in recovering nontuberculous mycobacterium from clinically infected patients.

Introduction

Transport system devices continue to undergo improvements in their ability to maximise the absorption of clinical specimens during collection and maintain the viability of bacterial pathogens during transport and subsequent recovery in the laboratory.
In the last decade flocked swabs have become increasingly more popular for the collection of specimens requiring bof pathogens.
The present study is an assessment of the Copan ESwab, which consists of a flocked swab placed in 1.0 mL Liquid Amies broth, using nontuberculous mycobacterial isolates obtained from Public Health Ontario Laboratory (PHOL), Department of Mycobacteriology using the roll plate method as per the CLSI M40-2A document.
This is the first focused study examining the viability and recovery of nontuberculous acterial and viral detection. Flocked swabs differ from the traditional fiber swabs by having nylon fibers attached perpendicularly to a molded plastic applicator which prevents entrapment of the clinical specimen. This results in greater release of the specimen in the liquid transport medium or onto culture plates resulting in greater recovery mycobacteria using a swab transport system.

Material & Method

Mycobacterial strains: (clinical isolates all characterised by PHOL), *Mycobacterium abscessus* subsp. *Abscessus*, *M. fortuitum, M. chelonae* , *M. marium* , *M. haemophilum*
Transport Swab System: Copan Liquid Amies ESwab collection and transport system (Copan Diagnostic, Inc., Brescia, Italy)
Culture media: Middlebrook 7H10 agar + OADC supplement plates, Columbia Agar w/ 5% sheep blood plates, Lowenstein- Jensen media slants, Physiological saline (BioMedia Unlimited Ltd, Toronto)

METHOD
M40-A2 Roll Plate Method:

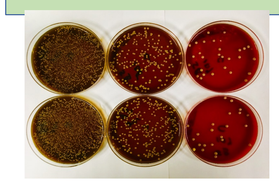
All challenge isolates were received on Lowenstein-Jensen (LJ) media slants from PHOL and stored at room temperature.
Prior to testing each isolate was sub-cultured onto blood agar plates (BAP) and incubated at 30ºC for 5 to7 days to check for purity and viability.
Once visible colonies were observed, a set of LJ slants were inoculated to use as working cultures which were stored and maintained at room temperature.
Organisms were also inoculated into vials with cryopreservative beads and stored at -20ºC for additional work if needed.
All organisms were sub-cultured three times prior to testing.
A heavy stock suspension was made from 5 to 7 days growth on blood agar culture plates (BAP) and vortexed for uniformity.
From this, suspensions were prepared in 4.0 mL saline plastic tubes, in triplicate and adjusted to match a 0.5 MacFarland turbidity standard (1.5 ×10^8 CFU/mL) using a Vitek nephelometer.
Each suspension was serially diluted 1:10 in normal saline to obtain working suspensions of ~ 1.5 ×10^7 CFU/mL to ~ 1.5×10^3 CFU/mL.
Controls were prepared by inoculating 100 uL of each tenfold serial dilution onto BAPs in duplicate.
The final working concentrations used to inoculate swabs were 10^6, 10^5, and 10^4 CFU.
100µL of each working suspension was transferred into 18 wells of a micro titre plate using an Eppendorf pipette.
Flocked swabs were placed into the wells and allowed to absorb the inoculum for approximately 10 seconds.
After inserting into their respective labelled ESwab transport device, the applicator sticks were snapped off at the score mark and the caps screwed on.
Each microorganism/device combination was performed in triplicate for each time point (0, 24, and 48 hr).
The 0 hr swabs were removed from the transport devices containing 1.0mL Amies broth after ~15 minutes incubation and discarded.
Using an Eppendorf pipette, 100µL of the inoculum was transferred onto the center of a blood agar plate containing 5% sheep blood, streaked for isolation, and incubated at 30°C in O_2.
The remaining ESwabs were incubated at room temperature for 24 and 48 hrs.
Results were calculated by taking an average of the colony counts from triplicate tests.
Counts at 24 and 48 hrs were compared to the 0 hr reference counts.

Results

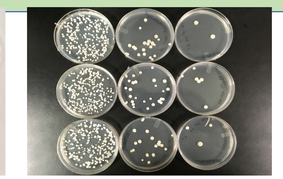
	M.abscessus			*M.chelonae*			*M.marinum*			*M.fortuitum*			*M.haemophilum*		
Concentration	10^6	10^5	10^4	10^6	10^5	10^4	10^6	10^5	10^4	10^6	10^5	10^4	10^6	10^5	10^4
0 hr	TNTC	335	36	TNTC	270	25	TNTC	166	9	205	12	1	TNTC	257	30
24 hr / RT	TNTC	370	43	TNTC	263	22	TNTC	235	22	290	17	3	TNTC	282	33
48 hr / RT	TNTC	276	27	TNTC	243	16	TNTC	TNTC	433	240	14	2	TNTC	298	34
24 hr / 4ºC	TNTC	293	35	TNTC	242	17	TNTC	148	7	184	12	1	TNTC	258	35
48 hr / 4ºC	TNTC	284	30	TNTC	230	20	TNTC	138	6	155	12	1	TNTC	257	28

Data expressed as an average CFU (performed in triplicate) , 10^6, 10^5, 10^4 = tenfold serial dilutions starting from a 0.5 McFarland, TNTC = too numerous to count

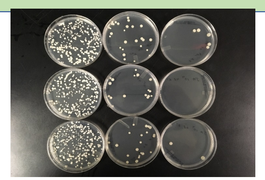
Compliance with M40-2A roll method was met by all five NTM isolates stored at 4ºC at both 24 and 48 hours of incubation. In addition, compliance was met with all five NTM isolates tested at room temperature storage at both 24 and 48 hours of incubation with the exception of *M. marinum* which showed overgrowth only after 48 hours of storage.



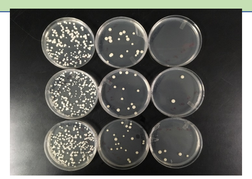
M fortiutum **control plates on Blood agar, 10^6, 10^5, and 10^4 CFU/uL**



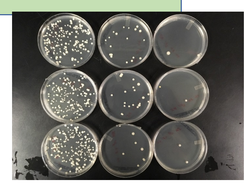
M fortiutum **0 hrs, 10^6, 10^5, and 10^4 CFU/uL**



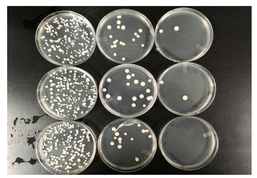
M fortiutum **24 hrs at room temperature, 10^6, 10^5, and 10^4 CFU/uL**



M fortiutum **48 hrs at room temperature, 10^6, 10^5, and 10^4 CFU/uL**



M fortiutum **24 hrs at 4ºC, 10^6, 10^5, and 10^4 CFU/uL**

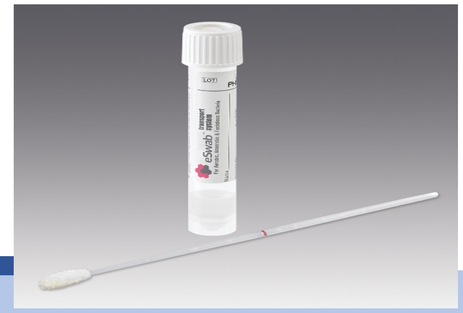


M fortiutum **48 hrs at 4ºC, 10^6, 10^5, and 10^4 CFU/uL**

Conclusion

For decades the use of routine rayon fiber swabs for the collection of specimens suspected of containing acid-fast bacilli have been considered less than optimal and indeed have often been rejected for mycobacterial culture. This is because the filamentous, sticky acid-fast bacilli get trapped inside of the fiber 'mattress' of the swab and are not readily dislodged onto culture media. Indeed, in theory our colony counts should have grown higher concentrations of organisms (e.g., 100 uL of 10^4/mL should produce ~1000 CFUs); but despite using fresh cultures, rigorous turbidity standardization (0.5 McFarland) and frequent vortexing, colony counts shown in Table 1 indicate a 10-fold decrease in expected organism recovery. Again, this is likely due to the nature of the organisms themselves. However, ESwab collection devices are known to work much differently from routine rayon swabs in both the collection of specimens and the delivery of those specimens onto culture media. As our results indicate the Copan ESwab collection and transport device appears to be an appropriate system for the maintenance, transportation, and recovery of difficult organisms such as nontuberculous mycobacteria. ESwabs were shown to maintain mycobacteria known to cause skin and soft tissue infections, with high efficiency, for up to 48 hrs at both room and refrigeration temperatures. Further study is required to determine the utility of the ESwab in recovering nontuberculous mycobacterium from clinically infected patient specimens.

Reference: Clinical and Laboratory Standards Institute. 2010. Quality control of microbiological transport systems; approved Standard -second edition. CLSI document M40-A2. CLSI, Wayne, PA.



Copan ESwab Collection & Transport System

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