C-20 Evaluation of Swab Transport Systems Using Standardized Roll-Plate Method

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ABSTRACT:

Background: Previous reported swab evaluation studies have incorporated many variations in their methodology, which made meaningful comparisons difficult. NCCLS has developed a method, as outlined in document M40-A, in an attempt to standardize the evaluation process. This study was undertaken to compare swab transport systems from Starplex and Copan, using the recommended roll-plate method, testing only facultative bacteria. **Methods:** A total of 12 ATCC and 1 clinical bacterial strain were included in this evaluation, including those as recommended by NCCLS M40-A. Each isolate was prepared to 0.5 McFarland standard, diluted from 10^{-2} to 10^{-5}. Starswab II (STR) from Starplex and Transystem M40 (COP) from Copan, were inoculated in triplicate using 100 µl, for time 0, 24 and 48 hours, held at 22ºC (RT) and 4ºC. Plates were inoculated on 3 planes as recommended and incubated as required. **Results:** Optimum initial dilution varied considerably between isolates, falling between 10^{-2} and 10^{-3}. *S. pneumoniae*, *H. influenzae* and *N. gonorrhoeae* were optimally recovered in both swab types at 4ºC. At RT, there was some variability in recovery between similar strains for these isolates, and acceptance criteria were decreased. Both swab systems had excellent recovery of *S. aureus*, *S. pyogenes*, *S. faecalis*, and *P. auruginosa* at 4ºC, but at RT there was some multiplication. **Conclusion:** The roll-plate method recommended by NCCLS M40-A is a reliable method for the evaluation of swab transport systems. Both swab types had good recovery of all bacterial types tested at 4ºC, meeting acceptance criteria. Additional work is required to determine the effect of multiplication of some isolates in mixed cultures at RT.

Introduction:

The transport of samples for bacterial isolation from patient to laboratory remains a considerable challenge. Multiple studies have been undertaken to determine the best swab type, as well as the best test method for evaluation. Previous swab evaluation studies have incorporated many variations in their methodology, which made meaningful comparisons difficult. NCCLS has developed a method, as outlined in document M40-A, in an attempt to standardize the evaluation process. This study was undertaken to compare swab transport systems from Starplex and Copan, using the standard roll-plate method, testing only facultative bacteria, Excluding strict anaerobes.

Methods:

A total of 12 ATCC and 1 clinical bacterial strain were included in this evaluation, as listed on Table 1. Each isolate was prepared to 0.5 McFarland standard, diluted from 10^{-2} to 10^{-5}. Starswab II (STR) (Starplex Scientific Inc., Etobicoke, Ontario, Canada) and Transystem M40 (COP) (Copan Italia, Brescia, Italy) were inoculated in triplicate using 100 µl, for time 0, 24 and 48 hours, held at 22ºC (RT) and 4ºC. Plates were inoculated on 3 planes as recommended and incubated as required

Table 1. Isolates used in Study

No.	Isolate	ATCC #	Number of subcultures from original Lyophilized stock *
1	*Streptococcus pneumoniae*	6035	8
2	*Streptococcus pneumoniae*	6035	3
3	*Haemophilus influenzae*	10211	8
4	*Haemophilus influenzae*	10211	3
5	*Neisseria gonorrhoeae*	Clinical strain	Fresh clinical isolate
6	*Neisseria gonorrhoeae* (bl+)	31426	8
7	*Neisseria gonorrhoeae*	43069	3
8	*Esherishia coli*	29212	8
9	*Staphylococcus aureus* (MRSA)	25923	8
10	*Staphylococcus aureus* (MSSA)	43300	8
11	*Streptococcus pyogenes* (Gp. A)	19615	8
12	*Enterococcus faecalis*	29212	8
13	*Psuedomonas aeruginosa*	27853	8

* The number of subcultures from the original lyophilized stock may be significant. The isolates with 8 subcultures were those used as quality control organisms in the routine laboratory on a regular bases. The isolates with 3 subcultures were from new lyophilized vials.

Results:

Table 2 indicates that in this study the optimal initial dilution varied considerable between isolates, falling between $1x10^{-2}$ to $1x10^{-5}$.

Table 3 lists the recovery of all isolates, at time 0, 24 and 48 hours, at both room temperature and 4ºC.

Table 2 Dilution from initial inoculum of 0.5 McFarland (~$1.5x10^8$ cfu/ml) that achieved optimum bacterial numbers at 0 time (30 - 300

No.	Isolate	ATCC	SWAB	dilution	CFU
1	*S. pneumoniae*	6305	STR	$1x10^{-3}$	100
			COP	$1x10^{-3}$	80
2	*S. pneumoniae*	6305	STR	$2x10^{-3}$	100
			COP	$2x10^{-3}$	60
3	*H. influenzae*	10211	STR	$2x10^{-4}$	100
			COP	$2x10^{-4}$	150
4	*H. influenzae*	10211	STR	$2x10^{-4}$	100
			COP	$2x10^{-4}$	100
5	*N. gonorrhoeae*	clinical	STR	$2x10^{-3}$	250
			COP	$2x10^{-3}$	250
6	*N. gonorrhoeae* (bl+)	31426	STR	$1x10^{-3}$	200
			COP	$1x10^{-3}$	100
7	*N. gonorrhoeae*	43069	STR	$2x10^{-4}$	150
			COP	$2x10^{-4}$	150
8	*E. coli*	29212	STR	$2x10^{-4}$	100
			COP	$2x10^{-4}$	100
9	*S. aureus* (MRSA)	43300	STR	$2x10^{-4}$	80
			COP	$2x10^{-4}$	50
10	*S. aureus* (MSSA)	25923	STR	$2x10^{-4}$	100
			COP	$2x10^{-4}$	100
11	*S. pyogenes*	19615	STR	$1x10^{-3}$	100
			COP	$1x10^{-3}$	100
12	*E. faecalis*	29212	STR	$1x10^{-3}$	100
			COP	$1x10^{-3}$	100
13	*P. aeruginosa*	27853	STR	$2x10^{-4}$	80
			COP	$2x10^{-4}$	100

Table 3. Recovery of isolates after 24 and 48 hours, at R.T., and 4ºC

				0 - time	24 hours		48 hours	
					R.T.	4ºC	R.T.	4ºC
No.	Isolate	ATCC	SWAB	CFU	CFU	CFU	CFU	CFU
1	*S. pneumoniae*	6305	STR	100	80	100	100	50
			COP	80	30	6	5	10
2	*S. pneumoniae*	6305	STR	100	50	100	0	100
			COP	60	30	20	2	20
3	*H. influenzae*	10211	STR	300	20	200	200	100
			COP	300	300	200	400	100
4	*H. influenzae*	10211	STR	300	50	250	0	250
			COP	150	300	300	TN	300
5	*N. gonorrhoeae*	clinical	STR	250	50	10	0	10
			COP	250	40	15	0	5
6	*N. gonorrhoeae*(bl+)	31426	STR	200	0	5	0	0
			COP	100	0	0	0	0
7	*N. gonorrhoeae*	43069	STR	300	0	200	0	80
			COP	300	80	80	0	50
8	*E. coli*	29212	STR	100	TN	300	TN	300
			COP	100	TN	80	TN	80
9	*S. aureus* (MRSA)	43300	STR	80	200	80	TN	80
			COP	50	100	50	TN	50
10	*S. aureus* (MSSA)	25923	STR	100	200	150	300	100
			COP	100	200	100	300	120
11	*S. pyogenes* (Gp. A)	19615	STR	100	200	80	TN	80
			COP	100	200	70	TN	80
12	*E. faecalis*	29212	STR	100	400	200	TN	200
			COP	100	500	100	TN	100
13	*P. aeruginosa*	27853	STR	80	TN	80	TN	50
			COP	100	TN	250	TN	100

STR = Starplex; COP = Copan; TN = Too Numerous to Count; bl+ = beta lactamase producer; R.T. = 22ºC; MRSA= Methicilin Resistant *S. aureus*; MSSA = Methicillin Susceptible *S. aureus*

Discussion:

S. pneumoniae: both systems had good recovery at both R.T. & 4ºC, although fresh QC isolate had lower recovery at R.T.

H. influenzae: both systems had good recovery at 4ºC., COP had increased recovery at R.T. after 24 hr, with overgrowth after 48 hr.; STR showed slight decline after 48 hr at R.T.

N. gonorrhoeae clinical strain: both systems had good recovery at 24hr, both at R.T. and 4ºC.; after 48 hr. reduced recovery at 4ºC., with no recovery at R.T.

N. gonorrhoeae 31426 (bl+): STR had low recovery after 24 hr. at 4ºC , but COP had no recovery. Neither system grew after 24 hr. at R.T.

N. gonorrhoeae 43069: both systems had good recovery after 48 hr at 4ºC; COP had reduced recovery at 24 hr at R.T., but STR had no recovery

E. coli, S. aureus, S. pyogenes, P. aeruginosa: good recovery for both systems at 4ºC, with considerable overgrowth at R.T.

Conclusions:

- Roll Plate method is a reliable method to evaluate efficacy of swab transport systems
- ten-fold increase of initial dilutions may be excessive for some isolates (creating either too heavy, or too light an inoculum at time zero)
- variable recovery rates, both inter and intra species; frequency of subculture may affect recovery
- Both Copan's M40 and Starplex's StarswabII swab systems had reliable recovery of isolates tested
- Better recovery of isolates at 4ºC than at R.T., particularly with more fastidious strains
- Transport at R.T. may result in overgrowth of some isolates, particularly with non-fastidious strains

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