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Evaluation of Liquid-Based Swab Transport Systems against the New Approved CLSI M40-A2 Standard

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Following revised information pertaining to newer swab types and testing protocols in the new CLSI M40-A2 standard, we evaluated three liquid swab transport systems for the recovery of aerobic, anaerobic, and fastidious organisms at room temperature and at 4°C. All tested liquid swab transport systems were fully compliant with the M40-A2 standard, with acceptable performance at both temperatures after the full specified holding period, using both qualitative (roll-plate) and quantitative (swab elution) methods.

Microbiology laboratory diagnosis relies on the recovery of bacterial isolates from clinical specimens. Tissue biopsy and fluid aspiration methods are preferred for collection of clinical samples; however, swab transport systems (STSs) are commonly used due to their low cost and ease of use and the ability to maintain viability for aerobic, anaerobic, and fastidious microorganisms over extended times (1, 2). The second edition of the Clinical and Laboratory Standards Institute (CLSI) M40-A2 standard on the quality control (QC) of microbiological transport systems was published in June 2014 (3), replacing the previous M40-A standard published in 2003 (4). The new M40-A2 standard provides revised testing protocols for liquid transport systems using swab types such as foam swabs and newer "flocked" fiber swabs (3). Routinely, clinical laboratories utilize the roll-plate method to inoculate swab transport devices onto medium plates. For swab validation, however, the M40-A2 standard describes two methods, i.e., a qualitative method (the roll-plate method) and a quantitative method (the swab elution method). The M40-A2 standard expects manufacturers to perform both methods of testing for flocked fiber and foam swabs used in conjunction with liquid media, to ensure the sensitivity of the devices and reliability in clinical settings. The M40-A2 document recommends that end users test swabs by both methods for validation assessments or choose the method that suits their laboratory environment. These new revisions and other additions, such as testing at two different temperatures, would ensure improved accuracy and facilitate better diagnosis.

(This work was presented in part at the 115th General Meeting of the American Society for Microbiology, New Orleans, LA, 30 May to 2 June 2015 [5].)

The STSs used in this study were manufactured and supplied by Medical Wire and Equipment (Corsham, United Kingdom). The STSs included Sigma Transwab PurFlock (flocked swab), Sigma Transwab PurFlock Minitip (flocked swab), and Sigma Transwab (foam swab) swabs. The swabs were used in conjunction with 1 ml of liquid Amies transport medium (E&O Laboratories Ltd., Burnhouse, United Kingdom). Ten American Type Culture Collection (ATCC) bacterial strains (Table 1) were assessed for viability and recovery in accordance with the M40-A2 approved standard. Microorganisms were cultured on plated media (Table 1) and incubated at 37°C under the atmospheric conditions specified in Table 1. Agar plates were incubated under aerobic, anaerobic, or 5% CO_2 conditions for 18 to 24 h (a maximum of 48 h for fastidious bacteria and anaerobes). To determine bacterial viability, the methods described in the M40-A2 standard, i.e., the roll-plate (qualitative) and swab elution (quantitative) methods, were followed accordingly.

TABLE 1 Growth conditions for M40-A2 test microorganisms

Microorganism[a]	Atmosphere	Medium[b]	Incubation time (h)
Pseudomonas aeruginosa ATCC BAA-427	Aerobic	Tryptic soy agar	48
Streptococcus pyogenes ATCC 19615	5% CO_2	Columbia blood agar	48
Streptococcus pneumoniae ATCC 6305	5% CO_2	Columbia blood agar	48
Haemophilus influenzae ATCC 10211	5% CO_2	Chocolate agar	48
Bacteroides fragilis ATCC 25285	Anaerobic	Columbia blood agar	48
Peptostreptococcus anaerobius ATCC 27337	Anaerobic	Columbia blood agar	48
Fusobacterium nucleatum ATCC 25586	Anaerobic	Columbia blood agar	48
Propionibacterium acnes ATCC 6919	Anaerobic	Columbia blood agar	48
Prevotella melaninogenica ATCC 25845	Anaerobic	Columbia blood agar	48
Neisseria gonorrhoeae ATCC 43069	5% CO_2	Chocolate agar	24

[a] ATCC, American Type Culture Collection.
[b] Agar was supplied by E&O Laboratories Ltd. (Scotland).

For the roll-plate method, inocula were prepared to approximately 1.5×10^8 CFU/ml (0.5 McFarland standard) in 0.85%

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TABLE 2 Bacterial recovery and overgrowth for foam and flocked swabs over 48 h at room temperature and 4°C, using the roll-plate (qualitative) method

Bacteria and swab type	Temperature	Bacterial recovery (CFU)[a]			Compliant[b]
		0 h	24 h	48 h	
Pseudomonas aeruginosa ATCC BAA-427					
Purflock	RT	117	NA	NA	NA
	4°C	9	52	90	Yes
Minitip	RT	83	NA	NA	NA
	4°C	32	57	83	Yes
Foam	RT	141	NA	NA	NA
	4°C	20	87	109	Yes
Haemophilus influenzae ATCC 10211					
Purflock	RT	179	19	5	Yes
	4°C		22	12	Yes
Minitip	RT	175	14	6	Yes
	4°C		18	6	Yes
Foam	RT	168	11	7	Yes
	4°C		42	11	Yes
Streptococcus pneumoniae ATCC 6305					
Purflock	RT	156	46	30	Yes
	4°C		89	46	Yes
Minitip	RT	145	76	18	Yes
	4°C		89	32	Yes
Foam	RT	225	131	74	Yes
	4°C		216	202	Yes
Streptococcus pyogenes ATCC 19615					
Purflock	RT	154	32	6	Yes
	4°C		79	24	Yes
Minitip	RT	195	56	9	Yes
	4°C		67	19	Yes
Foam	RT	201	43	12	Yes
	4°C		108	23	Yes
Prevotella melaninogenica ATCC 25845					
Purflock	RT	112	105	13	Yes
	4°C		27	21	Yes
Minitip	RT	59	25	12	Yes
	4°C		41	21	Yes
Foam	RT	73	95	38	Yes
	4°C		92	80	Yes
Bacteroides fragilis ATCC 25285					
Purflock	RT	124	85	67	Yes
	4°C		99	82	Yes
Minitip	RT	123	89	54	Yes
	4°C		76	70	Yes
Foam	RT	187	108	80	Yes
	4°C		105	74	Yes
Peptostreptococcus anaerobius ATCC 27337					
Purflock	RT	289	134	45	Yes
	4°C		198	61	Yes
Minitip	RT	276	203	104	Yes
	4°C		207	125	Yes
Foam	RT	301	176	105	Yes
	4°C		165	135	Yes
Propionibacterium acnes ATCC 6919					
Purflock	RT	189	54	9	Yes
	4°C		52	27	Yes
Minitip	RT	165	43	11	Yes
	4°C		54	19	Yes
Foam	RT	187	78	31	Yes
	4°C		69	52	Yes
Fusobacterium nucleatum ATCC 25586					
Purflock	RT	209	143	86	Yes
	4°C		187	104	Yes
Minitip	RT	215	168	78	Yes
	4°C		186	124	Yes
Foam	RT	297	215	108	Yes
	4°C		246	178	Yes
Neisseria gonorrhoeae ATCC 43069					
Purflock	RT	247	8	NA	Yes
	4°C		13	NA	Yes
Minitip	RT	173	14	NA	Yes
	4°C		17	NA	Yes
Foam	RT	273	31	NA	Yes
	4°C		65	NA	Yes

[a] NA, not applicable.
[b] The M40-A2 compliance criteria were yields of ≥5 CFU (or 1-log-unit increase for *P. aeruginosa* at 4°C only) after the specified holding period, using the same dilution as for the time zero plates.

physiological saline, using an 18- to 24-h culture for each microorganism. Final working dilutions of 1.5×10^6 to 1.5×10^4 CFU/ml were prepared, and the dilutions were dispensed in triplicate into a 96-well plate, in 100-μl aliquots for Sigma Transwab PurFlock swabs, 20-μl aliquots for Sigma Transwab Purflock Minitip swabs, or 50-μl aliquots for Sigma Transwab swabs. The swabs were immersed in the aliquots, and the dilutions were absorbed for 10 s. The swabs were then placed in the liquid transport medium and maintained at room temperature (RT) (approximately 24°C) or 4°C for 48 h (24 h for *Neisseria gonorrhoeae*). After 0, 24, and 48 h, the swabs were removed, rolled directly onto their respective agar plates, and incubated under the required atmospheric conditions (Table 1) for 24 to 48 h, according to the CLSI M40-A2 standard. Enumerated colonies were counted from each plate, and CFU values were determined. The dilution that yielded an inoculum density closest to 250 colonies at time zero was the only dilution used and counted at 24 and 48 h. For overgrowth studies with *Pseudomonas aeruginosa*, the suspensions were diluted an additional 1:10, to approximately 1.5×10^3 CFU/ml, before being dispensed in triplicate into a 96-well plate; this was to allow measurable yields after incubation.

For the swab elution method, the inocula were prepared in a

TABLE 3 Bacterial recovery and overgrowth for foam and flocked swabs over 48 h at room temperature and 4°C, using the swab elution (quantitative) method

Bacteria and swab type	Temperature	Bacterial recovery (CFU)[a]			Log-unit change[b]
		0 h	24 h	48 h	
Pseudomonas aeruginosa ATCC BAA-427					
Purflock	RT	4.53×10^7	NA	NA	NA
	4°C		2.37×10^8	1.34×10^8	0.47
Minitip	RT	3.67×10^7	NA	NA	NA
	4°C		8.07×10^7	1.37×10^8	0.57
Foam	RT	3.27×10^7	NA	NA	NA
	4°C		8.73×10^7	3.30×10^8	1.00
Haemophilus influenzae ATCC 10211					
Purflock	RT	1.26×10^7	2.04×10^6	4.27×10^5	1.47
	4°C		1.23×10^6	6.80×10^5	1.27
Minitip	RT	2.56×10^7	6.00×10^5	5.13×10^5	1.70
	4°C		8.67×10^5	5.9×10^5	1.64
Foam	RT	3.27×10^7	1.10×10^6	2.17×10^5	2.18
	4°C		1.16×10^6	1.19×10^5	2.44
Streptococcus pneumoniae ATCC 6305					
Purflock	RT	3.47×10^6	9.47×10^5	5.27×10^5	0.82
	4°C		1.53×10^6	1.05×10^6	0.52
Minitip	RT	3.20×10^6	1.33×10^6	7.07×10^5	0.66
	4°C		1.60×10^6	8.40×10^5	0.58
Foam	RT	6.27×10^6	6.40×10^6	1.37×10^6	0.66
	4°C		1.73×10^6	6.10×10^6	0.01
Streptococcus pyogenes ATCC 19615					
Purflock	RT	3.73×10^6	5.00×10^5	6.40×10^4	1.77
	4°C		8.40×10^5	3.00×10^4	2.09
Minitip	RT	3.57×10^6	1.60×10^6	2.37×10^6	0.18
	4°C		3.53×10^6	2.53×10^6	0.15
Foam	RT	8.30×10^6	4.53×10^6	2.67×10^6	0.49
	4°C		6.70×10^6	7.37×10^6	0.05
Prevotella melaninogenica ATCC 25845					
Purflock	RT	1.04×10^7	4.30×10^6	4.57×10^6	0.36
	4°C		5.87×10^6	2.80×10^6	0.57
Minitip	RT	6.23×10^6	8.67×10^6	4.53×10^6	0.14
	4°C		6.20×10^6	3.80×10^6	0.21
Foam	RT	1.03×10^7	5.20×10^6	9.33×10^6	0.04
	4°C		6.07×10^6	6.50×10^6	0.20
Bacteroides fragilis ATCC 25285					
Purflock	RT	1.73×10^8	1.06×10^7	7.01×10^6	1.39
	4°C		3.46×10^7	5.43×10^6	1.50
Minitip	RT	9.13×10^7	9.23×10^6	3.40×10^6	1.43
	4°C		3.77×10^6	5.61×10^6	1.21
Foam	RT	9.83×10^7	1.63×10^7	7.10×10^6	1.14
	4°C		4.57×10^7	9.41×10^6	1.02
Peptostreptococcus anaerobius ATCC 27337					
Purflock	RT	9.85×10^7	5.05×10^6	4.75×10^5	2.32
	4°C		9.04×10^6	7.36×10^5	2.13
Minitip	RT	8.84×10^7	9.85×10^6	1.02×10^6	1.94
	4°C		1.85×10^7	8.71×10^6	1.01
Foam	RT	2.56×10^8	7.01×10^6	2.04×10^6	2.10
	4°C		9.56×10^7	2.30×10^7	1.05
Propionibacterium acnes ATCC 6919					
Purflock	RT	6.29×10^7	9.23×10^6	2.40×10^5	2.42
	4°C		3.04×10^7	8.72×10^6	0.86

(Continued on following page)

TABLE 3 (Continued)

Bacteria and swab type	Temperature	Bacterial recovery (CFU)[a]			Log-unit change[b]
		0 h	24 h	48 h	
Minitip	RT	6.76×10^7	1.99×10^7	1.86×10^6	1.56
	4°C		8.30×10^6	9.86×10^6	0.84
Foam	RT	7.04×10^7	6.25×10^6	4.31×10^6	1.21
	4°C		1.21×10^7	6.96×10^6	1.00
Fusobacterium nucleatum ATCC 25586					
Purflock	RT	8.67×10^7	4.43×10^6	1.02×10^5	2.93
	4°C		6.21×10^7	3.65×10^6	1.38
Minitip	RT	6.07×10^7	9.06×10^6	3.43×10^5	2.25
	4°C		8.91×10^6	5.61×10^6	1.03
Foam	RT	4.35×10^8	4.71×10^7	9.09×10^6	1.68
	4°C		9.09×10^7	5.41×10^7	0.91
Neisseria gonorrhoeae ATCC 43069					
Purflock	RT	7.5×10^4	8.6×10^1	NA	2.94
	4°C		1.4×10^2	NA	2.73
Minitip	RT	4.5×10^4	1.4×10^2	NA	2.51
	4°C		7.9×10^3	NA	0.76
Foam	RT	8.13×10^6	4.67×10^5	NA	1.24
	4°C		1.20×10^6	NA	0.83

[a] NA, not applicable.
[b] The M40-A2 compliance criteria were no greater than a 3-log-unit decrease at 4°C or room temperature or a 1-log-unit increase for *P. aeruginosa* at 4°C only. The log-unit change was calculated as log(48-h value) − log(time zero value).

manner similar to that for the roll-plate method; however, the initial suspensions were diluted 1:10 and dispensed in triplicate into a 96-well plate, in 100-μl aliquots for Sigma Transwab PurFlock swabs, 20-μl aliquots for Sigma Transwab Purflock Minitip swabs, or 50-μl aliquots for Sigma Transwab swabs. The swabs were then placed in 1 ml of liquid Amies transport medium and maintained at RT (approximately 24°C) or 4°C for 48 h (24 h for *N. gonorrhoeae*). After 0, 24, and 48 h, the swabs were removed and a 10-fold serial dilution, to approximately 1.5×10^2 CFU/ml, was prepared with the liquid Amies transport medium. From each of the dilutions, 50 μl was dispensed onto the respective agar plates (Table 1) using a spiral plater (Don Whitley Scientific, York, United Kingdom). The agar plates were then incubated under the required atmospheric conditions for 24 to 48 h, and the colonies were enumerated.

The M40-A2 standard indicates that, for bacterial recovery from STSs using the roll-plate method, there should be ≥5 CFU after the specified holding period for specimens held at 4°C or RT, from the same dilution as used in time zero plate counts, in order for the viability assessment to be considered acceptable. In overgrowth studies, any specimen held at 4°C should yield no more than a 1-log-unit increase in CFU between time zero and the end of the specified holding period. In our study, all three Sigma Transwab systems met the acceptability criteria for viability studies, as all tested microorganisms yielded ≥5 CFU after the specified holding periods (Table 2). In addition, all Transwab systems met the criteria for overgrowth at 4°C, with no more than 1-log-unit increases for *P. aeruginosa* (Table 2).

For the swab elution method, the M40-A2 standard indicates that, for compliance regarding viability, any specimen held at 4°C or RT should yield no more than a 3-log-unit decrease in CFU between time zero and the end of the specified holding period and, for assessment of overgrowth, any specimen held at 4°C should yield no more than a 1-log-unit increase in CFU between time zero and the end of the specified holding period. Table 3 demonstrates that all three Sigma Transwab systems tested in this study met the viability criteria of the M40-A2 standard, with no more than 3-log-unit decreases in CFU for all microorganisms after the specified holding periods; this included *N. gonorrhoeae*, which was incubated for only 24 h. Results also showed that the M40-A2 criteria for overgrowth at 4°C were met, with no more than 1-log-unit increases being observed for *P. aeruginosa* (Table 3).

The M40-A2 standard was revised as a result of numerous study data and incorporated redefined testing protocols to include new swab types and better defined temperatures for QC testing (3). Prior to the recently published M40-A2 standard, swab transport systems, including the swab tip formats used in this study, were evaluated for viability and recovery using only one test method, i.e., the swab elution method or the roll-plate method (2, 6). To our knowledge, our study is the first evaluation of STSs using both methods since the revision and publication of the M40-A2 standard. Other studies that were published recently utilized either a single method of assessment (the roll-plate method) (7) or a different method (a high-throughput homogenizer) (8), not indicated in the M40-A2 document. In our study, all three swab formats tested were compliant with the M40-A2 criteria for viability studies. This is in contrast to the data reported by Avolio and Camporese (7), which suggested that one of the swab formats tested in our study, the Sigma Transwab (foam) format, failed the CLSI acceptance criteria; we addressed this in our letter to the editor (9).

The three Sigma Transwab systems were found to have acceptable performance at both temperatures after the full specified holding period, using both qualitative (roll-plate) and quantitative (swab elution) methods. In addition, we recommend that commercially available liquid medium transport systems used in conjunction with foam or flocked swabs be internally evaluated using both qualitative and quantitative methods, to ensure the

sensitivity of the system, the reliability of the results in clinical settings, and compliance with the M40-A2 standard.

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