POSTER BOARD 1084 ASM 2015 NEW ORLEANS 1ST JUNE 2015

Using the new CSLI M40-A2 Standard Coupled with Molecular Criteria to assess the performance of Sigma Transwab® PurFlock® (Standard and Mini-tip) with Liquid Amies Medium

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Abstract

Background: Sigma-Transwab® PurFlock®- is a liquid medium format transport swab designed for use on automated processing platforms. The PurFlock® swab is intended to improve absorption and release of the specimen. In 2014 the revised standard CLSI M40-A2 included new provisions for the evaluation of liquid medium transport swabs with novel bud types such as foam or flock. In this study, Sigma Transwab® PurFlock® (standard and minitip) was assessed to M40-A2, and a new step was added evaluating the ability to recover genomic DNA from the medium after the holding period.

Materials: The 10 bacterial strains specified in CLSI M40-A2 were used. Inoculated devices were held at controlled room temperature (RT) and 4° C. Viable counts were determined at 0, 24 and 48 h. Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit, quantified by the Nanodrop 1000 and amplified by standard Polymerase Chain Reaction (PCR) and universal primers intended for the M40-A2 bacteria (*16S* ribosomal *DNA*).

Results: Sigma Transwab® PurFlock® (standard and minitip) were able to recover all specified strains at both 4° C and RT in accordance with M40-A2. Genomic DNA was successfully recovered after holding in the liquid Amies medium with high quality and yield after 24 hr. Preliminary data from the PCR analysis shows that the universal primer is able to amplify the 16S ribosomal DNA from the different bacterial genera.

Conclusion: Sigma Transwab® PurFlock® (standard and minitip) recovered all of the organisms specified in CLSI M40-A2. DNA integrity was maintained after holding in liquid Amies medium. The ability to amplify genomic DNA of the different genera on the M40-A2 standard using the universal primers can support quicker laboratory diagnosis, reduce confirmation times for bacterial infections and rapid identification tests.

Background

Successful transport and sampling of bacteria is essential for diagnosis and treatment of patients. Swabs are an efficient method of collecting bacterial samples, the material and transport medium are important. The Σ-Transwab® PurFlock is a transport system containing Liquid Amies Medium allowing maintenance of aerobes, anaerobes and fastidious organisms without overgrowth; and a Flocked swab, having high levels of absorbency and in turn a high release.

The new M40-A2 is the recognised standard for Swab Transport Systems (STS). Furthermore, this new M40-A2 standard provides revised testing protocols for transport systems using newer swab types such as flock swabs that were introduced after publication of the original M40-A standard. These amendments are essential to further accurate and current diagnosis. The new standard also recommends that both quantitative and qualitative methods be used when testing foam or flock used in conjunction with liquid transport media due to the versatility of the STS; it can be used to inoculate agar directly via swab or liquid media or used by automated equipment. Use of both quantitative and qualitative methods ensures reliable performance under laboratory usage and accurate sensitivity.

Objectives

In this study, two liquid STS; Sigma-Transwab® PurFlock® and Sigma-Transwab® Purflock Minitip® were assessed using quantitative and qualitative methodologies in accordance with CLSI M40-A2 standard.

A new step was added evaluating the ability to recover genomic DNA from the medium after a 24hr holding period.

Methods and Materials

M40-A2 Compliance

Two STS were used in this study; Sigma-Transwab® PurFlock® and Sigma-Transwab® Purflock Minitip® both manufactured and supplied by Medical Wire and Equipment, Corsham, UK.

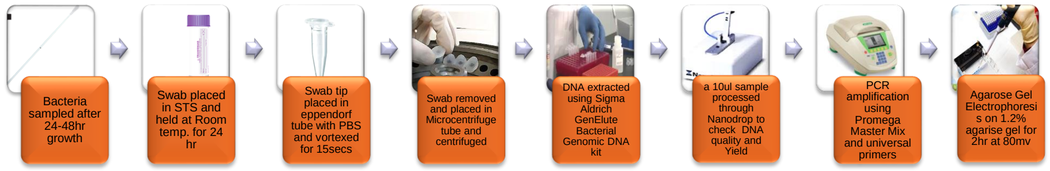
The new M40-A2 standard recommends that for assessment of viability, ten bacteria organisms comprising of aerobes, anaerobes and fastidious organisms are used. The organisms were cultured on the appropriate agar and incubated at 37°C in the required atmospheric conditions and times as specified in M40-A2.

Both quantitative (Roll Plate) and qualitative (Swab Elution) methods were used in accordance with the newly implemented recommendations by M40-A2.

Roll Plate	Swab Elution
Bacterial suspension $1.5x10^8$	Bacterial suspension $1.5x10^8$
Serial dilutions up to 10^{-3}	Diluted 10^{-1}
Aliquots dispensed in triplicate 100µl (Purflock) and 20µl (Minitip)	Aliquots dispensed in triplicate 100µl (Purflock) and 20µl (Minitip)
Swabs immersed and aliquots absorbed	Swabs immersed and aliquots absorbed
Held in STS for up to 48hr at 4°C and Room temp.	Held in STS for upto 48hr at 4°C and Room temp.
Swab removed and rolled onto agar plates	STS vortexed, swab removed and transport media serial diluted up to 10^{-3}
	50µl* inoculated onto agar using spiral plater

*inoculum amount used for Don Whitley Spiral Plater

Molecular Analysis



Results

Table 1. M40-A2 Viability and overgrowth compliance for PurFlock swabs using Quantitative and Qualitative Methods

Bacteria	Swab	Temp	Quantitative (Roll Plate) CFU				Qualitative (Swab Elution) CFU/ml			
			0hr	24hr	48hr	M40-A2 Compliance	0hr	24hr	48hr	M40-A2 Compliance
Pseudomonas aeruginosa ATCC® BAA-427	Purflock	Room temp.	117	OG	OG	✓	$4.53x10^7$	$1.50\ x10^9$	$1.67\ x10^9$	✓
		4°C	9	52	90	✓		$2.37\ x10^8$	$1.34\ x10^8$	✓
	Minitip	Room temp.	83	OG	OG	✓	$3.67\ x10^7$	$2.17\ x10^9$	$1.93\ x10^9$	✓
		4°C	32	57	83	✓		$8.07\ x10^7$	$1.37\ x10^8$	✓
Haemophilus Influenzae ATCC® 10211	Purflock	Room temp.	179	19	5	✓	$1.26\ x10^7$	$2.04\ x10^6$	$4.27\ x10^5$	✓
		4°C		22	12	✓		$1.23\ x10^6$	$6.80\ x10^5$	✓
	Minitip	Room temp.	175	14	6	✓	$2.56\ x10^7$	$6.00\ x10^5$	$5.13\ x10^5$	✓
		4°C		18	6	✓		$8.67\ x10^5$	$5.9\ x10^5$	✓
Streptococcus pneumoniae ATCC® 6305	Purflock	Room temp.	156	46	30	✓	$3.47\ x10^6$	$9.47\ x10^5$	$5.27\ x10^5$	✓
		4°C		89	46	✓		$1.53\ x10^6$	$1.05\ x10^6$	✓
	Minitip	Room temp.	145	76	18	✓	$3.20\ x10^6$	$1.33\ x10^6$	$7.07\ x10^5$	✓
		4°C		89	32	✓		$1.60\ x10^6$	$8.40\ x10^5$	✓
Streptococcus pyogenes ATCC® 19615	Purflock	Room temp.	154	32	6	✓	$3.73\ x10^7$	$5.00\ x10^5$	$6.40\ x10^4$	✓
		4°C		79	24	✓		$8.40\ x10^5$	$3.00\ x10^4$	✓
	Minitip	Room temp.	195	56	9	✓	$3.57\ x10^6$	$1.60\ x10^6$	$2.37\ x10^6$	✓
		4°C		67	19	✓		$3.53\ x10^6$	$2.53\ x10^6$	✓
Prevotella melaninogenica ATCC® 25845	Purflock	Room temp.	112	105	13	✓	$1.04\ x10^7$	$4.30\ x10^6$	$4.57\ x10^6$	✓
		4°C		27	21	✓		$5.87\ x10^6$	$2.80\ x10^6$	✓
	Minitip	Room temp.	59	25	12	✓	$6.23\ x10^6$	$8.67\ x10^6$	$4.53\ x10^6$	✓
		4°C		41	21	✓		$6.20\ x10^6$	$3.80\ x10^6$	✓
Bacteroides fragilis ATCC® 25285	Purflock	Room temp.	124	85	67	✓	$1.73\ x10^8$	$1.06\ x10^7$	$7.01\ x10^6$	✓
		4°C		99	82	✓		$3.46\ x10^7$	$5.43\ x10^6$	✓
	Minitip	Room temp.	123	89	54	✓	$9.13\ x10^7$	$9.23\ x10^6$	$3.40\ x10^6$	✓
		4°C		76	70	✓		$3.77\ x10^6$	$5.61\ x10^6$	✓
Peptostreptococcus anaerobius ATCC® 27337	Purflock	Room temp.	289	134	45	✓	$9.85\ x10^7$	$5.05\ x10^6$	$4.75\ x10^5$	✓
		4°C		198	61	✓		$9.04\ x10^6$	$7.36\ x10^5$	✓
	Minitip	Room temp.	276	203	104	✓	$8.84\ x10^7$	$9.85\ x10^6$	$1.02\ x10^6$	✓
		4°C		207	125	✓		$1.85\ x10^7$	$8.71\ x10^6$	✓
Propionibacterium acnes ATCC® 6919	Purflock	Room temp.	189	54	9	✓	$6.29\ x10^7$	$9.23\ x10^6$	$2.40\ x10^5$	✓
		4°C		52	27	✓		$3.04\ x10^7$	$8.72\ x10^6$	✓
	Minitip	Room temp.	165	43	11	✓	$6.76\ x10^7$	$1.99\ x10^7$	$1.86\ x10^6$	✓
		4°C		54	19	✓		$8.30\ x10^6$	$9.86\ x10^6$	✓
Fusobacterium nucleatum ATCC® 25586	Purflock	Room temp.	209	143	86	✓	$8.67\ x10^7$	$4.43\ x10^6$	$1.02\ x10^5$	✓
		4°C		187	104	✓		$6.21\ x10^7$	$3.65\ x10^6$	✓
	Minitip	Room temp.	215	168	78	✓	$6.07\ x10^7$	$9.06\ x10^6$	$3.43\ x10^5$	✓
		4°C		186	124	✓		$8.91\ x10^6$	$5.61\ x10^6$	✓
Neisseria gonorrhoeae ATCC® 43069	Purflock	Room temp.	247	8	n/a	✓	$7.5x10^4$	$8.6x10^1$	n/a	✓
		4°C		13	n/a	✓		$1.4x10^2$	n/a	✓
	Minitip	Room temp.	173	14	n/a	✓	$4.5x10^4$	$1.4x10^2$	n/a	✓
		4°C		17	n/a	✓		$7.9x10^3$	n/a	✓

Results

Table 2. Results of extracted DNA purity using Nanodrop 1000 for 10 M40-A2 compliant bacteria after a 24hr holding period in STS.

Bacteria	260/280 Ratio* (Purity of DNA)	260/230 Ratio+ (Purity of Nucleic acids)
Pseudomonas aeruginosa	1.98	2.23
Streptococcus pyogenes	1.58	2.55
Streptococcus pneumonia	1.44	1.09
Haemophilus influenzae	1.94	1.32
Bacteroides fragilis	1.87	2.55
Peptostreptococcus anaerobius	1.66	1.47
Fusobacterium nucleatum	1.58	1.8
Propionibacterium acnes	1.6	0.84
Prevotella melaninogenica	1.84	1.39
Neisseria gonorrhoeae	1.84	1.96

*260/280 is the ratio of absorbance at 260 and 280nm used for assessment of DNA purity. A ratio of ~1.8 is generally accepted as pure for DNA
+ 260/230 is a secondary measurement of nucleic acid purity. This is commonly in the range 1.8-2.2

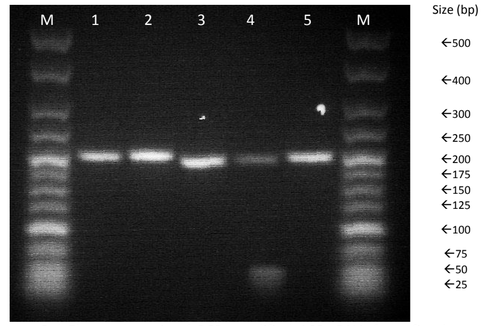


Figure 1. Agarose Gel Electrophoresis of PCR amplified 16S rDNA from five bacteria. Lane M, Molecular Weight Marker; lane 1, *Haemophilus Influenzae*; lane 2, *Neisseria gonorrhoeae*; lane 3, *Streptococcus pneumonia*; lane 4, *Streptococcus pyogenes*; lane 5 *Pseudomonas aeruginosa*

Discussion

The Purflock Σ-Transwab® met CLSI acceptance criteria for all M40-A2 bacteria stored at both temperatures after the specified holding periods for both Qualitative (Roll Plate) and Quantitative (Swab Elution) methods.

The data from the Nanodrop shows that DNA from all M40-A2 organisms can successfully be extracted after the 24hr holding period from Liquid Amies Swab Transport System. The purity of the DNA indicates no interference from Liquid Amies media.

The Agarose Gel Electrophoresis shows that PCR is able to amplify the selected genetic region of 16S Ribosomal DNA on all M40-A2 organisms and generate the correctly sized PCR product. This can be utilised to support quicker laboratory diagnosis and reduce confirmation times for bacterial infections.

References

1. Clinical and Laboratory Standards Institute (CLSI). *Quality Control of Microbiological Transport Systems; Approved Standard- Second Edition*. CLSI document M40-A2

***Acknowledgements**: The swab devices used in this study were provided by MWE.* www.mwe.co.uk