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Recovery of Genomic DNA and isolation of 16S Ribosomal DNA from Sigma Transwab® system with viability acceptance in accordance with CLSI M40-A2 standard

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Introduction

Successful transport and sampling of bacteria is essential for diagnosis and treatment of patients. Swabs are an efficient method of collecting bacterial samples, of which the material and transport medium are important. Sigma-Transwab® PurFlock is a liquid medium format transport swab designed for use on automated processing platforms. The medium within is liquid Amies and the swab is flock, which allows for greater absorption and release of bacterial cells.

In 2014 the revised standard CLSI M40-A2 included new provisions for the evaluation of liquid medium transport swab transport systems (STS) with novel bud types such as foam and flock. 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.

Aims

In this study the ability of the Sigma Transwab PF® to recover genomic DNA was assessed; The quality, yield and purity of the DNA was determined using the Nanodrop 1000, PCR was used to demonstrate amplification of 16S ribosomal DNA primers.

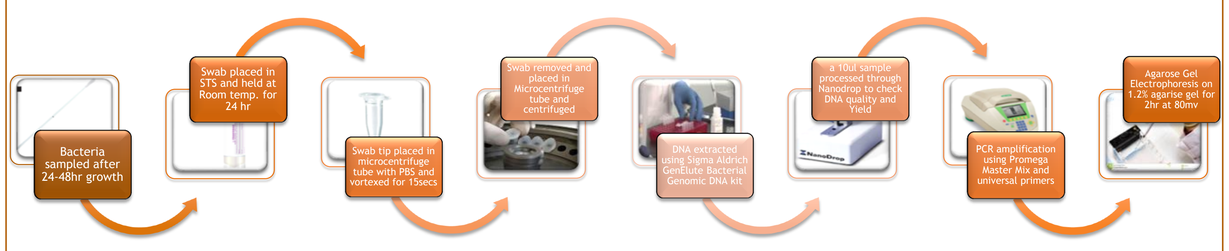
In addition Sigma Transwab® (foam tip) and Sigma Transwab® PF were evaluated for viability and recovery of all recommended 10 bacteria strains according to the CLSI M40-A2 swab elution and roll plate method.

Methods

The Transwabs® used in this study were; Sigma-Transwab® and Sigma-Transwab® PurFlock®. Manufactured and supplied by Medical Wire and Equipment, Corsham, UK.

The new M40-A2 standard is used for assessment of viability for ten bacteria comprising of aerobes, anaerobes and fastidious organisms. 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.

Molecular Analysis



M40-A2 Viability Studies

Roll Plate Method

-- Bacterial suspension $1.5x10^8$
-- Serial dilutions up to 10^{-3}
-- Aliquots dispensed in triplicate 100µl (Purflock) and 20µl (Minitip)
-- Swabs immersed and aliquots absorbed
-- Held in STS for up to 48hr at 4°C and Room temp.
-- Swab removed and rolled onto agar plates

Swab Elution method

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

Results

Table 1. Results of extracted DNA purity using Nanodrop 1000 for the ten 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

Results cont.

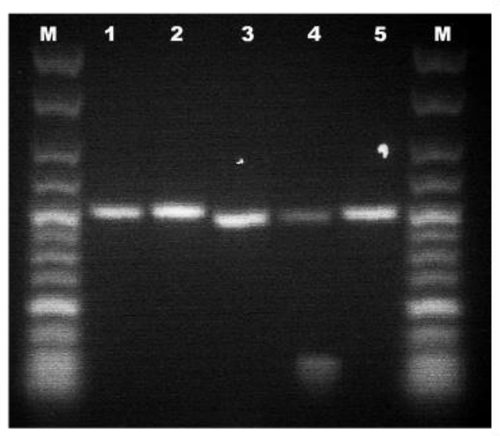


Figure 1. Agarose Gel Electrophoresis of PCR amplified 16S rDNA from five bacteria. Lane M, Molecular Weight Marker; lane 1, *Haemophilus Influenzae*; lane 2, *Neisseria gonorrhoeae*; lane 3, *Streptococcus pneumonia*; lane 4, *Streptococcus pyogenes*; lane 5 *Pseudomonas aeruginosa*

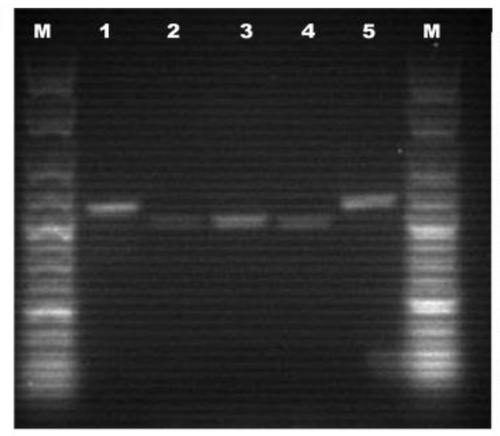


Figure 2. Agarose Gel Electrophoresis of PCR amplified 16S rDNA from Anaerobic bacteria. Lane M, Molecular Weight Marker; lane 1, *Bacteriodes fragilis*; lane 2, *Fusobacterium nucleatum*; lane 3, *Peptostreptococcus anaerobius*; lane 4, *Propionibacterium acnes* ; lane 5 *Prevotella melaninogenica*

Table 2. M40-A2 Viability and overgrowth compliance for Purflock swabs using Qualitative and Quantitative Methods

Bacteria	Swab	Temp	Qualitative (Roll Plate) CFU				Quantitative (Swab Elution) CFU/ml			
			0hr	24hr	48hr	M40-A2 Compliance	0hr	24hr	48hr	M40-A2 Compliance
Pseudomonas aeruginosa ATCC® BAA-427	Purflock	Room temp.	n/a	n/a	n/a	n/a	$4.53x10^7$	$1.50 x10^9$	$1.67 x10^9$	✓
		4°C	9	52	90	✓		$2.37 x10^8$	$1.34 x10^8$	✓
	Foam	Room temp.	n/a	n/a	n/a	n/a	$3.27 x10^7$	$1.30 x10^9$	$3.77 x10^8$	✓
		4°C	20	87	109	✓		$8.73 x10^7$	$3.3 x10^8$	✓
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	✓		$1.23 x10^6$	$6.80 x10^5$	✓
	Foam	Room temp.	168	11	7	✓	$3.27 x10^7$	$1.10 x10^6$	$2.17 x10^5$	✓
		4°C		42	11	✓		$1.16 x10^6$	$1.19 x10^5$	✓
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	✓		$1.53 x10^6$	$1.05 x10^6$	✓
	Foam	Room temp.	225	131	74	✓	$6.27 x10^6$	$6.40 x10^6$	$1.37 x10^6$	✓
		4°C		216	202	✓		$1.73 x10^6$	$7.60 x10^6$	✓
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	✓		$8.40 x10^5$	$3.00 x10^5$	✓
	Foam	Room temp.	201	43	12	✓	$8.30 x10^6$	$4.53 x10^6$	$2.67 x10^6$	✓
		4°C		108	23	✓		$6.70 x10^6$	$7.37 x10^6$	✓
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	✓		$5.87 x10^6$	$2.80 x10^6$	✓
	Foam	Room temp.	73	95	38	✓	$1.03 x10^7$	$5.20 x10^6$	$9.3 x10^6$	✓
		4°C		92	80	✓		$6.07 x10^6$	$6.5 x10^6$	✓
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	✓		$3.46 x10^7$	$5.43 x10^6$	✓
	Foam	Room temp.	187	108	80	✓	$9.83 x10^7$	$1.63 x10^7$	$7.10 x10^6$	✓
		4°C		105	74	✓		$4.57 x10^7$	$9.41 x10^6$	✓
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	✓		$9.04 x10^6$	$7.36 x10^5$	✓
	Foam	Room temp.	301	176	105	✓	$2.56 x10^8$	$7.01 x10^6$	$2.04 x10^6$	✓
		4°C		165	135	✓		$9.56 x10^7$	$2.30 x10^7$	✓
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	✓		$3.04 x10^7$	$8.72 x10^6$	✓
	Foam	Room temp.	187	78	31	✓	$7.04 x10^7$	$6.2 x10^6$	$4.31x10^6$	✓
		4°C		69	52	✓		$1.2 x10^7$	$6.96x10^6$	✓
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	✓		$6.21 x10^7$	$3.65 x10^6$	✓
	Foam	Room temp.	297	215	108	✓	$4.35 x10^8$	$4.71 x10^7$	$9.09 x10^6$	✓
		4°C		246	178	✓		$9.09 x10^7$	$5.41 x10^7$	✓
Neisseria gonorrhoeae ATCC® 43069	Purflock	Room temp.	247	8	n/a	✓	$7.5 x10^4$	$8.6x10^1$	n/a	✓
		4°C		13	n/a	✓		$1.4x10^2$	n/a	✓
	Foam	Room temp.	**267**	**52**	n/a	✓	$8.13 x10^6$	$4.67 x10^5$	n/a	✓
		4°C		**65**	n/a	✓		$1.20 x10^6$	n/a	✓

Discussion

DNA Extraction using the Sigma Aldrich GenElute Bacterial Genomic DNA kit for all ten M40-A2 organisms was successful.

Nanodrop results showed that DNA from all M40-A2 organisms can successfully be extracted after the 24hr holding period from Liquid Amies Swab Transport System. PCR effectively amplified the selected genetic region of 16S Ribosomal DNA from all M40-A2 organisms using the designed primers, generating the correctly sized PCR product as shown by the Agarose Gel Electrophoresis results in fig. 1 & 2.

In addition the Sigma 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 success of DNA extraction using molecular methods suggests that swabs are a useful addition to support quicker laboratory diagnosis and reduce confirmation times for bacterial infections and can be used in conjunction with molecular and conventional processing platforms.

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*

Sigma Transwab® PurFlock® (Standard & MiniTip) is not sold in US.