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Microorganism Performance Evaluation of Puritan Liquid Amies Collection and Transport System

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Introduction:

Proper specimen collection and transport plays a critical role in laboratory diagnosis of infectious diseases such as those associated with aerobic, anaerobic, and fastidious bacteria. Therefore, bacteriological transport systems are commonly used to facilitate the diagnosis of bacteria-related infections specifically, when there is a delay between specimen collection and laboratory processing.[1] Puritan Liquid Amies Collection and Transport System is a self-contained ready to use system that allows for the safe and efficient transport of clinical samples to the testing laboratory. This facilitates bacterial detection and isolation, thus aiding in diagnosis of diseases associated with such infectious organisms.

Bacterial viability studies are routinely performed as a means to validate the performance of swab transport devices. Two commonly used techniques for this purpose are the Roll-Plate Method and the Swab Elution Method.[2] The Roll-Plate Method is typically used as a way to inoculate swab transport systems onto solid media; this reflects the standard inoculation approach used in many laboratories as a qualitative method. Conversely, the Swab Elution Method allows for semi-quantitative evaluation of the transport systems ability to sustain viable organisms, but fails to reflect standard laboratory practices.[2] For this reason, both methods were used when evaluating the performance of Puritan Liquid Amies Collection and Transport System.

The performance characteristics of Puritan Liquid Amies Collection and Transport System were determined using the procedures outlined in the Clinical Laboratory Standards Institute (CLSI) M40-A document.[2] A variety of aerobic, anaerobic, and fastidious organisms were included in this study. The test organisms were comprised of the ten ATCC strains that are recommended in the CLSI M40-A document for determining performance characteristics of swab transport systems.[2] To determine the performance characteristics of Puritan Liquid Amies Collection and Transport Systems, bacterial viability studies were performed. These studies were conducted at two different temperatures to reflect refrigerated (4-8°C) and room temperature (20-25°C) conditions. The swabs from each transport system were inoculated with a specified volume of select bacterial concentrations. These swabs were then placed in their respective transport vials and held for 0, 24, and 48 h. At the designated time intervals, the swabs were removed and processed. These studies were conducted using both the Roll-Plate and Swab Elution Methods.

Materials and Methods:

Test Organisms and Media:

The ten ATCC strains recommended in the CLSI M40-A document were used in this evaluation. This includes: *Pseudomonas aeruginosa* ATCC BAA-427, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 6305, *Haemophilus influenzae* ATCC 10211, *Bacteroides fragilis* ATCC 25285, *Peptostreptococcus anaerobius* ATCC 27337, *Fusobacterium nucleatum* ATCC 25586, *Propionibacterium acnes* ATCC 6919, *Prevotella melaninogenica* ATCC 25845, and *Neisseria gonorrhoeae* ATCC 43069 (test organisms were obtained from PML Microbiologicals, Inc.: Wilsonville, OR). All isolates were grown on an appropriate agar medium. This included Columbia agar with 5% sheep blood (bioMérieux: Marcy l'Etoile, France), Chocolate agar (Becton Dickinson: Sparks, MD), and *Brucella* agar with 5% sheep blood (Becton Dickinson). The test organisms were incubated at 37°C under appropriate atmospheric conditions (anaerobic conditions were achieved using the BD GasPak EZ Gas Generating Container Systems).

Testing Set up and Procedure:

The ten ATCC strains, recommended in the CLSI M40-A document, were used in an equivalency study to compare the performance of two liquid Amies based transport systems. The test device, Puritan Liquid Amies Collection and Transport System was compared to a predicate device, the BD Liquid Amies Elution Swab (ESwab) Collection and Transport System manufactured by Copan Diagnostics Inc. The testing of each device was completed using the swabs included in each transport kit. Each round of testing was performed in duplicate with transport vials being held at room temperature (20-25°C) and refrigerated temperatures (4-8°C) for the duration of the study. Viability studies were performed at 0, 24, and 48 h using the Roll-Plate Method and Swab Elution method outlined in the CLSI M40-A2 document.

Roll-Plate Method:

To begin, a bacterial suspension was prepared from each fresh culture (18-24 hours old) in a separate vial containing 10 mL of 0.85 % sterile saline and verified by 0.5 McFarland Standard (DensiCHEK PLUS, bioMérieux) to obtain 1.5 x 10^8CFU/mL suspensions. From the starting suspensions, a series of five ten-fold dilutions were made resulting in final concentrations of 1.5×10^7, 1.5×10^6, 1.5×10^5, 1.5×10^4, and 1.5×10^3 CFU/ml. 100 µl aliquots of the desired concentrations were then transferred into the wells of a 96-well microtiter plate. The appropriate swabs were then immersed in the corresponding wells and allowed to absorb for approximately 15-20 seconds. Following absorption, the swabs were removed from the wells and immediately placed in vials containing Puritan Liquid Amies Collection and Transport System medium or the predicate device's medium. The swabs were held in the transport medium, at

the desired temperatures, for 0, 24, and 48 h. At each time point the swabs were removed from the transport vials and used to inoculate the entire surface of an appropriate solid agar plate. The vials were then discarded and the plates were incubated at 37°C in the appropriate environment for 24-48 h or until countable colonies were visible. Manual colony counts were conducted at all three time intervals for each swab-organism combination and the average was recorded. Three dilutions were analyzed for each test organism and the dilution yielding zero-time colony counts nearest to 300 CFU was reported.

Swab Elution Method:

To begin, a bacterial suspension was prepared from each fresh culture (18-24 hours old) in a separate vial containing 10 mL of 0.85 % sterile saline and verified by 0.5 McFarland Standard (DensiCHEK PLUS, bioMérieux) to obtain 1.5 x 10^8CFU/mL suspensions. This suspension was further diluted 1:10 in sterile saline resulting in an inoculum concentration of approximately 1.5 x 10^7 CFU/ml. 100 µl aliquots of the 1.5 x 10^7 CFU/ml inoculum were then transferred into the wells of a 96-well microtiter plate. The appropriate swabs were then immersed in the corresponding wells and allowed to absorb for approximately 15-20 seconds. Following absorption, the swabs were removed from the wells and immediately placed in vials containing Puritan Liquid Amies Collection and Transport System medium or the predicate device's medium. The swabs were held in the transport medium, at the desired temperatures, for 0, 24, and 48 h. At each time interval, the swabs were vortexed in the transport vials for at least 15 s and discarded. A 100 µl aliquot of the liquid Amies medium was then added to 0.9 ml of sterile saline. Serial tenfold dilutions were then prepared in 0.9 ml of sterile saline, resulting in final concentrations of approximately 10^5, 10^4, 10^3, 10^2, and 10^1 CFU/ml. A 100 µl aliquot was removed from each dilution and immediately placed on the surface of an appropriate agar media. A sterile spreader was then used to inoculate the entire surface of the agar. All plates were incubated at 37°C in appropriate atmospheric conditions. After incubation, manual colony counts were conducted at all three time intervals and the average CFU was recorded. The average CFU at 48 h of storage (24 h for *N. gonorrhoeae*) was then compared to the average CFU at 0 h. According to the CLSI M40-A document, for a swab system to be considered acceptable for room temperature storage, there can be no more than a 3 $\log_{10}$ (1 x 10^3 CFU ± 10%) decline in CFU from the 0 h result. For storage at refrigerated temperatures, there can be no more than a 3 $\log_{10}$ (1 x 10^3 CFU ± 10%) decline or a 1 log increase in CFU from the 0 h result.[2]

Note: There is no CLSI M40-A interpretation for *N. gonorrhoeae* beyond 24 h of storage; this applies to both the Roll-Plate method and Swab Elution method.

Results:

Table 1. Performance evalution of Puritan Liquid Amies Collection and Transport System vs. Predicate Device Roll-Plate Method, 20-25°C

Organism	0.5 McFarland microorganism suspension diluted with saline	Product Lot Numbers	Average CFUs Recovered: Time 0 h	Average CFUs Recovered: Time 24 h	Average CFUs Recovered: Time 48 h
Pseudomonas aeruginosa ATCC BAA-427	Diluted 10^{-4}	Puritan - 111101	308	310	81
		Puritan - 110907	241	251	111
		Puritan - 111209	267	260	106
		BD - 527045	304	208	91
Streptococcus pyogenes ATCC 19615	Diluted 10^{-4}	Puritan - 111101	250	204	77
		Puritan - 110907	194	210	131
		Puritan - 111209	245	191	77
		BD - 527045	262	222	85
Streptococcus pneumoniae ATCC 6305	Diluted 10^{-4}	Puritan - 111101	134	101	34
		Puritan - 110907	200	88	65
		Puritan - 111209	171	164	74
		BD - 527045	130	88	29
Haemophilus influenzae ATCC 10211	Diluted 10^{-4}	Puritan - 111101	264	254	82
		Puritan - 110907	236	136	48
		Puritan - 111209	250	198	61
		BD - 527045	278	302	95
Bacteroides fragilis ATCC 25285	Diluted 10^{-3}	Puritan - 111101	320	265	109
		Puritan - 110907	200	117	64
		Puritan - 111209	270	285	105
		BD - 527045	310	210	91
Peptostreptococcus anaerobius ATCC 27337	Diluted 10^{-3}	Puritan - 111101	265	118	41
		Puritan - 110907	260	130	85
		Puritan - 111209	225	150	18
		BD - 527045	254	128	46
Fusobacterium nucleatum ATCC 25586	Diluted 10^{-3}	Puritan - 111101	199	105	26
		Puritan - 110907	265	109	40
		Puritan - 111209	213	281	33
		BD - 527045	160	90	31
Propionibacterium acnes ATCC 6919	Diluted 10^{-4}	Puritan - 111101	280	161	57
		Puritan - 110907	279	96	29
		Puritan - 111209	202	196	65
		BD - 527045	248	171	40
Prevotella melaninogenica ATCC 25845	Diluted 10^{-3}	Puritan - 111101	271	121	29
		Puritan - 110907	264	96	21
		Puritan - 111209	289	165	16
		BD - 527045	246	119	20
Neisseria gonorrhoeae ATCC 43069	Diluted 10^{-4}	Puritan - 111101	264	150	
		Puritan - 110907	226	131	
		Puritan - 111209	258	158	
		BD - 527045	196	131	

Table 2. Performance evalution of Puritan Liquid Amies Collection and Transport System vs. Predicate Device Roll-Plate Method, 4-8°C

Organism	0.5 McFarland microorganism suspension diluted with saline	Product Lot Numbers	Average CFUs Recovered: Time 0 h	Average CFUs Recovered: Time 24 h	Average CFUs Recovered: Time 48 h
Pseudomonas aeruginosa ATCC BAA-427	Diluted 10^{-4}	Puritan - 111101	308	240	46
		Puritan - 110907	241	113	54
		Puritan - 111209	267	281	128
		BD - 527045	304	176	64
Streptococcus pyogenes ATCC 19615	Diluted 10^{-4}	Puritan - 111101	250	200	41
		Puritan - 110907	194	111	78
		Puritan - 111209	245	102	81
		BD - 527045	262	119	22
Streptococcus pneumoniae ATCC 6305	Diluted 10^{-4}	Puritan - 111101	134	61	16
		Puritan - 110907	200	35	35
		Puritan - 111209	171	122	59
		BD - 527045	130	73	14
Haemophilus influenzae ATCC 10211	Diluted 10^{-4}	Puritan - 111101	264	134	45
		Puritan - 110907	236	83	38
		Puritan - 111209	250	136	47
		BD - 527045	278	111	61
Bacteroides fragilis ATCC 25285	Diluted 10^{-3}	Puritan - 111101	320	220	52
		Puritan - 110907	200	103	33
		Puritan - 111209	270	230	96
		BD - 527045	310	190	36
Peptostreptococcus anaerobius ATCC 27337	Diluted 10^{-3}	Puritan - 111101	265	101	49
		Puritan - 110907	260	96	39
		Puritan - 111209	225	158	10
		BD - 527045	254	88	26
Fusobacterium nucleatum ATCC 25586	Diluted 10^{-3}	Puritan - 111101	199	85	14
		Puritan - 110907	265	67	21
		Puritan - 111209	213	181	41
		BD - 527045	160	60	4
Propionibacterium acnes ATCC 6919	Diluted 10^{-4}	Puritan - 111101	280	186	23
		Puritan - 110907	279	77	25
		Puritan - 111209	202	164	108
		BD - 527045	248	193	26
Prevotella melaninogenica ATCC 25845	Diluted 10^{-3}	Puritan - 111101	271	114	19
		Puritan - 110907	264	121	16
		Puritan - 111209	289	77	46
		BD - 527045	246	89	23
Neisseria gonorrhoeae ATCC 43069	Diluted 10^{-4}	Puritan - 111101	264	119	
		Puritan - 110907	226	90	
		Puritan - 111209	258	160	
		BD - 527045	196	99	

Table 3. Performance evalution of Puritan Liquid Amies Collection and Transport System vs. Predicate Device Swab Elution Method, 20-25°C

Organism	0.5 McFarland microorganism suspension diluted with saline	Product Lot Numbers	Average CFUs Recovered: Time 0 h	Average CFUs Recovered: Time 24 h	Average CFUs Recovered: Time 48 h	Log_{10} Decline
Pseudomonas aeruginosa ATCC BAA-427	Diluted 1:10	Puritan - 111101	$1.0x10^6$	$1.2x10^6$	$2.1x10^5$	-0.68
		Puritan - 110907	$1.2x10^6$	$1.3x10^6$	$6.1x10^5$	-0.29
		Puritan - 111209	$2.7x10^6$	$1.9x10^6$	$1.8x10^6$	-0.18
		BD - 527045	$2.8x10^6$	$3.0x10^6$	$2.6x10^6$	-0.03
Streptococcus pyogenes ATCC 19615	Diluted 1:10	Puritan - 111101	$2.1x10^6$	$2.7x10^6$	$7.4x10^5$	-0.45
		Puritan - 110907	$2.6x10^6$	$9.1x10^5$	$3.5x10^5$	-0.87
		Puritan - 111209	$7.6x10^5$	$1.0x10^6$	$2.5x10^5$	-0.48
		BD - 527045	$3.1x10^6$	$1.9x10^6$	$6.8x10^5$	-0.65
Streptococcus pneumoniae ATCC 6305	Diluted 1:10	Puritan - 111101	$2.2x10^6$	$1.8x10^6$	$5.5x10^5$	-0.60
		Puritan - 110907	$1.4x10^6$	$8.8x10^5$	$3.1x10^5$	-0.65
		Puritan - 111209	$2.1x10^6$	$1.1x10^6$	$9.1x10^5$	-0.36
		BD - 527045	$1.8x10^6$	$1.6x10^6$	$1.0x10^6$	-0.26
Haemophilus influenzae ATCC 10211	Diluted 1:10	Puritan - 111101	$2.6x10^6$	$7.8x10^5$	$7.1x10^5$	-0.56
		Puritan - 110907	$2.1x10^6$	$1.4x10^6$	$5.1x10^5$	-0.61
		Puritan - 111209	$3.1x10^6$	$2.0x10^6$	$1.5x10^6$	-0.32
		BD - 527045	$3.0x10^6$	$9.2x10^5$	$1.3x10^6$	-0.36
Bacteroides fragilis ATCC 25285	Diluted 1:10	Puritan - 111101	$1.7x10^6$	$1.2x10^6$	$2.1x10^5$	-0.91
		Puritan - 110907	$9.9x10^5$	$5.4x10^5$	$2.8x10^5$	-0.55
		Puritan - 111209	$2.9x10^6$	$2.1x10^6$	$1.3x10^6$	-0.35
		BD - 527045	$3.0x10^6$	$2.3x10^6$	$1.9x10^6$	-0.20
Peptostreptococcus anaerobius ATCC 27337	Diluted 1:10	Puritan - 111101	$3.1x10^6$	$1.5x10^6$	$4.1x10^5$	-0.88
		Puritan - 110907	$2.0x10^6$	$1.3x10^6$	$4.2x10^5$	-0.68
		Puritan - 111209	$1.9x10^6$	$7.0x10^5$	$3.9x10^5$	-0.69
		BD - 527045	$1.8x10^6$	$7.4x10^5$	$4.1x10^5$	-0.64
Fusobacterium nucleatum ATCC 25586	Diluted 1:10	Puritan - 111101	$2.0x10^6$	$8.6x10^5$	$2.5x10^5$	-0.90
		Puritan - 110907	$1.9x10^6$	$7.8x10^5$	$2.1x10^5$	-0.96
		Puritan - 111209	$2.6x10^6$	$6.1x10^5$	$3.0x10^5$	-0.94
		BD - 527045	$3.3x10^6$	$7.6x10^5$	$2.9x10^5$	-1.06
Propionibacterium acnes ATCC 6919	Diluted 1:10	Puritan - 111101	$2.3x10^6$	$1.2x10^6$	$7.7x10^5$	-0.48
		Puritan - 110907	$2.0x10^6$	$9.9x10^5$	$6.2x10^5$	-0.51
		Puritan - 111209	$1.0x10^6$	$6.2x10^5$	$2.1x10^5$	-0.68
		BD - 527045	$1.9x10^6$	$4.8x10^5$	$2.7x10^5$	-0.85
Prevotella melaninogenica ATCC 25845	Diluted 1:10	Puritan - 111101	$1.8x10^6$	$8.9x10^5$	$5.6x10^5$	-0.51
		Puritan - 110907	$1.5x10^6$	$5.3x10^5$	$3.5x10^5$	-0.63
		Puritan - 111209	$1.9x10^6$	$4.2x10^5$	$1.7x10^5$	-1.05
		BD - 527045	$2.3x10^6$	$1.9x10^5$	$2.7x10^5$	-0.93
Neisseria gonorrhoeae ATCC 43069	Diluted 1:10	Puritan - 111101	$1.1x10^6$	$1.0x10^5$		-1.04
		Puritan - 110907	$9.9x10^5$	$4.6x10^5$		-0.33
		Puritan - 111209	$1.3x10^6$	$1.3x10^5$		-1.00
		BD - 527045	$1.4x10^6$	$1.0x10^5$		-1.15

Table 4. Performance evalution of Puritan Liquid Amies Collection and Transport System vs. Predicate Device Swab Elution Method, 4-8°C

Organism	0.5 McFarland microorganism suspension diluted with saline	Product Lot Numbers	Average CFUs Recovered: Time 0 h	Average CFUs Recovered: Time 24 h	Average CFUs Recovered: Time 48 h	Log_{10} Decline
Pseudomonas aeruginosa ATCC BAA-427	Diluted 1:10	Puritan - 111101	$1.0x10^6$	$9.5x10^5$	$5.0x10^5$	-0.30
		Puritan - 110907	$1.2x10^6$	$9.5x10^5$	$3.0x10^5$	-0.6
		Puritan - 111209	$2.7x10^6$	$4.3x10^5$	$8.8x10^5$	-0.49
		BD - 527045	$2.8x10^6$	$6.5x10^5$	$1.1x10^6$	-0.41
Streptococcus pyogenes ATCC 19615	Diluted 1:10	Puritan - 111101	$2.1x10^6$	$7.7x10^5$	$2.5x10^5$	-0.92
		Puritan - 110907	$2.6x10^6$	$5.0x10^5$	$2.3x10^5$	-1.05
		Puritan - 111209	$7.6x10^5$	$1.0x10^6$	$6.1x10^5$	-0.1
		BD - 527045	$3.1x10^6$	$9.6x10^5$	$8.6x10^5$	-0.56
Streptococcus pneumoniae ATCC 6305	Diluted 1:10	Puritan - 111101	$2.2x10^6$	$1.1x10^6$	$1.3x10^5$	-1.23
		Puritan - 110907	$1.4x10^6$	$6.8x10^5$	$1.3x10^5$	-1.03
		Puritan - 111209	$2.1x10^6$	$1.1x10^6$	$1.6x10^6$	-0.12
		BD - 527045	$1.8x10^6$	$7.8x10^5$	$1.0x10^6$	-0.26
Haemophilus influenzae ATCC 10211	Diluted 1:10	Puritan - 111101	$2.6x10^6$	$4.1x10^5$	$3.4x10^5$	-0.88
		Puritan - 110907	$2.1x10^6$	$1.0x10^6$	$2.0x10^5$	-1.02
		Puritan - 111209	$3.1x10^6$	$3.8x10^5$	$4.2x10^5$	-0.87
		BD - 527045	$3.0x10^6$	$5.0x10^5$	$3.4x10^5$	-0.95
Bacteroides fragilis ATCC 25285	Diluted 1:10	Puritan - 111101	$1.7x10^6$	$8.1x10^5$	$7.8x10^5$	-0.34
		Puritan - 110907	$9.9x10^5$	$6.1x10^5$	$2.0x10^5$	-0.69
		Puritan - 111209	$2.9x10^6$	$7.9x10^5$	$9.9x10^5$	-0.47
		BD - 527045	$3.0x10^6$	$9.6x10^5$	$1.1x10^6$	-0.44
Peptostreptococcus anaerobius ATCC 27337	Diluted 1:10	Puritan - 111101	$3.1x10^6$	$5.5x10^5$	$1.3x10^5$	-1.38
		Puritan - 110907	$2.0x10^6$	$7.7x10^5$	$1.5x10^5$	-1.12
		Puritan - 111209	$1.9x10^6$	$6.1x10^5$	$9.7x10^5$	-0.29
		BD - 527045	$1.8x10^6$	$5.1x10^5$	$9.7x10^5$	-0.27
Fusobacterium nucleatum ATCC 25586	Diluted 1:10	Puritan - 111101	$2.0x10^6$	$2.4x10^5$	$1.4x10^5$	-1.15
		Puritan - 110907	$1.9x10^6$	$3.0x10^5$	$1.8x10^5$	-1.02
		Puritan - 111209	$2.6x10^6$	$5.0x10^5$	$5.8x10^5$	-0.65
		BD - 527045	$3.3x10^6$	$4.2x10^5$	$4.4x10^5$	-0.88
Propionibacterium acnes ATCC 6919	Diluted 1:10	Puritan - 111101	$2.3x10^6$	$7.5x10^5$	$4.4x10^5$	-0.72
		Puritan - 110907	$2.0x10^6$	$4.6x10^5$	$4.9x10^5$	-0.61
		Puritan - 111209	$1.0x10^6$	$9.6x10^5$	$4.5x10^5$	-0.35
		BD - 527045	$1.9x10^6$	$7.1x10^5$	$4.9x10^5$	-0.59
Prevotella melaninogenica ATCC 25845	Diluted 1:10	Puritan - 111101	$1.8x10^6$	$3.0x10^5$	$3.2x10^5$	-0.75
		Puritan - 110907	$1.5x10^6$	$3.5x10^5$	$1.7x10^5$	-0.95
		Puritan - 111209	$1.9x10^6$	$3.0x10^5$	$1.2x10^5$	-1.2
		BD - 527045	$2.3x10^6$	$4.8x10^5$	$1.9x10^5$	-1.08
Neisseria gonorrhoeae ATCC 43069	Diluted 1:10	Puritan - 111101	$1.1x10^6$	$2.3x10^5$		-0.68
		Puritan - 110907	$9.9x10^5$	$6.7x10^5$		-0.17
		Puritan - 111209	$1.3x10^6$	$1.6x10^6$		-0.09
		BD - 527045	$1.4x10^6$	$1.2x10^6$		-0.07

Summary and Conclusions:

The purpose of this study was to compare the performance of the Puritan Liquid Amies Collection and Transport System with a similar device that is currently on the market. The performance evaluation was carried out at two different temperatures using both the Roll-Plate and Swab Elution method as described in the CLSI M40-A document. Based on the evaluation of the results, it can be concluded that the Puritan Liquid Amies Collection and Transport System for aerobic, anaerobic, and fastidious bacteria is equivalent in performance to the predicate device.

Resources:

1. Van Horn, K.G., C.D. Audette, D. Sebeck, K.A. Tucker. Tucker. 2008. Comparison of the Copan eSwab System with Two Amies agar Swab Transport for Maintenance of Microorganism Viability. J. Clin. Microbiol. 46: 1655-1658.
2. Clinical Laboratory Standard Institute (CLSI). 2003. Quality Control of Microbiological Transport Systems; Approved Standard. M40-A vol. 23 No. 34

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