Engineering Validation of Puritan's Liquid Amies Transport System and Copan's WASP Automation

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ABSTRACT

INTRODUCTION: The objective of this evaluation was to assess the acceptability of Puritan's liquid Amies transport tubes (Puritan Medical Products Co., Maine, USA) for use on Copan's automated WASP equipment (Copan Diagnostics Inc., Brescia, Italy) specifically designed for the Copan E-Swab system. Results were compared with those obtained using the E-Swab system run in parallel. Because of the similarity of the two brands of transport tubes (photo #1), we decided to test the two kits together on the WASP (photo #2). The WASP is designed with many built in sensors to monitor each stage of handling of transport tubes by attaching unique bar code labels to plates, holding the tubes for recognizing the specimen, determining whether sufficient sample is present, and uncapping and rejecting any faulty specimen. The purpose of this evaluation therefore, was to determine if the instrument was able to differentiate similar brands in order to offer laboratories the flexibility of using other cost effect alternatives. Puritan Transport System kits were provided by Puritan Medical Products Co. LLC. Copan's E-Swab kits and 0.85% physiological saline tubes were purchased from Bio-Media Unlimited Ltd. Choc and BAP agar plates were obtained from Oxoid Canada and test organisms were obtained from ATCC: NG ATCC 43069, HIN ATCC 10211, SPY ATCC 19615, SPN ATCC 6305, BFR ATCC 25285, FNU ATCC 25586, PME ATCC 25845, PAN ATCC 27337, PAC ATCC 6919 and MRSA ATCC 8610. (1) 36 transport devices per test isolate were unwrapped, tubes removed from the sleeve, premade barcode labels attached to tubes and arranged in a rack. (2) 100µl aliquots of the working suspension were transferred into wells of a round bottom microtitre plate using an Eppendorf pipette. (3) Three Copan flocked swabs followed by three Puritan swabs were immersed into 100 ul of the organism suspension in the wells containing the inoculum. (4) All the swabs were removed and inserted into their respective transport device in the order they were placed in the wells. (5) The same procedure was followed for all four time periods (0, 2, 24 and 48 h) with each organism/device combination performed in triplicate. (6) Batches of the same dilution tubes were immediately placed on the WASP for processing/ inoculation onto choc or blood agar plates and incubated in CO_2 and/or ANO_2 for a minimum of 48 to 72 h. **RESULTS:** MRSA: Both Pur and Cop swabs appeared to grow rapidly after 0 h baseline but Cop swabs seemed to grow more rapidly. SPN: Pur swabs had higher colony counts than Cop. NG: Pur had higher counts and grew at 24 h at the highest dilution; Cop had no growth at 24 h. HIN: Mixed counts but Pur appeared to do better overall. BFR: Pur had higher counts at 0 and 2 h, Cop had higher counts at 24 and 48 h. PME: Pur had higher counts at 0 and 2 h , no growth for both at 24 and 48 h. PAC: Pur had higher counts at 0, 2, 24 and 48 h. PAN: Same growth for both at 0 h , Pur higher at 2 h, no growth for both at 24 and 48 h. FNU: Pur had higher counts at 0 h, no growth for both at 24 and 48 h. SPY: Counts for Pur and Cop were similar. **CONCLUSIONS:** Puritan's transport tubes appear to be interchangeable with Copan's tubes when using the WASP system and generally result in higher colony counts.

MATERIALS AND METHOD

MATERIALS AND METHODS: (organisms, swabs and media) For this study Puritan Liquid Amies Transport System kits were provided by Puritan Medical Products Co. LLC and Copan Liquid Amies Transport System (E-Swab) kits and 3.0mL 0.85% physiological saline tubes were purchased from Bio-Media Unlimited Ltd, Woodbridge Ontario. Chocolate agar plates and Blood agar with 5% sheep blood plates were obtained from Oxoid Canada. CLSI M40A QC *recommended* organisms were obtained from ATCC: *Neisseria gonorrhoeae* (NG) ATCC 43069, *Haemophilus influenzae* (HIN) ATCC 10211 Streptococcus *pyogenes* (SPY) ATCC 19615 *Streptococcus pneumonia* (SPN) ATCC 6305 *Bacteriodes fragilis* (BFR) ATCC 25285, *Fusobacterium nucleatum* (FNU) ATCC 25586, *Prevotella melaninogenica* (PME) ATCC 25845, *Peptostreptococcus anaerobius* (PAN) ATCC27337 *propionibacterium acnes* (PAC) ATCC 6919 and Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 8610.

INOCULUM PREPARATION: Each test organism was reconstituted from a lyophilized ATCC culture and subcultured twice to chocolate agar or 5% sheep blood agar plates. A fresh 18 - 24 hour culture of each bacterial strain, (48 hour for anaerobes) was used to prepare inoculum suspensions that matched 0.5 McFarland turbidity standards (~ 1.5 $\times 10^8$ cfu/ml) prepared in 0.85 % sterile physiologic saline (pH 6.8-7.2) using a DensiCHEK™ turbidity meter (bioMerieux). From this working suspension six 1:10 serial dilutions were prepared: 1:10, 1:100, 1:1000, 1:10.000, 1:100,00 and 1:1,000,000 representing 1.5× 10^7, 1.5 $\times 10^6$, 1.5$\times 10^5$, 1.5$\times 10^4$, 1.5 $\times 10^3$ and 1.5 $\times 10^2$ CFU/mL respectively Three dilutions (10^5, 10^4, and 10^3) were used for testing. From the serially diluted suspensions, duplicate plates of 100uL of 10^5, 10^4, 10^3 and 10^2 dilutions were used as control. The DensiCHEK™ turbidity meter was first validated before actual testing by preparing five log10 serial dilutions in 3.0 ml saline tubes from the working suspension and plating out 100 µl in duplicate.

INOCULATION PROCEDURE: 36 transport devices per test isolate were unwrapped, tubes removed from the sleeve, premade barcode labels attached to tubes and arranged in a rack. 100µl aliquots of the working suspension were transferred into wells of a round bottom microtitre plate using an Eppendorf pipette. Three Copan flocked swabs followed by three Puritan swabs were immersed into 100ul of the organism suspension in the wells containing the inoculum. Immediately starting with Copan swabs, all the swabs were removed and inserted into their respective transport device in the order they were placed in the wells. The same procedure was followed for all four time periods (0, 2, 24 and 48 h) with each microorganism/ device combination performed in triplicate. Batches of the same dilution tubes were immediately placed on the WASP for processing/inoculation on chocolate or blood agar plates and incubated in CO2 and/or anaerobically for a minimum of 48 to 72 h. Table 1.

Species	ATCC No.	Plate Media	Incubation Temp °C	Incubation atmosphere	Testing Time (hours)
Neisseria gonorrhoeae	43069	Chocolate agar	35-37	5% CO_2	0,2, 24
Haemophilus influenzae	10211	Chocolate agar	35-37	5% CO_2	0,2, 24, 48
Streptococcus pyogenes	19615	5% sheep blood agar	35 -37	5% CO_2	0,2, 24, 48
Streptococcus pneumoniae	6305	5% sheep blood agar	35-37	5% CO_2	0,2, 24, 48
MRSA	8610	5% sheep blood agar	35-37	5% CO_2	0,2 ,24, 48
Bacteroides fragilis	25285	5% sheep blood agar	35-37	Ano_2	0,2, 24, 48
Peptostreptococcus anaerobius	27337	5% sheep blood agar	35-37	Ano_2	0,2, 24, 48
Fusobacterium nucleatum	25586	5% sheep blood agar	35-37	Ano_2	0,2, 24, 48
Propionibacterium acnes	6919	5% sheep blood agar	35-37	Ano_2	0,2, 24, 48
Prevotella melaninogenica	25845	5% sheep blood agar	35-37	Ano_2	0,2,24, 48

RESULTS

RESULTS: MRSA: Both Copan and Puritan swabs appear to grow rapidly after 0 h baseline but Copan swabs seem to grow more rapidly. SPN: Puritan swabs have higher counts than Copan. NG: Puritan has higher counts and grows at 24 h at the highest dilution. Copan has no growth at 24 h. HIN: Mixed counts but Puritan appears to do better overall. BFR: Puritan has higher counts at 0 and 2 h, Copan has higher counts at 24 and 48 h. PME: Puritan has higher counts at 0 and 2 h, no growth for both at 24 and 48 h. PAC: Puritan has higher counts at 0, 2, 24 and 48 h. PAN: Same growth for both at 0 h, Puritan higher at 2 h, no growth for both at 24 and 48 h. : Puritan has higher counts at 0 h, no growth for both at 24 and 48 h. SPY: Counts for Puritan and Copan are similar.

Organism		0hrs				2hrs				24hrs				48hrs			
		10^5	10^4	10^3	10^2	10^5	10^4	10^3	10^2	10^5	10^4	10^3	10^2	10^5	10^4	10^3	10^2
MRSA	Puritan	147	9	1	ND	140.3	16.3	0.6	ND	tntc	10.3	2	ND	tntc	tntc	1.3	ND
	Copan	194.6	23.6	2	ND	151.3	19.3	2	ND	tntc	70.3	6.3	ND	tntc	tntc	tntc	ND
Streptococcus pneumoniae	Puritan	56.6	4.6	0	ND	78.6	8.3	1.6	ND	39	5	0	ND	14	1.6	0	ND
	Copan	45.6	4	0	ND	48.6	5	0.3	ND	23.6	4	0	ND	14.6	0.3	0.3	ND
Neisseria gonorrhoeae	Puritan	94.3	5	0	ND	52	3.6	0	ND	3.3	0	0	ND				ND
	Copan	78.3	4	0	ND	45	3.6	0	ND	0	0	0	ND				ND
Haemophilus influenzae	Puritan	>500	51	5.3	ND	>500	63	4.3	ND	89.3	18.6	1	ND	33.6	9	0.3	ND
	Copan	>500	62.3	3	ND	>500	47.3	5.3	ND	112.6	12.3	0.6	ND	5	1.3	0.6	ND
Bacteroides fragilis	Puritan	~300	55.6	6	ND	~300	44.3	5.6	ND	~150	13.3	0.6	ND	~150	13.6	1.5	ND
	Copan	~300	45	5.6	ND	~300	36	1.6	ND	~150	21.3	2.3	ND	~150	16.6	2.3	ND
Prevotella melaninogenica	Puritan	154.6	25.6	4.6	ND	104.3	17.6	2	ND	0	0	0	ND	0	0	0	ND
	Copan	133.6	17.6	1.3	ND	99.3	12	1.6	ND	0	0	0	ND	0	0	0	ND
Propionibacterium acnes	Puritan	>500	68	6.6	ND	>500	75	7.6	ND	>300	41.6	4.6	ND	>300	40.3	2.6	ND
	Copan	>500	52	5.6	ND	>500	52.6	7.6	ND	>300	34.6	4.3	ND	>300	21.3	1.6	ND
Peptostreptococcus anaerobius	Puritan	91	8	1.6	ND	66.6	11	0	ND	0	0	0	ND	0	0	0	ND
	Copan	93.3	8	0.3	ND	14.3	2.3	0.3	ND	0	0	0	ND	0	0	0	ND
Fusobacterium nucleatum	Puritan	164	15.3	4.6	ND	163.6	14.6	6	ND	0	0	0	ND	0	0	0	ND
	Copan	134.3	13	3	ND	141.3	12	4	ND	0	0	0	ND	0	0	0	ND
Streptococcus pyogenes	Puritan	>300	49.3	5	ND	>300	50.6	5.3	ND	~150	12	1	ND	19.6	1.6	0	ND
	Copan	>300	41	4	ND	>300	45.3	3	ND	~150	9.5	0.6	ND	18	0	0	ND

CONCLUSION

- The WASP equipment readily accepted the Puritan liquid Amies transport tubes and performed as well as E-Swab tubes.
- The WASP equipment performed well the majority of the time but stopped frequently at random.
- There was a marked difference in the absorption by the two brands of flocked swabs. The results of which have been presented in a previous study.
- The results demonstrate that, in general, the colony counts are higher using the Puritan tubes than Copan tubes.

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