Urine Monovette® with Boric Acid

Simulated use -

Study showing stabilization of specific microorganisms in urine

prepared by:

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PROTOCOL

Testing of Laboratory Generated Microbial Suspensions

Purpose:
To demonstrate that the Urine Monovette® boric acid will curtail the growth yet maintain the viability of specific microorganisms commonly found in human urine. Microbial suspensions made from single organisms will be studied. These suspensions will be incubated at room temperature in the Urine Monovette®.
Appropriate controls will also be included.

Materials and Equipment:
Pipettor
Pipette tips 0 - 200 µl
Pipette tips 100 - 1000 µl
Culture Medium - Pooled and sterile-filtered urine
Inoculation spreader
Blood agar plates
MacConkey agar plates
Screw-capped tubes (approx. 15 ml volume)
Urine Monovettes boric acid
Incubator 37°C
Refrigerator (approximately 4°C)
McFarland tube 0.5

PROCEDURE

The urine to be used in these cultures will be a pooled supply that has been sterile filtered soon after collection. The minimum volume of suspension should be such that all devices can be properly filled.

1. The microorganisms to be tested will be inoculated into the sterile urine such that the target concentration is approximately 10^4 to 10^6 microorganisms per ml.

2. The suspension will be drawn into the Urine Monovettes boric acid to their nominal volumes. Two control test tubes containing no preservative will also be filled with 10 ml of the same urine suspension. One tube will serve as the positive control where growth is not inhibited. The other control tube will demonstrate the inhibition of growth using refrigerator temperatures (approximately 4°C). All devices including the control tubes will be done in duplicate.

3. After the appropriate incubation period, the suspension in each device will be diluted using an appropriate dilution scheme to reach a final count of 50 to 200 microorganisms per plate. A volume of 200 - 500 microliters will be plated in triplicate on Blood Agar Plates at 0 hr, 24 hr and 48 hr. The plates are to be incubated at 37°C and counted at approximately 24 hr. The average count for each suspension will be calculated and the number reported as the log base 10, for example 250,000/ml is reported as 5.4.

4. The incubations are carried out as follows:

Urine Monovette® with Boric Acid	Room temperature*
Control tube without preservative	Refrigerator temperature*
Positive control without preservative	Room temperature**

*room temperature = approximately 25°C
**refrigerator temperature = approximately 4°C

5 The following microorganisms will be used and are known to be found in urine specimens[1]. Testing of these microorganisms will be done in duplicate with a new suspension for each duplicate.

Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis
Streptococcus faecalis
Candida albicans

Criteria for Equivalence:

The criteria for judging equivalence will be to show that the Urine Monovette® boric acid performs well in its principal function. This function is to inhibit the growth of microorganisms in urine and yet preserve their viability so that their numbers can be counted. There should be no more than 0.5 log base 10 difference between the counts obtained in the Urine Monovette® as compared to the refrigerated control. If the Urine Monovette® compares favorably to that standard (no more than 0.5 log difference), it will have met the criteria.

[1] B. D. Davis, R. Dulbecco et. al. *Microbiology*, Lllippincott, 4th edition, 1990.

E. Jawetz, J. L. Melnick and E. A. Adelberg, Review of Medical Microbiology, 17th edition, Appleton & Lange 1987.

REPORT

Date: November 15, 2002

Testing of Laboratory Generated Microbial Suspensions.

As described in the protocol, the microorganisms listed below were tested in the Sarstedt Urine Monovette® boric acid.

Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis
Streptococcus faecalis
Candida albicans

The urine inoculated with the appropriate microorganism was drawn into each device and cultured at room temperature. Two controls with the same suspension were cultured at 4°C without preservative and at room temperature without preservative. After 24 hr and 48 hr of culture the organisms were plated on appropriate agar and the resulting colonies were counted after 24 hr. The numbers from the Urine Monovette® were compared to the numbers from the 4°C control tube. If the numbers from the Monovette® are within 0.5 log of the numbers from 4°C control then the criteria for equivalence are met.

RESULTS:

Individual results are shown in the attached tables 1 through 3. The results from the Urine Monovette® for each organism were within 0.5 log of the 4°C control.

The results of the *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus faecalis* and *Candida albicans* showed complete agreement between the Urine Monovette® and the 4°C control at 24 hr and 48 hr.

The numbers from these organisms are found in the attached tables. The counts from the Urine Monovette® for each organisms were within 0.5 log of the 4°C control tube. Thus, the Urine Monovette® boric acid did meet the criteria described in the protocol for each of these organisms.

CONCLUSION:

For all organisms evaluated, the number of microorganisms in the Urine Monovette® boric acid were stabilized over a period of 48 hours when stored at room temperature. It was shown that the maintenance/control of the organisms in the Urine Monovette® is equivalent to 4°C stabilization.

Table 1

Organism and Date of Study	Starting Conc. & Log	Conc. after incubation and comparison of Urine Monovette® boric acid to 4°C			
		24 hr	Log Difference	48 hr	Log Difference
Klebsiella pneumoniae	3.42×10^5 5.53				
	Incubation condition	Conc Log		Conc Log	
2-4-02	Urine Monovette® boric acid	2.33×10^5 5.37		1.55×10^5 5.19	
	4°C	4.06×10^5 5.61	< 0.5	2.82×10^5 5.45	< 0.5
	Room Temp.	2.85×10^8 8.45			
Klebsiella pneumoniae	Starting Conc. & Log				
	3.26×10^5 5.51				
2-25-02	Incubation condition				
	Urine Monovette® boric acid	5.01×10^5 5.71		4.49×10^5 5.65	
	4°C	6.28×10^5 5.80	< 0.5	6.04×10^5 5.78	< 0.5
	Room Temp.	7.35×10^7 7.87		2.05×10^8 8.31	
Proteus mirabilis	Starting Conc. & Log				
	6.56×10^5 5.82				
2-11-02	Incubation condition				
	Urine Monovette® boric acid	7.63×10^5 5.88		7.76×10^5 5.89	
	4°C	7.23×10^5 5.86	< 0.5	5.76×10^5 5.76	< 0.5
	Room Temp.	3.02×10^7 7.48		3.02×10^7 7.48	
Proteus mirabilis	Starting Conc. & Log				
	3.14×10^5 5.50				
3-4-02	Incubation condition				
	Urine Monovette® boric acid	1.29×10^6 6.11		6.04×10^5 5.78	
	4°C	8.01×10^5 5.90	< 0.5	5.09×10^5 5.72	< 0.5
	Room Temp.	1.62×10^8 8.21		2.52×10^8 8.40	

Table 2

Organism and Date of Study	Starting Conc. & Log	Conc. after incubation and comparison of Urine Monovette® boric acid to 4°C			
		24 hr	Log Difference	48 hr	Log Difference
Escherichia coli	7.99×10^5 5.90				
2-4-02	Incubation condition	Conc Log		Conc Log	
	Urine Monovette® boric acid	2.03×10^5 5.31		1.89×10^5 5.28	
	4°C	5.79×10^5 5.76	< 0.5	4.79×10^5 5.68	
	Room Temp.	2.40×10^8 8.38		2.37×10^8 8.37	
Escherichia coli	Starting Conc. & Log				
	4.33×10^5 5.64				
2-18-02	Incubation condition				
	Urine Monovette® boric acid	2.11×10^5 5.32		1.46×10^5 5.16	
	4°C	3.94×10^5 5.60	< 0.5	3.86×10^5 5.59	< 0.5
	Room Temp.	6.43×10^7 7.81		4.04×10^8 8.61	
Streptococcus faecalis	Starting Conc. & Log				
	4.33×10^5 5.64				
2-25-02	Incubation condition				
	Urine Monovette® boric acid	5.89×10^5 5.77		6.40×10^5 5.81	
	4°C	5.88×10^5 5.77	< 0.5		
	Room Temp.	3.61×10^7 7.56		6.42×10^7 7.81	
Streptococcus faecalis	Starting Conc. & Log				
	5.25×10^5 5.72				
2-25-02	Incubation condition				
	Urine Monovette® boric acid	6.89×10^5 5.84		7.27×10^5 5.86	
	4°C	5.49×10^5 5.74	< 0.5		< 0.5
	Room Temp.	1.08×10^8 8.03		5.04×10^7 7.70	

Table 3

Organism and Date of Study	Starting Conc. & Log	Conc. after incubation and comparison of Urine Monovette® boric acid to 4°C			
		24 hr	Log Difference	48 hr	Log Difference
Candida albicans	4.37 X 10^4 4.64				
	Incubation condition	Conc Log		Conc Log	
3-5-02	Urine Monovette® boric acid	1.55 X 10^4 4.19		3.02 X 10^4 4.48	
	4°C	4.70 X 10^4 4.67	< 0.5	4.53 X 10^4 4.66	< 0.5
	Room Temp.	1.80 X 10^6 6.26		1.10 X 10^7 7.04	
Candida albicans	Starting Conc. & Log				
	7.24 X 10^4 4.86				
3-11-02	Incubation condition				
	Urine Monovette® boric acid	5.04 X 10^4 4.70		4.70 X 10^4 4.67	
	4°C	8.90 X 10^4 4.95	< 0.5	8.78 X 10^4 4.94	< 0.5
	Room Temp.	1.91 X 10^6 6.28		6.27 X 10^5 5.80	