THE PRESERVATION OF URINE SPECIMENS FOR DELAYED BACTERIOLOGICAL EXAMINATION

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SUMMARY: A trial to determine a suitable method of preserving specimens of urine for delayed bacteriological examination is described. The results are discussed and the use of 1.8 per cent boric acid is recommended.

Introduction

The investigation of suspected bacilluria often presents technical difficulties even in the well equipped hospital, because bacteriological examination, if it is to be reliable, requires the specimen to be dealt with by the laboratory staff as soon as possible after voiding. In practice this usually means that the patient of a small medical unit without laboratory facilities has either to be sent to the nearest hospital or the specimen taken to the laboratory. When the specimen can be dealt with within the hour or refrigerated for short periods no problem arises, occasionally however the only way the specimen can be delivered is by post with consequent delay of a day or more. Under such circumstances the results can rarely be relied upon.

Since the work of Kass (1956) gave significance to bacterial counts per ml of mid-stream urine specimens, a number of attempts have been made to devise a method of preventing the multiplication of organisms in such specimens whilst they are in transit to the laboratory.

It was therefore decided to examine by a viable count technique a number of methods for the preservation of urine. Initially the use of Stuart's transport medium was considered, 5 ml of urine being added to a 'Universal container' which was then filled to the brim with the transport medium. This had been modified by increasing by one fifth all chemicals in the formula (to compensate for urine added) and the reduction of agar concentration. Viability studies showed that within twenty-four hours increases ranging from 10 fold to 1,000 fold occurred (P. D. Meers and D. Goode unpublished).

Odegaard (1967) suggested the use of 10 per cent Edathamil Calcium Disodium (E.D.T.A.) and stated that a satisfactory bacteriostatic action was obtained when using strains of *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

It had been previously suggested that the effect of E.D.T.A. was bacteriocidal (MacGregor and Elliker 1958), also that it was bacteriostatic (Zemjanis and Hoyt 1960). Gray and Wilkinson (1965) by studying the action of E.D.T.A. on isolated cell wall suspensions concluded that the action of E.D.T.A. appeared to involve the removal or displacement of a metal cation from the cell wall, causing leakage of cell solutes and loss of viability.

Porter and Brodie (1969) recommended the use of 1.8 per cent boric acid as a preservative in urine where specimens are to be sent through the post. In a trial involving 130 specimens this method was compared with dip-inoculation slide transport medium. The method of preservation by boric acid was found convenient and satisfactory.

While Gray and Wilkinson (1965) suggest that E.D.T.A. may well eventually sterilize suspensions on standing, it was thought that preservation by E.D.T.A. at 10 per cent concentration would provide a useful comparative assessment of preservation by 1.8 per cent boric acid.

Design of the trial

Urine was obtained as a mid-stream specimen from healthy volunteers and inoculated with sufficient of an overnight culture to give an anticipated viable count of between 100,000 per ml and 1×10^6 per ml.

The specimen was then distributed in 100 ml amounts into three sterile bottles. The first having no preservative, the second with 10g E.D.T.A. and the third with 1.8g of boric acid. Further experiments were carried out with the specimens added to plastic Universal containers with the same preservatives. The bottles were well shaken to dissolve the chemicals; E.D.T.A. it was found, took some appreciable time to dissolve completely.

A viable count by the technique of Miles and Misra (1938) was carried out on the untreated specimen to obtain a base line figure.

Viable counts were further carried out at 4, 18, 24 and 48 hours from the start. The medium used throughout was MacConkey's bile salt agar.

The findings from preliminary experiments showed that the well known nutrient properties of healthy urine as a culture medium, allowed the count of untreated specimens to rise rapidly in the first four hours at room temperature and to continue this rise to a point where 300×10^6 viable organisms per ml were present after twenty-four hours. When untreated specimens were becoming visibly turbid to the naked eye after a few hours standing at room temperature no counts were performed, only check plating to establish that the increase in turbidity was due to the organism originally introduced.

Due to the number of viable counts involved, it was also decided that information of viability at 24 and 48 hours would be of the greatest value and the examination at other points was discontinued.

Traum and Hart (1916) in a study of preservation of milk by boric acid showed that tubercle bacilli were not destroyed. A very limited series of experiments were therefore conducted with urine artificially infected with tubercle bacilli (H.37 Rv). Portions from such specimens were stored at room temperature for seven day periods, both with and without the addition of 1.8 per cent boric acid as a preservative. Concentrations by the method of Petroff (4 per cent NaOH) were carried out on all specimens, and cultures made on Lowenstein-Jensen medium.

Consideration was also given during the trial to the possibility of using the 1 oz (28 ml) Universal container, produced in plastic with a conical shaping to the inside of the base, as a convenient dispensing measure for the boric acid crystals. It was thought that filling this conical depression at the base of the container level with crystals might give an approximately correct amount of the chemical.

Twenty containers were taken and dry boric acid added to the level of the top of the conical depression. The contents of the twenty containers were then weighed individually and all were found to be about 1.0g (lowest weight 0.82g, highest 1.15g. Nine readings were over 1.0g.) This would have given concentrations of 4 per cent boric acid which is

Table I

Comparison of viable counts of fresh urine inoculated with dilution of *Escherichia coli* faecal type I, with counts of the same bulk infected sample preserved with boric acid 1.8 per cent or with 10 per cent E.D.T.A. Counts per ml of 'near' urine.

Trial Number	Unpreserved				1.8 per cent boric acid			10 per cent E.D.T.A.		
	Hours from start				Hours from start			Hours from start		
	Zero	4	24	48	4	24	48		24	48
1	1.4×10^6	6.2×10^6	305×10^6	NT	1.2×10^6	1.1×10^6	1.1×10^6	0.8×10^6	0.3×10^6	0.2×10^6
2	1.16×10^6	8.25×10^6	390×10^6	NT	1.2×10^6	1.0×10^6	0.66×10^6	0.82×10^6	0.65×10^6	0.21×10^6
3	295,000	1.38×10^6	280×10^6	NT	285,000	250,000	179,000	200,000	162,000	79,500
4	1.72×10^6	NT	300×10^6	NT	NT	200,000	250,000	NT	30,000	Less than 50 ml
5	220,000	NT	over 300×10^6	NT	NT	*200,000	165,000	NT	*100,000	75,000
6	40,000	NT	VT	VT	NT	36,000	29,000	NT	2,000	950
7	195,000	NT	VT	VT	NT	173,000	145,000	NT	90,000	26,000
8	240,000	NT	VT	VT	NT	210,000	191,000	NT	153,000	115,000
9	263,000	NT	VT	VT	NT	243,000	215,000	NT	132,000	85,000
10	146,000	NT	VT	VT	NT	125,000	97,500	NT	63,000	37,500

*Period of 18 hours only. NT—Not tested. VT—Visibly turbid (Count over 250×10^6 per ml).

Table II
Bulk urine specimen inoculated with diluted suspension of *Proteus mirabilis*.
Samples compared—Unpreserved, preserved with 1.8 per cent boric acid, preserved with 10 per cent E.D.T.A.
All counts are per ml of 'neat' urine.

Trial Number	<i>Unpreserved</i>		1.8 per cent boric acid		10 per cent E.D.T.A.	
	<i>Hours from start</i>		<i>Hours from start</i>		<i>Hours from start</i>	
	<i>Zero</i>	<i>18</i>	<i>18</i>	<i>48</i>	<i>18</i>	<i>48</i>
1	500,000	VT	250,000	223,000	20,000	125,000
2	540,000	VT	495,000	430,000	25,000	35,000
3	105,000	VT	91,000	55,500	4,500	15,000
4	225,000	VT	197,500	184,000	20,500	32,000
5	45,000	†VT	†37,000	31,000	†10,500	3,000
6	345,000	VT	250,000	198,500	†18,000	71,000
7	820,000	VT	755,000	690,000	†27,000	61,000
8	92,500	VT	84,000	75,000	†7,000	15,100
9	76,500	VT	70,000	59,000	†5,450	2,200
10	235,000	VT	215,000	193,000	†241,000	99,000

†Period of 24 hours. NT—Not tested. VT—Visibly turbid (Count over 250×10^6 per ml).

too high and the method is not recommended. A possible answer for suitable collection containers would be the use of a pharmaceutical powder dispensing machine set to deliver 0.5g amounts of boric acid into Universal containers of 1 oz (28 ml).

Results

No specimen preserved with boric acid failed to yield a positive culture, and several unpreserved specimens were overgrown with *Bacillus* species. Little significant variation occurred between counts obtained at the start of each experiment and those from specimens preserved for twenty-four hours with 1.8 per cent boric acid (Tables I to III). At concentrations of boric acid below 1.5 per cent a significant rise occurred in viable

Table III

Comparison of viable counts of fresh urine inoculated with a dilution of a culture of *Streptococcus faecalis* with counts of the same bulk specimen preserved with 1.8 per cent boric acid.

All counts are per ml of 'neat' urine.

Experiment number	Unpreserved			Preserved with boric acid	
	Zero	24	48	24	48
1	151,000	VT	VT	134,000	125,000
2	76,000	VT	VT	65,500	62,500
3	305,000	VT	VT	350,000	325,000
4	92,000	VT	VT	95,000	76,000
5	490,000	VT	VT	425,000	400,000

VT—Visibly turbid, count over 250×10^6 per ml (Routine plating carried out to confirm causative organism). E.D.T.A. was not compared in this small series of experiments.

Table IV

Effect of varying the concentration of boric acid used as a preservative.

A single bulk specimen was inoculated with a dilution of *Escherichia coli*. This was then distributed into a number of Universal containers in 25 ml amounts and preserved with quantities of boric acid as shown. Viable counts were carried out at 24 and 48 hours.

As counts per ml of 'neat' urine.

	Concentration of boric acid as per cent	Viable count	
		24	48
Viable count of untreated specimen 275,000 per ml at start (heavy visible growth after 24 hours at room temperature)	1.0	600,000	1.1×10^6
	1.5	304,000	290,000
	2.0	270,000	285,000
	2.5	240,000	225,000
	3.0	155,000	132,000
	3.5	75,000	82,000
	4.0	70,000	67,000
	4.5	22,500	18,400
	5.0	18,100	14,200
	5.5	5,000	1,200
	6.0	No growth	No growth
	6.5	"	"
	7.0	"	"

No growth—No colonies obtained from 1/5th ml of a 1 in 10 dilution specimen.

organisms per ml while with concentrations below 2.5 per cent exerted a slowly progressive disinfectant action (Table IV).

A concentration of 2 per cent boric acid did not apparently prevent the recovery of *Mycobacterium tuberculosis* after seven days exposure in normal urine (Table V).

Table V

Preservation of *Mycobacterium* spp. in urine.

Bulk urine 600 ml was inoculated with 5.0 ml of saline into which had been suspended approximately 20 mg of H.37 Rv strain of *Mycobacterium tuberculosis*.

The specimen was well mixed and distributed in 25 ml portions into 20 Universal containers. These were kept on the bench in a warm room for seven days. Ten were preserved with 2 per cent boric acid, the remaining ten served as controls.

Preserved with boric acid		Unpreserved	
1 +	6 +	1 +	6 +
2 +	7 +	2 —	7 —
3 +	8 +	3 +	8 +
4 +	9 +	4 +	9 +
5 +	10 +	5 —	10 —

- + Positive culture obtained on Lowenstein-Jensen medium after concentration by Petroff's method.
— Lowenstein-Jensen medium showing marked change to deep green colour after a few days incubation and becoming overgrown with *Bacillus* spp. *Mycobacterium tuberculosis* not grown after eight weeks at 37°C.

Conclusions

The use of 1.8 per cent boric acid would seem to be a most satisfactory and very economic method of preserving urine specimens at room temperature for over twenty-four hours. Such specimens taken for bacteriological examination, would give clinically acceptable results after an overnight delay in delivery to the laboratory.

Some basic dispensing problems are obvious in the issue of containers for the collection of specimens of urine to be preserved with 1.8 per cent boric acid and the use of a dispensing machine set to deliver 0.5g amounts of the chemical is suggested for this purpose.

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