they were washed with wet swabs and suspended in 1 ml. of nutrient broth and inoculated on blood agar media and into mice. All tested slides failed to produce either culture or infection.

A similar experiment was performed with the same type of slides from which mercuric chloride was removed. Both the medium and mice failed to show the presence of *B. anthracis*.

Because unstained areas round the bacteria may simply be the space between a lightly stained surrounding medium, which retracted from the cells on drying and bacteria, the best way to demonstrate capsules is actually to stain them by some procedure which differentiates them from the cell itself. A modification of Leifson's method (1930) for staining flagella accomplishes this in the case of *B. anthracis*.

Staining Procedure

Fix the films in Zenker's fluid for one to two minutes and wash in water. Then flood slides with the solution A for 10 minutes. Wash with tap water and stain with solution B for five to 10 minutes. Wash in tap water, dry, and examine.

Solution A

$\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ (sat. aqueous solution)	..	20 ml.
Tannic acid (20% aqueous solution)	..	10 "
Distilled water	..	10 "
Ethyl alcohol 95%	..	15 "
Basic fuchsin (sat. solution in 95% ethyl alcohol)	..	3 "

The stain deteriorates after a week and should be freshly prepared.

Solution B

Methylene blue in powder	..	0.1 g.
Borax	..	1 "
Distilled water	..	100 ml.

Anthrax bacilli stained by the second method appear as dark blue bacilli surrounded by pale blue capsules. Background and red cells are stained red.

The first method (films are fixed in Zenker's solution for three to five minutes, washed with water, stained with methylene blue, and washed in water) is recommended for routine diagnostic work in the field by a veterinary surgeon or medical practitioner, but the second method by which capsules as well as bacteria are stained is the better for laboratory and teaching purposes.

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A Simplified Method for the Quantitative Bacterial Culture of Urine

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The value of quantitative bacterial culture of freshly voided clean mid-stream urine in assessing infection of the urinary tract is established (Kass, 1955; Boshell and Sanford, 1958; Monzon, Ory, Dobson, Carter, and Yow, 1958). However, the technique of serial dilution recommended is time consuming and requires a large number of petri dishes and pipettes, so that its use on a large scale in a clinical laboratory is impractical. It was therefore decided to compare the colony counts obtained by the dilution technique with those obtained by culture of a "standard" loopful of urine. The results with the latter are sufficiently accurate for the purpose of assessing urinary tract infection in clinical practice.

Collection of Specimens

The urine was collected in the course of an investigation into the prevalence of urinary tract infection in diabetic and general populations. There were 100 urines in all, from 39 men and 61 women. A high proportion of the subjects were middle-aged and elderly.

In males the glans penis was swabbed with 1:1,000 benzalkonium hydrochloride ("roccal") and a mid-stream specimen of urine collected. Female patients were prepared in the dorsal lithotomy position as for catheterization with the same antiseptic. The labia were separated, the patient was then encouraged to void urine and the specimen was collected from the mid portion of the urinary stream into a sterile container. Specimens were dealt with immediately after collection; if this was not possible they were stored at -20°C .

Method

The number of viable organisms per millilitre of urine was determined by the same observer with each specimen of urine as follows:

Dilution Technique.—Serial tenfold dilutions of urine from 1 in 10 to 1 in 1,000,000 were made in sterile normal saline and 1 ml. of each dilution mixed with 10 ml. of molten nutrient agar kept at 50°C ., then poured into a petri dish and allowed to set. The six plates were incubated aerobically at 37°C . for 48 hours. The plate containing approximately 50–200 colonies was counted, and the result multiplied by the dilution gave the number of viable organisms per ml.

FIG. 1.—Log dilution loop count plotted against actual loop count from the same specimen (100 mid-stream urines).

Standard Loop Technique.—One loopful of undiluted freshly voided urine was spread over a nutrient agar plate. The loop (platinum wire 22 standard gauge, 3 mm. internal diameter) was removed vertically from the sample and both sides rubbed over the surface of the medium, taking care not to reinfect the loop with the original inoculum. After 48 hours' aerobic incubation at 37° C. the colonies were counted. Anaerobic culture on blood agar was performed in the early stage of the investigation. No anaerobes were isolated and this was discontinued.

Results

The results are shown in Fig. 1, in which the log of the bacterial count by the dilution method is plotted against the actual counts obtained by the loop method. Seventeen of the urine specimens cultured by the dilution method gave bacterial counts in excess of 100,000 organisms/ml. and in 16 of these the corresponding loop count was in excess of 200. The one exception, a count of 191,000 organisms by the dilution method, grew 161 colonies by the loop method.

Six counts by the dilution method were in the range of 10,000 to 100,000 organisms/ml. In five of these the corresponding loop count was between 95 and 185. In one, a dilution count of 31,000/ml. grew only two colonies by the loop method. All counts of less than 1,000 organisms/ml. by the dilution method yielded counts of less than 60 by the loop method.

All authors are agreed that dilution counts of over 100,000 organisms/ml. on voided mid-stream urine are indicative of urinary tract infection. The significance of counts between 10,000 and 100,000 organisms/ml. is less certain (Kass, 1956; Huvos and Rocha, 1959), and they should be repeated. These results show that, using the loop method, colony counts of more than 200 are indicative of urinary tract infection, counts between 90 and 200 are in the doubtful range and should be repeated, and all counts below 90 are probably the result of contamination.

Summary

The results of enumeration of viable organisms in freshly voided urine were compared by counting the colonies after serial dilution and by spreading a standard loopful of the same urine over the surface of an agar plate. The simpler loop technique appears to be sufficiently accurate for clinical purposes.

We wish to thank Miss M. A. Butcher for technical assistance.

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CORRECTION

Dr. Meynell writes: 'In the paper by O'Sullivan, Fitzgerald, Malins, and Meynell (*J. clin. Path.*, 13, 527),

under the paragraph entitled "Collection of Specimens", the statement at the end "if this was not possible they were stored at $-20^{\circ}\text{C}.$ " is wrong and should read "were stored at $+4^{\circ}\text{C}.$ " In this work no specimen had been stored. All were dealt with immediately after collection. We have since compared counts obtained following immediate inoculation; those after storing at $+4^{\circ}\text{C}.$ overnight and those after storing at $-20^{\circ}\text{C}.$ overnight. The first two are practically the same. At $-20^{\circ}\text{C}.$ viable counts are quite strikingly reduced. Although $-20^{\circ}\text{C}.$ may be satisfactory for storing bacteria and filtrable viruses it should not be used for viable counts.'

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