Effect of Delay on Culture of Urine

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One hundred random urine specimens which were submitted for culture were planted using a calibrated loop within 2 h of collection and 2 and 4 h later after standing at room temperature. Colonies were counted after an 18- to 24-h incubation at 37°C. Fifteen of the specimens demonstrated increases in counts exceeding $1 \times \log_{10}$; four increased from less than 10^5 /ml to greater than 10^5 /ml (three by the 4-h culture and a fourth by the 6-h culture), supporting the concept that delays of greater than 2 h in inoculating cultures may produce results which could cause errors in diagnosis.

It has been recommended and generally accepted that specimens for urine culture should be processed within 2 h of collection (A. L. Barry et al. [ed.]. 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections, p. 1-8. American Society for Microbiology, Washington, D.C.). Most urine specimens contain up to 10^2 organisms/ml as a result of contamination with urethral flora. Urine collected by suprapubic aspiration is generally sterile. In upper or lower urinary tract infection, bacteria are able to multiply in the bladder urine. This results in populations exceeding 10^5 bacteria/ml in most instances (3). Urine specimens that are inoculated with up to 10^2 organisms/ml by passage through the urethra might, after a period of delay, through bacterial multiplication, reach counts of 10^5 bacteria/ml or more, suggesting urinary tract infection. Recent publications have provided conflicting evidence that this is true. Shrestha (4) concluded that growth was not altered by delay in culture for up to 24 h. Jefferson et al. (2) reported that delay of over 2 to 4 h made a significant change in 5% of specimens. The purpose of this study was to correlate colony counts after varying periods of delay between collection and culture to corroborate one or the other of these reports.

One hundred random specimens of urine submitted for culture and received by the laboratory within 2 h of the stated time of collection were inoculated onto blood and MacConkey agar in bi-plates. Each half of the bi-plate was inoculated with urine by a 0.001-ml calibrated platinum loop. The urine was streaked in a single line parallel to the division of the bi-plate and then, without flaming, spread by making multiple streaks transversely through the initial line of inoculation. This method has been shown to produce results comparable to

that obtained by pour plate methods (1). Subsequently, the specimens were allowed to stand at room temperature. At intervals of 4 and 6 h from the stated time of collection, each specimen was again inoculated in the same manner. Plates were incubated aerobically at 37°C for 18 to 24 h, after which the plates were examined and the colonies were counted. When counts of less than 10^5 /ml were observed, presumptive identification was based on gross colony morphology only.

No growth was observed in 53 of the specimens; 47 cultures were positive. Among 32 of the positive cultures there was no difference in the counts observed over the 6-h period. Changes in counts exceeding $1 \times \log_{10}$ or greater were observed in 15 of the specimens. Four of these demonstrated counts which increased from $<10^5$ /ml to $>10^5$ /ml in the 4- and 6-h cultures. Three increased to this number by the 4-h culture and a fourth by the 6-h culture. All contained potential urinary tract pathogens (betahemolytic and nonhemolytic streptococci; *Escherichia coli*; nonfermentative gram-negative bacillus; *Klebsiella/Enterobacter*). Three of the four were pure cultures. In one, *Escherichia coli* was accompanied by a nonhemolytic streptococcus of 10^3 colonies/ml. The remaining 11 specimens showed changes of $1 \times \log_{10}$ or greater, but these were consistently above or below 10^5 /ml in all three cultures. In nine the change occurred by the 4-h culture and in the remainder by the 6-h culture.

Four of 47 specimens of urine that showed less than 10^5 bacteria/ml when cultured within 2 h of collection showed greater than 10^5 bacteria/ml when cultured after 4 and 6 h of room temperature incubation. Application of current standards of interpretation would result in a false impression of evidence of infec-

tion in these instances. These observations support the report of Jefferson et al. (2) and other currently recommended and accepted standards (Barry et al. [ed.]. 1975. Cumitech 2) and disagree with the observation of Shrestha (4).

LITERATURE CITED

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