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The Use of Acridine Orange Staining and Image Analysis to Detect Bacteriuria

J. Scholefield[1], R. Manson[1], R. J. Johnston[1] and R. Scott[2]

[1] Department of Bioscience Biotechnology, Food Science Division, Rapid Methods Microscopy Unit, University of Strathclyde, Glasgow
[2] Department of Urology, Royal Infirmary, Glasgow, UK

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Summary. Fifty urine samples from a variety of urological patients were analysed using the fluorochrome acridine orange in an automated system. The results were compared with standard colony counts. The method has a potential value in the detection of significant infection particularly in population studies.

Key words: Bacteriuria, Rapid detection, Acridine Orange, Fluorescence, Image analysis.

Introduction

Detection and enumeration of microorganisms in a variety of menstrua has been carried out using the fluorochrome acridine orange [1, 4, 5, 7]. However, all of these studies employed acridine orange at acidic or neutral pH and none of them used this fluorochrome to detect bacteriuria. Earlier work by authors [6] did include the examination of microorganisms in urine but this study was carried out on fixed smears of urine and used acidic acridine orange.

In this preliminary study an alkaline solution of acridine orange was used to stain wet mounts of urine from 50 hospital patients and an image analysis system was employed to detect the numbers of bacteria present in each preparation.

Methods

A total of 50 urine specimens freshly taken from patients in urological wards were used in this study. Twenty-four of the urines were midstream specimens and the remainder catheter specimens.

To ensure an alkaline condition for the acridine orange staining procedure a borate buffer was added to the urine. This buffer mixture was composed of 0.02 M disodium tetraborate containing 4 g of EDTA, 0.1 ml of Triton x100 and 77 ml of formaldehyde 37–39% (w/vol) per litre of solution, with the pH finally adjusted to 9.8 using 1 M sodium hydroxide. The acridine orange solution was prepared as a 0.5% (w/v) aqueous solution in distilled water and stored in an amber bottle at room temperature. Both solutions were filtered through a 0.45 micron pore size filter before use.

A volume of 0.8 ml of the borate buffer solution was added to 0.6 ml of the urine and gently mixed before the addition and mixing of 25 μl of acridine orange solution. The combined mixture was then held at 40 °C for 5 min before removing an aliquot into a Thoma bacterial counting chamber. The sample was examined using an Orthoplan microscope (E. Leitz Instruments Ltd.), fitted with an HBO W/4 light source and a Ploem incident light illumination system. The system used TRITC excitation filter, a TK580 dichroic and K590 barrier filter. A modified Hitachi CC television camera with a silicon diode detector and a x50 water immersion objective supplied the data to the Optomax OM1 image analyser (Micro-Measurements Ltd.). A minimum of 100 fields was counted for each sample and only those particles at or above a pre-selected brightness level were counted. Colony counts were performed on the urine samples using the standard calibrated loop method on cystine lactose deficient medium CLED (Oxoid Ltd.). The inoculated plates were counted after 18 h incubation at 37 °C.

Results

Of the 26 specimens producing less than x 10^5 colony forming units (CFU)/ml on the CLED agar the direct fluorescent microscopy method (DFM) correctly identified 18. The 8 false positive samples i.e. those with a DFM count greater than x 10^5/ml and a plate count of less than 10^5 CFU/ml included 3 samples where the administration of antibiotics was known. The 18 specimens with both DFM and CFU counts less than x 10^5/ml included 9 specimens from patients known to be receiving antibiotics. The false positive rate represents 31% of the total number of specimens with plate counts of less than x 10^5 CFU/ml.

Twenty-four specimens produced plate counts of greater than x 10^5 CFU/ml and the DFM method correctly identified 22 of these, but failed to identify 2 giving a false negative rate of 8.3%. One of the false negative specimens gave a colony count of 1.6 x 10^5 CFU/ml of a *Proteus* sp. and the other specimen a count of 2.5 x 10^5 CFU/ml of a coagulase negative staphylococcus. Both of these organisms were suc-

cessfully detected in other specimens by DFM as were *E. coli, Pseudomonas aeruginosa, Streptococcus faecalis* and *Klebsiella* sp.

Discussion

In this preliminary study the method has produced a false negative rate of 8.3% and although any false negative results are highly undesirable most of the current rapid systems for detecting bacteriuria do show some low rate of false negative samples [2, 3]. The false positive rate of 31% is also of the same order experienced with other rapid systems.

The detection of bacteriuria using alkaline acridine staining of urine in wet mounts and enumeration with an image analysis system has been shown to have potential and its use with an automatic DFM instrument (Bactoscan) [7], in a survey of bacteriuria in schoolgirls, will be described in a subsequent publication.

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R. Scott
Department of Urology
Royal Infirmary
Glasgow G4 OSF
UK