

when re-examined. However, the difference is not statistically significant. Unfortunately, *Ureaplasma urealyticum* cultures were not performed during the study period. The finding of Bowie et al. (6) that chlamydia-positive urethritis responds better to treatment than chlamydia-negative urethritis could not be confirmed in the present study.

Both erythromycin stearate and lymecycline in the dosages used in this study seem to be effective in the treatment of non-gonococcal urethritis regardless of whether the culture for *Chlamydia trachomatis* is positive or negative. Ten to 15 days of treatment seems to be sufficient, providing the partner is treated simultaneously.

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Acridine Orange Staining of Urethral and Cervical Smears for the Diagnosis of Gonorrhea

Urban Forsum¹ and Anders Hallén²

¹Institute of Clinical Bacteriology and ²Department of Dermatology, University of Uppsala, Uppsala, Sweden

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Abstract. Smears of urethral and cervical discharge for direct microscopical examination of gonococci were stained with acridine orange and methylene blue and the findings compared with culture of direct inoculated plates. These two staining methods yielded similar results.

Acridine orange staining seems a valuable alternative due to its sharp image contrast.

Key words: Identification of gonococci; Acridine orange staining; Methylene blue staining

Direct microscopical examination of stained urethral and cervical smears allows the trained physician to make a positive diagnosis of gonorrhea immediately at the patient's first visit. Correct treatment can then be given without delay. The risk of false-positive diagnosis is small. About 90% of gonococcal infections in men are detected (3), whereas in women only 50–60% of infections are detected (1, 3). This is partly due to the presence of other microorganisms and mucus that makes the intracellular diplococci less prominent.

Kronvall & Myhre (4) 1977 described a simple staining method for bacteria in clinical specimens using acridine orange at low pH. Cells and bacteria are easily differentiated by their green vs. orange colour when observed by fluorescence microscopy. We were interested to see whether this contrast staining would yield more positive findings, particularly in women, compared with the routine methylene blue staining.

MATERIAL AND METHODS

Patients seen in the venereal diseases clinic in whom gonorrhea might be suspected on clinical and epidemiological grounds (heavy purulent urethral discharge and/or a known contact with gonorrhea) were selected. The final material consisted of 83 patients, 55 women and 28 men. From each patient two sets of smears were prepared, from the urethra in men and from the urethra and the cervix in women. One smear was immediately stained with methylene blue and examined. The other was later stained with acridine orange *ad modum* Kronvall & Myhre (4) and examined in a Zeiss fluorescence microscope with incident light and blue band activation. The finding of monomorphic, intracellular, coffeebean-shaped diplococci was considered the sole criterion for making a diagnosis of gonorrhea. Swabs for culture were taken at the same time as the smears and plated immediately on GC agar plates at room temperature.

The GC agar used had the following composition: GC agar base (BBL) 10 g, aq. dest. to 1 000 ml. In addition to a plate with this mixture, another plate using the same composition but with the addition of VCN-inhibitor (BBL) 10 ml/l was used. After the inoculation, the plates were immediately placed in a candle jar and sent within 4 h to the laboratory, where they were incubated for 48 h at 37°C with 5% CO₂ and 95% humidity and then examined for gonococci. Oxidase-positive colonies with a morphology

Table I. Findings of gonococci by staining with methylene blue (MB), acridine orange (AO), and with culture (Cult.) in 28 men with suspected gonorrhea

MB	AO	Cult.	N
+	+	+	15
-	-	-	9
-	-	+	0
+	+	-	2
-	+	+	0
+	-	+	0
+	-	-	0
-	+	-	2
Total			28

suggestive of gonococci were confirmed as gonococci by the immunofluorescence technique described by Forsum (2).

RESULTS

48 of the 83 patients had culture-proven gonococcal infections. 64% of these infections were detected by both staining methods. A further 6% were found by either staining method. The male group is rather small, but all the 15 culture-proven infections were found by both stainings (Table I). Two men were positive by both stainings yet had negative cultures. The acridine orange staining gave positive results in another 2 men with negative cultures, possibly false-positive microscopy.

33 of the 55 women had culture-proven infections and both stainings detected 48% of these (Table II). A further 9% were detected by either staining method.

In 90% of all cases the two staining methods were in agreement.

Table II. Findings of gonococci by staining with methylene blue (MB), acridine orange (AO), and with culture (Cult.) in 55 women with suspected gonorrhea

MB	AO	Cult.	N
+	+	+	16
-	-	-	22
-	-	+	11
+	+	-	0
-	+	+	3
+	-	+	3
+	-	-	0
-	+	-	0
Total			55

DISCUSSION

The methylene blue staining technique is extremely simple and rapid. It takes about one minute to have a smear stained and ready for the microscope. The staining needs a minimum of laboratory facilities. An ordinary light microscope with a magnification of $\times 1000$ is adequate. All material is stained in shades of blue, but different kinds of epithelial cells and leukocytes are easy to identify. The gonococci are identified by morphology only, but this is easily done, with some experience.

The acridine orange staining is also fairly simple, but takes about 5 minutes to perform. It is necessary to have a microscope equipped for incident light fluorescence.

The orange staining of the gonococci is brilliant, but not specific. The epithelial cells and leukocytes are less distinctly stained and not so easy to identify. Another disadvantage is the slight initial haze in the viewing field that makes the microscopical examination more time consuming.

In this study both staining methods detected the same number of infections, although not in the same individuals. The methylene blue technique is preferable for most clinical purposes, but the technically more demanding acridine orange staining is a valuable alternative because of its sharp image contrast.

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