Practical Substitution for the Indole, Methyl Red, Voges-Proskauer, Citrate System

ALEXANDER VON GRAEVENITZ

Clinical Microbiology Laboratories, Yale-New Haven Hospital, and Section of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06504

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A substitution for the indole, methyl red, Voges-Proskauer, citrate system is proposed. The scheme employs indole and ampicillin susceptibility tests routinely and uses selectively three other media for six reactions.

The indole, methyl red, Voges-Proskauer, citrate (IMViC) system for the identification of coliforms (3) does not meet the needs of a modern clinical laboratory. Even if rapid methods are used, IMViC checks of every coliform organism are expensive, time-consuming, and may lead to misidentification (Table 1).

Several substitutes have been offered recently (1, 4). I propose a rapid diagnostic scheme for lactose-fermenting gram-negative rods which serves to economize without shortening the spectrum of possible diagnoses and unduly delaying the report. MacConkey, deoxycholate, or endo agar may be used as isolation and differential media. Media and tests selectively used for the identification of pink colonies include: Trypticase for indole check, 10-μg ampicillin disc for Kirby-Bauer sensitivity testing on Mueller-Hinton Agar (BBL); motility-ornithine medium (decarboxylase medium base with 1% L-ornithine and 0.3% agar), lysine-iron agar, Koser citrate medium (Difco); and dimethyl-p-phenylenediamine-hydrochloride (Eastman Organic Chemicals, Rochester, N.Y.) for the Kovacs oxidase test. The indole and motility-ornithine medium may be combined, if necessary, in the motility-indole-ornithine medium (1). Incubation is at 37°C for 24 hr. Triple Sugar Iron Agar was not routinely used.

An earlier survey of 785 prompt lactose fermenters from clinical specimens had shown that approximately 99% of the nonmucoid-growing strains belonged to the genera Escherichia, Citrobacter, Arizona, Aeromonas, and Erwinia. Of the mucoid-growing strains, 94% belonged to the genera Klebsiella, Enterobacter, and Citrobacter, and 6% to Escherichia (5). This distribution justified different diagnostic approaches to mucoid and nonmucoid strains. Furthermore, use could be made of the intrinsic ampicillin resistance of Aeromonas (6) and of atypical Enterobacter cloacae (7) as compared to the variability of ampicillin susceptibility in Escherichia coli.

**Nonmucoid strains.** Nonmucoid strains (Table 2) are initially checked for indole formation and ampicillin susceptibility, the latter constituting part of sensitivity testing in most laboratories. Indole-positive, ampicillin-sensitive strains are called E. coli. Ampicillin-resistant ones are further checked for oxidase and reactions in decarboxylase media. Indole-negative strains are tested in the decarboxylase medium irrespective of their ampicillin susceptibility. Yellow pigmented strains suggest Erwinia species which are negative for H2S and both decarboxylases.

**Mucoid strains.** Mucoid strains (Table 3) are simultaneously checked for indole formation, reactions in decarboxylase media, and ampicillin susceptibility.

Citrate tests are subsequently used only to separate: (i) Klebsiella from ornithine-negative, nonmotile E. coli, and (ii) ampicillin-resistant E. coli from atypical E. cloacae (7). The extended waiting period for routine citrate tests is thus largely avoided.

The system cannot differentiate between certain Erwinia and lysine-negative Klebsiella or H2S-negative Citrobacter strains. Tests for gelatin liquefaction, Voges-Proskauer reaction, and motility at 22°C serve for differentiation. Furthermore, rare strains will be missed, i.e., indole-positive Erwinia, Citrobacter, and Enterobacter; ornithine-positive Klebsiella and Citrobacter (H2S-negative); and lactose-fermenting strains of Salmonella, Proteus, Serratia, Enterobacter liquefaciens, and Aeromonas punctata.

The accuracy of the system was checked by submitting 110 mucoid and 192 nonmucoid prompt lactose fermenters to all tests listed (incubation for 48 hr) plus a Voges-Proskauer reaction on PathoTec VP Strips (Warner-Chilcott). The system misdiagnosed one indole-positive, H2S-
### Table 1. Possible diagnoses in the indole, methyl red, Voges-Proskauer, citrate system

| Indole | Methyl red | Voges-Proskauer | Citrate | Identification |
|--------|------------|-----------------|---------|----------------|
| +      | +          | -               | -       | +              |
| +      | +          | -               | +       | + Atypical Enterobacter cloacae |
| +      | -          | +               | +       | Escherichia coli, Aeromonas (Plesiomonas) shigelloides |
| +      | +          | -               | +       | Klebsiella, Aeromonas hydrophila |
| -      | +          | -               | +       | Aeromonas hydrophila |
| -      | +          | -               | -       | Klebsiella ozaenae, Citrobacter, Arizona, Erwinia |
| -      | -          | +               | -       | Klebsiella ozaenae, Erwinia |
| -      | -          | +               | +       | Klebsiella, Enterobacter cloacae, Enterobacter aerogenes, Erwinia |

### Table 2. System for nonmucoid lactose fermentersa

| OD | Mot | LD | H2S | Ox | Identification |
|----|-----|----|-----|----|----------------|
| +  | +   | +  | -   | +  | Aeromonas (Plesiomonas) shigelloides |
| +  | +   | +  | -   | -  | Escherichia coli |
| +  | +   | -  | -   | -  | E. coli, atypical Enterobacter cloacae |
| -  | +   | +  | -   | -  | Aeromonas hydrophila |
| -  | +   | +  | -   | -  | Klebsiella, E. coli |

**Indole-positive, ampicillin-resistant**

| OD | Mot | LD | H2S | Ox | Identification |
|----|-----|----|-----|----|----------------|
| +  | +   | +  | -   | +  | Arizona |
| +  | +   | +  | -   | -  | Enterobacter aerogenes |
| +  | +   | -  | -   | -  | Enterobacter cloacae |
| -  | +   | +  | -   | -  | Citrobacter |
| -  | +   | +  | -   | -  | Klebsiella |
| -  | +   | +  | -   | -  | Klebsiella ozaenae, Erwinia |

**Indole-negative, ampicillin different reactions**

| OD | Mot | LD | H2S | Ox | Identification |
|----|-----|----|-----|----|----------------|
| +  | +   | +  | -   | +  | Arizona |
| +  | +   | +  | -   | -  | Enterobacter aerogenes |
| +  | +   | -  | -   | -  | Enterobacter cloacae |
| -  | +   | +  | -   | -  | Citrobacter |
| -  | +   | +  | -   | -  | Klebsiella |
| -  | +   | +  | -   | -  | Klebsiella ozaenae, Erwinia |

**Abbreviations:** OD, ornithine decarboxylase; Mot, motility (in ornithine decarboxylase medium); LD, lysine decarboxylase (in lysine-iron agar); H2S, hydrogen sulfide production (in lysine-iron agar); Ox, oxidase; +, positive; −, negative; +/−, positive or negative.

### Table 3. System for mucoid lactose fermentersa

| Ind | OD | Mot | LD | H2S | Am | Identification |
|-----|----|-----|----|-----|----|----------------|
| +   | +  | +  | -  | -   | d  | Escherichia coli |
| +   | +  | +  | -  | -   | s  | E. coli |
| +   | +  | +  | -  | -   | r  | E. coli, atypical Enterobacter cloacae |
| +   | -  | +  | -  | -   | d  | E. coli |
| +   | -  | +  | -  | -   | d  | E. coli |
| +   | +  | -  | +  | -   | d  | E. coli, Klebsiella |
| -   | +  | +  | -  | -   | d  | Citrobacter |
| -   | +  | +  | -  | -   | d  | Enterobacter aerogenes |
| -   | +  | +  | -  | -   | d  | E. cloacae |
| -   | +  | +  | -  | -   | d  | Citrobacter, Erwinia |
| -   | +  | +  | -  | -   | d  | Klebsiella |
| -   | +  | +  | -  | -   | d  | Klebsiella ozaenae, Erwinia |

**Abbreviations:** Ind, indole formation; OD, ornithine decarboxylase; Mot, motility (in ornithine decarboxylase medium); LD, lysine decarboxylase (in lysine-iron agar); H2S, hydrogen sulfide production (in lysine-iron agar); Am, ampicillin; d, different reactions; r, resistant; s, sensitive, +, positive; −, negative; +/−, positive or negative.

**Check citrate test.**
negative Citrobacter and three late indole-forming E. coli strains. In two cases, it failed to differentiate between Klebsiella and Erwinia. It is believed that the economic advantages of the system would outweigh these few diagnostic shortcomings.

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