

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

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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- μ g ampicillin disc for Kirby-Bauer sensitivity testing on Mueller-Hinton Agar (BBL); motility-ornithine medium (decarboxylase medium base with 1% 1-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 <i>Enterobacter cloacae</i>
+	+	-	-	<i>Escherichia coli</i> , <i>Aeromonas (Plesiomonas) shigelloides</i>
+	-	+	+	<i>Klebsiella</i> , <i>Aeromonas hydrophila</i>
+	-	+	-	<i>Aeromonas hydrophila</i>
-	+	-	+	<i>Klebsiella ozaenae</i> , <i>Citrobacter</i> , <i>Arizona</i> , <i>Erwinia</i>
-	+	-	-	<i>Klebsiella ozaenae</i> , <i>Erwinia</i>
-	-	+	-	<i>Klebsiella ozaenae</i> , <i>Erwinia</i>
-	-	+	+	<i>Klebsiella</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter aerogenes</i> , <i>Erwinia</i>

TABLE 2. System for nonmucoid lactose fermenters^a

Indole-positive, ampicillin-resistant					Indole-negative, ampicillin different reactions					
OD ^b	Mot	LD	H ₂ S	Ox	Identification	OD	Mot	LD	H ₂ S	Identification
+	+	+	-	+	<i>Aeromonas (Plesiomonas) shigelloides</i>	+	+	+	+	<i>Arizona</i>
+	+/-	+	-	-	<i>Escherichia coli</i>	+	+	+	-	<i>Enterobacter aerogenes</i>
+	+	-	-	-	<i>E. coli</i> , atypical <i>Enterobacter cloacae</i> ^c	+	+	-	-	<i>Enterobacter cloacae</i>
						+/-	+	-	+	<i>Citrobacter</i>
-	+	+	-	-	<i>E. coli</i>	-	+	-	-	<i>Citrobacter</i> , <i>Erwinia</i>
-	+	-	-	+	<i>Aeromonas hydrophila</i>	-	-	+	-	<i>Klebsiella</i>
-	+/-	-	-	-	<i>E. coli</i>	-	-	-	-	<i>Klebsiella ozaenae</i> , <i>Erwinia</i>
-	-	+	-	-	<i>Klebsiella</i> , <i>E. coli</i> ^c					

^a Indole-positive ampicillin-sensitive strains are called *Escherichia coli*.

^b 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.

^c Check citrate test.

TABLE 3. System for mucoid lactose fermenters^a

Ind	OD	Mot	LD	H ₂ S	Am	Identification
+	+	+/-	+	-	d	<i>Escherichia coli</i>
+	+	+/-	-	-	s	<i>E. coli</i>
+	+	+/-	-	-	r	<i>E. coli</i> , atypical <i>Enterobacter cloacae</i> ^b
+	-	+/-	-	-	d	<i>E. coli</i>
+	-	+	+	-	d	<i>E. coli</i>
+	-	-	+	-	d	<i>E. coli</i> , <i>Klebsiella</i> ^b
-	+	+	-	+	d	<i>Citrobacter</i>
-	+	+	+	-	d	<i>Enterobacter aerogenes</i>
-	+	+	-	-	d	<i>E. cloacae</i>
-	-	+	-	-	d	<i>Citrobacter</i> , <i>Erwinia</i>
-	-	-	+	-	d	<i>Klebsiella</i>
-	-	-	-	-	d	<i>Klebsiella ozaenae</i> , <i>Erwinia</i>

^a 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.

^b 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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