Simplified Procedure for Provisional Identification of Shigella—like Organisms and of Hydrogen Sulphide producing Enterobacteria

P. BHAT
Wellcome Research unit*, Christian Medical College Hospital, Vellore, Tamil Nadu, India.

ABSTRACT
Simplified methods with employment of a minimum number of tests for biochemical screening and identification of various enterobacteria and certain other aerobic gram negative fermenting rods which may superficially resemble each other are described. The advantages of adopting such methods in laboratories confronted by the usual practical problems of a busy diagnostic service are discussed.

INTRODUCTION
The procedures followed in any laboratory depend on several factors ranging from availability of facilities to that of personnel. This is particularly so in the processing of stool samples. Whatever may be the procedure adopted, it should be the aim of any diagnostic laboratory to be able to follow procedures which are simplified but at the same time effective enough to make a provisional identification of the organisms in as short a time as possible. This is important for a diagnostic laboratory as it helps the clinician. Over a number of years, the procedures followed in this laboratory have been found to meet with these requirements. These form the basis of the following report.

METHODS
A battery of standard plating media is used when stool specimens are studied. In brief it includes MacConkey agar (MA), desoxycholate citrate agar (DCA), Salmonella-Shigella agar (SSA), bismuth sulphite agar (BSA), xylose lysine desoxycholate citrate agar (XLD) and selenite enrichment broth (SF). Following overnight incubation SF is subcultured onto SSA. Thiosulphate citrate bile salts sucrose agar (TCBS) is used when cholera is suspected and blood agar (BA) is included when cholera or enteropathogenic E.coli diarrhoeal disease (EPEC) of the younger age is suspected (Chart I).

<table>
<thead>
<tr>
<th>Media used in the examination of stools</th>
<th>Stool specimens / rectal swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>DCA</td>
</tr>
<tr>
<td></td>
<td>XLD</td>
</tr>
<tr>
<td></td>
<td>BSA</td>
</tr>
<tr>
<td></td>
<td>TCBS</td>
</tr>
<tr>
<td></td>
<td>BA</td>
</tr>
<tr>
<td></td>
<td>SF</td>
</tr>
<tr>
<td></td>
<td>SSA</td>
</tr>
</tbody>
</table>

* Wellcome Trust in collaboration with the World Health Organisation.
Suspicious looking colonies from MA, DCA, SSA, XLD, BSA and TCBS are screened using the preliminary screening media which consist of mannitol motility medium (MMM) triple-sugar-iron agar medium (TSI) and peptone water (PW). When facilities are available a simultaneous inoculation of lysine-iron-agar (LIA) would minimize the time in completing provisional identification of the organisms (Chart II).

**CHART II**

<table>
<thead>
<tr>
<th>Preliminary screening media</th>
<th>Suspicious-looking colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLF&lt;sup&gt;a&lt;/sup&gt; on Pink colonies on Typical Typical yellow colonies on</td>
<td></td>
</tr>
<tr>
<td>MA on XLD black colonies on TCSI</td>
<td></td>
</tr>
<tr>
<td>DCA on BSA</td>
<td></td>
</tr>
</tbody>
</table>

**Preliminary screening media**

<table>
<thead>
<tr>
<th>MMM</th>
<th>TSI</th>
<th>PW</th>
<th>LIA</th>
</tr>
</thead>
</table>

NLF<sup>a</sup> — Non-lactose fermenting

Following overnight incubation MMM is examined for evidence of mannitol fermentation and motility. TSI for evidence of glucose, lactose or sucrose fermentation and hydrogen sulphide production and PW for indole production. LIA is examined for the production of lysine decarboxylase, lysine deaminase, and hydrogen sulphide. The interpretation of reactions in these media are shown in Tables 1, 2, 3. A few biochemical tests also are done to further differentiate the organisms when indicated. They are as follows:

1. Oxidase test<sup>8</sup> to differentiate Enterobacteriaceae from the other aerobic fermenting rods, i.e. *Plesiomonas shigelloides*, *Aeromonas hydrophila* and the vibrios
2. Urease test in Christensen's urease agar
3. Phenyl pyruvic acid (PPA) using phenylalanine malonate broth<sup>11</sup>
4. Decarboxylase tests for lysine, arginine and ornithine<sup>10</sup>
5. Citrate utilization test using Simmons' citrate
6. Fermentation of dulcitol, lactose, sucrose, mallose. All these tests were done as described earlier<sup>6</sup>
7. Pigment production on nutrient agar (NA)<sup>7</sup>
8. Gelatin liquefaction agar<sup>9</sup>

**Sero logical confirmation**

This is done by the slide agglutination test using the specific antisera.
RESULTS
Tables 1, 2, 3 show the interpretation of changes observed in MMM, TSI and LIA, respectively.

**TABLE 1**
Interpretation of changes observed in MMM *

<table>
<thead>
<tr>
<th>Appearance of medium</th>
<th>Interpretation of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red in colour, clear</td>
<td>Uninoculated medium</td>
</tr>
<tr>
<td>Turbidity along stab-line</td>
<td>Non-motile organism</td>
</tr>
<tr>
<td>Turbidity confined to aerobic top layer;</td>
<td>Non-motile organism unable to use NO₈ as H₂ acceptor</td>
</tr>
<tr>
<td>not extending to anaerobic lower layers</td>
<td></td>
</tr>
<tr>
<td>Lateral spreading of turbidity from stab-line in</td>
<td>Motile organisms — sluggish to highly motile</td>
</tr>
<tr>
<td>varying degrees</td>
<td></td>
</tr>
<tr>
<td>Yellowish throughout</td>
<td>Fermentation of mannitol</td>
</tr>
<tr>
<td>Slight deepening of original red colour</td>
<td>Non-fermentation of mannitol</td>
</tr>
</tbody>
</table>

* Bhat et al 1971a4

**TABLE 2**
Interpretation of changes observed in TSI *

<table>
<thead>
<tr>
<th>Appearance of medium</th>
<th>Interpretation of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange red in colour</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>Alkaline (deeper red) slant and butt</td>
<td>No sugars fermented</td>
</tr>
<tr>
<td>Acid (yellow) on slant and in butt</td>
<td>Lactose/or sucrose fermented along with glucose</td>
</tr>
<tr>
<td>Acid (yellow) in butt only, alkaline (deep red) slant</td>
<td>Glucose only fermented</td>
</tr>
<tr>
<td>Bubbles in butt or slant</td>
<td>Gas formed during fermentation</td>
</tr>
<tr>
<td>Blackening in butt or at the junction of butt and slant</td>
<td>Hydrogen sulphide produced</td>
</tr>
</tbody>
</table>

* Modified from Bailey and Scott, 1966

**TABLE 3**
Interpretation of changes observed in LIA *

<table>
<thead>
<tr>
<th>Appearance of medium</th>
<th>Interpretation of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light purple in colour, clear</td>
<td>Uninoculated medium</td>
</tr>
<tr>
<td>Acid (yellow) butt; alkaline (violet) slant</td>
<td>Fermentation of glucose without lysine decarboxylase production</td>
</tr>
<tr>
<td>Alkaline (violet) butt; alkaline (violet) slant</td>
<td>Fermentation of glucose with production of lysine decarboxylase</td>
</tr>
<tr>
<td>Acid (yellow) butt; red slant</td>
<td>Fermentation of glucose with oxidative deamination</td>
</tr>
<tr>
<td>Bubbles formed in but, often suppressed</td>
<td>Production of gas when glucose is fermented</td>
</tr>
</tbody>
</table>

* Bhat et al 1969a
### Biochemical differentiation of *Shigella* and *Shigella*-like organisms in the preliminary screening media

<table>
<thead>
<tr>
<th></th>
<th>MMM</th>
<th>TSI</th>
<th>PW</th>
<th>LIA</th>
<th>Oxidase test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mannitol</td>
<td>Motility</td>
<td>Butt</td>
<td>Slant</td>
<td>H₂S</td>
</tr>
<tr>
<td><em>E. coli; A-D</em></td>
<td>—</td>
<td>—</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>E. agglomerans</em></td>
<td>+/−</td>
<td>+/−</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>Providencia</em></td>
<td>—</td>
<td>—</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>P. rettgeri</em></td>
<td>+/−</td>
<td>+/−</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>P. shigelloides</em></td>
<td>—</td>
<td>—</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>K</td>
<td>—</td>
</tr>
<tr>
<td><em>V. cholerae</em> &amp; <em>El Tor</em></td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>A/K</td>
<td>—</td>
</tr>
</tbody>
</table>

— = Negative  
A = Acid  
V = Variable  
+ = Positive  
K = Alkaline  
R = Red (Oxidative deamination)  
K⁺ = Lysine decarboxylase positive  
* = Not belonging to Enterobacteriaceae

Also organisms which do not belong to Enterobacteriaceae which may have *Shigella*-like reaction in the TSI. All *Shigella* species are non-motile and do not produce lysine decarboxylase or lysine deaminase. Thus organisms like *Providencia*, and *Proteus rettgeri* irrespective of their presence or absence of motility would be eliminated as they both produce lysine deaminase. Likewise *P. shigelloides* and vibrios would be ruled out as they are positive for lysine decarboxylase activity. These and *Aeromonas* are also oxidase positive whereas all members of Enterobacteriaceae are oxidase negative.

This narrows down the list to anaerogenic *E. coli*, including the Alkalescens - Dispar (A-D) group and also the anaerogenic species of *Enterobacter agglomerans* formerly known as *Erwinia* or *Chromobacterium typhle-flavans*. It may be seen, that in the absence of motility and lysine decarboxylase activity, the organisms closely resemble mannitol fermenting and indole producing *Shigella*.

At this juncture, serological confirmation by using the various *Shigella* grouping antisera (Burroughs Wellcome & Co., London) in a slide agglutination test would help to
confirm the organisms. In the case of *Shigella*, a positive slide agglutination reaction would be evident (Chart III).

**CHART III**

Biochemical and serological identification of *shigellae* *

**SHIGELLA**

<table>
<thead>
<tr>
<th>Acid in glucose</th>
<th>Mannitol Negative</th>
<th>Mannitol Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole -</td>
<td>Indole +</td>
<td>Lactose -</td>
</tr>
<tr>
<td>Sh. dysenteriae 1</td>
<td>Sh. dysenteriae 2</td>
<td>Indole -</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Indole+</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Sh. flexneri 6</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Sh. flexneri 1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Sh. sonnel</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Sh. boydii</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

* Modified from Bailey and Scott, 1966

If in the living state, the serological test is negative, a slide agglutination is repeated on the same culture following heating as follows: a saline suspension is made and heated at 100 C for half hour to remove the heat labile surface antigens. This is especially so in the case of A-D and a polyvalent A-D O antiserumis** to be used.9

When cultures are non-agglutinable it is to be usually considered as not a *Shigella* or A-D and needs to be identified.

A preliminary differentiation of anaerogenic *E. coli* and *E. agglomerans* is done as shown in Table 5.

***(Burroughs Wellcome & Co., London.)*
### TABLE 5

Biochemical differentiation between *Enterobacter agglomerans* and anaerogenic *E. coli*

<table>
<thead>
<tr>
<th>Organism</th>
<th>NA</th>
<th>Decarboxylase</th>
<th>Simmons' citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lysine</td>
<td>Arginine</td>
</tr>
<tr>
<td><em>E. agglomerans</em></td>
<td>Yellow pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anaerogenic <em>E. coli</em></td>
<td>No pigment</td>
<td>V</td>
<td>V</td>
</tr>
</tbody>
</table>

NA = Nutrient agar  — = Negative  + = Positive  V = Variable

Procedure for the identification of *Shigella* and *Shigella*-like organisms in the absence of LIA is shown in Table 6.

### TABLE 6

Differentiation of *Shigella* and *Shigella*-like organisms without LIA

<table>
<thead>
<tr>
<th>Organism</th>
<th>M M M</th>
<th>TSI</th>
<th>PW for Indole</th>
<th>Oxidase</th>
<th>Urease</th>
<th>PPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mannitol</td>
<td>Motility</td>
<td>Butt</td>
<td>Slant</td>
<td>H₂S</td>
<td>Odour</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>+/—</td>
<td>—</td>
<td>A</td>
<td>K</td>
<td>—</td>
<td>V</td>
</tr>
<tr>
<td>Providence</td>
<td>—</td>
<td>—/+</td>
<td>A</td>
<td>K</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td><em>P. rettgeri</em></td>
<td>+/—</td>
<td>+</td>
<td>A</td>
<td>K</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td><em>P. shigelloides</em></td>
<td>—</td>
<td>+</td>
<td>A</td>
<td>K</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>Parity</td>
<td>K</td>
<td>+</td>
</tr>
<tr>
<td>Vibrios</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>A/K</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

A = Acid  K = Alkaline  — = Negative  + = Positive  V = Variable

II. **Simplified procedures for hydrogen sulphide producing enterobacteria:**

Usually when only TSI reactions are determined and found to indicate fermentation of glucose only, with or without gas, as shown by an alkaline slant and acid butt and formation of H₂S, and when parallel tests for indole production using PW and mannitol fermentation in the MMM are also available, these cultures are considered to be strains of H₂S producing *Proteus*, *Salmonella*, *Arizona* or *Bethesda-Bellerup* (BB) or *Edwardsiella tarda*. Simultaneous determination of TSI and LIA reactions yield additional valuable information for a more complete preliminary differentiation (see Table 7). As shown in this table *Salmonella* and/or late lactose fermenting *Arizona* have identical reactions in the preliminary screening media. At this point a slide agglutination test is done using the polyvalent O
### TABLE 7

Identification of organisms producing identical reactions in TSI * with simultaneous inoculation of MMM, PW and LIA

<table>
<thead>
<tr>
<th>Organism</th>
<th>TSI</th>
<th>MMM</th>
<th>PW</th>
<th>LIA</th>
<th>Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Butt</td>
<td>Slant</td>
<td>H₂S</td>
<td>Odour</td>
<td>Mannitol</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Ag/A</td>
<td>K</td>
<td>+</td>
<td>—</td>
<td>+/−</td>
</tr>
<tr>
<td><em>Arizona</em></td>
<td>Ag</td>
<td>K</td>
<td>+</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td><em>B. B</em></td>
<td>Ag</td>
<td>K</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>Ag</td>
<td>K</td>
<td>+</td>
<td>Fishy</td>
<td>—</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>Ag</td>
<td>K</td>
<td>+</td>
<td>Fishy</td>
<td>—</td>
</tr>
<tr>
<td><em>Ed. tarda</em></td>
<td>Ag</td>
<td>K</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>K</td>
<td>−/+</td>
<td>Aromatic</td>
<td>−</td>
<td>—</td>
</tr>
</tbody>
</table>

* On a few occasions, H₂S producing *Pseudomonas* species has been isolated

A = Acid
AG = Acid and gas
K = Alkaline
− = Negative
+ = Positive
R = Red (Oxidative deamination)
K* = Lysine decarboxylase positive

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salmonella antiserum (purchased from Burroughs Wellcome & Co., London) including Vi also, to screen the common *Salmonella* strains encountered in the humans. If positive, the specific O group is determined using individual grouping antiserum to screen O groups, from A to H. In this laboratory, further identification is done by sending the culture to a reference laboratory excepting in the case of *S. paratyphi* A and *S. typhi*. If inagglutinable

### TABLE 8

Biochemical tests found useful for differentiation of *Salmonella, Arizona* and CBB

<table>
<thead>
<tr>
<th>Organism</th>
<th>Malonate</th>
<th>Dextrose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Christensen’s urease</th>
<th>Gelatin liquefication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>−</td>
<td>A</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>Arizona</em></td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>CBB</td>
<td>−/+</td>
<td>A</td>
<td>V</td>
<td>V</td>
<td>4 / 4</td>
<td>−</td>
</tr>
</tbody>
</table>

A = Acid
4 = Positive
− = Negative
V = Variable
d = delayed

then a few biochemical tests are done as shown in Table 8 to differentiate between *Salmonella, Arizona* and Citrobacter including the BB strains (CBB). In Table 9, the biochemical tests which help to differentiate *P. vulgaris* and *P. mirabilis* are listed.
### TABLE 9

Biochemical tests found useful to differentiate *P. vulgaris* and *P. mirabilis*

<table>
<thead>
<tr>
<th>Test</th>
<th><em>P. vulgaris</em></th>
<th><em>P. mirabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>A(A)</td>
<td>(A) —</td>
</tr>
<tr>
<td>Maltose</td>
<td>A(A)</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

A = Acid, (A) = Acid production delayed
— = Negative, + = Positive

In TSI, H₂S might not be produced because of rapid fermentation of lactose or sucrose and development of pronounced acidity throughout the medium. Organisms producing acidity throughout the entire TSI medium, without formation of H₂S and indole might or might not be strains of *E. coli*, *Klebsiella-Enterobacter*, H₂S producing *Proteus*, *Arizona* or CBB strains. Simultaneous inoculation of LIA and TSI aids differentiation, along with MM and PW for indole production (Table 10.)

### TABLE 10

Identification of organisms producing acid throughout TSI with gas, with simultaneous inoculation of MM, PW and LIA

<table>
<thead>
<tr>
<th>Organism</th>
<th>TSI Butt</th>
<th>TSI Slant</th>
<th>TSI Odour</th>
<th>MM Butt</th>
<th>MM Slant</th>
<th>MM Mannitol</th>
<th>MM Motility</th>
<th>PW Indole</th>
<th>PW Butt</th>
<th>PW Slant</th>
<th>PW Gas</th>
<th>LIA Butt</th>
<th>LIA Slant</th>
<th>LIA Odour</th>
<th>LIA H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>AG</td>
<td>A</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>K⁺/A</td>
<td>K</td>
<td>+/—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>A+/-</td>
<td>+</td>
<td>+/—</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>K⁺/A</td>
<td>K</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>AG</td>
<td>A</td>
<td>—</td>
<td>+</td>
<td>+</td>
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<td>K⁺/A</td>
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<tr>
<td><em>P. vulgaris</em></td>
<td>A+/-</td>
<td>+</td>
<td>+/—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>R</td>
<td>V</td>
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<tr>
<td><em>Arizona</em></td>
<td>AG</td>
<td>A</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>K⁺</td>
<td>K</td>
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<tr>
<td>CBB</td>
<td>AG</td>
<td>A</td>
<td>—</td>
<td>+</td>
<td>+</td>
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<td>K</td>
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A = Acid, K = Alkaline, — = Negative, + = Positive, AG = Acid and Gas, R = Red (Oxidative deamination), K⁺ = Lysine decarboxylase positive, V = Variable

Over a number of years this methodology has been followed by the microbiology laboratory of the Wellcome Research Unit, C.M.C. Hospital. This has been found useful to screen aerobic fermenting rods and to provisionally identify them within a short time without using too many biochemical tests. The number of primary plating media listed may be rather too many and it may not be possible to include all. In such circumstances XLD can be the one single medium of choice for isolating *Shigella* and DCA can be dispensed with²; BSA also can be excluded as *Arizona* group of organisms is extremely rare in diarrhoeal diseases of man² and SSA can replace BSA in the isolation of salmonellae (unpublished data, P. Bhat 1975).
ACKNOWLEDGEMENT

I wish to thank all my colleagues in the microbiology laboratory of the Wellcome Research Unit whose cooperation has made this report possible. I am also grateful to Prof. S. J. Baker for his encouragement and advice.

REFERENCES


3 BHAT, P. AND RAJAN, D. Comparative evaluation of deoxycholate citrate medium and xylose lysine deoxycholate medium in the isolation of shigellae. Amer. J. clin. Pathol. 1975 (accepted for publication)


