THE LABORATORY DIAGNOSIS OF TYPHOID FEVER

AN OUTLINE OF THE METHODS USED AT THE GOVERNMENT PATHOLOGICAL LABORATORY, CAPE TOWN

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The bacteriological diagnosis of typhoid fever is, for all practical purposes, dependent upon the recovery of *Salmonella typhi* from specimens of blood, faeces and urine, and to a lesser degree upon the measurement in the patient's blood of the specific antibody response to the flagellar (H) and somatic (O) antigens.

The techniques used at the Government Pathological Laboratory, Cape Town, for the isolation of *S. typhi* will be outlined. We have found these methods also suitable for the isolation of the other members of the salmonella-shigella group.

The essence of the scheme is to isolate non-lactose-fermenting organisms (into which category the salmonella-shigella organisms fall) from the specimen, to separate off from these the large group of proteus organisms by utilizing the fact that they rapidly hydrolyse urea, and then submitting the remainder to tests for motility, the ability to ferment dextrose, mannite, sucrose and salicin, to produce indole, and to form sulphide. An assessment of these biochemical properties permits of a presumptive diagnosis, and confirmation is obtained by agglutination tests with specific antiserum.

ISOLATION AND IDENTIFICATION OF S. TYPHI FROM STOOL AND URINARY SPECIMENS

Stools. On receipt at the laboratory each specimen of stool is plated onto selective differential media, viz. (*a*) McConkey's agar and (*b*) salmonella-shigella agar (Difco). About 1 g. of faeces is also emulsified into a tube of selenite enrichment broth which, after about 18 hours' incubation, is subcultured onto a plate of salmonella-shigella agar.

Urines. Equal quantities of the urine and of double-strength selenite broth are mixed and incubated for about 18 hours. Subculture is then made onto a salmonella-shigella agar plate.

Subsequent procedures. After 18-24 hours' incubation, all the McConkey and salmonella-shigella agar plates are examined for non-lactose-fermenting colonies with characteristics suggestive of pathogens. At least 6 suspicious colonies are then picked off and seeded into tubes of Singer's urea broth.[1] These tubes are incubated for about 6 hours and are then examined. Tubes showing an alkaline change from the rapid hydrolysis of the urea are discarded as containing proteus organisms. From those tubes which show no signs of urease activity or of lactose fermentation, subcultures are made into the following tubes of differential media: Kligler's iron agar (Difco), semi-solid mannite medium, sucrose-lactose-salicin medium and tryptone water. After 18-24 hours' incubation these tubes are examined for changes indicative of the presence of intestinal pathogens. The presumptive diagnosis of the type of organism suggested by the reactions in these tubes is then confirmed by slide agglutination tests with the appropriate specific antisera.

If *S. typhi* is identified, pure cultures of the organism on plain agar slopes are submitted to the Phage-Typing Unit of the Institute of Pathology, Pretoria, for phage typing.

This scheme we have found to be economic and reliable.

Kligler's iron agar[2] is a useful differential tube medium in that in a single tube of medium it is possible to confirm that the organism is a non-lactose-fermenter, and to decide whether it ferments dextrose (and whether with acid only or acid and gas) and produces hydrogen sulphide.

The semi-solid mannite medium is equally useful in that in one tube it can be decided whether the organism is motile, whether it ferments mannite and whether with acid only or acid and gas.

SLS medium, which contains sucrose, lactose and salicin, is useful in discarding organisms of the paracolon group, which may closely resemble salmonellas but can be differentiated by the fact that paracolons sooner or later always ferment one or more of these sugars, which the salmonellas never do.

Tryptone water is used to test for indole production (Kovac's reagent is used in the test).

The use of Singer's urea broth has the advantage that it not only indicates urease activity of the seeded organism but also, by virtue of the fact that it contains lactose, acts as a confirmatory test for the absence of lactose fermentation of the organism.

ISOLATION OF S. TYPHI FROM BLOOD CULTURES

As the chance of isolating *S. typhi* from blood cultures is virtually 100% in the first week of the disease, too much stress cannot be placed on the desirability of undertaking this procedure in all cases of clinically suspected typhoid fever, and it is regrettable that this most useful laboratory investigation is not called for more frequently. Blood specimens for culture are submitted in bile-salt broth media (5 c.c. of the blood in 100 c.c. of the broth), and on arrival at the laboratory are incubated overnight and then plated onto McConkey's agar, which, after 24 hours' incubation, is examined for characteristic growth. If this is present, tubes of Kligler's iron agar are inoculated and suspicious growths are then identified by slide agglutination tests. Absence of growth on primary plating necessitates repeated platings from the original broth on the 2nd, 4th and 8th days, before final discarding as negative. Usually, if *S. typhi* is present, it will be isolated on the primary plating. Occasionally only, clot cultures are performed from clotted blood specimens submitted for Widal tests.

ISOLATION OF S. TYPHI FROM CHRONIC URINARY AND FAECAL CARRIERS

As we feel a little sceptical about the value of the Vi test in tracing carriers, encouragement is given to the epidemiological approach to the problem and, once a few persons are suspect on epidemiological grounds, repeated specimens of stool and urine are submitted for cultural examination. Supplies of selenite broth are supplied to the health officials undertaking the investigations, and are seeded by them with the requisite amount of faecal material. These specimens, together with the urinary specimens, are forwarded to the laboratory, where the broths are subcultured onto a salmonella-shigella agar plate, and growths with the colonial characteristics resembling those of *S. typhi* are passed through Singer's urea media and thence subcultured only onto a Kligler's iron agar. Characteristic findings are confirmed by slide agglutination. The urinary specimens are dealt with as described above.

Where specimens of stool may be long delayed in transit, they may be preserved by the addition of Sach's solution,[3] obtainable from this laboratory on request. We have, however, seldom found this necessary with *S. typhi* specimens.

FORMULAE OF MEDIA

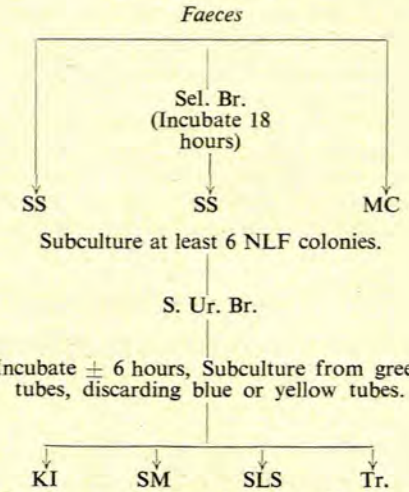
S.L.S. Medium. Peptone 10 g., sodium chloride 5 g., agar 3 g., distilled water 1,000 ml. These ingredients are steamed to dissolve, and after cooling the following are added:

Sucrose 10 g., lactose 10 g., salicin 5 g. Adjust the pH to 7·8. Add 10 ml. of Andrade's indicator and 4 ml. of a 0·4% brom-thymol-blue solution. The medium is dispensed in 5-ml. amounts in tubes and the tubes are steamed for 3 successive days for 15 minutes.

Semi-solid Mannite Medium: Proteose peptone 5 g., sodium chloride 5 g., agar 5 g., distilled water 1,000 ml. Adjust the pH to 7·8, and then add 10 g. of mannite, and 4 ml. of a 0·4% solution of brom-cresol purple. The medium is dispensed in 5-ml. amounts in tubes and the tubes autoclaved at 15 lb. pressure for 25 minutes.

Singer's Urea Medium: Difco tryptone 20 g., sodium chloride 5 g., distilled water 1,000 ml. These ingredients are steamed to dissolve and then cooled. The pH is adjusted to 7·3 and 4 ml. of cresol-red solution, 10 ml. of brom-thymol-blue solution and 10 ml. of thymol-blue solution are added. 100 ml. of 10% lactose solution and 80 ml. of 20% urea solution are then added by Seitz filtration. The medium is dispensed in 3-ml. amounts and incubated for 24 hours, and then refrigerated until required.

SCHEME FOR ISOLATION AND IDENTIFICATION OF PATHOGENIC ENTEROBACTERIACEAE



Incubate 18-24 hours. Do Gram's stain. Classify according to biochemical reactions. Confirm diagnosis of suspected pathogens by slide agglutination tests with appropriate specific diagnostic antisera.

DIAGNOSIS BY BLOOD CULTURE

1. Collect 5 c.c. of blood in 100 c.c. of B.Br. and incubate.
2. Subculture onto MC at end of 24 hours and, if necessary, at end of 2nd, 4th and 8th days before finally discarding as negative.
3. Subculture NLF colonies onto KI.
4. Confirm diagnosis of tubes, giving suggestive biochemical reactions by slide agglutination tests.

KEY

Sel.Br.=selenite enrichment broth. SS=salmonella-shigella agar (Difco). MC=McConkey agar. S.Ur.Br.=Singer's urea broth. KI=Kligler's iron agar (Difco). SM=semi-solid mannite agar. SLS=sucrose-lactose-salicin medium. Tr.=Tryptone water. B.Br.=bile broth. NLF=non-lactose-fermenters.

INTERPRETATION OF BIOCHEMICAL REACTIONS

S.Ur.Br. Blue colour=urea hydrolysis. Yellow colour=lactose fermentation.

KI. Yellow slope and butt=lactose fermentation. Red slope and yellow butt=dextrose fermentation. Splitting of agar=gas formation. Blackened butt=H_2S production.

SM. Growth confined to line of stab=non-motile. Growth diffuse=motile. Yellow colour=mannite fermentation. Fine gas bubbles=gas production.

SLS. Red colour=fermentation of sucrose, lactose or salicin.

Tr. Test with Kovac's reagent for indol formation.

DIFFERENTIAL PRESUMPTIVE DIAGNOSIS ACCORDING TO BIOCHEMICAL REACTIONS

Organism	*Motility*	*Dextrose*	*Mannite*	*Sucrose Lactose Salicin*	*Indol*	*H_2S*
S. typhi	+	A	A	—	—	+
Other salmonella	+ (−)	AG (A)	AG (A)	—	—	+ (−)
Sh. paradysenteriae (Newcastle) ..	—	AG	AG	—	—	—
Sh. dysenteria (Shiga)	—	A	—	—	—	—
Sh. ambigua (Schmitz)	—	A	—	—	+	—
Sh. sonnei.. ..	—	A	A	A (very slow)	—	—
Sh. paradysenteriae (Flexner and Boyd)	—	A	A	—	− (+)	—
Sh. alkalescens ..	—	A	A	—	+	—
Proteus group ..	+	AG	—	+	+ (−)	+ (−)
Proteus morganii..	+	A (A)	—	—	+	—
Pseudomonas aeruginosa	+	A (−)	—	—	—	—
Alcaligines faecalis	+	—	—	—	—	—
Paracolobactrum	+ (−)	AG	AG (−)	+	+ (−)	− (+)

A=acid formation only AG=acid and gas formation

TESTS FOR (*a*) 'FREEDOM FROM INFECTION' OF CONVALESCENTS AND (*b*) DETECTION OF CARRIERS

Faeces

1. Forward to laboratory in Sel. Br. Incubate up to 18 hours.
2. Subculture onto SS. Incubate 18-24 hours.
3. Subculture at least 6 non-lactose-fermenting colonies onto S. Ur. Br. and incubate 6 hours.
4. Discard blue or yellow tubes. Subculture green tubes. onto KI and incubate 18-24 hours.
5. Examine KI tubes. Discard tubes which do not show red slope with yellow butt and no gas and (usually) blackening.
6. Do Gram stains of smear.
7. Do slide agglutination tests with Vi, H and O typhoid antisera.

Urine

1. Mix equal quantities of urine and Sel. Br. Incubate 18 hours.
2. Subculture onto SS and proceed as for faeces.

SUMMARY

An outline is given of the laboratory methods used in the Government Pathological Laboratory, Cape Town, for the isolation of *S. typhi* from specimens submitted.

The various media used are very briefly described, and a scheme for the isolation and identification of pathogenic enterobacteriaceae is given.

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REFERENCES

1. Singer, J. (1950): Amer. J. Clin. Path., **20**, 880.
2. *Difco Manual*, 9th ed. Michigan: Difco Laboratories Inc., p. 165.
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