Technical method

The isolation and identification of *Vibrio cholerae*

A. L. Furniss and T. J. Donovan  *From the Public Health Laboratory, Maidstone*

Cholera is not endemic in Britain but cases have been occurring amongst people returning from places abroad where the disease is present. A diagnosis of cholera is therefore unlikely unless the patient has been abroad within the last two weeks or has been in very close contact with a known vibrio excreter.

The watery stool of a severe case of cholera may give an almost pure growth of vibrios, and enrichment and selective techniques are hardly necessary. In most cases, however, vibrios are present in small numbers together with other organisms; this means that enrichment and selective media are essential.

**Collection of Sample for the Laboratory**

A specimen of faeces should be sent to the laboratory as soon as possible. It should not be sent by post because of the delay. Transport medium is not necessary in Britain where laboratories are so accessible.

**Direct Examination of Stool**

The microscopic appearance of even a severe cholera stool is not diagnostic; isolation of the organisms is of fundamental importance. Treatment, if necessary, will depend on the degree of dehydration and will not await a laboratory report.

**Enrichment**

Vibrios will multiply more rapidly in alkaline peptone water than other organisms, but the selective effect is lost if subculture is delayed until other organisms have multiplied or if the peptone water is too heavily inoculated. This period of selective advantage for the vibrios may be prolonged if the alkaline water is incubated at 20°C.

**Selective Media**

Monsur’s medium (Monsur, 1963) is an excellent selective medium for vibrios, but has the disadvantage that it is not commercially available.

The base can be prepared in the laboratory and stored until required; the potassium tellurite can then be added just before pouring to give a final concentration of 1 in 200 000. Familiarity with the medium is necessary to gain full advantage from it.

Thiosulphate-citrate-bile-salt-agar (TCBS) is commercially available and easy to use (Kobayashi, Enomoto, Sakazaki, and Kuwahara, 1963). It is recommended at present for routine use in Britain for the isolation of vibrios.

**In the Laboratory**

Inoculate the following: (1) About 2 ml faeces into 20 ml alkaline peptone water, pH 8 (first peptone water), and incubate for five to eight hours. (2) Thiosulphate-citrate-bile-salt-agar plate with a heavy inoculum of faeces (first TCBS plate). (3) The usual media for the isolation of shigellas, salmonellas, etc.

After five to eight hours’ incubation from the first alkaline peptone water inoculate the following: (4) a new peptone water (second peptone water) with about 1 ml of fluid from the top of the first peptone water. This second peptone water is to be incubated for a further five hours or overnight. (5) A new TCBS plate (second TCBS plate) with a heavy inoculum. (6) Nutrient agar which may be found useful as a source of colonies for agglutination.

**SECOND DAY**

If the first and second TCBS plates are negative, inoculate a third TCBS plate from the top of the second peptone water.

**EXAMINATION OF TCBS PLATES**

Cholera vibrios appear as yellow colonies because of the fermentation of sucrose and the surrounding

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mediumized (2-3 mm diameter after 18 hours' incubation), yellow colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. cholerae</em></td>
<td>Large, yellow colonies</td>
</tr>
<tr>
<td><em>V. cholodonicus</em></td>
<td>Large, deep green colonies</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>No growth or small, colourless or pale green colonies</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>No growth or small, yellow or greenish colonies</td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>No growth</td>
</tr>
<tr>
<td><em>Enterococci</em></td>
<td>Small, yellowish-white colonies</td>
</tr>
</tbody>
</table>

**Table**  *Colonial appearance of organisms on TCBS agar after overnight incubation at 37°C*
medium may also be turned yellow. It should be noted that the yellow colour may fade. (Some freeze-dried strains do not grow on TCBS agar and some have lost their ability to ferment sucrose overnight. Typical strains that have been recently isolated are available from the authors.)

**RECOMMENDED IDENTIFICATION PROCEDURE**

Typical yellow colonies should be subcultured to a nutrient agar plate which is incubated at 37°C for five to eight hours. After this time there is normally sufficient growth for the following tests.

1 **OXIDASE TEST (KOVACS, 1956)**

This should be done on colonies on nutrient agar and not on growth from a medium which contains a fermentable carbohydrate, as does TCBS.

2 **AGGLUTINATION WITH TYPE ‘0’ 1 CHOLERA ANTISERUM (‘POLYVALENT’)**

It should be possible to use fresh colonies on TCBS agar for slide agglutination but after some time the colonies become so sticky as to make agglutination impossible. It is, therefore, preferable to check the agglutination from the growth on nutrient agar.

3 **GRAM STAIN TO CHECK THE PURITY OF THE CULTURE**

Curvature of the bacterial axis may or may not be recognizable; this is not a diagnostic feature.

The following biochemical media are then heavily inoculated from the growth on the nutrient agar plate:

4 **DECARBOXYLASE MEDIA (MÖLLER, 1955)**

Modified by the addition of 1% sodium chloride, ie, lysine, arginine, ornithine, and a blank. Such media with added sodium chloride are still satisfactory for use with enterobacteria.

5 **PEPTONE WATER SUGARS WITH ANDRADE’S INDICATOR**

Glucose, sucrose, arabinose, mannose, and simple peptone water.

6 **TUBES OF HUGH & LEIFSON’S OXIDATION-FERMENTATION MEDIUM (HUGH & LEIFSON, 1953)**

In addition, TCBS, blood agar (for purity check), a nutrient agar slope (to be sent for typing), and electrolyte-deficient medium (see below) should be inoculated and incubated at 37°C. Most cholera vibrios grow sufficiently for the results to be read after overnight incubation.

Vibrios are characteristically actively motile, oxidase-positive, Gram-negative bacilli. Other characters are: acid with no gas in glucose, acid in sucrose, acid in mannose, arabinose negative, indole positive, arginine negative, lysine positive, ornithine positive.

**OTHER TESTS**

Vibrios are sensitive to the vibriostatic agent O129 (2,4-diamino-6, 7 di-isopropyl pteridine) (Shewan et al, 1954). This sensitivity can be tested by means of a disc placed on a lawn of the culture. One hundred and fifty mg O129 compound is dissolved in a 1:1 alcohol-ether mixture; discs are impregnated with a drop of this solution and dried (Caselitz, 1966).

We have ourselves little experience of the so-called ‘string test’ (Smith, 1970), but think it may be of help in identifying *Vibrio cholerae* colonies. A colony is emulsified in a drop of 0.2% desoxycholate in distilled water on a slide. Cholera and allied vibrios will characteristically become viscid so that when the point of the wire is withdrawn from the slide the drop is adherent enough to be elongated momentarily as a ‘string’.

**USE OF ELECTROLYTE-DEFICIENT MEDIUM IN THE IDENTIFICATION OF VIBRIOS**

This medium, CLED, was originally designed to prevent the swarming of proteus (Mackey and Sandys, 1966, modified by Bevis, 1968). It has been found to be of great practical value in differentiating vibrios. *V. cholerae* will grow on CLED. The salt-requiring vibrios, which include *V. parahaemolyticus* and *V. alginolyticus*, will not grow on CLED because they will not grow in the absence of sodium chloride. *V. cholerae* can multiply in the absence of sodium chloride, although like all vibrios its growth is enhanced by the addition of sodium chloride.

**TYPING OF VIBRIOS**

Although identification of cholera vibrios is practicable in the general diagnostic laboratory, all strains of vibrios isolated, whether agglutinating or not, should be sent to a Reference Laboratory as soon as possible; serotyping and phage typing can be undertaken. Biotyping of strains from the present pandemic shows them as El tor biotype, but the occurrence of variants makes it clear that the idea of two polar forms of immutable biotypes is false. There is much still to be done adequately typed.

**References**


Hugh, R., and Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by...
A new selective medium for pathogenic vibrios: TCBS (modified
Kovacs, N. (1956). Identification of _Pseudomonas pyocyanea_ by the
oxidase reaction. _Nature (Lond.),_ 178, 703.
Mackey, J. P., and Sandys, G. H. (1966). Diagnosis of urinary infe-
Møller, V. (1955). Simplified tests for some amino acid decarboxylases
and for the arginine dihydrolase system. _Acta path. microbiol.
sand.,_ 36, 158-172.
rapid differentiation of certain non-pathogenic, asporogenous
bacilli. _Nature (Lond.),_ 173, 208-209.

**Book reviews**

In the now well known Monograph Series published by the Public Health Labora-
try Service it is the intention to publish collections of papers on various labora-
tory techniques that experience has shown to work well. This is the first group of
such papers and like other monographs in the series will find its way into most
microbiology laboratories. It contains a selection of descriptions of very different
techniques.

Readers will find precise instructions for success with Elek plates for the
demonstration of diphtheria toxin, and adherence to the suggestions will no
doubt help to reduce the number of occasional failures with this technique.
Methods for the isolation and characteri-
ization of _Listeria monocytogenes_ are collected together and are here for easy
reference. The way that we should go
about the isolation and identification of mycobacteria is carefully and comprehen-
sively described including a host of
practical tips. This is an authoritative
collection of advice from the Tuberculosis Reference Laboratory. A further two
subjects are dealt with: the slide tests for
glandular fever and finally there is a note
on the isolation and identification of
genital tract lactobacilli.

_D. M. JONES_

The publication of this MRC memoran-
dum is most welcome, especially as the
previous edition, which was published in
1955, had been out of date for well over a
decade. The present monograph is an
ideal introduction for anyone concerned
with patients suffering from haemostatic
disorders. Brief and always to the point,
the author covers the clinical and labora-
tory diagnosis of hereditary and acquired
coaulation defects and their treatment.
Stress is laid on the need to treat these
patients at special centres and the author
succeeds in showing that, although the
scene has been revolutionized by increased
supplies of concentrate, there is much more
to the treatment of haemophilia than the
administration of blood products. At 34p
the pamphlet is cheap enough and anyone
connected with the management of
bleeding disorders will benefit from having
a copy, whether they be laboratory tech-
nician, nurse, physiotherapist, or doctor.
I have only one adverse criticism and this
refers to Appendix 2 which lists the
haemophilia centres in the UK. Although
the list, as it stands, is far better than no
list at all it is a pity that, even at the time
of publication, it was (like so many other
printed lists) partly out of date and full of
little errors.

_KATHARINE M. DORMANDY_

**Tumors of the Extra-Adrenal Paraganglion System (Including Chemoreceptors).** By George G. Glenner and Philip M. Grim-
ley. (Pp. 90; illustrated. $4.50.) Washington,
D.C.: Armed Forces Institute of
Pathology, 1974.
The fascicles comprising the Armed
Forces Institute of Pathology 'Atlas of
tumor pathology' have long since estab-
lished themselves as essential companions
to the practising histopathologist, and
this new issue on neoplasms of the extra-
adrenal paraganglia is a most useful
addition to the series.
The first 38 pages are devoted to the
anatomy, histology, cytology, electron
microscopy, histochemistry, cytochem-
istry, and physiology of the normal para-
ganglia, and a classification of these
structures into a number of anatomical
groups or 'families' is suggested. Tumours
and tumour-like lesions of the extra-
adrenal paraganglia are then described.
The text is concise, but contains much
useful clinical, as well as pathological
information. The illustrations are of high
quality.

This publication maintains the high
standards already set by earlier additions
to the 'second series' of AFIP fascicles. It
represents a most valuable compendium of
up-to-date knowledge concerning the
paraganglia and their tumours.

_N. F. C. GOWING_

**Cardiomyopathies (Recent Advances in Studies on Cardiac Structure and Metabo-
5p.) London: Medical and Technical
This is the proceedings of an international
symposium on cardiomyopathies, and like
other reports of its kind, covers the field
widely. There is more than the usual
quota of pathology, with less considera-
tion of clinical and therapeutic problems.
Pathologists will find this of value as a
reference work. It would be a pity if
weakening of links between this country
and Africa, such a rich source of many
types of cardiomyopathy, held back the
development and spread of knowledge
in this subject.

_R. A. B. DRURY_