Bacteriological Diagnosis of Cholera under Field Conditions

by K. A. Monsur, Director, Institute of Public Health, Dacca; and Consultant Bacteriologist, Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan

In countries where laboratory facilities are very meagre the bacteriological diagnosis of cholera in outlying areas always presents a difficult problem. Recent studies of media at our laboratory show that it is possible to make an accurate bacteriological diagnosis of cholera even in very remote areas. The two media found most useful are (1) a solid alkaline, bile salt, tellurite, gelatin-agar plate which acts as a highly selective medium for *Vibrio cholerae*, and (2) a liquid alkaline, bile salt, peptone, tellurite medium which acts as both an enrichment and a preservative fluid. With these two media the bacteriological diagnosis of cholera in rural areas can be approached from two different angles:

(1) on-the-spot diagnosis by a mobile team in the field, supplied with media sent out periodically from a base laboratory; and

(2) central laboratory diagnosis of specimens posted in a preservative fluid medium; reliable results can be had even after a week or more.

Materials and methods

The technician is supplied from a base laboratory with prepared solid medium in flasks and with sterile Petri dishes. Plates are poured in the field by melting the medium over boiling water. Before plates are poured, the requisite quantity of potassium tellurite solution is added to the melted medium. The medium is highly inhibitory to other organisms, especially the anthracoids, and accidental contamination should not be troublesome. If plates are poured in small lots and used in a day or two, refrigeration is not necessary. The plates may be inoculated from rectal swabs directly or after enrichment in liquid medium. *V. cholerae* grows abundantly on the plate at room temperature and incubation is not essential. Because of the marked inhibitory effect of the medium on other organisms and the large numbers in which vibrios are generally present

---


1243b
in the stool in the early stages of cholera the growth is generally pure and abundant. It is usually possible to confirm the diagnosis serologically in the field by slide agglutination.

Previous workers have taken advantage of the fact that *V. cholerae* is a proteolytic organism for its isolation on gelatin-agar plates. On gelatin-agar each colony of *V. cholerae* develops a characteristic halo." Useless though this gelatin-agar is for the isolation of *V. cholerae*, it is non-selective and is not inhibitory to competing organisms. During a study intended to develop a preservative fluid for *V. cholerae* it was found that sodium taurocholate and potassium tellurite greatly enhanced the chances of isolation of *V. cholerae* from material heavily contaminated with other organisms. The medium prepared by combining these ingredients with those of gelatin-agar plates is highly selective for *V. cholerae*. The organism grows abundantly on this plate and gives typical colony morphology, permitting easy identification." The composition of the medium is as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium taurocholate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Gelatin (Difco)</td>
<td>30.0 g</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
</tbody>
</table>

The medium is sterilized by autoclaving and potassium tellurite is added to a final concentration of 0.5–1 × 10⁻⁵ (from one part in 200 000 to one part in 100 000) before pouring plates.

Studies at the Pakistan-SEATO Cholera Research Laboratory have shown that at least two fluid media not only enrich but also preserve *V. cholerae*. One of these is alkaline-peptone water containing 10.0 g trypticase, 10.0 g sodium chloride and 1.0 g sodium carbonate per litre, with a pH of about 9.2. The other is the same as the selective medium described above with omission of the two solidifying agents, gelatin and agar. This medium is now used as a preservative for specimens coming to the laboratory from a distance.

The medium is distributed in 2-ounce screw-capped bottles in 25-ml quantities and potassium tellurite is added to the medium at the time of inoculation to a final concentration of 1 × 10⁻⁵. Separate addition of potassium tellurite in the field can be avoided by pretreating the rectal swabs used for collecting specimens with the proper amount of potassium tellurite beforehand. These swabs, if kept well dried, have given satisfactory results when used up to three months after preparation.

**Observations**

The solid medium has a high pH—about 8.5; this, together with the sodium taurocholate and potassium tellurite, makes the medium highly inhibitory to most organisms. On the other hand, *V. cholerae* grows very well, producing characteristic colonies which attain a large size (3-4 mm) in 48 hours with typical black centres and well-defined haloes around each colony. As a rule the colonies can easily be identified at 24 hours by their characteristic greyish appearance and surrounding halo. Quantitative studies with artificially infected stool suspensions have shown that the medium is capable of detecting a single viable *V. cholerae* in the inoculum, even though it be heavily contaminated with other faecal organisms.

The two liquid media have a pH of about 9.2. Experiments were carried out in which stool suspensions were added to a series of screw-capped bottles containing 25-ml quantities of these media. The bottles were then inoculated with serial dilutions of cultures of *V. cholerae* and a loopful from each of these inoculated bottles was plated out on different media at various intervals. The results of these experiments, when compared with the viable count of the original peptone-water culture of *V. cholerae* used for inoculation, indicate that both media are capable of detecting even a single viable organism per ml of stool suspension. The purity of the final growth and the persistence of *V. cholerae* in the presence of other organisms seem to be better in the medium containing bile salts and potassium tellurite than in plain alkaline peptone-water.

These two liquid media were tested with swabs from cholera patients. Rectal swabs from patients were put in screw-capped bottles containing the medium to be tested and the caps were screwed down tightly. In most cases two pairs, and in some three pairs, of bottles were used. One of each pair contained plain alkaline peptone-water and the other had bile salt and potassium tellurite added. One loopful from each of the first pair of bottles was plated out some hours after incubation and, if this was negative, again the following morning. The remaining pair were kept at room temperature without being opened and were plated out on the

---

seventh day. When a third pair of bottles was available these were tested after 15 days. In the few instances in which it was not possible to follow this routine rigidly, only the relevant information has been included in the analysis of data. During the period from May to December 1961, (a) of 73 positive cultures put in alkaline peptone-water and kept at room temperature 62 were positive and 11 negative on the seventh day; (b) of 74 positive isolations put in alkaline, bile-salt, tellurite peptone-water and kept at room temperature 71 were positive and three were negative on the seventh day. There was then a loss of 11 in 73 in plain alkaline peptone-water and of three in 73 in alkaline, bile-salt, tellurite peptone-water. This result fits the experimental observations described above. The growth obtained from alkaline, bile-salt, tellurite peptone was definitely purer than that obtained from plain alkaline peptone-water. Alkaline, bile-salt, tellurite peptone succeeded in growing the organism after overnight incubation in every case in which the other medium was successful. From the point of view of rapidity of isolation, alkaline peptone-water seems to give a slightly earlier positive result on a larger number of cases than does alkaline, bile-salt, tellurite peptone, although in every case the latter medium gave a similar positive, or perhaps a better, growth within 24 hours. Enrichment and preservation of the culture for a prolonged period (about a week or so) are best achieved with alkaline, bile-salt, tellurite peptone, whereas plain alkaline peptone-water is preferred for a rapid diagnostic growth.

**Conclusion**

Two additional tools are available, to be used singly or together for the field diagnosis of cholera. A mobile team can now make a spot diagnosis with plates and simultaneously send control specimens to base laboratories for confirmation. When field teams are not available, specimens from suspect outbreaks may be sent routinely by local lay representatives to base laboratories by post or otherwise. Under this system a base laboratory can serve an extensive area with reliable diagnoses on material sent in by non-technical representatives.

These methods offer new possibilities for the early diagnosis of outbreaks of cholera, thus permitting health authorities to take effective steps early in an outbreak before extensive spread occurs.

Lysogénie et lysotypie de *V. cholerae* et *V. El Tor*

d'origines géographiques diverses

par J. Gallut & P. Nicolle, Institut Pasteur, Paris

Depuis 1947, à l'occasion de l'épidémie d'Egypte, nous avons rassemblé une série de bactériophages cholériques, dont certains ont été isolés par nous-mêmes à partir de souches lysogènes au cours d'une étude systématique sur la fréquence de la lysogénie parmi des souches de *Vibrio cholerae*, de *Vibrio El Tor* et de vibrons non agglutinables (NAG) et dont les autres nous furent donnés par M. d'Hérelle et le Dr Regamey.

Nous pûmes ainsi étudier les diverses images de réactions lytiques fournies par les souches de la collection du Service du Choléra de l'Institut Pasteur sous l'action de cette série de phages. Les différences observées nous permirent rapidement de penser qu'une lysotypie était possible. Cette étude a été interrompue pendant une dizaine d'années, puis reprise récemment.

Asheshov et ses collaborateurs avaient constaté que les souches de vibrons cholériques isolées en Inde pouvaient être réparties en quatre groupes suivant leurs images variées de sensibilité aux préparations adaptées du bactériophage du type A. Mais aucune tentative systématique n'avait été entreprise pour mettre au point une méthode de lysotypie de ces germes, capable de fournir d'utilles indications aux épidémiologistes.

Le Comité d'Experts du Choléra de l'OMS, dans son rapport de 1952, avait recommandé qu'une