NOTE

VIBRIO AGAR: A NEW PLATING MEDIUM FOR ISOLATION OF *VIBRIO CHOLERAE*

Although large varieties of agar media have been reported for the isolation of *Vibrio cholerae*, TCBS agar and alkaline bile salt agar are the most commonly used.

TCBS agar was devised by Kobayashi et al. (1963) and confirmed to be satisfactory in an extensive field trial during the Joint Japan-Philippines-WHO Cholera Studies. TCBS agar is a highly selective medium for *V. cholerae*, but the selectivity is sometimes insufficient to suppress the growth of *Proteus*, *Aeromonas* and enterococci, especially, when the plate is used for secondary isolation of the vibrio after enrichment of the fecal specimens in alkaline peptone water. In those instances, differentiation of the *V. cholerae* colony from those of contaminants is somewhat difficult because the latter also forms sucrose-fermenting yellow colony resembling that of *V. cholerae*. In addition to this, the *V. cholerae* colony growing on TCBS agar plate shows some difficulty to be agglutinated with *V. cholerae* O-1 antiserum. Due to the high selectivity of the agar, some strains of *V. cholerae* especially classical and non-agglutinable strains, fail to grow on the agar plate. Many workers recommended, therefore, that alkaline bile salt agar be used together with TCBS agar to avoid such unpredictable lapses.

Alkaline bile salt agar is less selective and not a differential medium and much experience is necessary to use this agar. Although *V. cholerae* may be detected on alkaline bile salt agar with great ease when the oblique light technique is used, the technique is very time-consuming and therefore cannot be employed for the observation of a large number of plates in routine examination.

Recently, a new plating medium, Vibrio agar, has been developed by "Nissui Seiyaku Co." in Japan for the isolation of *Vibrio parahaemolyticus*. However, the agar was somewhat insufficient for growth and differentiation of *V. cholerae*. During the course of the Cholera Carrier and Serological Studies in Calcutta under the joint auspices of the Indian Council of Medical Research, West Bengal Government Health Department and WHO in 1969, the authors revised the formula of the original Vibrio agar. The final formula of the revised Vibrio agar is as follows:

Yeast extract (Difco), 5 g; trypticase (BBL), 4 g; proteose peptone (Difco), 3 g; sucrose, 20 g; sodium thiosulfate (5 H₂O), 6.5 g; sodium citrate (2 H₂O), 10 g, sodium deoxycholate, 1 g; sodium chloride, 10 g; ox bile (depigmented solids), 5 g; sodium lauryl sulfate, 0.2 g; water blue, 0.2 g; cresol red, 0.02 g; agar, 15 g; distilled water, 1,000 ml. Final pH 8.5.

The selectivity of the revised Vibrio agar was less than that of TCBS agar, but more than that of alkaline bile salt agar. The *V. cholerae* colony on Vibrio agar was bluish gray, translucent and sticky; it was larger than the colonies of other organisms, and also than the *V. cholerae* colony on TCBS agar. Colonies of sucrose-fermenting contaminants such as *Proteus* and *Aeromonas* were more blue and smaller

on Vibrio agar and easily distinguishable from the vibrio colony, while the *V. cholerae* colony could not be differentiated in appearance from that of contaminants on TCBS agar. In addition, the stickiness of the *V. cholerae* colony seemed to be an important distinguishable character. Sometimes, *Klebsiella* grew on Vibrio agar forming a large colony which could be easily differentiated from that of *V. cholerae* because of its mucoid and opaque appearance. The *V. cholerae* colony on Vibrio agar was more easily agglutinated with *V. cholerae* O-1 antiserum than that on TCBS agar.

The revised Vibrio agar, which was sent from Nissui Seiyaku in dehydrated form, was compared with TCBS agar (Eiken) and alkaline bile salt agar for the isolation of *V. cholerae* by using 400 stool specimens obtained from the cholera field in Calcutta. Stool swabs or rectal swabs were collected into alkaline peptone water or Monsur's tellurite bile salt broth, and after 6 to 8 hr incubation, each of a loopful of the culture was streaked onto the three plates mentioned above. After overnight incubation at 37 C, suspicious colonies on each of the plates were tested with *V. cholerae* O-1 antiserum by slide agglutination. Colonies agglutinated with the antiserum were then subcultured onto/into TSI agar, SIM medium, VP medium and lysine decarboxylase medium for further biochemical and serological tests for the identification of *V. cholerae*.

The results of the isolation of *V. cholerae* on the three plates are summarized in Table 1.

Table 1. Comparison of Vibrio agar, TCBS agar and alkaline bile salt agar for isolation of *V. cholerae* from 400 stool specimens

Isolation of <i>V. cholerae</i> on			Number of specimens (%)
Vibrio agar	TCBS agar	Alkaline bile salt agar	
+	+	+	92(23.0)
+	+	—	31(7.7)
+	—	+	3(0.8)
—	+	+	0
+	—	—	14(3.5)
—	+	—	2(0.5)
—	—	+	0
—	—	—	258(64.5)
Total			400(100)

These results suggest that the revised Vibrio agar is a satisfactory medium for the isolation of *V. cholerae*. It was considered that more frequent detection of *V. cholerae* on Vibrio agar than on TCBS agar was due to the easily differential appearance of the *V. cholerae* colony from that of other organisms. This fact would be important for the bacteriologist who is inexperienced in the examination of cholera specimens.

It should be noted that all positive cases on alkaline bile salt agar also gave positive results on either, or both, Vibrio agar and TCBS agar. The results may

show that alkaline bile salt agar is not at all efficient as a medium for the cholera studies without application of the oblique light technique. The results obtained here were quite opposite to a study by De et al. (1968) who reported that better isolation of *V. cholerae* was obtained on alkaline bile salt agar rather than on TCBS agar.

Although more frequent detection of *V. cholerae* was given on Vibrio agar than on TCBS agar in this study, the authors considered that TCBS agar is not necessarily inferior to Vibrio agar. It is possible that more frequent isolation of *V. cholerae* may be obtained on TCBS agar if another study were carried out elsewhere. In fact in the comparative study, two of the 400 specimens examined here gave positive results only on TCBS agar. It may be difficult, therefore, to measure any superiority between TCBS and Vibrio agar.

On the basis of these results, it is recommended that Vibrio agar should be employed together with TCBS agar, instead of alkaline bile salt agar, in routine cholera studies.

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