

## A HIGHLY SELECTIVE GELATIN-TAUROCHOLATE-TELLURITE PLATE FOR THE ISOLATION OF VIBRIO CHOLERAE

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### INTRODUCTION

For a long time it has been known that *Vibrio cholerae* produces characteristic colonial morphology in gelatin plates. However, gelatin plates never became popular for the isolation of *V. cholerae* mainly because of the difficulty encountered with the low temperature of incubation required for these plates. Goodner overcame this difficulty very nicely by incorporating gelatin in agar. On gelatin agar plates, gelatinase-producing organisms cause a characteristic halo or zoning around the colonies. *V. cholerae* is a gelatinase producer and the colonies of *V. cholerae* on gelatin agar plates are surrounded by a well marked halo. This halo, along with the characteristic colonial appearance on gelatin agar plates, makes the isolation of a colony of *V. cholerae* from a mixture of colonies of bacteria a relatively simple affair.

Previous workers have reported that a combination of sodium taurocholate and potassium tellurite in appropriate concentration greatly enhances the chance of *V. cholerae* from material heavily contaminated with other bacteria. While at the Jefferson Medical College, in the course of an investigation on a preserving fluid for *V. cholerae*, we were concerned with the effect of these substances. It was felt worthwhile to try the selective action of sodium taurocholate and potassium tellurite incorporated into gelatin agar plates with a view to obtaining a combined effect useful in isolation and preliminary identification of *V. cholerae*. The present communication records the results of observations on a selective medium containing these reagents.

### MATERIALS AND METHODS

After various preliminary trials, a medium composed as follows was found to give the most satisfactory results:

Trypticase(*)	...	...	...	10.0 gms.
Sodium chloride	...	...	...	10.0 gms.
Sodium taurocholate	...	...	...	5.0 gms.
Sodium carbonate	...	...	...	1.0 gm.
Gelatin(**)	...	...	...	30.0 gms.
Agar Agar	...	...	...	15.0 gms.
Distilled water	...	...	...	1.0 litre

\* Baltimore Biological Laboratories

\*\* Difco.

The medium was sterilised by autoclaving at 20 lbs. pressure for 20 minutes. Potassium tellurite was added to a final concentration of 1 in 200,000 before

pouring plates. The pH of the medium was in the neighbourhood of 8.5 but no deliberate attempt at pH adjustment was made. All cultures were incubated at a temperature of 33 C., which is the practice we have followed for *V. cholerae*.

### RESULTS

a) *Colonial appearance:* At 24 hours the colonies of *V. cholerae* resemble those on gelatin agar plates with a definite halo around them, but tend to be somewhat black in the centre, which is most evident when seen against a white background. Generally speaking, the size of the colonies tends to be somewhat larger than those on plain gelatin agar plates. At 48 hours the colony has become larger and developed a well-marked black centre with a transparent periphery. The zoning around the colonies also becomes more prominent. Colonies can easily be identified even when crowded by colonies of contaminating micro-organisms.

b) *Inhibitory effect on V. cholerae and other bacteria:* As would be expected from the composition of the medium, it is highly inhibitory for most intestinal bacteria but *V. cholerae* grows luxuriantly. No detectable inhibitory effect of this medium on *V. cholerae* could be demonstrated either from colonial appearance or by comparison of viable counts on *V. cholerae* carried out side by side with non-selective media such as nutrient agar or gelatin agar.

Stool suspensions were inoculated with *Proteus* and a measured volume of serial peptone water dilutions of *V. cholerae* were added. When a measured volume of such an artificially infected stool suspension was streaked on gelatin-taurocholate-gelatin plates the number of colonies of *V. cholerae* that could be isolated from the plates closely approached the maximum that could be expected from a viable count of the original peptone water culture of the inoculum added to the stool. The value of the plates in directly isolating *V. cholerae* from materials heavily contaminated with other bacteria is thus obvious.

### CONCLUSION

Gelatin-taurocholate-tellurite plates appear to be useful for the isolation of *V. cholerae* from materials heavily contaminated with other enteric bacteria. Their use in field trials is therefore indicated.

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