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THE ISOLATION OF STREPTOCOCCI FROM MIXED CULTURES[1]

GEORGE H. CHAPMAN

Clinical Research Laboratory, 604 Fifth Avenue, New York 20, N. Y.

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The two culture media to be described have been found useful for the isolation of streptococci, particularly from mixed cultures. The tellurite streptococcus medium has the following composition:

Water	1,000 ml
Bacto tryptose	20 g
Bacto agar	15 g
Sucrose	50 g
Glucose	1 g
K_2HPO_4 anhydrous, Merck reagent	4 g

Adjust to pH 7.0 and add 0.8 ml of 0.10 per cent crystal violet (C.C.), based on the dye content of the sample, not on its crude weight, and 7.5 ml of 1.0 per cent trypan blue. Sterilize as usual. When ready to pour, add 0.40 ml of a stock solution of 5.0 per cent unheated potassium tellurite which is manipulated with aseptic precautions. Incubate the cultures exactly 24 hours.

Streptococcus salivarius usually produces pale blue opaque colonies from 2 to 5 mm in diameter. Most of them are smooth, raised ("gum drop") and glistening, while a few are rugose. *Streptococcus mitis*, and possibly most hemolytic streptococci, produce blue colonies about 0.2 mm in diameter. Like *Streptococcus salivarius* they vary considerably in morphology and size but the two species can be easily differentiated from each other. Enterococci produce dark-brown or black, smooth, slightly raised (not "gum drop") colonies from 0.5 to 1.5 mm in diameter. With the exception of a few coliforms in an occasional instance, other bacteria do not grow on the medium. The coliforms that are inhibited are actually killed; and consequently about 97 per cent of the transplants, even from feces, are pure cultures of streptococci, most of the others being either pure or mixed cultures of staphylococci. We have never observed "spreaders."

The second medium employs sodium azide as the inhibiting agent. This inhibits all but coliforms, which however are reduced in number and size. A higher concentration of azide will give greater inhibition of coliforms but will inhibit streptococci also. The medium is particularly suitable for *Streptococcus mitis*, each colony of which is surrounded by a large halo. Some enterococci produce haloes but the colonies may be differentiated in most cases by

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the larger size and blue color. Few *Streptococcus salivarius* colonies produce a halo. The composition of this azide violet blood agar medium is:—

Water	1,000 ml
Bacto tryptose	20 g
Glucose	1 g
Sucrose	5 g
Sodium chloride	5 g
Agar	15 g
"S.T.37"	0.5 ml
Sodium azide	0.06 g

Adjust to pH 6.8 and add 1.0 ml of 0.10 per cent crystal violet (C.C.) based on the dye content of the sample and not on its crude weight. Sterilize as usual. When ready to pour add the desired amount of blood. Incubate the cultures 26 to 28 hours (not "overnight").

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