A MODIFIED TELLURITE MEDIUM FOR THE DETECTION AND ISOLATION OF *CORYNEBACTERIUM DIPHTHERIAE* IN ROUTINE DIAGNOSTIC WORK

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Many attempts have been made during the last few years to produce a medium which would assist the bacteriologist in the detection and isolation of *Corynebacterium diphtheriae* (Douglas, 1922; Allison & Ayling, 1929; Clauberg, 1931; Anderson *et al.* 1931; Gaze, 1933; and others).

In each instance potassium tellurite is an essential constituent of the medium. That prepared by McLeod is one of the most useful since in addition to facilitating isolation, three distinct types, *gravis*, intermediate and *mitis*, may be readily recognized by colonial appearance. The medium contains 7–10 per cent of freshly drawn rabbit's blood, the supply of which presents considerable difficulties where large and continuous supplies of medium are required; in many small laboratories such a supply is not obtainable. It was this drawback which led to the introduction of the modified tellurite medium (Gaze, 1933) to which glucose is added and in which ox serum replaces rabbit's blood. It has proved very satisfactory for the detection and isolation of *C. diphtheriae*, but on it the three types could not be differentiated by the appearance of the colonies, and some diphtheroids were rather difficult to distinguish from *C. diphtheriae*. Various attempts were made to improve the medium, and on the ground that many diphtheroids ferment saccharose, it seemed worth while to try replacing glucose by saccharose, at the same time adding an indicator. The majority of diphtheroids fermented the saccharose causing a pink coloration of the medium, while the growth of *C. diphtheriae* left the medium unchanged. There are, however, some diphtheroids, usually found in nasal swabs, which do not ferment saccharose, but these have a distinctive colony appearance which serves readily to distinguish them from colonies of *C. diphtheriae*. Careful observation also showed that on this medium the three types of *C. diphtheriae* produced distinctive colonies.

The appearance of *Corynebacterium diphtheriae* colonies on this medium

Gravis *type*

After 24 *hours' incubation*, a well-separated colony is approximately 2 mm. in diameter, and has a somewhat raised centre with a slightly irregular edge. The surface is usually dullish and finely granular. The central part is pale greyish

black with a lighter grey periphery which is quite translucent at the edge. The peripheral portion shows some tendency towards radial striations.

After 48 *hours' incubation*, the diameter of the colony is increased to 3–5 mm., the raised centre is darker than at 24 hours and the periphery is definitely grey. The surface of the colony is granular and striations are usually but not always well marked. The appearance of colony is usually described as a "daisy head".

Strains of the *gravis* type (starch fermenters) do occur, notably in Hull and certain parts of Scotland, which yield colonies not conforming to the above description but rather resembling the *mitis* type of colony with, however, a roughened surface.

Intermediate type

After 24 *hours' incubation*. Tiny flattened umbonate colony with regular edge, smooth surface and approximately 0·5 mm. in diameter. The central part is dark brown gradually fading in intensity to a clear translucent periphery.

After 48 *hours' incubation*. Colony up to 1·5 mm. in diameter. The coloration is more intense and the clear periphery is reduced in size. In addition the edge is often finely irregular and a number of fine raised concentric rings may be noticed on the surface of the colony. Very occasionally the surface becomes finely granular.

Mitis *type*

After 24 *hours' incubation*, the colony is approximately 1 mm. in diameter, low and convex with a regular edge and smooth surface. The central part is usually blackish, the periphery a pale grey.

After 48 *hours' incubation*, the diameter is increased to about 2–3 mm. The colony becomes much blacker, the surface shiny and only the extreme periphery remains of pale colour.

Points bearing on the examination of plate culture when using this medium

Gravis colonies are sometimes difficult to identify after 18 hours' growth; the typical characters of the colony appear after 24 to 48 hours' incubation, at the end of which time the typical "daisy head" appearance is usually present.

Mitis colonies apart from increase in size and blackness show little change after 24 hours' incubation. It is, however, with the *mitis* type of colony that confusion is most likely to arise; some strains of Hofmann's bacillus and also certain members of the non-saccharose-fermenting group of diphtheroids yield a somewhat similar colony, but careful examination usually shows that the diphtheroid colonies at 24 hours are absolutely black, very shiny, and show no clear periphery.

The intermediate colonies remain very small and can easily be recognized by their lighter colour and small umbonate shape both at 24 and 48 hours.

The medium

The medium is prepared according to the following methods:

A. Add 1½ lb. of minced ox heart to 1000 c.c. of tap water at 48° C. and keep at this temperature for 1 hour. Then squeeze out the juice through lint or muslin and filter through Chardin filter paper in the ice chest overnight. Do *not* make up the volume with water. To every 1000 c.c. of this filtrate add 20 g. Witte's peptone and 5 g. sodium chloride A.R. Warm to 45° C. until both salt and peptone are dissolved. It is better to add the peptone gradually to the extract and stir in, otherwise lumps will occur and these take much longer to dissolve. The reaction of this fluid is then adjusted as follows. Heat 50 c.c. to 80–90° C. for 5–10 min. Filter through paper and allow to cool. Determine by the usual method the amount of $N/1$ NaOH required to bring 10 c.c. to pH 7·6. Then add to the bulk of fluid the amount of alkali calculated on the basis of this titration. Filter through Chardin filter paper or Seitz K clarifying disc, and afterwards through a Seitz E.K. filter disk (into a sterile filter flask). The filtrate is stored in 100 c.c. quantities in sterile flasks or bottles and tested for sterility by incubating at 37° C. for 24 hours.

B. Make a 5 per cent solution of agar fibre in water. It is not necessary to adjust the reaction or filter. Place 100 c.c. quantities into 250 c.c. flasks and autoclave at 115° C. for 20 min.

C. Make a 1 per cent solution of potassium tellurite (B.D.H.) in distilled water. *This must be made fresh each week.*

D. Ox serum sterilized by filtration.

To a flask containing 100 c.c. of A add 8 c.c. of potassium tellurite solution (this need not be sterilized), 15 c.c. of sterile ox serum, 2 g. of saccharose (B.D.H. A.R.) and 2 c.c. of Andrade's indicator. Heat the mixture to 75–80° C. and keep at this temperature for about 10 min. During this heating process the mixture should be well shaken to avoid clumping. Add this mixture to a flask of melted 5 per cent agar (B) also at 75° C. and keep at this temperature for 10 min. The medium can now be put into sterile tubes for slopes or cooled to 45° C. for plates. The finished medium is semi-opaque pale buff in colour, with a slight pinkish tint. The sterile filtered broth, ox serum and potassium tellurite solution can be kept in the ice chest and used when required. Other brands of peptone have been tried but found to be inferior to Witte's. To obtain the colony appearances as described in this paper it seems to be essential to use Witte's peptone and British Drug Houses' saccharose A.R. and potassium tellurite.

Results

For the past 12 months parallel examinations have been carried out in the laboratory on 16,000 swabs sent in for routine examination. In all these experiments tellurite slopes were used as these were found to be more convenient than plates for the number of tests to be made. Each swab was first inoculated

on to a Loeffler's serum slope and then on to a tellurite slope, which were incubated at 37° C. The next day films for microscopic examination were made from the serum slopes and stained by Neisser's method, the tellurite slopes were examined with a hand lens and the results recorded. These examinations were made by two different observers and when all were examined the results compared. When a positive result had been recorded on the tellurite medium and a negative on Loeffler's serum, the colonies on the tellurite medium were subcultured on to a serum slope for microscopic examination next day. The reason for this subculturing is that the tellurite tends to alter the morphology of the diphtheria bacilli. In this way many positive results were obtained from swabs which otherwise would have been reported negative (see Tables I and II).

Table I. *The results of culturing* 16,000 *nose or throat swabs on Loeffler's medium and saccharose-serum-tellurite medium*

Medium used	Number of swabs	%
Negative on Loeffler and tellurite	14,039	87·70
Positive on Loeffler and tellurite	1,514	9·46
Positive on tellurite only	440	2·75
Positive on Loeffler only	7	0·049

Table II. *Comparing the number of positive swabs detected by the saccharose-serum-tellurite medium with the number detected by Loeffler's medium*

Medium used	Number of positive swabs	%
Loeffler and tellurite	1514	77·20
Tellurite only	440	22·38
Loeffler only	7	0·36

In all cases when positive results were obtained on tellurite only, the strain was isolated and tested for virulence, the colony formation noted, and biochemical reactions made for type (see Tables III and IV). During the whole

Table III. *The percentage of virulent and avirulent strains from various sources in* 440 *swabs that were positive on tellurite medium only*

Origin of swabs			Number virulent	%	Number avirulent	%
264 clinical cases	Throat	205	189	92·19	16	7·8
	Nose	59	47	79·66	12	20·34
176 contact cases	Throat	87	74	85·05	13	14·94
	Nose	89	77	86·52	12	13·47
Total		440	387	85·85	53	14·13

Table IV. *The source and number of virulent and avirulent strains of each type, from* 440 *swabs that were positive on tellurite medium only*

Origin of swabs			Virulent			Avirulent		
			Gravis	*Mitis*	Inter-mediate	*Gravis*	*Mitis*	Inter-mediate
264 clinical cases	Throat	205	136	31	22	2	9	5
	Nose	59	35	6	6	2	8	2
176 contact cases	Throat	87	53	14	7	2	8	3
	Nose	89	55	7	15	1	8	3
Total		440	279	58	50	7	33	13

of these experiments only three errors were made in the identification of the type of *C. diphtheriae* by examining colonies on plates with a hand lens after 24 hours' incubation. Table V compares the earlier modification of the tellurite

Table V. *Comparing the results obtained with the saccharose-serum-tellurite medium, the medium containing glucose and with Loeffler's medium*

	4000 swabs on modified medium containing glucose (Gaze, 1933)		16,000 swabs on the saccharose-serum-tellurite medium	
Medium used	Number positive	%	Number positive	%
Loeffler and tellurite	548	89·25	1514	77·20
Tellurite only	61	9·93	440	22·38
Loeffler only	5	0·81	7	0·36

medium (Gaze, 1933) with the medium described in this paper. It must be mentioned that the two sets of experiments have not been done with the same swabs, as it was considered that there was not sufficient material on one swab to inoculate three tubes of medium equally; the procedure has always been to inoculate the Loeffler's slope first. This table shows clearly the large increase in the number of positives on this new medium as compared with the glucose medium. Although not a strict comparison, it will be seen that for practical purposes the number of positives obtained on examination of a large series of swabs was markedly increased by using the new modified medium. The seven swabs that were positive on Loeffler's slopes only were from clinical cases and all the strains were virulent. Four were *gravis*, two *mitis*, and one intermediate type.

The medium has been extremely valuable in the examination of contact cases from schools and hospitals. In such cases Loeffler's medium and tellurite slopes were inoculated in the usual way and incubated. The next morning the tellurite slopes were examined with a hand lens and any suspicious cultures noted. From the serum slopes corresponding to these suspicious cultures films were made, stained and examined microscopically. The serum slopes corresponding to the tellurite slopes that are completely negative are very unlikely to be positive and our experience shows that by disregarding them only 0·36 per cent of all positive swabs would be missed (see Table V). There is something to be said for reporting such slopes as negative without further (microscopic) examination when the swab is from a suspected carrier. The proportion of carriers that would be missed if this is done is very small and may be offset against the large increase in the number detected by the saccharose-tellurite medium. We have already mentioned that the seven swabs negative on tellurite and positive on Loeffler were all from clinical cases; in no instance did this occur with a swab from a carrier, though it may have been a matter of chance.

We do not ourselves feel justified in using the tellurite medium alone. We prefer to base a diagnosis on the microscopic appearance of the bacilli from

Loeffler's medium. Cultures that are positive on tellurite only are inoculated on to a slope of Loeffler's medium and examined next day.

The use of tellurite plates instead of tubes is an advantage, as single colonies can be picked off without delay if a virulence test is required.

SUMMARY

1. A further improvement in the modified McLeod's medium (Gaze, 1933) has resulted from the substitution of saccharose for glucose with the addition of Andrade's indicator.

2. Of the total positive swabs, the saccharose-containing medium detected about 22 per cent which were negative on Loeffler's medium, whereas it failed to detect only 0·36 per cent.

3. On this medium colonies typical of the *gravis*, intermediate and *mitis* types are formed and can be easily differentiated with the aid of a hand lens. It is also invaluable for the isolation of pure cultures.

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