A COMPARISON OF BLOOD AND EGG MEDIA FOR THE RAPID ISOLATION OF MYCOBACTERIUM TUBERCULOSIS

BY

G. A. DUNLOP AND B. D. LOWE

From the Pathological Department, Ransom Sanatorium, Notts

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The rapid isolation of Mycobacterium tuberculosis (Maccabe and Gould, 1954) on a culture medium consisting of human blood, saponin, and agar has been described. It was stated that on this medium growth of M. tuberculosis was more rapid than on Löwenstein-Jensen medium, and that the medium was superior to Löwenstein-Jensen.

In an attempt to confirm this finding saponated blood agar was prepared here, and a small series of microscopically positive sputa was inoculated on to this medium in parallel with a laboratory-prepared Löwenstein-Jensen medium.

Subcultures of M. tuberculosis isolated from sputa were also compared on the two media.

Culture Media

Saponated Blood Agar.—Prepared as originally described: 5 ml. of 10% saponin is added to 95 ml. of citrated Group O human blood, the mixture heated to 55° C. and added to an equal amount of 4% agar solution at 45° C. Quantities, each of 10 ml., are sloped in sterilized "universal" containers.

Two varieties of saponated blood agar were prepared, one using saponin, B.D.H., the other using saponin, white, B.D.H.

Löwenstein-Jensen Medium (Mackie and McCartney, 1948).—The medium was modified by the addition of 0.5% of dextrose to the mineral salt solution and by inspissation once only at 75°–80° C. for 30 minutes; 10 ml. is placed in sterilized "universal" containers.

Tests

In the first series of cultures for comparison of saponated blood agar (S.B.A.) and Löwenstein-Jensen medium (L.-J.) the S.B.A. contained the brown saponin.

Thirty microscopically positive sputa, concentrated by Petroff’s method, were inoculated simultaneously on to two slopes each of S.B.A. and L.-J. and incubated at 37° C. for two weeks.

A second series was later begun in which the S.B.A. contained the white saponin. In this series 24 microscopically positive sputa were similarly inoculated on to S.B.A. and L.-J. slopes and incubated for two weeks.

Subcultures from positive primary cultures were also set up on both media.

Results

First Series.—It was found that growth of M. tuberculosis was supported by the S.B.A. on primary culture, but neither early appearance of colonies nor luxuriance of growth could compare favourably with that of the L.-J. medium.

Only five of the 30 sputa yielded growth on S.B.A. in two weeks as against 24 on L.-J. medium.

There was also a high rate of contamination on S.B.A.: 12 of the 30 sputa rapidly became overgrown and useless compared with five contaminated cultures on L.-J. medium.

The possibility of the addition of penicillin to the S.B.A. to reduce the amount of contamination was considered, but was not attempted in either series.

Subcultures from 12 positive primary cultures on L.-J. medium were set up in parallel on S.B.A. and L.-J. media. Obvious growth appeared on each type of medium in an average of eight days, the amount of growth being similar at first, but later more luxuriant on L.-J. medium after further incubation to two or three weeks.

Second Series.—The results from primary cultures on the second type of S.B.A. (white saponin) were much better than those on the first type.

Twenty-one of the 24 sputa yielded growth on both S.B.A. and L.-J. media, in 16 cases obvious growth occurring at the same time on both types of medium.

Of the remaining eight sputa, four yielded growth one to three days earlier on L.-J. medium, and in one case only growth appeared earlier on S.B.A. than on L.-J. medium. Three specimens yielded contaminants only.
Subcultures, as in the first series, showed close similarity in the time of the first appearance of definite growth and in the amount of that growth.

Discussion

Although the number of sputa in both series is small, it would suggest that rapid primary growth of *M. tuberculosis* on saponated blood agar is not always readily producible in routine clinical laboratory work.

The superiority of white saponin over the ordinary commercial grade of saponin is indicated by a comparison of the two series.

One serious disadvantage of saponated blood agar was the high rate of contamination. The degree of contamination was frequently much worse on S.B.A., the whole surface of the slope being covered with bacterial growth and ruined in two or three days. Contaminating organisms on L.-J. usually spread upwards from the base of the slope and sometimes enabled colonies of *M. tuberculosis* to be isolated from the top of the slope. It is probable that the addition of penicillin, as used in penicillin-glycerol-blood agar (Tarshis, 1953), would reduce the number of contaminated slopes.

A further point in favour of the L.-J. medium was the ease with which developing colonies of *M. tuberculosis* could be distinguished from the original inoculum and from the majority of contaminants. On S.B.A. medium, the debris of the original inoculum tended to mask small colonies which at first could not be differentiated from the dark background.

The average time for the appearance of typical surface growth on modified Löwenstein-Jensen medium was 9.3 days and on saponated blood agar of the second series was 10 days, a difference of no significance; but in all the cultures, growth on Löwenstein-Jensen was more luxuriant and more easily identified in the earlier stages.

An interesting observation was the early appearance of typical developing cord-like colonies in the liquid at the base of the L.-J. slope (condensation water and primary inoculum). These have appeared as early as five days after inoculation, and can be seen readily using the 4 in. objective in a Ziehl-Neelsen-stained film.

Summary

The growth of tubercle bacilli from microscopically positive sputa was compared on saponated blood agar using two types of saponin, and a modified Löwenstein-Jensen medium.

It was found that saponin (white) yielded a better blood agar medium than the brown saponin, but that both varieties of the medium were inferior to a modified Löwenstein-Jensen medium for primary isolation of *M. tuberculosis*.

REFERENCES

