

THE USE OF PHENOL RED AND BROM-CRESOL PURPLE
AS INDICATORS IN THE BACTERIOLOGICAL
EXAMINATION OF STOOLS.

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There have been so many new media proposed during the past few years for the isolation from stools of organisms belonging to the typhoid-dysentery group that one hesitates to bring forward a new one. Believing that the indicators phenol red and brom-cresol purple possess distinct advantages over those formerly used in stool bacteriology, we have felt justified in pointing out their applicability in this particular domain of bacteriological technique.

A critical analysis of all the media advocated for the culture of stools is considered beyond the scope of this paper, but a brief mention of some of those more widely used does not seem amiss. In this country Endo's medium, prepared according to the author's formula or according to the modifications advocated by Kendall (1) or Robinson and Rettger (2), has been used perhaps more extensively than any other when a non-restraining medium was desired, although this medium has disadvantages which are well known to all workers in this field. The methylene blue-eosin agar of Holt-Harris and Teague (3) has found favor with some workers because it avoided some of the faults of the Endo medium. Meyer and Stickel (4) consider that either the medium of Holt-Harris and Teague or their own eosin-China blue medium prepared with peptic or tryptic digests is superior to the Endo agar, litmus agar, or Congo red agar "for the direct isolation and detection of dysentery bacilli from stool specimens." The extensive work of Krumwiede (5) and his coworkers upon the usefulness of brilliant green agar as a selective restraining medium has shown that this medium can be successfully applied in routine work when dealing with members of the typhoid-paratyphoid group. Teague and Clurman (6) advocate the use of a meat extract agar containing brilliant green and eosin as a selective medium for the isolation of members of the typhoid-paratyphoid group, and Meyer and Stickel¹ state that eosin and

¹ Meyer and Stickel (4), p. 67.)

brilliant green used in connection with their peptic digest agar "permits the detection of a higher percentage of viable typhoid bacilli than any other one thus far introduced into bacteriologic technic." Kligler (7) finds that the Endo medium after the addition of sulfite reaches a hydrogen ion concentration ranging from pH 8.6 to 8.8 and states that this degree of alkalinity is unfavorable to the growth of the Shiga bacillus. He also advocates the use of neutral red with the brilliant green plate of Krumwiede and considers this the best among several indicators that he tried. Kligler thinks also that the reaction of the medium has a marked influence upon the restraining activity of the brilliant green. It may be mentioned at this point that Krumwiede (8) objects to the use of eosin in connection with brilliant green because of the resultant eye strain from the irritating color produced by this combination. This objection would seem to hold good only where large numbers of plates are to be examined by one worker. Morishima (9) has recently advocated the use of a combination of phenol red and China blue as an indicator for lactose plates in the culture of stools.

In this communication it is proposed to record our experience with the use of phenolsulfonephthalein (phenol red) and dibromo-ortho-cresol-sulfonephthalein (brom-cresol purple) as indicators in stool bacteriology. To Clark and Lubs (10), largely, belongs the credit for the introduction of these indicators into bacteriological technique. They suggest that these indicators might be serviceable in the preparation of indicator plates (11), but so far as our survey of the literature has gone we have not been able to find any reference to the use of these indicators in stool bacteriology except in the case of the combination of phenol red with China blue, as advocated by Morishima.

Method.

In studying these indicators to determine their availability for stool work the basis of the medium used was a 1.5 and a 3 per cent beef extract agar. To this agar were added varying amounts of lactose from sterile solution and varying amounts of indicator, in order to determine the optimum amount of each of these ingredients. The reaction of the medium also was varied in order to ascertain the pH at which sharpest color differentiation was had. Plates were seeded with mixtures of *Bacillus coli* and members of the typhoid-dysentery group, also with stools inoculated with members of the latter. When the optimum amounts of the constituents had been ascertained, brilliant green was added to the agar in the proportions

recommended by Krumwiede, and the degree of restraining activity noted as compared with controls consisting of agar with brilliant green alone and agar with the indicator alone.

RESULTS.

In the case of both indicators a 3 per cent beef extract agar cleared with egg to which 1 per cent lactose had been added from sterile 20 per cent solution gave the best results. With the thicker agar there was less diffusion of acid. Thick plates, about 20 cc. of agar to a plate, were poured. We shall discuss the two indicators separately.

Phenol Red.—The optimum reaction of the agar, if this indicator is to be used, is a hydrogen ion concentration represented by a pH of 7.6 to 7.8. The optimum amount of indicator is 10 cc. of a 0.04 per cent aqueous solution for every 100 cc. of agar. The indicator may be sterilized by autoclaving. Plates poured from agar with a pH of 7.6 to 7.8 and containing the amount of indicator mentioned will assume on cooling a color best described as ranging between salmon pink and old rose. Such plates are clear, and upon them lactose-fermenting organisms produce vivid greenish yellow colonies with a surrounding zone of green. The typhoid bacillus, the paratyphoid bacillus (A and B), and the dysentery bacilli (Shiga, Flexner, and Hiss Y), all produce pink colonies. These organisms evidently produce alkali, for the surrounding medium assumes a decidedly deeper pink than the uninoculated portions of the plate. This alkali production with consequent intensification of the pink color of the medium in the neighborhood of the typhoid-dysentery colonies is a helpful factor in the differentiation of the latter. In thickly seeded plates the diffusion of the acid in the vicinity of the lactose-fermenting colonies is often sufficient to mask this change in the direction of alkalinity exhibited by the typhoid-dysentery colonies, but where good distribution is obtained the color change is most striking. Even in thickly seeded plates where typhoid and colon colonies abut upon one another, a difference in the color of the two types of colonies is observable. The colon colonies assume a bright green or yellow-green color and are opaque, whereas the typhoid colonies in such cases are more translucent and possess a bluish green color. The dye does not mask the corrugated, woolly appearance of the typhoid

colonies which is characteristic of them and upon which Krumwiede lays great stress in their recognition. On the contrary, we are inclined to feel that the dye perhaps enhances this characteristic. We have had no opportunity to study freshly isolated strains belonging to the dysentery group, and it is possible that such strains may be inhibited by this indicator; however, it does not inhibit the growth of freshly isolated strains of *Bacillus typhosus* or *paratyphosus*. The indicator itself is not reduced by the bacteria. We have found it very satisfactory when used in connection with sugar broth or Russell's double sugar.

Brom-Cresol Purple.—In our experience this indicator, when used in connection with lactose plates, gives a sharper color differentiation than does phenol red and hence is preferable to the latter. It is purple in alkaline solution, changing to yellow in the presence of acid. The color change takes place in the zone of pH 5.2 to 6.8. The optimum reaction of the agar if this indicator is to be employed is a pH of 7.2 to 7.4. The optimum amount of indicator to use is 5 to 8 cc. of a 0.04 per cent aqueous solution for every 100 cc. of agar. This solution may be sterilized in the autoclave. Plates poured from agar with a reaction of pH 7.2 to 7.4 and with the amount of indicator mentioned assume a deep blue color and are clear. Upon these plates the lactose-fermenting organisms produce greenish yellow colonies with a yellow zone, and the non-lactose fermenters produce bluish colonies. The typical appearance of the typhoid colonies is not obscured by this indicator. Even on thickly seeded plates the colonies of the non-lactose-fermenting organisms tend to preserve a bluish appearance, being bluish green rather than a brilliant yellow, the color that the colon bacillus colonies assume. It is not an infrequent experience on thickly seeded plates to see a round yellow colon colony with a wedge-shaped bluish green sector occupying a portion of the circle and representing a typhoid colony which has refused to be masked by the larger, acid-producing colony of colon bacilli. This indicator did not show any restraining influence upon any of our laboratory cultures of *Bacillus dysenteriae*, including the Shiga variety. Whether or not it would exercise any restraint on freshly isolated cultures we are not able to say.

Combination of Phenol Red or Brom-Cresol Purple with Brilliant Green.—To judge from our rather limited experience either indicator

may be used along with brilliant green in the amounts advocated by Krumwiede. We have had an opportunity to study only one sample of brilliant green, the source of which was unknown but which was active in its restraining power upon *Bacillus coli*. Neither indicator, so far as could be determined, exercised any inhibiting effect upon this restraining power of the brilliant green. The only change noticed was in the color of the plates, the phenol red-brilliant green plates being brownish red in color and the brom-cresol purple-brilliant green plates being bluish purple. The characteristic color changes produced by the lactose-fermenting colonies were not altered by the brilliant green.

DISCUSSION.

Since the introduction of lactose indicator plates for the bacteriological examination of stools for the detection of members of the typhoid-dysentery group, numerous attempts have been made to find a medium which was entirely satisfactory. As pointed out by Hiss and Zinsser (12) the best results are apt to be attained when an individual becomes thoroughly familiar with one type of medium and restricts himself to it. Endo's medium has the disadvantage of tending to vary with different batches, some being satisfactory and others useless. There can be little question but that the selective restraining media have been highly successful in skilled hands when large numbers of stools have had to be examined. Few workers, however, in carrying out routine examinations with such media, are willing entirely to dispense with a non-restraining medium such as the Endo plate, but prefer to use it in addition as a control. This is particularly apt to be the case in laboratories serving hospitals, where the proportion of stool cultures to other bacteriological examinations is not great and where the workers cannot be expected to attain the degree of skill in stool bacteriology which is attained by those carrying out an extensive series of bacteriological examinations of stools. For the less experienced worker, let us say, a non-restraining medium is essential, if only as a check, and a medium which does not obscure the characteristic appearance of the typhoid colonies and at the same time shows a sharp color differentiation should be satisfactory for the purpose.

It is because we believe that the indicators phenol red and brom-cresol purple may be successfully employed with lactose to give a

satisfactory differential plate for members of the typhoid-dysentery group when a non-restraining medium is desired and that such a medium is superior to the customary Endo plate that we have ventured to add another medium to the already long list of media proposed for the isolation of typhoid and dysentery bacilli from stools. We think the medium proposed is superior to Endo's because it gives sharper differentiation than the latter, is easier to prepare, uniform batches can be obtained, and it can be kept indefinitely if not allowed to evaporate. Of the two indicators, we prefer brom-cresol purple to phenol red.

CONCLUSIONS.

1. Either phenol red or brom-cresol purple may be used as indicators in the preparation of lactose agar plates for the isolation of members of the typhoid-dysentery group of bacteria from stools. Of the two, brom-cresol purple gives sharper differentiation and is to be preferred.

2. These indicators exercise no restraining influence upon the growth of cultures of the typhoid bacillus or paratyphoid bacillus freshly isolated from the human body, or of laboratory cultures of *Bacillus dysenteriae*.

3. Both indicators may be successfully employed with brilliant green in the isolation of the typhoid-paratyphoid group from stools without sacrificing the restraining activity of the brilliant green upon other bacteria.

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