THE DETECTION MICROSCOPICALLY OF COLONIES OF SHIGELLA
IN STOOL CULTURES

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Early recognition of the presence of Shigella in the stool of a patient facilitates use of the most specific therapy and proper consideration of epidemiologic implications.

The positive diagnosis of a Shigella infection requires isolation of the etiologic agent from the stool of the patient and its identification by biochemical and serological studies which usually require a minimum of 36 to 48 hours. In order to shorten this time interval, it seemed possible to adapt to our needs a method of study used recently by Cooper et al. (1953), in studying the microscopic characteristics of colonies of Shigella when viewed under oblique transmitted illumination. In that study, it was possible to recognize colonies of Shigella with considerable certainty and to differentiate quite reliably between virulent and nonvirulent colony types.

Previous investigators using oblique illumination for microscopic study of colonies of microorganisms have been: Huddleson and Baltzer (1950) studying colonies of bacteria in various genera when grown on a medium containing 2,3,5-triphenyl tetrazolium chloride; Landy (1950) for distinguishing between V and W form colonies of Salmonella; White and Wilson (1951) for observing differences in smooth and non-smooth clones of Brucella; Eigelsbach et al. (1951, 1952) for detecting variant colonies of Bacterium tularensis and studying immunogenic differences among variants; Branham (1953) for differentiation of Shigella sonnei, Phase I and Phase II; and Foshay and Fleming (1951) in observing differences in texture and color of parent and variant colonies of Shigella flexneri II.

MATERIALS AND METHODS

A comparative study was planned in order to determine, first, whether it was possible to detect with the microscope colonies of Shigella on media streaked with stool from patients acutely ill with diarrhea and, second, to determine whether the microscopic method was more rapid than the standard method.

Rectal swabs in glycerine preservative solution (Connecticut State Department of Health, 1945), submitted to the Bacteriology Laboratory of the Children’s Hospital for study for intestinal pathogens, were inoculated by the personnel of that laboratory on media according to their standard method, and on two modified media used in this study for microscopic observation of the colonies. For the hospital laboratory the rectal swab was inoculated on two plates of MacConkey agar (Difco), two plates of S-S (Shigella-Salmonella) agar (Difco), one plate of Brilliant Green agar (Difco), and in one tube of tetrahionate broth (Difco). For the microscopic study of colonies the same rectal swab used before it was placed in the tetrahionate broth for inoculating the surfaces of one plate each, 15 to 17 ml, of the two modified agar media. These media were MacConkey agar and S-S agar from which the neutral red indicator had been omitted in order to obtain a clearer medium which would facilitate light transmission and favor microscopic observation of the details of colony structure and color. All cultures were incubated at 37 C. The length of incubation varied from 12 to 24 hours, depending upon the time of culturing.

In the hospital laboratory the tetrahionate broth culture, after incubating overnight, was subcultured on one plate each of MacConkey agar, S-S agar, and Brilliant Green agar. The primary plate cultures streaked initially with the rectal swabs and the plate cultures streaked with

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the tetrathionate broth culture were each inspected after incubation overnight. Colonies resembling those of intestinal pathogens were picked and inoculated into tubes of triple sugar iron agar slants (Baltimore Biological Laboratories). After incubating overnight, the triple sugar iron cultures which gave reactions similar to those produced by Shigella or Salmonella, acid or acid and gas, respectively, in the butt of the tube, and an alkaline slant were used for preparing slide agglutination tests with Shigella or Salmonella group agglutinating antisera (Lederle) and for subculturing into a number of media to obtain information regarding their biochemical activities. If agglutination occurred with one of the group sera, and biochemical reactions were compatible, then a subculture was sent to the Laboratories of the Ohio State Department of Health for determination of the specific type by further serological studies. With these standard methods now widely used, the earliest time at which the laboratory could make a preliminary report of the presence of Shigella or Salmonella, based on positive serological findings with group agglutinating antisera, was 36 hours if the rectal swab were cultured at 9:00 PM the first day.

For the microscopic study the colonies on the plates of the modified media were examined microscopically following incubation overnight, using a Bausch and Lomb binocular dissecting microscope equipped with 10× oculars and 0.7×, 2.0×, and 3.0× objectives. A mirror from a Zeiss monocular microscope was placed concave side up on the table approximately equidistant from and in line with both the microscope and the lamp. The source of illumination was an Erb and Gray microscope lamp, model 1 (figure 1). The narrow beam of light from the lamp was reflected by the mirror obliquely through the colonies under observation. The angle of incidence of the light with the mirror was 41 degrees which did not permit the passage of the light beam directly into the objectives of the microscope. Colonies were described only as they appeared when in the center of the field of the microscope.

Colonies giving colonial appearances typica

Figure 1. Arrangement of microscope, mirror, and microscope lamp for study of colonies with oblique illumination.
of Salmonella or Shigella were picked directly from the plate for agglutination with Salmonella or Shigella group agglutinating antisera (Lederle). If the colony were too small to permit identification in this manner, it was picked to a small amount of brain heart infusion (Difco) and incubated 2 to 3 hours followed by centrifugation. The bacteria obtained by centrifuging were used in the slide agglutination test. With this microscopic method the earliest practical time at which the presence of Shigella could be reported was 13 hours if the rectal swab was cultured at 9:00 PM the previous day. Colonies recognized as being those of Shigella or Salmonella were picked to triple sugar iron medium and then to various media for determining their biochemical activities. Final serological typing of Salmonella and Shigella was made on subcultures sent to the Laboratories of the Ohio State Department of Health.

RESULTS

The various types of bacteria found in the stool cultures developed colonies on the two modified media which could be differentiated best on the modified MacConkey’s medium where there was less tendency to resemble colonies of other species.

Colonies of Escherichia coli on modified MacConkey’s agar were convex, heavy, opaque, dull white to pale brown, with a dry appearing surface. Occasionally some dull red color was seen near the periphery, shading into the predominant white. A white zone of precipitated bile usually was seen surrounding the colony. On modified S-S agar the appearance was similar but more uniformly opaque and mucoid. On both modified media, colonies of Aerobacter were slightly more mucoid and surrounded by a smaller zone of precipitated bile than was seen around colonies of E. coli.

Colonies of Proteus morganii on modified MacConkey agar showed a wide upper margin predominantly light purple with a suggestion of pink; a smooth central blue disc surrounded by an indistinct yellowish-white band; a lower light blue margin and indistinct convolutions (cross-slicing) around the margin which resembled the small hills and valleys of a relief map. Colonies on modified S-S agar were wide, flat and smooth, predominantly dull-green, slightly blue with tan-colored centers, narrow bright golden-red border around the upper half, and a narrow cross-sliced marginal area.

Colonies of Proteus mirabilis on modified MacConkey agar were usually large, flat, pale blue and spreading with dull salmon-pink centers, narrow greenish-golden margins around the upper half, and peripheral bands showing cross-slicing. On the modified MacConkey agar, colonies showed obvious spreading tendencies as seen in thin rust-colored films extending outward from the main portion of the colonies. Colonies on modified S-S agar were more convex. The narrow upper margin was predominantly red or pink and the wider green lower margin extended as a full circle around the colonies just inside the narrow red upper margin. The centers of young colonies were usually dull tan which became black with prolonged incubation.

Colonies of Paracolobactrum on modified MacConkey agar were somewhat flattened, predominantly pale blue, and contained a few pink or golden spots. Colonies of aerogenic and anaerogenic forms could be differentiated since the aerogenic type usually presented smooth, almost mucoid surface textures while the anaerogenic type was more granular and had some peripheral cross-slicing. The anaerogenic colonies were usually more colorful with light blue central areas surrounded by greenish-golden upper margins and greenish-blue lower edges. On the modified S-S agar, the appearance of these colonies was variable and easily confused with other colony types, particularly Proteus and occasionally Shigella.

Colonies of Salmonella on the modified MacConkey agar were slightly flattened or convex with narrow red margins and dull-green centers with small central dull pinkish-tan discs. Fine cross-slicing extended over nearly the entire colony. On modified S-S agar, colonies of various species of Salmonella were variable in their appearance, and since too few Salmonella types were isolated by this technique, we are not presenting specific descriptions at this time.

Colonies of the flexneri group of Shigella on modified MacConkey agar were quite constant in appearance, generally of smooth texture, somewhat granular, slightly convex with even and regular margins. The substance of the colonies was predominantly golden-pink with a dull blue upper margin and a crescent shaped golden-green lower segment. On modified S-S agar, colonies
were smooth and convex with regular margins, dull-blue upper halves and brighter pale-pink lower halves.

There were two colony forms of *S. sonnei* on the modified MacConkey agar. The more common type of colony was smooth and convex with a discrete margin. The less common type was similar except that pseudopodia-like outgrowths extended from the central convex section. The colors in each were nearly identical with those seen in colonies of *S. flexneri*. The colonies of *S. sonnei* could be differentiated from those of *S. flexneri* by their more “fuzzy” surface texture which made the colonies appear slightly out of focus. In contrast, the colonies of *S. flexneri* appeared in sharp focus. On modified S-S agar, the two types of *S. sonnei* colonies were indistinguishable from each other and from the *S. flexneri* types.

**Efficiency of the microscopic technique.** Since a total of 115 fecal specimens from 99 patients was cultured simultaneously in the diagnostic laboratory according to the commonly accepted standard technique and on the special modified media in petri dishes for subsequent microscopic study, a comparison is possible of the relative efficiency of the two methods. The comparison shows that a total of 21 patients infected with *Shigella* were detected by the two methods, 16 of these were detected equally well by each method, 2 were detected only by the microscopic method, and 3 were detected only by the standard method. Two additional patients infected with *Salmonella* were detected by each method. The conclusion is that the two methods were about equally efficient for the detection of patients infected with *Shigella*. The important differences between the two methods are found in the shorter time required for the microscopic method, 13 hours in contrast to 36 hours with the standard method, and in the total amount of media used, 230 petri dishes of media, 2 per specimen, with the microscopic method in contrast to 920 petri dishes of media, 8 per specimen, using the standard method. Our experience would indicate that perhaps both modified media are not necessary but desirable since the appearance of a colony typical of *Shigella* on one medium strengthens one’s feelings regarding the appearance of a colony on the other medium not quite typical of *Shigella*. There was also a considerable saving with the microscopic method in the number of tubes of differential media used for biochemical determinations since there was greater accuracy in the selection of colonies.

**DISCUSSION**

No attempt is made to explain the mechanism of color induction in bacterial colonies as observed by a technique of oblique transmitted lighting for microscopic study. However, since marked changes in the colors of a colony are observed with a change in the angle of incidence of the light beam or a change of the position of the colony within the microscopic field, it would seem highly probable that the colors may be attributed to the varying refractive powers of the various colony types.

The descriptions given here of the appearance of the various types of colonies would not necessarily be the same in the hands of other investigators, due to individual differences in color interpretation. However, the various colonies can be distinguished readily with a minimum of experience by previously familiarizing oneself with the appearance of colonies of known genera most likely to be seen in material under study.

It must be emphasized that the most critical point of this technique is the importance of maintaining a constant relationship between the microscope, the mirror, the source of illumination, and the position of the colonies within the microscopic field.

**SUMMARY**

Through the use of a microscope, obliquely transmitted illumination, and the modification of media normally used for the isolation of intestinal pathogens it was found that colonies of *Shigella* could be recognized and were readily distinguishable from colonies of most other bacteria commonly observed in stool cultures.

A comparative study was planned in cooperation with the bacteriology laboratory of the Children’s Hospital, Cincinnati, Ohio, so that rectal swabs, as submitted for culture, were streaked on the routine media used by the laboratory of the hospital and also on two modified media to be used in this study. These two media were S-S (*Salmonella-Shigella*) agar and MacConkey’s agar, each modified by omission of the indicator.

Studies with the modified media revealed that colonies of *Escherichia coli*, *Aerobacter aerogenes*, the Paracolon group, *Proteus*, *Shigella*, and
Salmonella were distinctly different when observed microscopically with the oblique illumination method.

A total of 21 patients infected with Shigella were detected by the two methods; 16 of these were detected equally well by each method; 2 were detected only by the microscopic method; and 3 were detected only by the standard method.

Two additional patients infected with Salmonella were detected by each method.

There was a significant difference in the total quantity of media used by the two methods. The microscopic method required 230 petri dishes of media and few tubes of media for biochemical determinations. The standard method required 920 petri dishes of media and many tubes of media for biochemical determinations.

In the hospital laboratory, cultural and serological studies required a minimum of 36 hours for group serological identification of Shigella and Salmonella. By the microscopic method, similar identification was accomplished in a minimum of 13 hours.

REFERENCES
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