

Evaluation of Lacto-Phenol Cotton Blue for Wet Mount Preparation of Feces

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The use of lacto-phenol cotton blue (LPCB) stain for wet mount preparation of stools to demonstrate intestinal parasites by routine microscopy was evaluated in this study. LPCB-stained trophozoites and cysts and helminthic ova could easily be detected and identified in LPCB wet mounts of stools. The stain is recommended for routine use in the wet mount preparation of stools in a parasitology laboratory.

Feces are the most frequent specimens collected and examined for demonstration of parasites of the gastrointestinal tract. The fecal specimens are usually examined in the laboratory by microscopic examination, which essentially consists of direct wet mount preparation, concentration, and permanent staining (6).

Direct wet mount preparation is an important component in the microscopy of feces. In a routine parasitology laboratory, two preparations of each specimen are usually made on each slide: one unstained preparation and another temporarily stained preparation. The saline wet mount is an unstained preparation made by using physiological saline. The advantage of saline preparation is that it helps to demonstrate the motility of trophozoites. However, definitive diagnosis of cysts or trophozoites is difficult with saline preparations, because internal structures are often poorly visible. To overcome this disadvantage, several stain solutions have been used for preparation of temporarily stained wet mounts of fecal specimens. These include Quensel stain (7), Nair's buffered methylene blue (5), Lugol's iodine stain (4), and D'Antoni's iodine stain (1). These solutions stain internal structures of parasites, thereby facilitating their detection and identification in the feces (2).

Lacto-phenol cotton blue (LPCB) is a stain routinely used in microbiological laboratories to prepare wet mounts of fungal culture for microscopic examination. The stain has not been used previously in any wet mount preparation of stools. In this communication, we report for the first time the evaluation and use of this stain as a temporary staining agent in the wet mount preparation of stools for demonstration of parasites.

We examined 190 stool specimens in our laboratory. Each specimen was examined microscopically by preparing an LPCB wet mount and also by preparing saline and iodine wet mounts as follows.

(i) LPCB wet mounts. LPCB wet mounts were prepared by mixing a drop of LPCB stain with a small volume of stool on a glass microscope slide and placing a coverslip on the mixture. LPCB contains 20 g of phenol crystals, 20 ml of lactic acid, 40 ml of glycerol, 0.05 g of cotton blue stain, and 20 ml of distilled water.

(ii) Saline wet mounts. Saline wet mounts were made by mixing a small volume of stool with a drop of physiological saline on a glass microscope slide and placing a coverslip over

the mixture. The stool smear was thin enough that newsprint could be read through the smear.

(iii) Iodine wet mounts. Iodine wet mounts were prepared by adding a small volume of stool to a drop of Lugol's iodine (diluted 1:5 with distilled water) on a glass microscope slide and placing a coverslip on the mixture.

The saline wet mount preparations were examined first and then the iodine wet mounts and lastly the LPCB wet mounts were examined. These wet mounts were microscopically screened initially by using a low-power (10 \times) objective and then using a high-power (40 \times) objective of a compound light microscope.

Direct microscopic examination of saline, iodine, and LPCB wet mount preparations of 190 stool specimens showed trophozoites, cysts, and ova of various parasites in 40 specimens.

The parasites demonstrated were *Entamoeba histolytica* cysts (11 stool specimens), *E. histolytica* trophozoites (1 specimen), *Giardia lamblia* cysts (8 specimens), hookworm ova (11 specimens), *Ascaris lumbricoides* eggs (11 specimens), *Trichuris trichura* eggs (4 specimens), *Hymenolepis nana* (10 specimens), and *Taenia* species eggs (1 specimen). These were seen in LPCB wet mounts and also in iodine and saline mounts.

In LPCB wet mounts of feces, trophozoites, cysts, and ova stained by LPCB showed the following features.

***E. histolytica*. (i) Cyst.** The cytoplasm is stained deep blue. The cyst wall is stained lightly but is clearly defined. It is surrounded by a clear halo. Nuclei are clearly visible (Fig. 1A).

(ii) Trophozoite. The cytoplasm of the trophozoite, like that of the cyst, is stained deep blue. The nucleus is clearly visible. A pseudopodium is stained light blue. Motility of the trophozoite is not seen.

***G. lamblia* cyst.** The cytoplasm is stained deep blue. The clear space between the cytoplasm and the cyst wall is lightly stained and is refractile. The cyst wall is surrounded by a clear halo. The axostyle is stained deep blue. Nuclei are visible (Fig. 1B).

Hookworm ovum. Blastomeres are stained deep blue. The space between the eggshell and segmented ovum is stained light blue. The eggshell is not stained or very lightly stained but clearly discernible (Fig. 1C).

***A. lumbricoides* fertilized egg.** The outer corticated, thick wall is stained deep blue. The eggshell is stained lightly. The ovum is stained deep blue, while the polar space between the eggshell and the ovum is stained light blue (Fig. 1D).

***T. trichura* egg.** The double eggshell is stained deep blue. Mucus plugs are not stained but well-defined. The unseg-

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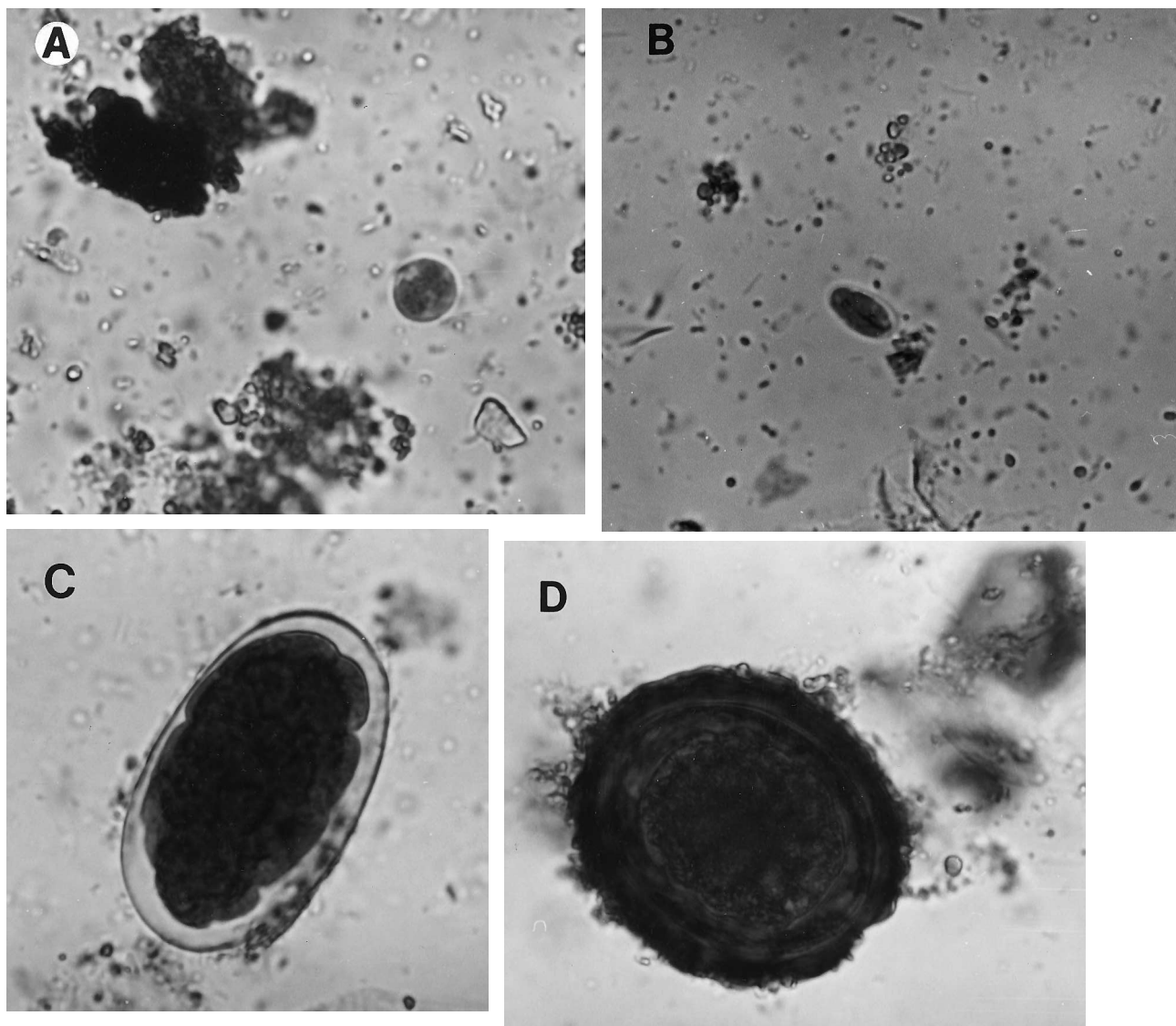


FIG. 1. LPCB-stained *E. histolytica* cyst (A), *G. lamblia* cyst (B), hookworm egg (C), and *A. lumbricoides* egg (D). Magnification for all panels, $\times 400$.

mented ovum is deeply stained and distinctly differentiated from the eggshell.

***H. nana* egg.** Two distinct membranes of the egg are unstained. The space between the two membranes is stained light blue. The embryophore is stained deep blue. Hooklets are faintly visible.

***Taenia* egg.** The eggshell is not stained. The embryophore is stained light blue. The oncosphere is stained deep blue. Hooklets are faintly visible.

In LPCB wet mounts, cysts and trophozoites of *E. histolytica* were demonstrated in 12 stool specimens. Polymorphonuclear leukocytes were few or absent in these stool preparations, as most of these cells were lysed in LPCB stain. Also in the LPCB wet mounts, vegetable cells, artifacts, and muscle fibers, etc., were stained blue. This facilitated their accurate identification without causing any confusion with cysts or ova.

The LPCB stain is a combined fixative, staining, and clearing agent and is being widely used in wet mount preparations of fungal specimens (8). It contains cotton blue, which stains not only the fungal elements but also the internal structures of

parasites in the stool. Phenol and lactic acid, which kill viable fungi, also kill viable trophozoites and possible cysts and ova. Finally, glycerol in LPCB provides a semipermanent preparation.

In the present study, LPCB stain, like iodine stain, stained internal structures of trophozoites, cysts and ova, thus facilitating their recognition and identification in stools. The LPCB stain appears to offer many advantages over saline or iodine wet mounts. One problem with saline wet mounts is that polymorphonuclear leukocytes are frequently mistaken for *E. histolytica* cysts, while macrophages (monocytes) are frequently mistaken for *E. histolytica* trophozoites (3). However, in LPCB wet mounts, since most of the polymorphonuclear leukocytes are lysed, as observed in this study, intact blue-stained trophozoites could easily be detected and identified. It is a fact that in iodine wet mounts, cysts are stained poorly with the iodine and are less refractile than unstained cysts in the saline preparation; therefore, they may easily be overlooked. In contrast, cysts in LPCB wet mounts are stained deep blue. It is difficult to overlook these blue cysts even during screening of stool

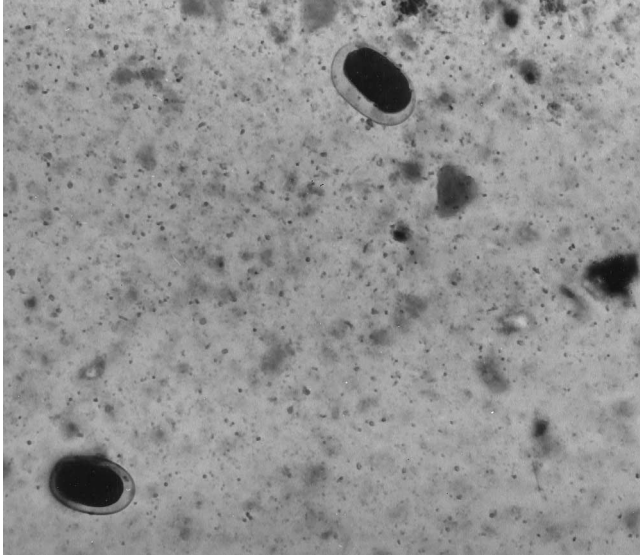


FIG. 2. LPCB-stained hookworm eggs (magnification, $\times 100$).

specimens with a low-power objective. This is a particularly important consideration because LPCB-stained fecal smears can be screened for the parasites rapidly and easily without causing any eyestrain (Fig. 2). This will be an advantage particularly for students being trained in microscopic examinations of stools for parasites. A noted disadvantage of saline wet mounts is that colorless, non-bile-stained ova of hookworms and *H. nana*, etc., are frequently missed in stool examinations, particularly during screening with a low-power objective. However, in LPCB wet mounts, helminthic ova are stained such a deep blue that it is difficult to miss them even during low-power microscopic screening. Furthermore, as demonstrated in this study, vegetable cells (Fig. 3), mucus (Fig. 4), and other artifacts in the stool are clearly stained with LPCB stain. They are still recognizable as artifacts when the LPCB stain is used. The only disadvantage of the LPCB stain is that bile-stained helminthic ova, such as ova of *A. lumbricoides* and *T. trichura*, lose

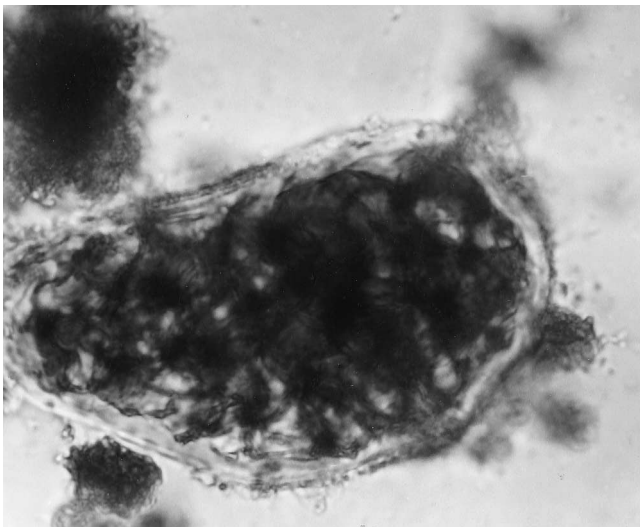


FIG. 3. LPCB-stained vegetable cell (magnification, ca. $\times 300$).

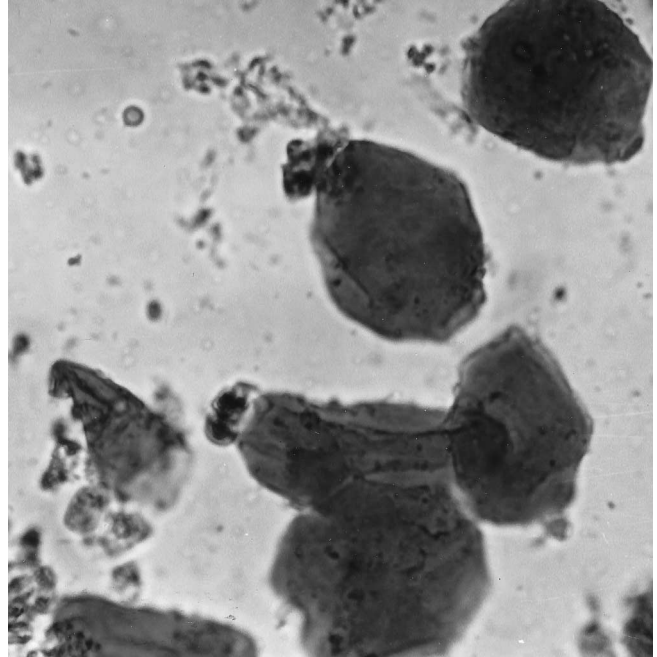


FIG. 4. LPCB-stained mucus (magnification, $\times 400$).

their natural brown color and are stained blue. This problem, of course, occurs with any wet mount preparation using temporary stains. For example, in iodine wet mounts, both bile-stained and non-bile-stained helminthic ova are stained brown. This disadvantage of the LPCB mount can be obviated by examination of a saline wet mount of the same stool to detect bile-stained eggs.

Various temporary staining solutions have been used for wet mount preparations of the stool, including Quensel stain (7), Nair's buffered methylene blue (5), and Lugol's (4) and D'Antoni's (1) iodine stains. To the best of our knowledge, the LPCB stain has not previously been used or evaluated as a temporary staining solution for wet mount preparation of stool samples. The LPCB wet mount is simple, the reagents are inexpensive and are easily available commercially, and the method facilitates the detection and identification of protozoal cysts and trophozoites and helminthic ova in stools. We therefore recommend the use of LPCB stain along with saline wet mounts for routine microscopic examination of stools in parasitology laboratories.

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