Agar media are prepared and poured into the dishes in the conventional fashion. After use, the dishes may be sterilized by the use of chemical disinfectants, by incineration, or by autoclaving and then discarded.

The disposable dishes have been employed for routine work in this research laboratory and in the bacteriology section of the clinical laboratories at the Latter Day Saints Hospital, Salt Lake City, Utah. Comparative tests have shown that results similar to those obtained with the usual glass product are obtained with the paper dishes.

Certain advantages of the disposable paper and plastic petri dish are significant; for example, the problems accompanying accumulations of dirty dishes are relieved; experiments involving the use of larger than usual numbers of dishes can be carried out without burden to the budget or inventory.

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Sterile Microdissection and Isolation of Malarial Oocysts

In connection with the study of the growth of malarial oocysts *in vitro* (*1*), the following technique was developed for sterile dissection and successive transfer of the oocysts in hanging drops (*2*).

A chamber of dimensions 35 by 40 by 22 cm (Fig. 1) was made with three walls of sheet metal, a plastic sleeve in front for insertion of one's hands, and a Lucite top with an opening for the body of a dissecting microscope (*3*). This opening was sealed by an elastic rubber membrane.

In addition to the fluorescent light in the chamber, two study lamps were used on the outside, each with a water-filled 50-ml flask on the Lucite top to focus the light on the object. A little ammoniated copper sulfate was added to the water for better contrast.

The illumination of the object was further strengthened by replacing the glass-plate stage of the dissecting microscope with a piece of Bakelite, in the center of which a hole was drilled that corresponded in place and size to the visual field of the low-power objectives. With this device, good contrast and a well-defined outline of the object were obtained.

Micro dissecting instruments were made from fine steel wire with ends ground into a point or a cutting edge. A pair was held by soft copper wire sheathed in plastic, one member being

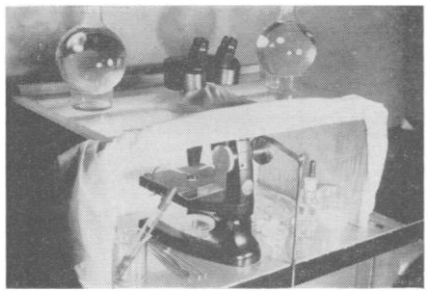

Fig. 1. Chamber equipped for sterile microdissection.

fastened through the slit provided for an arm rest at either side of the stage. The handle, made from glass rod, was held by many turns of the wire, which was looped at the base so that the instrument could be directed to any position or else bent down under the stage while it was not in use.

A foot-pedaled focusing device was provided in order to free the operator's hands for dissection. This device was composed of a ring fastened by a screw to the focusing knob and hinged to a steel rod, which was in turn hinged to the foot pedal.

The chamber was first sprayed with 75-percent alcohol and then equipped with all necessary sterile articles and materials so that it would be self-sufficient after the operation was under way. These articles were a dropping bottle of Ringer's solution, a test tube fitted with a medicine dropper and containing the culture medium, a covered dish of hexylresorcinol (1:1000), petri dishes with glass slides and coverslips, dissecting instruments, and depression slides ringed with petrolatum.

A previously sterilized covered jar containing petrolatum and a section of glass tubing with a diameter slightly larger than the circle of the depression slide was heated until the petrolatum melted. The jar was then sprayed with alcohol and placed quickly in the chamber. Rings of petrolatum were made by setting the glass tubing on the depresison slides, which were then placed upside down on a rack in the petri dish.

The chamber was also provided with sterile tissue paper contained in a small box taped against the side wall. The slit opening of this box was covered by a glass plate hinged above with adhesive tape. Pieces of paper to wipe off the tips of the dissecting instruments could be easily pulled out from the slit; the glass plate served as a protection against contamination.

After the afore-mentioned articles had been placed in the chamber, the chamber was sprayed with alcohol a second time and the operation proceeded as follows. The mosquito to be dissected was immobilized by carbon dioxide and then brushed free of scales. Both the oral and the anal openings were sealed by touching them to a fresh surface of Duco cement. After the cement had hardened, the insect was placed in the chamber and dipped for a few seconds in hexylresorcinol. It was then washed through 8 or 9 drops of Ringer's solution on slides and was ready for dissection.

The Duco cement not only prevented contamination of the Ringer's solution by the contents of the digestive tract but also kept the insect from floating; thus it made the dissection easier. In dissection, care had to be taken not to cut through the digestive tract. Usually, two operations could be made on a single stomach, which nevertheless continued to contract hours after it had been cut provided that the Ringer's solution was changed frequently.

Neither a micropipet nor a microspatula was successful in transferring oocysts. But by means of a "ferry," the oocysts were transferred successfully from drop to drop of Ringer's solution for washing, and from drop to drop of medium for culturing. The "ferry" was a fragment of broken No. 1 coverslip about 1 mm^2 in size. Such fragments were made beforehand in great numbers and only those with straight edges were chosen, since good straight edges alone could assure a sure grip with the fine points of the forceps. These selected glass fragments were sterilized in a small petri dish and kept inside the chamber.

After an oocyst had been cut out under the high power of the microscope, it was observed with low power and pushed by a microspatula to the center of the ferry. A slide was placed on the stage, and then a coverslip was placed on the slide. One or two drops of Ringer's solution and a drop of medium were placed on the slide for washing, and a drop of medium was put on the coverslip for culturing.

The ferry was picked up by a pair of fine forceps and washed in the drops of Ringer's solution and medium on the slide and was finally placed in the culture medium on the coverslip. It was then handled with the usual hanging drop technique. All of these steps had to be carried out without losing sight of the oocyst. By the techniques outlined here, oocysts of the size of 20 μ have been dissected and transferred successively to fresh medium for culturing.

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References and Notes

1. G. H. Ball, *Exptl. Parasitol.* 3, 358 (1954).
2. This work was aided by grant No. 1857 from the U.S. Public Health Service and was suggested by Gordon H. Ball.
3. Thanks are due to Thomas W. James for the design of the chamber.

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