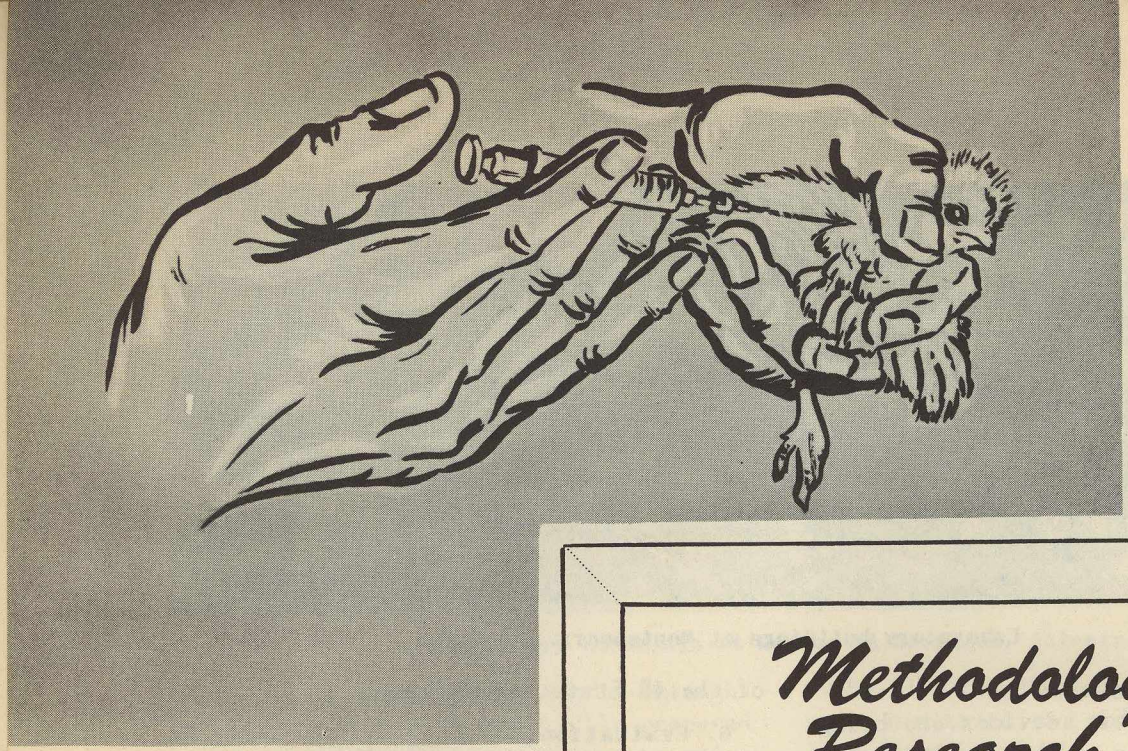


Methodology Research

Dr. M. M. Brooke, i/c Parasitology Branch

Methodology research as engaged in by the Laboratory Division is the first step toward its goal of improving laboratory diagnostic medicine. This methodology research consists of two portions: (1) the evaluation of existing diagnostic procedures, and (2) the development of new techniques when these are needed. Thus the research activities of the Laboratory Division are, for the most part, in the realm of applied research rather than in the field of theoretical or basic research.

A large number of rather varied problems are being investigated by the Bacteriology-Serology, Tuberculosis, Virus, Parasitology, and Entomology Laboratories of the Laboratory Division. A few representative examples will be used to illustrate the scope of methodology research.

One of the research problems of the Bacteriology Laboratories involves several kinds of media used for the isolation of diptheria bacilli. A comparison of some of the more promising of these media is being carried on by inoculating each throat culture received onto each of several media under investigation. This investigation will continue for some time, as new diagnostic media are developed by various investigators. The various tellurite plate media containing whole blood vary somewhat, but are, as a group, uniformly much better than the tellurite media containing only serum. Later, it is planned to study these media for initial cultivation directly from the patient's throat in an effort to improve upon the present Loeffler's medium.

Comparative tests are being run by the Serology Laboratories to determine which of several complement fixation techniques will give the optimal sensitivity and specificity. In connection with this study, comparative tests are being performed on commercial complement to determine its status.

The serological diagnosis of brucellosis has inherent limitations, but the technique is widely used and thus is being investigated.

The Tuberculosis Laboratory has undertaken an extensive program of methodology

research concerned with the microscopical diagnosis, cultural diagnosis, animal diagnosis, typing, and virulence testing of tubercle bacilli. These studies are directed toward both the evaluation of existing laboratory procedures and the development of new, more efficient methods. For example: A study to ascertain the efficacy of centrifugalization as a concentration procedure is under way.

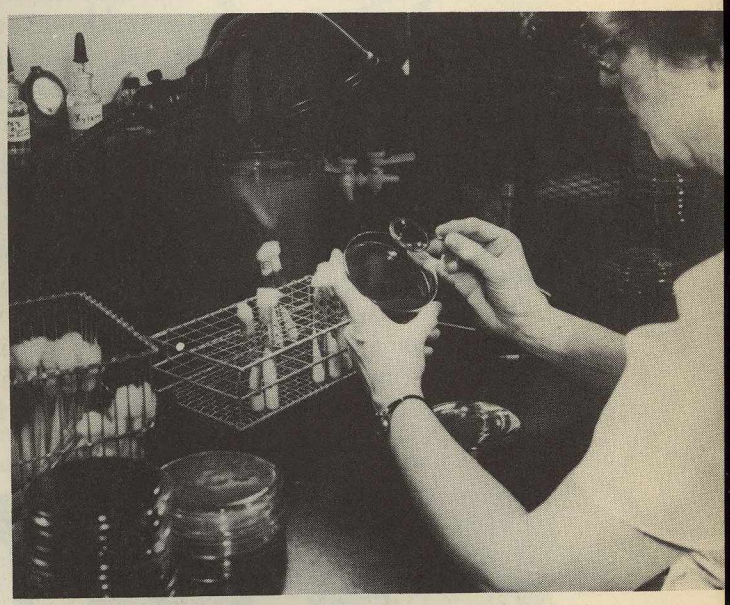
A major study has been the comparison of various tuberculosis culture media by inoculation of different media with the same material. The media being tested are modified Lowenstein's, Dubos' (both solid and liquid), Petraganinis', the Trudeau Society's, Sula's, Herrold's egg-yolk agar, and others.

Because of the difficulty of shipping or storing pathological materials over any considerable period of time without having the tubercle bacilli die, studies are being made of various methods of preserving the organisms in a viable state for transportation and storage.

The neurotropic virus diseases are most difficult to diagnose clinically and study epidemiologically. At present, the Virus Laboratory possesses stocks of all the more important viruses. Complement fixation tests for many of the neurotropic virus diseases lack specificity and sensitivity. Studies are being carried on to develop better antigens and to improve the various tests.

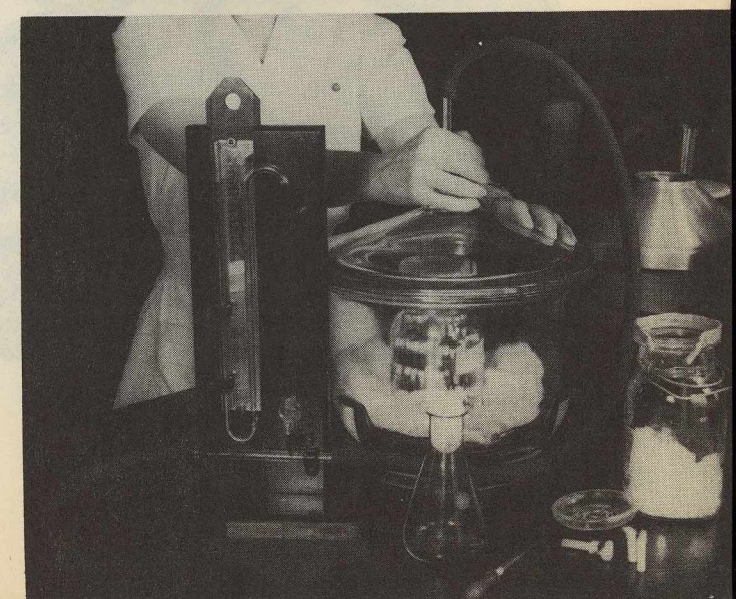
It is frequently difficult for physicians to get laboratory assistance in establishing the diagnosis of amebiasis. Shipping the stool to a distant laboratory for diagnosis has been unsatisfactory since the trophozoites are completely disintegrated by the time the specimen is examined. Recently the Parasitological Laboratory has developed a technique that shows promise of markedly improving the diagnosis of this disease. It has been found that by mixing a water-soluble resin, polyvinyl alcohol (PVA), with a fixative, an effective preservative is obtained.

Routine diagnostic serology in virus laboratory.



Checking diptheria cultures.

Equipment used for dessication of bacterial cultures.

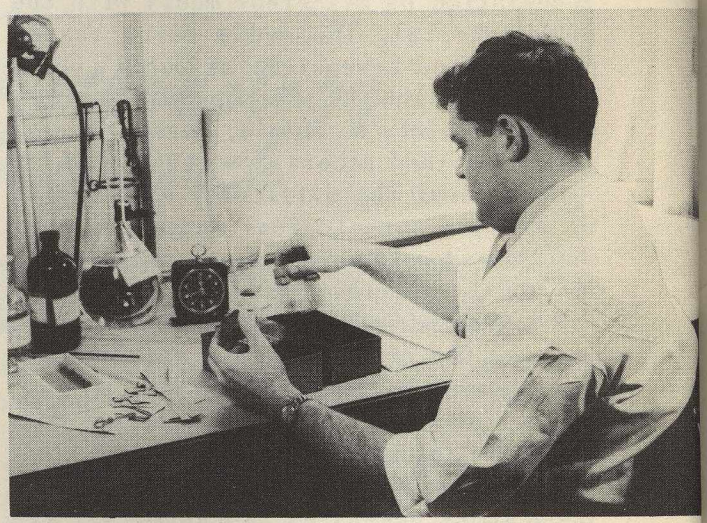


The diagnosis can be made from the preserved specimen immediately or months later. One of the distinct advantages of the PVA-fixative technique is that it makes possible the successful staining of organisms occurring in fluid specimens. To date it has been found that the technique triples the number of positive diagnoses.

The PVA-fixative technique illustrates a phase of methodology research devoted to the development of new techniques where none are available to meet the existing need.

Where large numbers of blood films have to be stained in connection with malaria surveys and other studies, it is a distinct advantage to employ a mass staining procedure. The one formerly used frequently permitted blood to wash off a positive film and to adhere to a normal blood film. If the transferred blood contained malarial parasites, a normal individual might be diagnosed as having malaria. After the discovery of this imperfection it was found that by adding a small quantity of a surface active agent (Triton X-30) all transfer was virtually eliminated.

Staining of fecal smears following preservation by PVA - Fixative technique.



Experimental staining procedures for thick blood film diagnosis of malaria.

Entomologists comparing specimens submitted for identification with museum collection.