The reference diagnostic service is available to all public health laboratories and all practicing physicians through their health departments with a bona fide diagnostic problem involving a communicable disease or infectious microorganisms. The service deals with rickettsial and viral infections, mycotic diseases, tuberculosis, parasitic infections, diptheriology, the bacterial enteric infections, leptospiroses, and general bacteriology, including any and all infectious diseases for which laboratory facilities are available.

In the process of organizing the growing reference diagnostic services, occasional relocation of laboratories has taken place, and thus it is sometimes not clear to what address various types of diagnostic specimens should be sent. To clarify this matter and to hasten the transmission of each specimen to its proper destination, a complete list has been prepared by the Office of State Services showing: (a) all diagnostic services currently available in the Communicable Disease Center; (b) the sort of material most likely to yield a positive result; and (c) method for preparing and shipping it, with EXACT ADDRESS for each sort of material. An abbreviated form of the list is reproduced here for convenience of the reader.

THE DIAGNOSTIC SERVICES

Some idea of the nature and scope of the reference diagnostic work may be gained from the following outline of the activities of the various laboratories.

THE VIRUS LABORATORIES

The Virus Laboratory receives from State laboratories, private physicians, and numerous other sources, human blood serum, autopsy specimens, brain material, spinal fluids, feces, nasal washings, etc. Serum neutralization tests have been done against the viruses of Eastern and Western equine encephalomyelitis, St. Louis encephalitis,

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Correct Addresses of Laboratory Division, Communicable Disease Center to Insure Prompt Delivery of Specimens Submitted for Reference Diagnostic Services.

(Copy of letter or other information as to service desired should accompany specimen)

SERVICES AVAILABLE	ADDRESS
BACTERIOLOGY	
Identification of unknown organisms other than those referable to laboratory units listed below.	U. S. Public Health Service Communicable Disease Center General Bacteriology Laboratory Chamblee, Georgia
Examination of throat cultures for isolation of *C. diphtheriae.* Identification and virulence testing of pure cultures suspected of being *C. diphtheriae.* Typing of cultures of *C. diphtheriae.*	U. S. Public Health Service Communicable Disease Center Diphtheria Laboratory Chamblee, Georgia
Cultures or stools for isolation and identification of *Salmonella* and *Shigella.* Bacteriophage typing of *S. typhi* and *S. paratyphi B.* Grouping of other enteric bacteria by biochemical methods, etc.	U. S. Public Health Service Communicable Disease Center Enteric Bacteriology Laboratory Chamblee, Georgia
Acid fast cultures for typing and determination of virulence. Streptomycin sensitivity testing on cultures. Examination of pathological material for tubercle bacilli (problem cases only: not accepted routinely)	U. S. Public Health Service Communicable Disease Center Tuberculosis Laboratory Chamblee, Georgia
SEROLOGY	
Serological tests for leptospirosis, rickettsioses, lymphogranuloma venereum — psittacosis group of viruses, infectious mononucleosis, brucellosis, etc. Host preference serology. Streptococcus typing.	U. S. Public Health Service Communicable Disease Center Immunology-Serology Laboratory Chamblee, Georgia
MYCOLOGY	
Examination of specimens for fungi. Identification of mycological cultures. Examination of tissue sections for pathological changes due to fungi.	U. S. Public Health Service Communicable Disease Center Mycology Laboratory Chamblee, Georgia
PARASITOLOGY	
Identification of intestinal parasites in amebiasis, hookworm infection, ascariasis, echinococcosis, tape worm infection, etc.	U. S. Public Health Service Communicable Disease Center Intestinal Parasites Laboratory 291 Peachtree Street Atlanta, Georgia
Identification of blood parasites in malaria, filariasis, leishmaniasis, trypanosomiasis, etc.	U. S. Public Health Service Communicable Disease Center Blood Parasites Laboratory 291 Peachtree Street Atlanta, Georgia
MEDICAL ENTOMOLOGY	
Identification of arthropods of medical importance, (mites, ticks, lice, bed bugs, fleas, mosquitoes, cockroaches, flies, etc.)	U. S. Public Health Service Communicable Disease Center Medical Entomology Laboratory 291 Peachtree Street Atlanta, Georgia
VIROLOGY	
Serum neutralization tests, agglutination tests, hemagglutination tests and complement fixation tests for viral diseases. *Examination of body fluids, secretions, excretions, tissues and arthropods for virus. Examination of human and animal tissues for pathological changes due to viral diseases.	U. S. Public Health Service Communicable Disease Center Virus Laboratory Box 436, Rt. 3, Federal Drive Montgomery 5, Alabama

*Such examinations are also available at the Division of Infectious Diseases, Microbiological Institute, National Institutes of Health, Bethesda, Maryland.

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Inoculation of embryonated eggs for virus cultivation.

lymphocytic choriomeningitis, herpes, and the Newcastle disease virus of chickens. Complement fixation tests have likewise been done against the three encephalitic viruses, as well as for lymphocytic choriomeningitis and mumps. Fecal specimens and nasal washings have been inoculated into monkeys for isolation of the poliomyelitis virus, using a method developed in this laboratory, which employs a mixture of penicillin and streptomycin for inhibiting bacterial but not viral growth. By this method the percentage of virus isolations has been greatly increased. Human and animal autopsy materials (brain or cord) have been received from various States. Eastern equine encephalomyelitis has most frequently been recovered from such specimens received from Southeastern States.

Diagnostic services for the influenza viruses are designed to detect outbreaks of influenza in the initial stages, and to endeavor to isolate new strains of influenza virus.

Serum neutralization and complement fixation tests have been done on materials collected for various surveys by the Epidemiology Division of the Communicable Disease Center. In this connection, entomological materials (flies, mosquitoes, and other arthropods) have been examined for the presence of viruses. The virus of Eastern equine encephalomyelitis has been recovered on several occasions in such studies.

Occurrences of particular interest in the course of the laboratory studies have been the finding of antibodies for the virus of Newcastle disease of chickens in the blood of a large number of human cases showing a particular type of neurologic syndrome; and the development of an improved technique for inoculation of bacterially infected fecal material into animals for recovery of the virus of poliomyelitis.

THE SEROLOGY-IMMUNOLOGY LABORATORIES

The work of these laboratories has consisted in large part of complement fixation tests on sera from rats trapped in connection with murine typhus control studies. In addition, complement fixation tests are made on sera from persons with undiagnosed febrile diseases, the antigens including Rocky Mountain spotted fever, Q fever, rickettsialpox, and murine typhus. Weil-Felix tests are also done on these sera.

The Serology Laboratories also carry on active studies on the host preference of mosquitoes of medical importance. The purpose is to determine the kind of animal on which disease-bearing mosquitoes prefer to feed. The antisera used in this work are prepared in our own laboratory.

Other services of this laboratory include

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complement fixation tests for the lymphogranuloma-psittacosis group of viruses. These are available upon request. The heterophile antibody test for infectious mononucleosis is also performed. The absorption technique for this test has recently been inaugurated in order to yield results of greater specificity.

In the near future, diagnostic complement fixation tests for amebiasis, histoplasmosis, and coccidioidomycosis, will be available. The agglutination tests for leptospirosis and brucellosis are being rendered.

A reference diagnostic service for the grouping and typing of hemolytic streptococci has been completed.

antigens for blastomycosis are at present available for distribution and, during the coming year, antigens for histoplasmosis and coccidioidomycosis will be made ready.

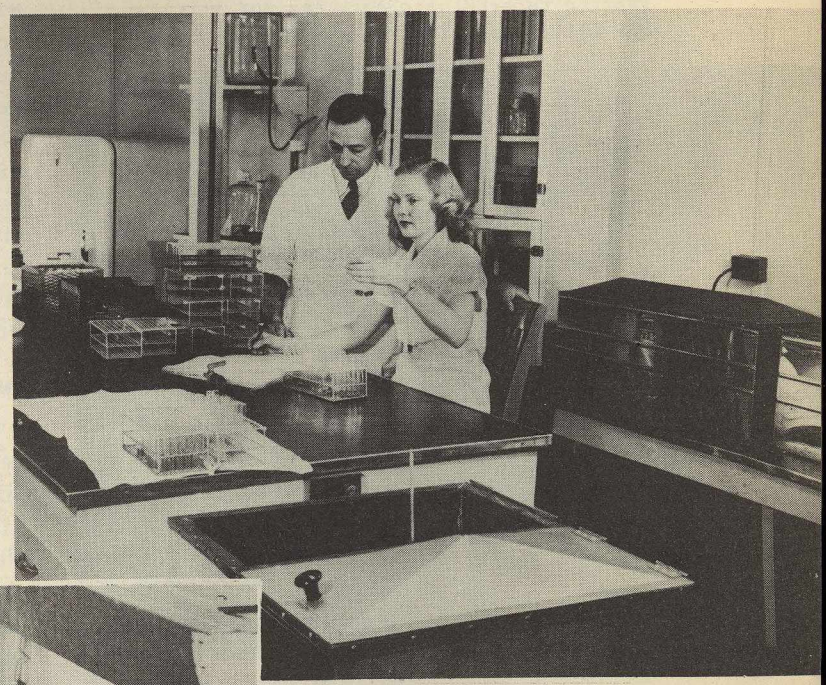
THE TUBERCULOSIS LABORATORIES

Reference diagnosis in tuberculosis is carried on at the Communicable Disease Center in collaboration with the Tuberculosis Control Division of the Public Health Service. The laboratory handles a large volume of work, the nature of which is shown by the following outline:

a. Routine microscopic diagnosis is made on specimens of sputum, gastric wash-

THE MYCOLOGY LABORATORIES

The mycological reference diagnostic service has been widely welcomed because mycological diagnosis is available in only a few laboratories. Three general types of diagnostic services are available; those now carried on are (a) the identification of fungi sent to the laboratory and attempts at isolation of pathogenic fungi from various types of clinical material, such as sputum, spinal fluid, blood, hairs, and nail and skin scrapings; (b) examination of histological slides for pathogenic fungi; (c) skin-testing



Reading results of diagnostic rickettsial serology tests.

Isolation of pathogenic fungi from clinical material.

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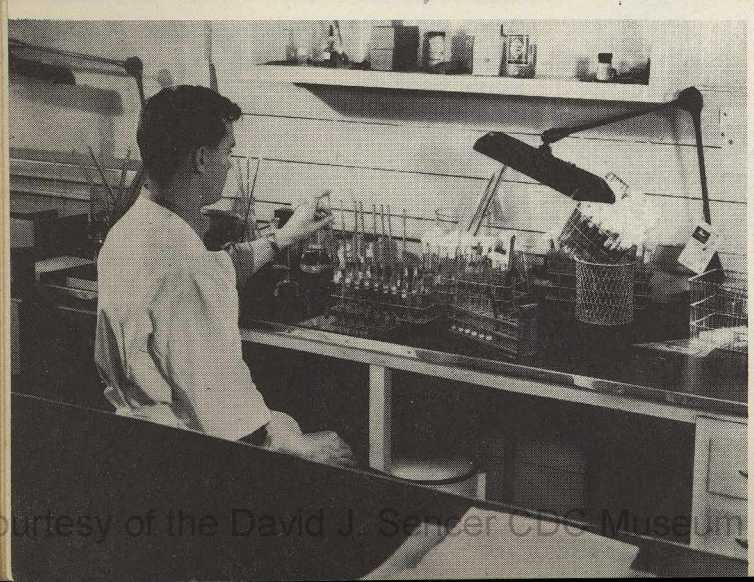
Incubator containing cultures from reference diagnostic material.

Testing tuberculosis cultures for streptomycin sensitivity.



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ings, and other body fluids. The microscopic diagnoses are supplemented by cultural methods and, when advisable, by animal inoculation.

b. Reference diagnostic service is rendered on cultures of acidfast bacilli from many sources. These are examined culturally and by animal inoculation when determination of type is requested.

c. The streptomycin sensitivity (or resistance) of the tubercle bacilli isolated from various patients is being determined. Tubercle bacilli that are not only resistant to, but ABSOLUTELY DEPENDENT on, streptomycin (veritable microbiological drug addicts!) have been found.

The following media have been evaluated with respect to their adaptability to routine streptomycin sensitivity assays: Lowenstein (Jensen-Holm modification), Herrold's egg-yolk agar, Dubos' Tweenalbumen, and Sula's. An evaluation of a simple egg-yolk medium recommended by the American Trudeau Society has also been started, especially in regard to its use in the sensitivity testing of primary cultures. A slide culture technique is also being investigated.

THE ENTERIC BACTERIOLOGY LABORATORIES

This important phase of diagnostic bacteriology has developed greatly in volume and degree of complexity. From qualitative biochemical reactions, it has now entered the fields of immunology and serology in their most profound qualitative and quantitative aspects. These facts are evident in the following brief list of reference diagnostic activities of the Enteric Bacteriology Laboratory:

1. Serological typing of *Shigella* and *Salmonella* cultures.
2. Grouping of paracolon bacteria, *Proteus*, etc., by biochemical methods. Certain groups are identified serologically.

Serological typing of *Salmonella* cultures.

3. Bacteriophage typing of *S. typhi*.

The Enteric Bacteriology Laboratories are now the U. S. national representatives for the International Committee on Bacteriophage Typing, with headquarters in London.

Not only are these services currently available on an international scale, but it is hoped that in the near future the following services may be added:

1. Distribution of bacteriophages for typing *S. typhi*.
2. Distribution of *Salmonella* typing serums. These materials will be distributed only to laboratories having the personnel and facilities for their proper use.
3. Serological grouping of *Klebsiella* cultures.

Several unusual observations made from reference diagnostic material are:

1. *S. typhi*, phage type E, was recognized among cultures isolated from cloacal swabs from chickens. The organism was isolated in the laboratories of the Dysentry Control Project in New Mexico.
2. Cultures from an outbreak of *S. anatum* infection among restaurant patrons were identified. There were seven cultures from patients, three from cooked turkey (which was the vehicle of infection), and one from a normal carrier who was a food handler in the restaurant. It is not often such a complete chain of evidence revealing the source and vector of the outbreak is compiled.
3. Three new *Salmonella* types were recognized.
   a. *S. corvallis* — VIII, XX:z4z23 — from turkeys.
   b. *S. colorado* — VI, VII: 1,w — 1,5 — from man, clinical condition unknown.
   c. *Unnamed type* — I, XL: R — 1,6 — from a food handler.
4. A culture isolated from the heart blood of a 5-month-old infant who died of enteric infection, was recognized as an Arizona paracolon, formula 10:1, 2, 10.
5. Cultures of *S. typhi* from several epidemics have been typed by bacteriophage. In every instance all the cultures from a given epidemic belonged to the same type. The types most frequently found are A, C, and E.

THE GENERAL BACTERIOLOGY LABORATORIES

Under this heading are comprised all bacteriological diagnostic problems not referable to the other laboratories discussed in this outline. For the past several months this laboratory has concerned itself largely with problems in the diagnosis of diphtheria. This disease has increased markedly in prevalence and severity during the last few months. Included in the large volume of work already handled has been the isolation of strains of corynebacteria from throat cultures, the determination of their identity, type (gravis, mitis, minimus), and virulence. Observations of special interest are the increase in numbers of gravis-type strains, and the occurrence of minimus-type strains and of virulent saccharose-fermenting strains in the southeastern part of the United States.

THE PARASITOLOGY LABORATORIES

The Parasitology reference diagnostic work has developed to a very large volume with many ramifications.

Some interesting problems in diagnosis not concerning diptheria were also encountered. For example, a culture of unknown nature from an undiagnosed disease condition turned out to be the glanders organism (*P. mallei*); a supposed diptheria culture was found to be a species of *Nocardia*; and from the sputum of a patient suspected of having a pulmonary mycosis, another species of *Nocardia* was isolated.

The types of specimens submitted to the Blood Parasite Laboratory are blood smears, thick and thin (thick blood smears to be examined for malarial parasites constitute the bulk of specimens submitted), whole blood, biopsied lymph nodes,

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PARASITOLOGICAL REFERRAL DIAGNOSIS

Specimens are received in the mail, marked for identification, and microscope slide preparations made in saline, iodine, and Quensel's stain. These preparations are sealed with paraffin and are then ready for diagnostic study under the microscope.

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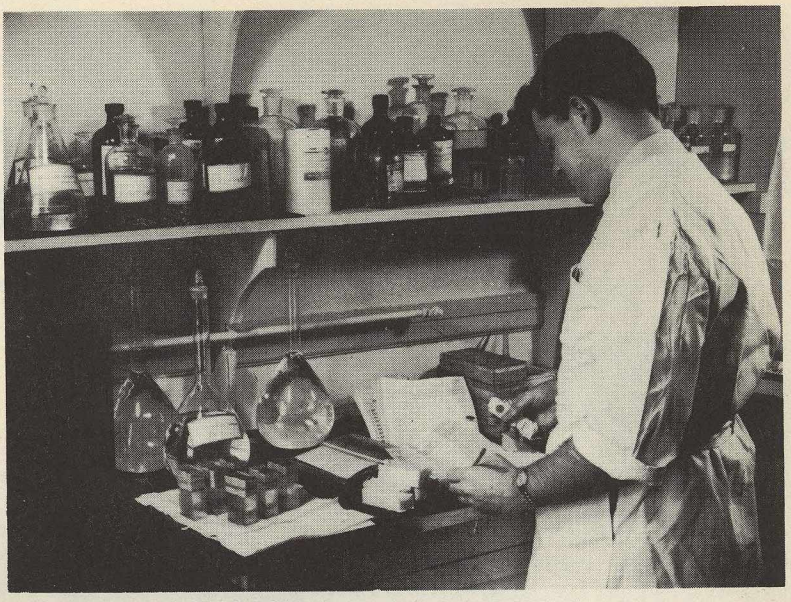
and bone marrow smears. The organisms diagnosed are malaria (*Plasmodium falciparum, P. vivax,* and *P. malariae*), *Wuchereria bancrofti, Trypanosoma cruzi,* and *Leishmania donovani.*

Specimens submitted to the Intestinal Parasite Laboratory include feces, urine, sputum, pus, adult worms, deer meat, tissue sections, pleural fluid, peritoneal fluid, fish, and cellulose-tape anal swabs. These organisms have been identified: *E. histolytica* and other intestinal protozoa, intestinal nematodes (roundworms), intestinal cestodes (tapeworms), exotic parasites (*Schistosoma haematobium* and *Paragonimus westermani*), hydatid cysts of *Echinococcus,* and *Trichinella.*

The materials for diagnosis come from every State in the Union and from foreign countries.

Reference diagnostic specimens often are the first evidence of unusual incidence of many diseases, as subsequent investigations have shown. Reference diagnostic services have frequently backed up local laboratory diagnoses. However, local laboratory errors have been corrected at times.



Checking slides preparatory to staining blood smears from malaria survey.

Ascaris in specimen submitted for diagnosis.

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