RA 428.3 .U55 1976

state hygienic laboratory manual of services



prepared for practicing physicians and public agencies of iowa



State Hygienic Laboratory

Medical Laboratories Building The University of Iowa Iowa City, Iowa 52242

William J. Hausler, Jr., Ph.D.

Director

R. M. A. Hahne, Ph.D.

Assistant Director

E. D. Renner, Ph.D.

Assistant Director

and

Principal Microbiologist

Y. W. Wong, M.A. Assistant Director and Principal Virologist

Office Hours: 8:00 a.m.-5:00 p.m., Monday-Friday Telephone: Area Code 319/353-5990

In case of emergency after hours, call 319/353-5990 and recorded instructions will be given regarding whom to contact.

Des Moines Branch Laboratory 405 State Office and Lab Building East 7th and Court Streets Des Moines, Iowa 50309 Telephone: 515/281-5371

State Library Of Iowa
State Documents Center
Miller Building
Des Moines, Iowa

TABLE OF CONTENTS

| LIST OF TABLES | . V | Viral and Rickettsial Diseases | 55 |
|-------------------------------------|-----|---|----|
| | | Rubella HI Test | 63 |
| LIST OF FIGURES | vii | ENVIRONMENTAL SCIENCE SERVICES | 70 |
| PREFACE | ix | Air Pollution Surveillance | 71 |
| | | Industrial Hygiene | 71 |
| GENERAL INSTRUCTIONS | xi | Radiological Health | |
| LABORATORY DIAGNOSTIC SERVICES | 1 | Drinking Water | 80 |
| Respiratory Tract Infections | | Mineral and Radiological Analysis of Water Supply Samples | 82 |
| Enteric Infections | | Fluoride Analysis and Proficiency Testing | 85 |
| Miscellaneous Bacteriology | | Surface Water and Wastewater Analysis | 87 |
| Reference Bacteriology | | Special Analytical Services | 88 |
| Food Specimens | | Effluent Quality Analysis Program | 88 |
| Staphylococcal Bacteriophage Typing | | Water Pollution Surveillance | 88 |
| Parasites and Ova | | Swimming Pools | 90 |
| Blood Parasites | | Milk Quality | 90 |
| Mycobacteria Examination | | OTHER LABORATORY SERVICES | 93 |
| Mycology | | Pediatric Lead Poisoning Program | 93 |
| Rabies Examination | 45 | Laboratory Consultation and Development | 95 |
| Syphilis Serology | 47 | Drug Analysis | 97 |
| Special Serology | 250 | | |
| Febrile Agglutination | 52 | | |

LIST OF TABLES

| Table | 4-1 | Potential clinical significance of various mycobacteria | 32 | Table | 8-2 | Summary of specimens to collect for laboratory diagnosis of viral | 00 |
|-------|-----|---|----|-------|------|--|----|
| Table | 5-1 | Types of specimen and suggested | | | | and rickettsial infection | 68 |
| | | media for isolation of the fungi- caused mycoses | 41 | Table | 8-3 | Summary of viral and rickettsial tests available | 69 |
| Table | 7-1 | Battery of agglutination tests for | | Table | 8-4 | Abbreviations and symbols | 69 |
| | | febrile diseases | 53 | Table | 12-1 | Tests available, surface water | |
| Table | 8-1 | Tests available for viral and rickettsial diagnosis | 66 | | | and wastewater | 87 |

5 7 1 1 1 1 1 1 1

ATTOCKED OF THE PERSON OF THE

LIST OF FIGURES

| Figure | 2-1 | Throat specimen collection kit | 3 | Figure | 4-10 | Final report | 33 |
|--------|------|--|-----|--------|----------|--|----|
| Figure | 2-2 | Procedure for obtaining throat swab | 4 . | Figure | 4-11 | TB drug susceptibility profile | 34 |
| Figure | 2-3 | Diphtheria specimen collection kit | 5 | Figure | 5-1 | Mycology specimen collection kit | 36 |
| Figure | 2-4 | Procedure for collection of | | Figure | 5-2 | Completed mycology data form | 37 |
| | | enteric specimen | | Figure | 5-3 | Identification of nebulized | 20 |
| Figure | 2-5 | Enteric specimen collection kit | 9 | | 6 | mycology sputum specimen | 39 |
| Figure | 2-6 | Slide examination kit | 10 | Figure | 5-4 | Fungal serology specimen collection kit | 40 |
| Figure | 2-7 | Miscellaneous data form | 11 | Figure | 5-5 | Negative mycology culture report | |
| Figure | 2-8 | Antibiotic sensitivity report form | 14 | Figure | | Positive mycology culture report | |
| Figure | 2-9 | Miscellaneous examination report form | 14 | .0 | (1-41) A | Procedure for obtaining syphilis serology blood specimen | |
| Figure | 2-10 | Reference Bacteriology examination request form | 15 | Figure | 7-2 | Syphilis serology specimen collection | 49 |
| Figure | 2-11 | Label for etiologic agents/ biomedical material | 16 | Figure | 7-3 | Serologic data form | 51 |
| Figure | 0.10 | Shipping containers for reference | | Figure | 7-4 | Out-of-state marriage form | 50 |
| rigure | 2-12 | bacteria specimens | 17 | Figure | 7-5 | Report of serologic results | 51 |
| Figure | 2-13 | Bacteriophage typing report form | 19 | Figure | 7-6 | Special serology data form | 52 |
| Figure | 3-1 | Parasites and ova specimen collection kit | 21 | Figure | 7-7 | Febrile agglutination specimen collection kit | 54 |
| Figure | 3-2 | Pinworm specimen collection kit | 23 | Figure | 7-8 | Febrile agglutination data form | 52 |
| Figure | 3-3 | Parasite and ova report form | 24 | Figure | 8-1 | Virus and rickettsial patient | F0 |
| Figure | 3-4 | Positive slide report for malaria | 27 | | | history form | |
| Figure | 3-5 | Positive IFA report for malaria | 27 | | | Virus and rickettsial data form | 55 |
| Figure | 4-1 | TB specimen collection kit | 29 | Figure | 8-3 | Procedure for collection of V & R fecal specimen | 58 |
| Figure | 4-2 | TB-gastric specimen collection kit | 30 | Figure | 8-4 | Procedure for collection of V & R | |
| Figure | 4-3 | Completed TB data slip | 28 | Figure | 0-1 | CSF specimen | 59 |
| Figure | 4-4 | Identification of nebulized sputum | 31 | Figure | 8-5 | Procedure for collection of V & R | 00 |
| Figure | 4-5 | Report of positive microscopic TB examination | 32 | Figure | 8-6 | V & R shipping unit | |
| Figure | 4-6 | Report of negative microscopic TB examination | 32 | O | | Procedure for obtaining V & R blood specimen | |
| Figure | 4-7 | Report of negative TB culture | 33 | Figure | 8-8 | Rubella HI data form | |
| Figure | 4-8 | Report of positive TB culture | 33 | | | Rubella HI Collection kit | |
| Figure | 4-9 | Presumptive TB identification | 33 | | | | |
| | | report | 00 | Figure | 11-1 | X-ray protection survey report— | 74 |

LIST OF FIGURES (cont'd)

| Figure 11-2 | X-ray protection survey report— | | Figure 12-2 | Water analysis data slip | 80 |
|----------------------|---|-------------|--------------------|------------------------------------|----|
| | radiographic | 75 | Figure 12-3 | Mineral analysis data slip | 84 |
| Figure 11-3 | X-ray protection survey report— fluoroscope | 76 | Figure 12-4 | Fluoride analysis collection kit | 86 |
| Figure 11-4 | X-ray protection survey report— | | Figure 12-5 | EQAP collection kit | 89 |
| | facility, dental | 77 | Figure 12-6 | Swimming pool water collection kit | 91 |
| Figure 11-5 | X-ray protection survey report- | 10000 | Figure 13-1 | Blood lead collection kit | 94 |
| radiographic, dental | 78 | Figure 14-1 | PKU blood test kit | 96 | |
| Figure 11-6 | X-ray protection survey report— scatter radiation measurements | 79 | Figure 14-2 | PKU report form | 95 |
| Figure 12-1 | Drinking water collection kit | 81 | | | |
| | | | | | |

THE REPORT OF THE PARTY OF THE

The purpose of this manual is to provide information about the services available from the State Hygienic Laboratory to all persons concerned with the health and well-being of Iowa citizens. Information regarding the examinations offered, methods to be used in collection and shipment of specimens, and procedures for reporting of laboratory results are included in each section of this manual.

When the first edition of this manual was circulated in March, 1973, it was felt that updating could be accomplished every few years by the addition and deletion of pages in a looseleaf format, thus saving the expense of a complete reprinting. However, when we recently began to evaluate the extent of revision necessary to update the manual, it was apparent that adding and deleting pages was in fact much too simplistic. Not only have whole programs been either discontinued or initiated, but within many existing programs methodologies and philosophies have changed so drastically that a complete revision appears to be the only proper way to keep those dependent upon the Laboratory abreast of its capabilities.

Therefore, every several years a new edition of this manual will be distributed to those using the

Laboratory's services.

Editor March, 1976

- 1. The value of a laboratory examination depends as much on the quality of the specimen submitted as it does on the technical method of examination. A POORLY COLLECTED AND IMPROPERLY PREPARED SPECIMEN CANNOT YIELD RELIABLE INFORMATION. Therefore, it is important that every specimen sent to the Laboratory be collected and shipped in accordance with the instructions furnished in this manual. Detailed directions are listed in the section of this manual explaining the services of the respective laboratory division performing the test desired.
- 2. Requests for tests can be made only on the proper data forms. If the desired tests require more than one data form, divide the specimens and fill out separate forms. Examples of the proper data forms are given in that section of the manual dealing with the desired test.
- 3. The data forms must be filled out completely using a typewriter or black, medium soft lead pencil. WRITE OR PRINT LEGIBLY. Your report will consist of an exact copy of the request form with the Laboratory's findings recorded.
- 4. Kits for the collection and shipment of specimens are sent free upon request. Since they are expensive, only those necessary for short periods of time should be requested. These kits are furnished for the sole purpose of submitting specimens to the State Hygienic Laboratory. THE USE OF THESE KITS FOR ANY OTHER PURPOSE CONSTITUTES A MISUSE OF STATE PROPERTY. Illustrations of each kit are included in the appropriate sections of this manual. Federal laws require that biological material sent through the mail be carefully packed to avoid breakage and to protect personnel involved in its handling. Specimen mailing containers are to be used as follows:

Tube Shippers

The labels provided for the tube shippers are of the pressure-sensitive adhesive variety and need not be moistened to make them stick. To seal the specimen mailer for return to the State Hygienic Laboratory, remove the paper-backing from the label by bending the label along one of the black diagonal lines on the paper-backing. When the crease is sharp enough, the paper-backing will loosen at the crease and can be easily removed. Wrap the label firmly around the specimen mailer so the ends of the label overlap slightly. The specimen kit is now sufficiently sealed for return to the Laboratory.

Bottle Shippers

Pre-addressed chipboard boxes are supplied to return the specimen kit to the State Hygienic Laboratory. The box has mailing lock-tabs to insure against the opening of the box during transit to the Laboratory. Please be certain the lock-tabs are secured before mailing.

- 5. Do not ship specimens on weekends. Specimens mailed to the Laboratory on either Friday or Saturday may remain in the post office through Sunday. In order to avoid spoilage and hemolysis, blood specimens should be refrigerated until they are mailed. This precaution is especially important during hot or very cold weather.
- 6. Laboratory records are confidential. Except on receipt of written permission, no information on an individual patient is given to persons other than the physician submitting the specimen or an authorized public health officer.
- 7. The kits obtained from the State Hygienic Laboratory contain pre-addressed return mailing boxes. If other mailing containers are used, the outside wrapper is to carry the name and address of the addressee, the return address and the name of the sender, and a notation that a "Specimen for Bacteriological Examination" is enclosed.

LABORATORY DIAGNOSTIC SERVICES

The Diagnostic Services of the State Hygienic Laboratory consist of three divisions: Microbiology, Serology and Virology. While we speak of certain laboratory examinations as diagnostic tests, it should be emphasized that a laboratory does not diagnose. It performs certain biochemical or biological tests to determine the presence or absence of certain substances or organisms in a given specimen. A laboratory does not and cannot interpret how the substances happen to be present. It is up to the physician to interpret the laboratory report in the light of his or her clinical findings.

The most conclusive test a laboratory can offer is the isolation and identification of pathogenic organisms. The failure to demonstrate the organisms may mean: (1) that they were not present in that particular sample, (2) that the sample was taken at the wrong stage of the disease, (3) that the organisms were too few in number to be detected, (4) that the pathogenic organism failed to survive in transit to the laboratory or (5) that the patient was not infected.

In some diseases, where it is impractical to attempt an isolation of the organisms, serologic tests may be employed as indirect evidence of infections by measuring the patient's antibody response. It should be pointed out that the antibody titer of a patient's serum merely reflects current or past exposure to a specific antigen. A single quantitative serologic test is no measure of the degree or extent of infection. Two or more specimens should be obtained to determine a significant rise or fall in antibody titer.

INTRODUCTION

Throat and nasopharyngeal cultures are important in the diagnosis of such infections as streptococcal sore throat, diphtheria and whooping cough. They are also useful in determining the focal point of infection in such diseases as scarlet fever, rheumatic fever and acute glomerulonephritis. In epidemiological studies, these cultures have been essential for the detection of carriers of beta hemolytic streptococcus, hospital Staphylococcus sp., Corynebacterium diphtheriae and other potential pathogens.

COLLECTION AND SHIPMENT OF SPECIMENS

Throat Cultures

The throat culture specimen must be taken properly, preferably at the onset of symptoms and *before* antibiotic therapy. Reculture may be taken seven to 10 days past treatment. For routine throat culture, the kit shown in Figure 2-1 is available on request.

- Collect specimens under good lighting; use a sterile, cotton-tipped swab as illustrated in Figure 2-2. Depress the tongue with a tongue blade and pass the swab firmly over the back of the patient's throat, tonsils or tonsillar fossae and any area of inflammation or exudation.
- 2. Place the swab in the test tube with the cotton stopper. Do not break the applicator stick when replacing in the tube.
- Complete the throat culture data form, place both in the styrofoam shipping container, attach the adhesive-backed mailing label and mail.

NOTE: If diphtheria is suspected, two swabs should be taken as described above. A diphtheria outfit (Figure 2-3) is provided for this purpose.

Nasopharyngeal Cultures

Nasopharyngeal culture specimens are recommended when attempting the isolation of pneumococci, meningococci or *Haemophilus influenzae*, because these organisms are found more commonly in the nasopharynx than in the nose or throat. Nasopharyngeal swabs are essential for the recovery of *Neisseria meningitidis* from suspected carriers or for the recovery of *Bordetella pertussis* from suspected cases of

whooping cough. They are most useful in culturing specimens from infants and small children, whose sputum specimens are not readily obtainable.

- To take these cultures, use a cotton-tipped chromel or stainless steel wire applicator (not supplied by Laboratory).
- 2. Insert the instrument through the nose to the posterior nasopharynx, leave it there for a few seconds and then deftly withdraw it.
- Care must be taken to avoid mouth and throat contamination of the swab.

Microorganisms Most Likely to Survive One- to Two-Day Shipment on Dry Swab

- 1. Group A beta hemolytic streptococcus. This is the causative agent for streptococcal pharyngitis and tonsillitis. The mucous membranes of the tonsils and pharynx show a redness and swelling, and, in many cases, there are white to yellow patches on the tonsils or tonsillar fossae. It is recommended that other household members be checked for carriers, as streptococcal infections are repeatedly diagnosed within the family.
- 2. Staphylococcus aureus. Staphylococcal sinusitis and sore throat are caused by this organism. It is known that 30-40 percent of normal individuals carry this organism in nasal secretions. A person with a draining sinus or any purulent discharge is a source for the epidemic spread of skin lesions, boils, abscesses, wounds, etc. This is especially critical in hospitals where susceptibility to the organism is greatest among infants and the chronically ill. This organism will survive in significant numbers if the specimen arrives in the laboratory within 24-48 hours. The number of organisms on the swab tends to decrease the longer the shipping distance.
- 3. Corynebacterium diphtheriae. This organism occurs primarily in the respiratory tract, rarely on the skin or in wounds of infected persons or normal carriers. The organism is transmitted by droplet or contact routes to susceptible persons where it infects the mucous membranes of the respiratory tract and there produces the exotoxin which, in turn, produces pathological symptoms.

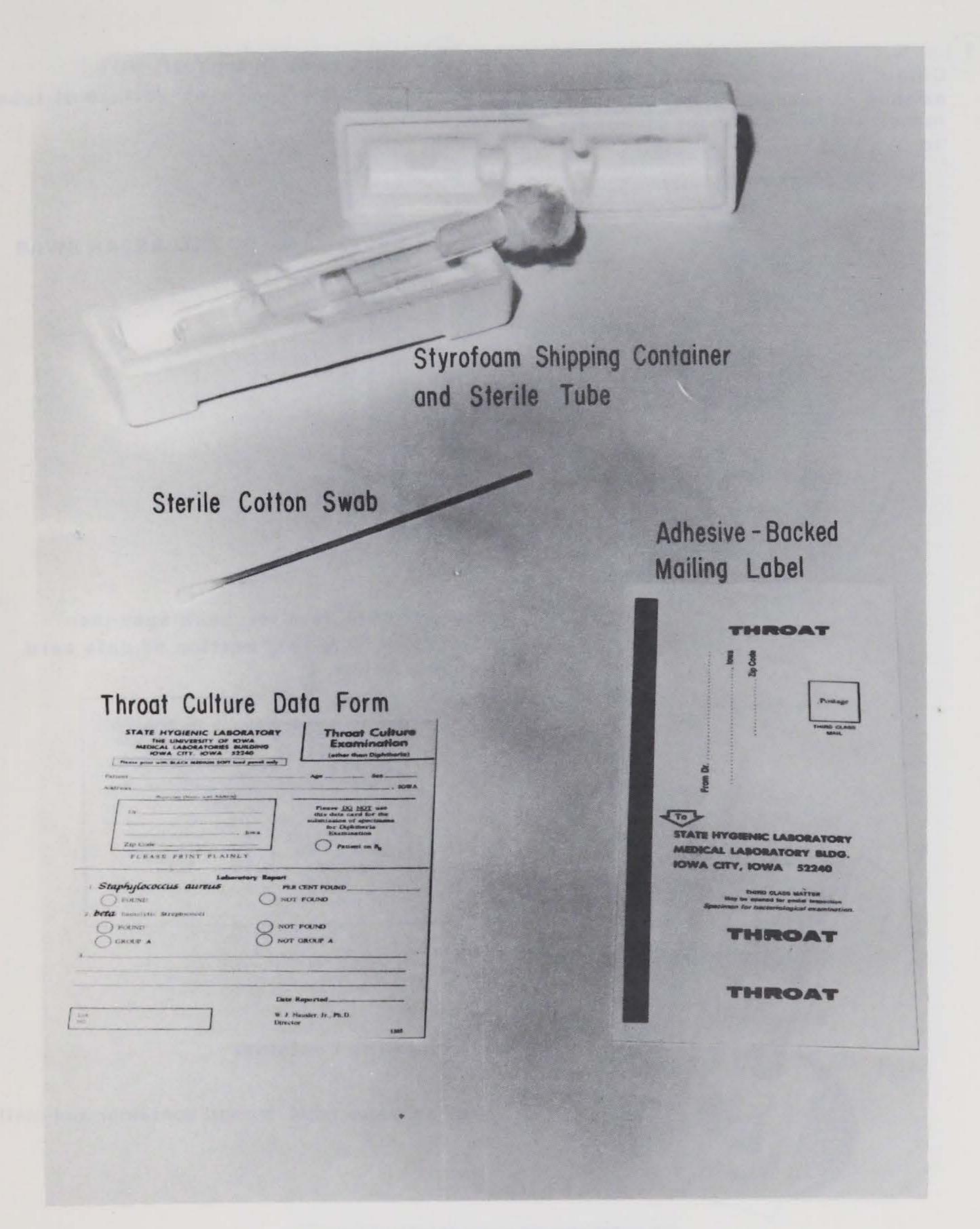
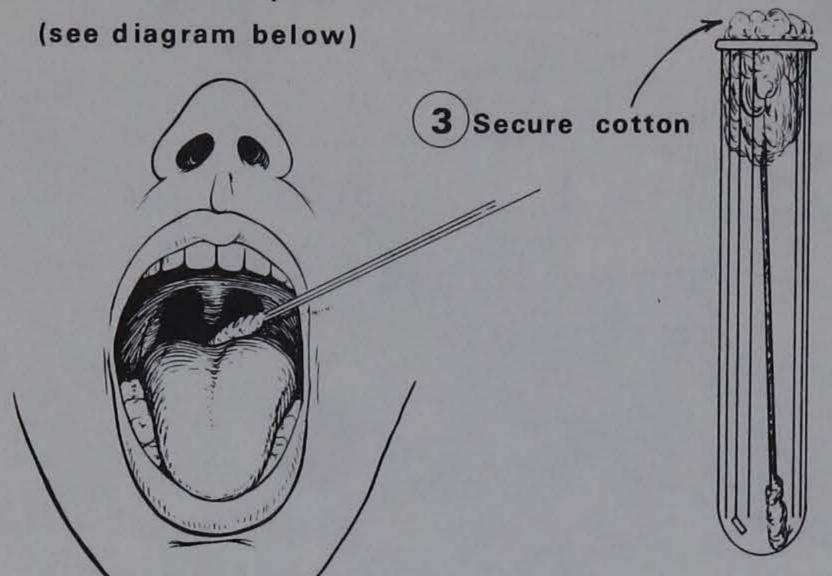


Figure 2-1 Throat specimen collection kit

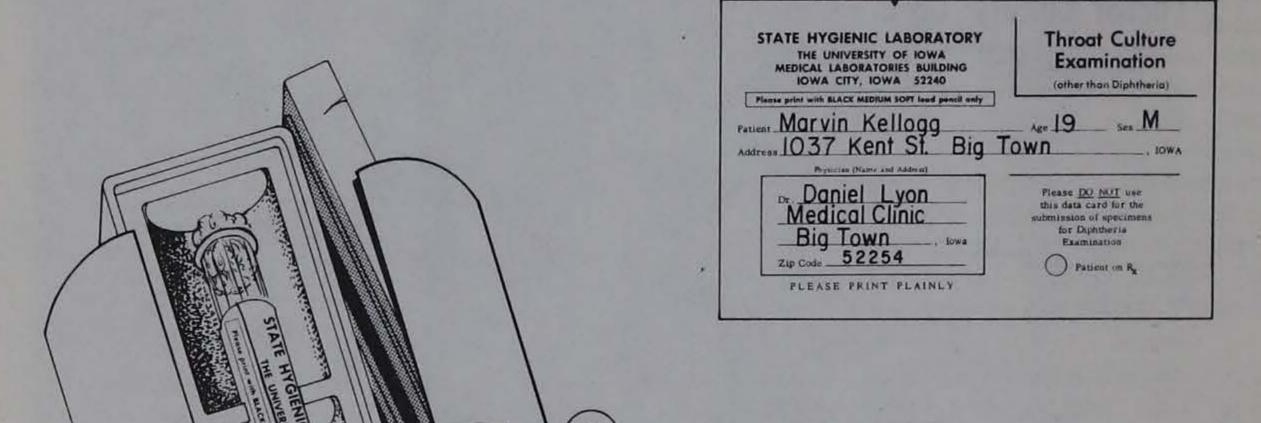
Obtain with sterile throat swab as soon as possible after onset of illness, material from posterior pharynx, tonsils and faucial pillars.

Place swab in tube. DO NOT touch any interior surface of tube.



4 DO NOT BREAK SWAB

5 Fill out data card for each specimen Complete only that portion of data card shown below



Place in mailing container

7) Wrap adhesive label around container and mail

Figure 2-2 Procedure for obtaining throat swab

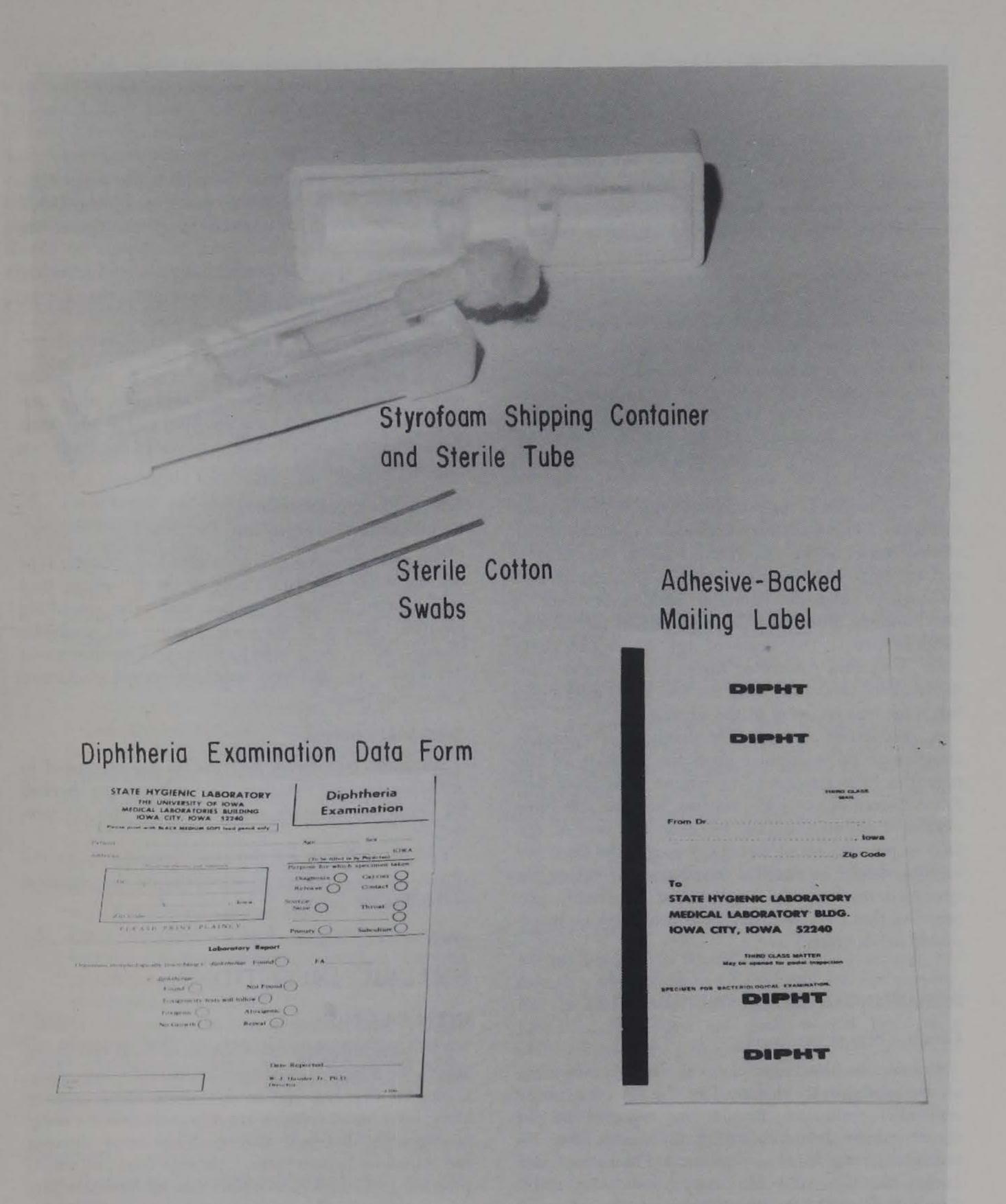


Figure 2-3 Diphtheria specimen collection kit

Microorganisms Best Cultured Locally

Some organisms will not remain viable on a dry swab for longer than a few hours and therefore are best handled by a local laboratory. Specimens cultured at the local level may be sent as pure culture isolates to the State Hygienic Laboratory for identification or confirmation. Instructions for the submission of subcultures by local laboratories are given on page 14.

- 1. Streptococcus pneumoniae. This organism was known formerly as Diplococcus pneumoniae and is found in the human throat, saliva and respiratory secretions of many individuals as well as in carriers. Pneumococcal pneumonia is a common disease among infants, the aged and debilitated. In suspected cases, a sputum specimen is highly recommended for culture purposes. However, a throat culture swab is acceptable, but only if processed within two hours of being taken.
- 2. Haemophilus influenzae and Haemophilus parainfluenzae. These are very fastidious organisms which
 can be found in the respiratory tract as normal flora
 and are capable of causing severe respiratory disease,
 meningitis, subacute bacterial endocarditis and a
 characteristic obstructive laryngotracheal infection in
 children two to five years of age which can prove
 fatal. A sputum culture or throat culture swab must
 arrive at the laboratory within a few hours after being
 taken for best recovery of the organism.
- 3. Bordetella pertussis. The diagnosis of whooping cough may be confirmed upon the isolation of this organism from respiratory secretions. This acute bacterial disease involves the trachea, bronchi and bronchioles of children, usually less than one year of age. In a suspected case of whooping cough, the local laboratory should be notified in advance of taking the specimen since special media must be freshly prepared so that the nasopharyngeal swab may be innoculated immediately.

REPORTING OF LABORATORY RESULTS

Group A beta streptococci

Colonies on blood agar plates showing beta hemolysis are subsequently examined by the FA (fluorescent antibody) technique. Results are reported on the throat culture data forms (Fig. 2-1). A positive FA indicates Group A beta streptococci. Fluorescent antibody which is negative for Group A indicates possible Group B, C or D beta streptococci, usually not implicated in serious pathogenic conditions of the nose and throat.

Antibiotic sensitivities are not done on Group A beta streptococci unless urgently requested by the physician.

Staphylococcus aureus

Staphylococcus colonies are reported in percentages from a nose or throat culture plate. Percentages of 75 percent or higher are considered of pathogenic significance.

Coagulase tests are performed to verify a Staphylococcus aureus, and antibiotic sensitivities are set up on the coagulase-positive organisms.

Pneumococci and Haemophilus organisms

A significant number of colonies of S. pneumoniae and/or Haemophilus influenzae organisms on a nose or throat culture plate are reported to the physician. An antibiotic sensitivity test is provided for these two organisms by special request only.

Enteric-like organisms and/or Pseudomonas-like organisms

If a predominance of Escherichia-Enterobacter type organisms are found in a nose or throat culture, they are reported to the physician. Since the consulting physician knows of the case history and antibiotic therapy, he or she is able to judge the significance of the report. An antibiotic sensitivity is not performed unless requested.

Bordetella pertussis

Suspected cultures of *B. pertussis* are incubated in our laboratory for five days. The cultures are checked for suspicious colonies and verified by the FA procedure.

Positive cultures are reported immediately to the physician; an antibiogram sensitivity on the organism will follow the report.

ENTERIC INFECTIONS

INTRODUCTION

The bacteriological examination of fecal specimens aids in the diagnosis of gastrointestinal infections manifested by diarrhea and/or dysentery. Stool cultures along with blood cultures are important aids for diagnosing acute diarrheal diseases. Since many diseases are spread by human carriers through food and drink, properly performed stool cultures on all food-handling personnel returning to work after an intestinal disorder strongly supplement public health control measures.

The organisms most frequently involved in enteric infections are Salmonella and Shigella sp. and enteropathogenic Escherichia coli. Saphrophytic organisms, including Proteus morganii and the Providencia group have been implicated as etiological agents of infant diarrhea as well as the ECHO and coxsackie viruses. Although not a problem in Iowa, Vibrio cholera is classified in this infectious disease category and should be considered when attempting diagnosis of gastroenteritis in recent international travelers.

COLLECTION AND SHIPMENT OF SPECIMENS

- Collect feces from patients as soon after onset of illness as is possible, and before the start of treatment. Transfer the specimen (about the size of a navy bean) with a tongue depressor blade to the phosphate buffered glycerol (PBG) supplied in the enteric kit (Figures 2-4, 2-5).
- 2. Mix thoroughly and secure cap tightly.
- Place bottle into the plastic bag supplied in the enteric kit and seal by rolling the top down and bending the wire tabs. In the case of a urine sample or liquid stool, no more than four ml should be added to the PBG. Rectal swabs may be used for infants, debilitated patients or large numbers of patients. When collecting a rectal swab be certain to insert swab past the sphincter muscle to obtain a fecal specimen. Isolation of bacteria from rectal swabs is best handled locally. However, if this is not possible, transfer the swab to the PBG and rotate vigorously. Discard the swab after pressing against the side of the bottle and proceed as with shipment of stool specimen. If a blood culture is necessary, contact the State Hygienic Laboratory (319/ 353-5990) for special instructions.
- Complete data form supplied in the kit by giving all requested information.
- Place the specimen and completed data form into the styrofoam shipping container. Use the pre-addressed mailing box which is provided, attach postage and mail.

NOTE: The enteric kits have expiration dates on them. The phosphate buffered glycerol is unsatisfactory for use if kept unrefrigerated longer than 60 days. If kits expire before use, return them to the State Hygienic Laboratory for replacement. Instructions for the submission of subcultures are given on page 14.

Remember:

- Don't ship a specimen without using a preservative.
- Don't collect an excessive volume of stool specimen.
- 3. Be sure to secure screw-cap tightly.

 Place the specimen bottle in the plastic bag, roll and bend wire tabs before putting it in the styrofoam mailer.

REPORTING OF LABORATORY RESULTS

Laboratory results will be reported by mail to the physician, veterinarian or laboratory director on the data form provided as soon as the results are known.

MISCELLANEOUS BACTERIOLOGY

INTRODUCTION

Miscellaneous Bacteriology provides bacterial examination of specimens obtained from wounds, abscesses, eyes, spinal fluid, etc. Three kits are available upon request for the bacterial examination of such miscellaneous sources: 1) Brucella blood culture kit (available by special request only), 2) miscellaneous culture kit (same as Figure 2-1 but has miscellaneous data form, Figure 2-7), 3) slide examination kit for Neisseria gonorrhoeae (Figure 2-6), and 4) gonorrhea culture kit (Transgrow). The Laboratory will examine subculture, and identify organisms isolated from commercial blood culture bottles and other commercial media which are not supplied by the Laboratory in kit form.

Services are also provided for identification, confirmation, toxin testing, sero-typing, etc., of subcultures submitted by laboratories within the state. Instructions for submission of subcultures are given on page 13 under Reference Bacteriology.

COLLECTION AND SHIPMENT OF SPECIMENS

Blood culture

For the isolation of *Brucella* only, the Laboratory will furnish a blood culture kit designed for this purpose. For routine blood cultures, however, commercial blood culture bottles are recommended. It is advisable that primary isolations from routine blood cultures be done in the local laboratory, and that only subcultures for additional study or confirmation be submitted to the State Hygienic Laboratory. Blood for *brucella* culture should be obtained when patient's temperature begins to rise, or during or just after a chill. In the collection of blood for cultures, observe the most rigid aseptic techniques, or contaminants may destroy the chance of isolating the causative or

COLLECTION OF ENTERIC SPECIMENS

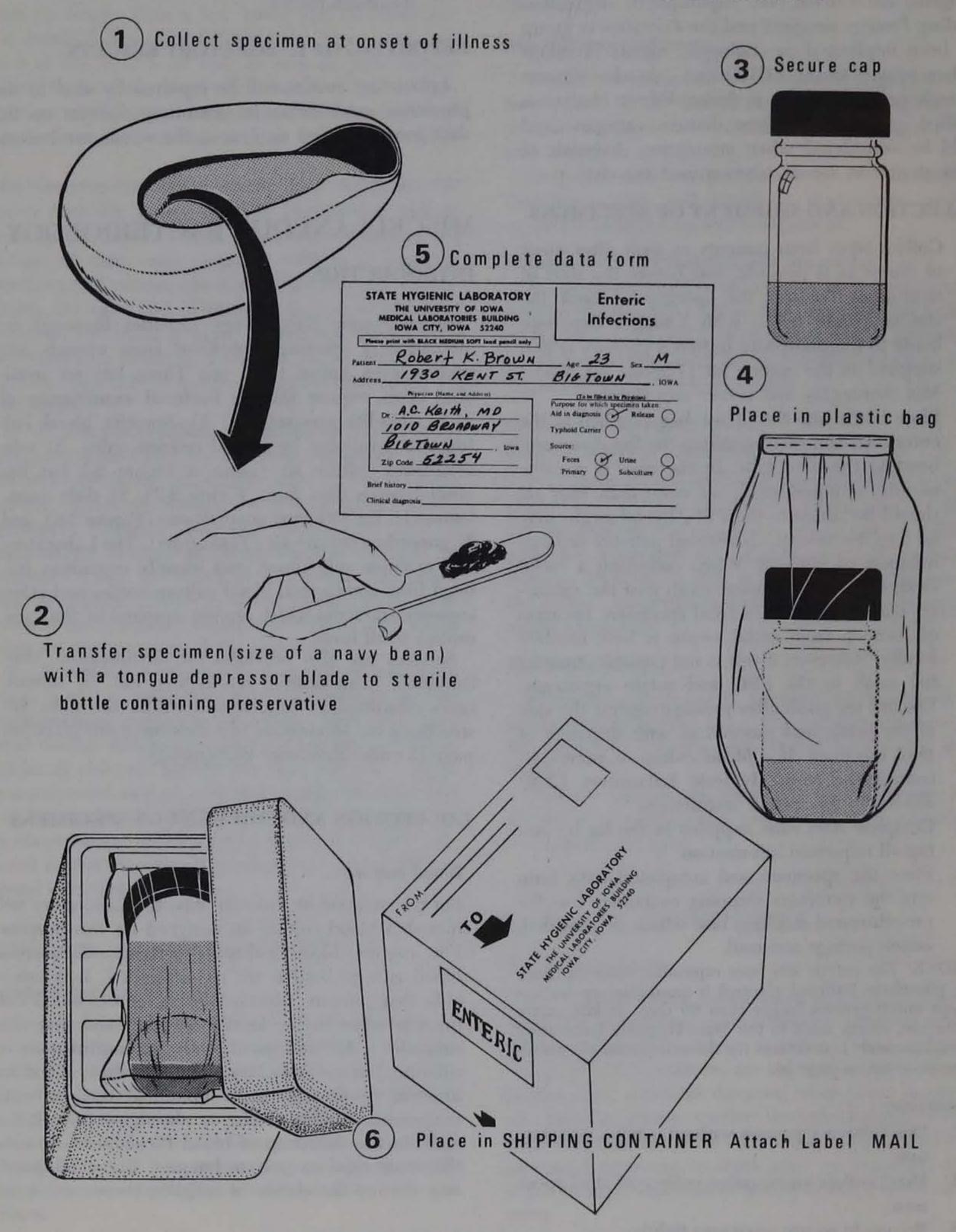


Figure 2-4 Procedure for collection of enteric specimen

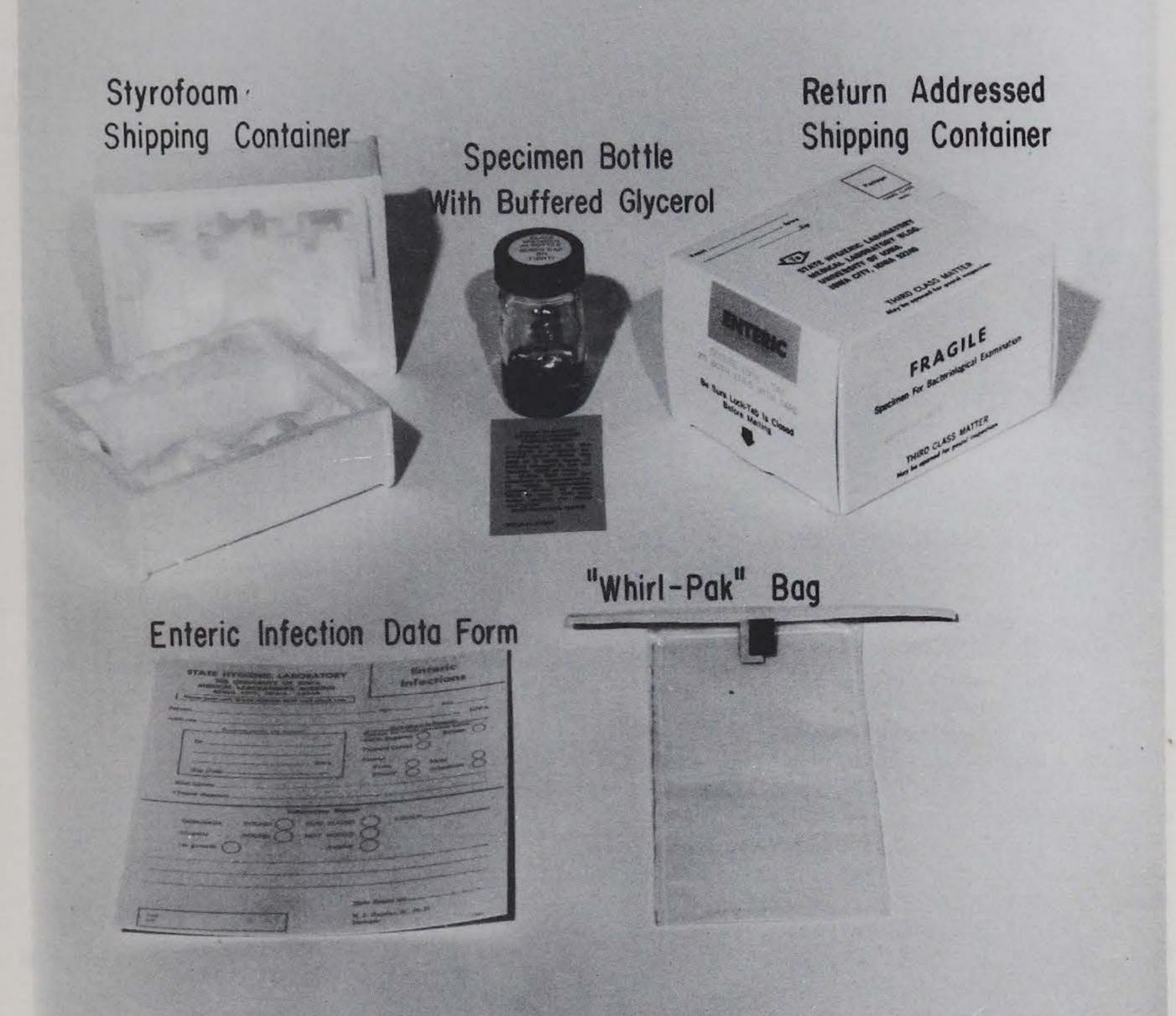


Figure 2-5 Enteric specimen collection kit

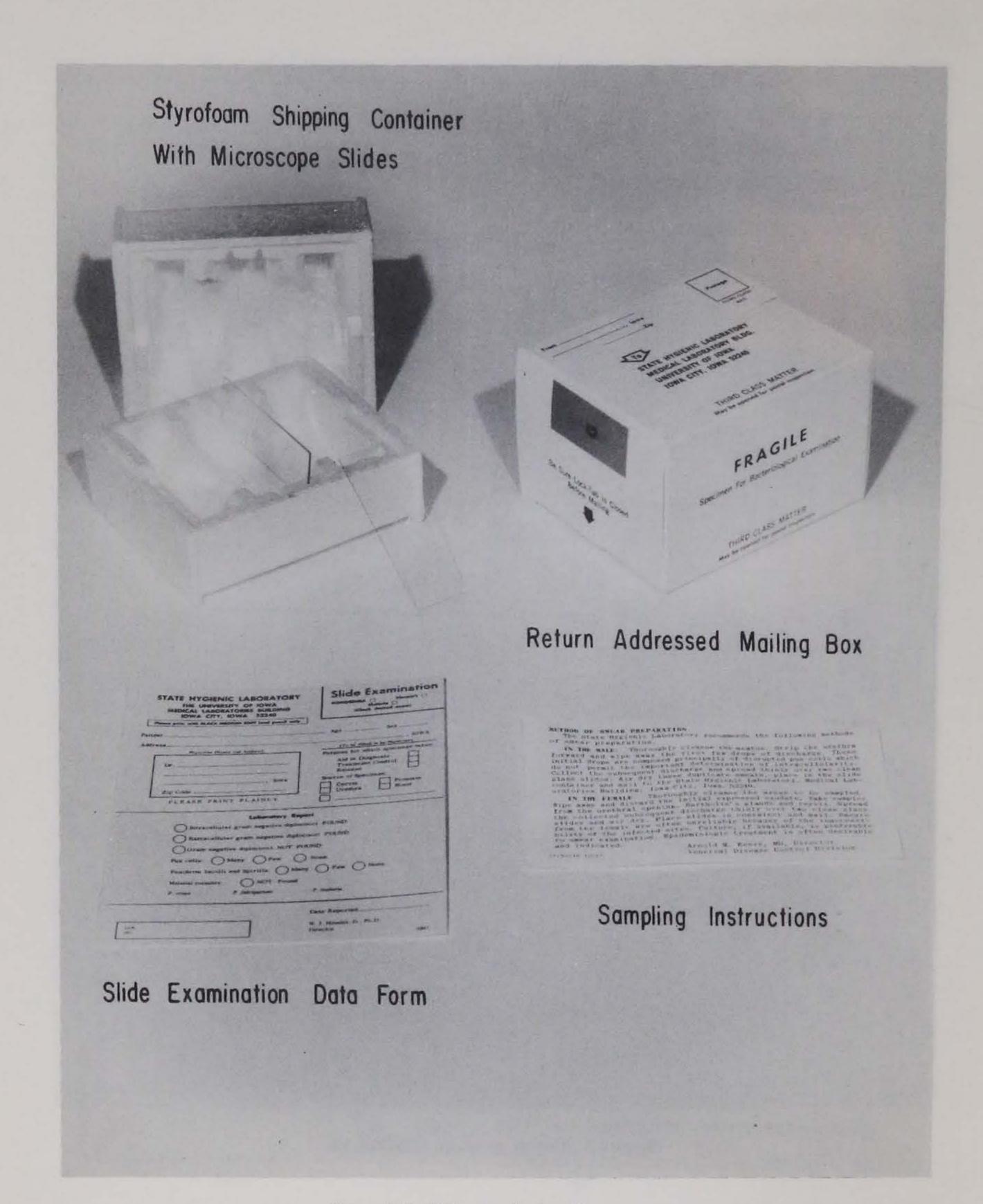


Figure 2-6 Slide examination kit

ganisms. After decontaminating the skin with tincture of iodine, followed by wiping with 70 percent alcohol. draw five ml of blood and inoculate into the broth.

Routine blood cultures should be collected aerobically and anaerobically into blood culture bottles and submitted to the Laboratory. These may be purchased commercially. Blood cultures should be taken before antibacterial treatment is given. If this is not possible, please inform the Laboratory of the drugs being used.

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Miscellaneous Examination |
|--|------------------------------|
| Please print with BLACK MEDIUM SOFT lead pencil only | |
| tient | Age Sex |
| dress | , iow/ |
| Physician (Name and Address) | |
| | Examination Desired; |
| Dr | |
| | O Primary Culture |
| , Iowa | Osubculture |
| Zip Code | Source of Specimen: |
| | |
| Laboratory Re | port |
| Laboratory Re | port |
| | port |
| | |
| | Date Reported |
| | |

Figure 2-7 Miscellaneous data form

Miscellaneous culture

Specimens from human sources for bacterial culture are widely varied and may yield almost any pathogenic organism. Since the primary culture is the most important single procedure in a bacteriological diagnosis, specimens should be cultured immediately, as many pathogenic organisms (e.g., Moraxella, etc.) are sensitive to various physical conditions. Cultures should be taken before antibacterial treatment is given. If this is not possible, the Laboratory should be informed of the antibacterial drugs being used. Specimens should neither be refrigerated nor allowed to dry out. (Since improper specimens received by the Laboratory due to delays in mailing may produce misleading reports, primary culturing in the local laboratory has many advantages.) Pure culture isolates thus obtained, can then be submitted to the State Hygienic Laboratory for identification, confirmation, typing, etc. However, if primary specimens are to be submitted to this laboratory, the services available and the collection methods are as follows:

- 1. Sputum specimens should be true sputum, not saliva. A nasopharyngeal specimen may frequently be substituted (when culturing from infants and small children). Insert, with a gentle rotary motion deep into both nares, a thin, flexible, sterile swab moistened in sterile saline or broth.
- 2. Skin cultures may be taken by thoroughly rubbing a sterile, moist (saline or broth) swab over the selected area.
- 3. Superficial lesions. Remove surface debris and pick up the exudate with a sterile swab. Moisten the swab with saline or broth first if the lesion is dry.
- 4. Draining sinus or fistula. Collect the specimen on a sterile swab inserted deep into the sinus, taking care not to touch the surrounding skin.
- 5. Wounds and abscesses. Swab specimens of abscesses should be taken from the outer edges of the infected area rather than from the middle of the thick purulent material. For wounds, remove any surface pus or scar and collect pus or exudate on a sterile swab from the deeper part of the wound. However, the most suitable materials from wounds and abscesses are tissues or fluid aspirated from the infected areas. Swab samples are less satisfactory, but, if used, at least three should be obtained.
- 6. Aspirated fluids. Prepare the skin as for a blood culture and collect the fluid with a sterile syringe and transfer the material to a sterile screw-capped tube (which can be centrifuged) for transport to the Laboratory. Spinal fluid should be kept warm during transit. Dripping the fluid directly onto chocolate agar plates may increase the isolation of fastidious organisms such as *Haemophilus*.
- 7. Conjunctival cultures. Pull the lower eyelid downward to expose the conjunctiva. While holding the upper lid to prevent blinking, rub a small sterile swab, moistened with broth, across the exposed conjunctiva.
- 8. Urines. Quantitative urine cultures are not performed at the State Hygienic Laboratory as overgrowth occurs and the counts will be invalid. These should be done at local laboratories.
- 9. Special examinations. Instructions for submission of material for special bacterial examination may be obtained by first contacting the director of the State Hygienic Laboratory. The Laboratory is prepared to render prompt consultation or assistance in any unusual outbreak of disease in the state.
- 10. Sterility testing of biologicals (vaccines. etc.) is available on a "cost for service" basis. Con-

tact the Office of the Director for additional information.

Slide and Culture Examination for the Diagnosis of Gonorrhea

Gonorrhea, unlike many other diseases, affects the male and female in totally different manners. This phenomenon is reflected in the type of specimen required for laboratory assistance in diagnosing this disease. The male exhibits, in most instances, a painful urethritis, and a slide with a film of urethral exudate is sufficient for diagnosis.

The State Hygienic Laboratory, in cooperation with the Iowa State Department of Health, has initiated a bacteriologic culture service to aid physicians in the detection and diagnosis of gonorrhea in females.

Because the gonorrhea organism is quite fragile and does not survive transportation in commonly used types of culture media, a special transport medium, Transgrow (TG), was developed. Transgrow is basically an improved Thayer-Martin medium "packaged" in high CO₂ environment, and is to be used for transport of suspected gonorrhea specimens to the Laboratory.

Thayer-Martin (TM) plates may also be utilized by physicians and clinics in the immediate Iowa City and Coralville area. These plate cultures must be brought to the Laboratory on the same day as the specimen is taken.

1. Specimen Collection in the Male-Urethral Smears. The urethral exudate in the symptomatic male is spread upon two slides, air dried (do not flame dry) and mailed to the laboratory in the "GC Slide Examination Kit" (Figure 2-6). The presence of intracellular, gram-negative diplococci in that smear is sufficient basis for a diagnosis of gonorrhea in the male. To obtain exudate for examination, retract the prepuce from the glans penis, and if it is unclean, wipe the meatus with sterile gauze. Digitally strip the penile urethra to obtain exudate on a swab. GENTLY ROLL THE SWAB ON BOTH SLIDES. Do not smear as trauma will disrupt the leucocytes thus rendering interpretation of the slide examination more difficult.

Urethral Cultures. If the male is presymptomatic, asymptomatic or a slide-negative epidemiological case, culture on specific gonococcal media (TM or TG) may be required. Culture may also be required if slide examination is inconclusive, a test of cure is required or if the case has legal implications. Specimens for culture are obtained by the judicious use of a sterile bacteriologic loop. The loop is inserted into the anterior urethra (one to 1½ inches), and the mucosa is gently scraped. The material obtained is immediately streaked on to the selective gonococcal me-

dium. The incubation and handling of the medium is described in detail in the section below entitled "Endocervical Cultures."

In male homosexuals, additional cultures should be obtained from the anal canal and/or the pharynx. Techniques for obtaining anal cultures are the same as described in the section below on anal cultures in the female except the male is in the standing, bent-over position.

2. Specimen Collection in the Female. To diagnose gonorrhea in the female, culture specimens should be obtained from the cervix and the anal canal (rectal culture). Specimens thus obtained are streaked upon appropriate gonococcal selective media. Cultures should be preincubated for 16-18 hours prior to shipment to the Laboratory. If Transgrow is used, bottles should be incubated in an upright position. Be sure to note this on the accompanying data slip as "preincubated." The survival of resultant growth during transport (mailing) is markedly better and will also decrease the time required for identification upon arrival to the laboratory. Gonorrheal cultures should be so shipped to minimize any delay in transit. Any delay might adversely affect the reliability of the bacteriological findings.

Test-of-cure cultures in the female should be handled in the same manner as described for diagnostic cultures.

Endocervical Cultures. The cervix is the best site to culture in the female. A speculum moistened with warm water (do not use any other lubricant) is used to expose the cervix. Cervical mucus is removed by using a cotton ball held in ring forceps. Insert a sterile cotton-tipped swab into the endocervical canal and move it from side to side allowing several seconds for absorption of organisms. Roll the swab from side to side over the surface of the medium. When Transgrow is used, keep the neck of the bottle in an elevated position to minimize CO2 loss. Remove the cap of the bottle only when ready to inoculate medium. Soak up all excess moisture in the bottle with specimen swab and then roll swab from side to side across medium, starting at the bottom of the bottle. The culture is then incubated for 16-18 hours at 35° C prior to shipment to the Laboratory. Be sure the cap is tightly closed on Transgrow bottles prior to mailing. If Thayer-Martin plates are used, roll swab directly on the surface of the medium in a large "Z" pattern to provide adequate exposure of swab to plate for transfer of organisms. The plates are then incubated in an inverted position in a candle jar until delivered to the Laboratory. A wide-mouth pickle or mayonnaise jar with any type of candle in it will provide the proper CO2 atmosphere for Thayer-Martin plates. Be sure to

light the candle and tighten the lid each time the jar

is opened to add or remove a plate.

Anal Cultures. The anal canal may harbor gono-cocci even though the patient may have negative endocervical cultures. For this reason, some authorities recommend an anal canal culture in addition to the endocervical culture in the asymptomatic female. The anal culture can usually be obtained after the cervical specimen without changing the position of the patient. Insert a sterile cotton-tipped swab approximately one inch into the anal canal. Move the swab from side to side to sample the crypts. Allow several seconds for absorption of organisms into the swab. If the swab is inadvertently pushed into feces, discard and sample again as the gonococcal medium cannot compensate for this degree of contamination. Inoculate the medium and handle as described above.

Urethral or Vaginal Cultures. In some instances the cervical technique may be unsatisfactory (e.g., molested children, hysterectomized females). In these instances, vaginal and/or urethral specimens should be

employed.

To obtain culture material from the female urethra, strip it toward the orifice to express any exudate present. Then use a sterile loop or sterile swab to collect the specimen and inoculate and incubate the medium as previously described. The vaginal culture would require the use of a speculum in order to sample the posterior vaginal vault. If the hymen is intact, the vaginal orifice is sampled with a sterile cotton-tipped swab. The medium is inoculated and incubated as described above.

3. Specimen Collection in Special Situations. Thayer-Martin culture is the method of choice in special situations such as suspected gonococcal conjunctivitis, arthritis or septicemia. The material is collected as one would culture from these sites normally and inoculated onto the specific selective gonococcal medium. In these extragenital special circumstances, a chocolate agar culture should also be employed as the selective medium might prove too inhibitory for the organism being sought.

Gram staining and fluorescent antibody staining of direct smears from conjunctivae, joint fluids or skin lesions can be used as an adjunct in the diagnosis of these manifestations of gonorrhea, particularly when partial therapy may prevent cultural recovery of organisms. However, it should be emphasized that fluorescent antibody conjugates are approved only as a confirmatory test for organisms grown on gonococcal selective media. They are not approved for stain-

ing of direct smears.

For any miscellaneous examination, it is important

that the laboratory request slip (Figure 2-7) be filled out carefully and completely, with the following information to accompany the specimen:

- 1. Name, age, sex and address of the patient
- 2. Examination desired
- 3. Type and source of specimen
- 4. Provisional diagnosis
- 5. Physician's or laboratory's name and address
- 6. Any further information that may be of value, such as clinical symptoms and antibiotics being given.

REPORTING OF LABORATORY RESULTS

As soon as all significant organisms have been isolated and antibiotic susceptibility tests have been performed on all the isolates, a copy of the antibiogram for each organism isolated is sent to the physician or laboratory, submitting the specimen (Figure 2-8). These reports are followed by a copy of the specimen report with the organisms isolated and listed as soon as the last organism is identified (Figure 2-9). Preliminary reports may occasionally be made on the finding of a particularly noted pathogen if all isolates have not been identified at that time, or if further tests are to be performed. Results are also occasionally telephoned immediately if the findings are considered especially urgent.

REFERENCE BACTERIOLOGY

INTRODUCTION

The Bacteriology Section has been reorganized with the creation of a separate unit for identification, confirmation, toxin testing, sero-typing, etc., of subcultures and other referred specimens, e.g., sera for Clostridium botulinum toxin. Together with this change, a new laboratory request form has been developed for the submission of these specimens (Figure 2-10). A separate sheet should be used for each isolate and filled out with as much information as is available. Starting with this information, we are able to determine which organism to examine in the case of a mixed culture, include special media necessary for certain organisms that may be suspected, assess the importance of various species of bacteria, and decrease the amount of work and time required to examine these cultures.

COLLECTION AND SHIPMENT OF SPECIMENS

Following are some recommended methods for submitting cultures:

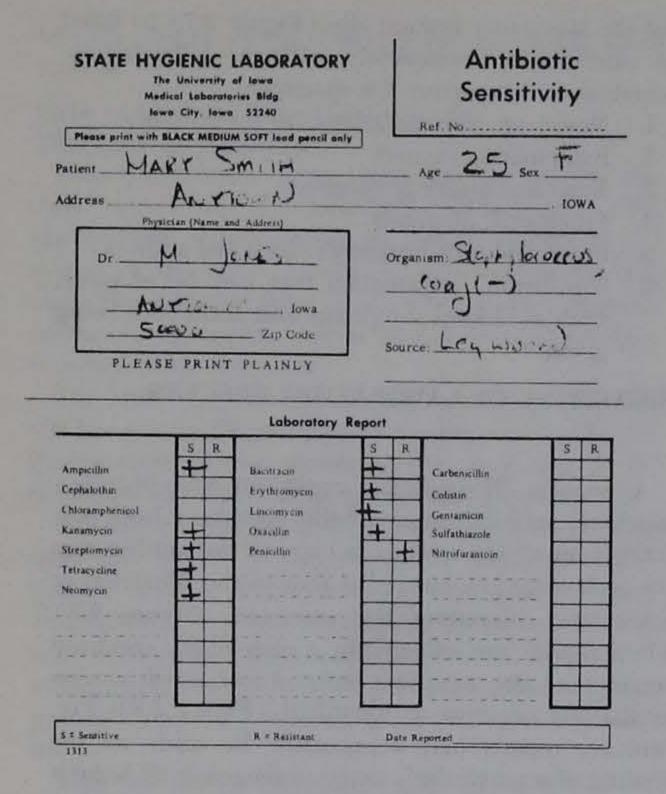


Figure 2-8 Antibiotic sensitivity report form

- 1. Only pure cultures should be sent, as many organisms are overgrown by contaminants during shipping. If it has been completely impossible to isolate an organism from a mixture, please specify this and note which organism is to be isolated so special selective techniques may be incorporated.
- Cultures submitted should be young, preferably 18-24 hours old or showing first sign of good growth. This will help ensure the viability of the culture as it is received by our laboratory.
- Enteric-like organisms may be shipped on any good plain infusion agar slants without added carbohydrate, e.g., heart infusion agar. Plates are not acceptable for shipping cultures.
- 4. Cultures for possible Neisseria gonorrhoeae or Neisseria meningitidis may be grown for 18-24 hours in tubes of chocolate agar, Thayer-Martin medium, or Transgrow bottles in a candle jar and sealed immediately upon removal to retain a CO₂ atmosphere. Shipping of an original Transgrow culture bottle after examination in the local laboratory is unsatisfactory because the suspected organisms may be overgrown by contaminants that had been temporarily suppressed or the organisms may be non-viable due to toxicity of the oxidase

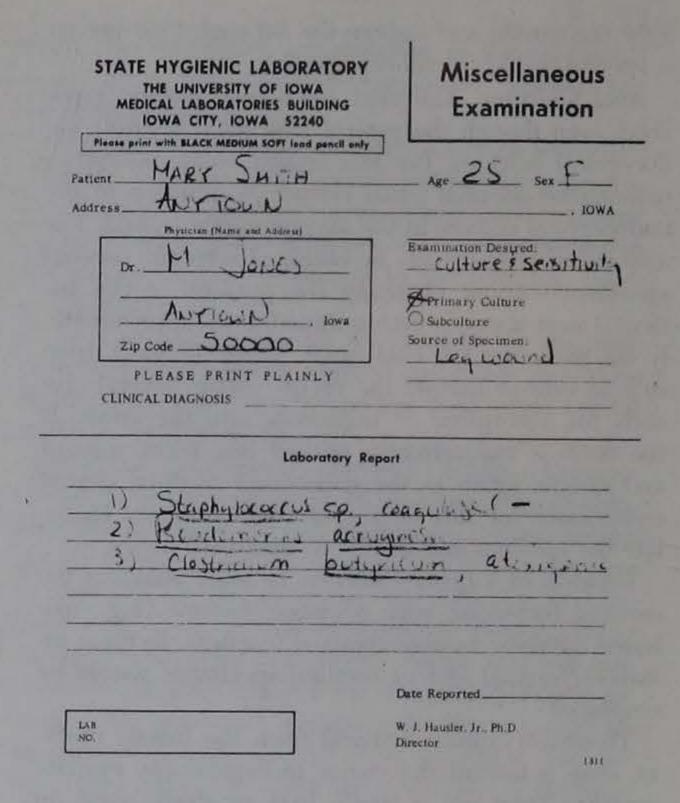


Figure 2-9 Miscellaneous examination report form

reagent or rapid death in relation to age of the culture. It is best to first transfer to a Thayer-Martin plate for isolation or directly transfer an isolated colony to a fresh Transgrow bottle for shipping.

- 5. Miscellaneous aerobic organisms are preferably shipped on a suitable slanted medium to obtain good growth without carbohydrates, or no more than 0.1% glucose if necessary for growth. However, a semi-solid transport medium (0.3-0.5% agar) such as Amies', Stuarts', Cary and Blair, or CTA without carbohydrate would also be satisfactory for transporting most organisms. For these a small vial (2 dram) is nearly filled with the medium and a swab of the culture inserted and cut off. Plates are not satisfactory for shipping cultures.
- 6. Anaerobes may be shipped in tubes of liquid or semi-solid (0.3-0.5% agar) carbohydrate-free media. Plates or slants are not acceptable. The medium should be freshly prepared and tubed 2-3 inches deep in screw-cap tubes. These should be boiled 10 minutes prior to inoculation to drive off dissolved oxygen. Some of the media suggested for use are plain chopped meat media (especially for

| NOTE: TYPE OR PRINT WITH BLACK FELT- | TIP PEN OR MEDIUM SOFT LEAD PENCIL ONLY | | | |
|--|---|--|--|--|
| STATE HYGIENIC LABORATORY | REFERENCE BACTERIA | | | |
| MEDICAL LABORATORY BLDG THE UNIVERSITY OF IOWA | EXAMINATION | | | |
| 10WA CITY, 10WA 52242 PH. 319/353-5990 | | | | |
| NAME AND ADDRESS OF | PATIENT IDENTIFICATION NAME: Last First Middle Initial | | | |
| PHYSICIAN OR ORGANIZATION: | NAME: Last First Middle Initial | | | |
| | ADDRESS: | | | |
| | BIRTHDATE: Mo Day Yr SEX: | | | |
| | CLINICAL DIAGNOSIS: | | | |
| HOSP. OR LAB. NO. | DATE OF ONSET: Mo Day Yr | | | |
| DATE SENT: Mo Day Yr | FATAL: Yes No | | | |
| LABORATORY EXAMINATION(S) REQUESTED: SPECIFIC AGENT SUSPECTED: MEDIUM SUBMITTED ON: | | | | |
| OTHER ORGANISMS FOUND: | | | | |
| DATE SPECIMEN TAKEN: ORIGIN: | ner (specify): | | | |
| Mo Day Yr Human Oth | ner (specify): | | | |
| SOURCE OF SPECIMEN (specify site): | | | | |
| TREATMENT: Drugs Used: None | IMMUNIZATIONS (Significant to Specimen): Mo Yr | | | |
| Date Degan. Date compretes. | | | | |
| MoYrMoYr | ASSOCIATED ILLNESS: | | | |
| | ASSUCTATED TELNESS, | | | |
| SYMPTOMS: | | | | |
| Community Illness Recent Travel (Location and Dates) | ingle Case Contact Carrier Epidemic | | | |
| Suspected Source of Infection (Note animal | and arthropod contacts). | | | |
| LIST BELOW OR ENCLOSE ALL AVAILABLE BIOCHEMICAL DATA (Include Primary Isolation Medium): | | | | |
| | | | | |
| | | | | |
| | | | | |
| SHL NUMBER: | DATE RECEIVED: | | | |
| LABORATORY REPORT: | | | | |
| | | | | |
| | | | | |
| W. J. Hausler Jr. PhD | DATE REPORTED: | | | |

Figure 2-10 Reference Bacteriology examination request form

Clostridia), thioglycollate, or CTA medium base. Prior to shipment, a %- to 1-inch overlay of melted 5% agar or paraffin should be added to actively growing cultures in either liquid or semi-solid media. Also anaerobic tubes or bottles into which liquid cultures can be injected via the rubber stopper are commercially available (from Robbins Division of Scott Laboratories and Hyland Laboratories). A second, less desirable commercial system consists of two "gassed-out" (O₂-free CO₂) tubes. One contains a swab and the other contains semi-solid modified Cary and Blair transport medium. The swab of the culture is inserted as rapidly as possible into the second tube while the latter is held in an upright position.

Some fastidious anaerobes can be shipped in a commercial blood culture medium such as the Vacutainer blood culture tubes. However, these media contain carbohydrates and should not be

used for gas producing organisms.
7. Shipping instructions. The screw-caps should be tightened and sealed with waterproof tape and each tube or vial (primary container) should be labeled with the patient's name and laboratory number (on the back of slanted media to avoid obstructing view of the growth).

The State Hygienic Laboratory does not supply mailing containers for the shipment to our laboratory of bacterial subcultures. Some laboratories are using the styrofoam mailer supplied by the Laboratory for other services (such as throat culture swabs) for the shipment of subcultures without the use of a secondary inner container. Furthermore, these cultures are often submitted in liquid media that leak or on media containing carbohydrates by which the organisms produce gas and force material from the tube into the mailer. These practices pose a serious threat of accidental infection to laboratory workers and mail handlers, and must be discontinued.

We list below material taken from Section 72.25 of Part 72, Title 42, of the Code of Federal Regulations relating to packaging and other requirements applicable to the transportation of etiologic agents.

A. Definitions

An "etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease.

B. Transportation of subcultures

No person may knowingly transport or cause to be transported, directly or indirectly, a subculture containing, or reasonably believed by such person to contain, one or more etiologic agents unless it is packaged and shipped in accordance with the following requirements:

1. Volume less than 50 ml.

Material shall be placed in a securely closed, watertight container (primary container) (test tube, vial, etc.) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength (Figure 2-11).

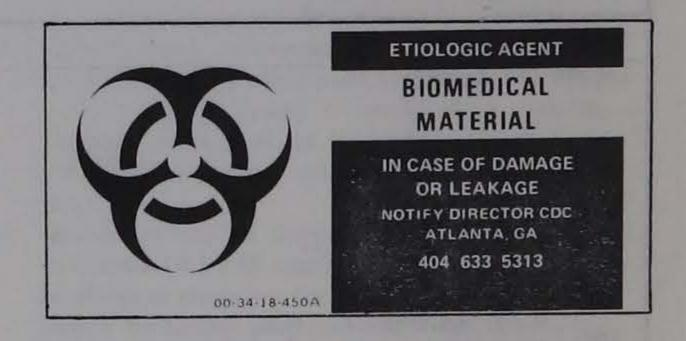
2. Request forms

The form(s) for reference bacteria examination should be enclosed between the secondary container and the outer shipping container.

3. Labels

The label for etiologic agents/biomedical material must be shown (red on white) on the outer shipping container (Figure 2-12).

Figure 2-11 Label for etiologic agents/biomedical material



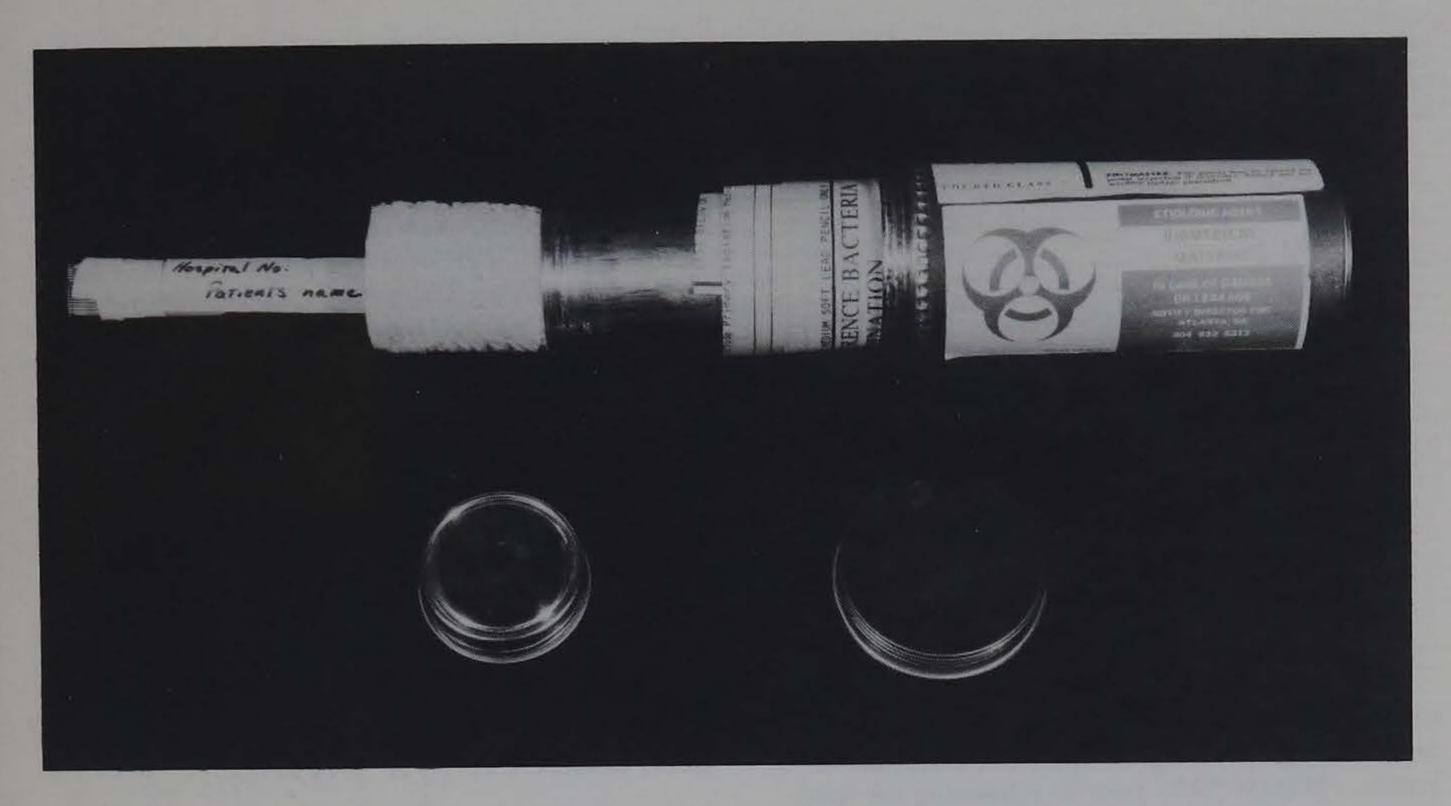


Figure 2-12 Shipping container for reference bacteria specimens

The styrofoam tube mailers used by the State Hygienic Laboratory are not to be considered a secondary container, and any shipper using these mailers for subcultures bears the responsibility of seeing that a proper secondary container is used and for ensuring that the contents are properly packaged and labeled to comply with the Code of Federal Regulations relating to the transportation of etiologic agents. Scientific Products and Cole-Parmer have available complete shipping containers including a metal or plastic container within an outer fiberboard container meeting U.S. postal regulations. Also Shamrock, Inc., P.O. Box 143, Bellwood, Illinois 60104 has the proper label for putting on the outer mailing container designating it as biomedical material/etiologic agents.

Since laboratory findings are adversely affected by delay in transit on weekends and holidays, it is best that whenever possible the sender hold the specimens and maintain them until they can be shipped to arrive during laboratory hours on work days. Also to avoid delay, designate Reference Bacteriology Unit in the mailing address.

REPORTING OF LABORATORY RESULTS

Reports of subcultures will be accompanied by a profile of the organism's characteristics as determined by the State Hygienic Laboratory. This profile can be used by the referring laboratory as a quality control

check on their procedures, reagents and media. If more than one organism is found, the organism most closely resembling the suspected agent will be identified. Occasionally more than one organism will be identified, such as in a mixture of two anaerobes, but if the culture is heavily mixed, the laboratory will be phoned to see which organism is to be identified if no specific agent or type of bacteria is specified. Also, occasionally a preliminary report is made if significant information has been obtained but further tests are yet to be done or if the culture has to be forwarded to the Center for Disease Control, Atlanta, Georgia, for further study. Reports of antibiotic susceptibility tests are not made except on special request.

FOOD SPECIMENS

INTRODUCTION

Laboratory analysis of food specimens is done to complete the investigation of food-borne disease outbreaks. The food specimen is examined for the bacteria or bacterial-formed toxin which caused the illness or death. Follow-up on the causative agent may lead to more rigid control of the processed food or feed during its preparation and prior to human or animal consumption. Examination for the following agents may be performed by the State Hygienic Laboratory:

Food Infection (bacterial)

Shigella sp.

Salmonella sp.

Brucella sp.

Clostridium perfringens

Corynebacterium diphtheriae

Bacillus cereus

Streptococcus faecalis

Streptococcus group A

Mycobacterium tuberculosis

Enteropathogenic E. coli

Vibrio parahaemolyticus

Food Infections (viruses)

Food Intoxications (bacterial)

Clostridial toxin

Staphylococcal toxin

Laboratory analysis of food specimens requires a certain amount of preparatory work. Therefore, it is requested that you contact the State Hygienic Laboratory (319/353-5990) prior to submitting the specimens. You will be informed of any special precautions to be taken concerning your specific situation.

COLLECTION AND SHIPMENT OF SPECIMENS

- If possible collect 100 gms (five or six oz) of food. Avoid contamination of specimen during collection. If the suspect food is commercially packaged, attempts should be made to obtain unopened packages.
- Frozen foods should remain frozen for transport to the Laboratory. Other perishable foods, not frozen, should be refrigerated and kept cold in transit to the Laboratory
- Do not take food samples from their original container and place in an unsterile container for transport to the Laboratory. Use only containers known to be sterile.
- Food samples showing any decomposition or other evidence of mishandling subsequent to collection cannot be accepted for microbiological analysis by the Laboratory.
- Sample identification should include the following:
 - a) Place and time of collection.
 - b) Method of collection if indicated.
 - c) Reason for submission.
 - d) Other pertinent information.
- The sample must be sealed in its container in such a manner that the container cannot be opened without breaking the seal. The name of

the person who collected and sealed the sample should be written on the seal along with the date and time of sealing.

7. A cover letter including sample history and other pertinent information should accompany the sample. Include the following:

a) Number of people affected.

- b) Elapsed time between ingestion of the food and the onset of symptoms.
- c) Symptoms observed or reported.
- d) Reasons for suspecting the food.

REPORTING OF LABORATORY RESULTS

The laboratory findings will be reported by telephone followed by a letter as soon as they are known. Reports will be sent to the physician or veterinarian who submitted the specimens.

STAPHYLOCOCCAL BACTERIOPHAGE TYPING

INTRODUCTION

Bacteriophage typing is used as an epidemiologic tool to determine the source, avenue of spread and relative severity of a specific infectious disease problem. Staphylococcal phage typing may be used to assist the infectious disease committees of local hospitals to ascertain the source(s) of hospital staphylococcal outbreaks. Phage typing is also employed to determine the chain of events culminating in a food poisoning episode. If the same phage type is found in the patient, the suspect food and the food handler, then the avenue of the food poisoning incident is known, and steps can be taken to prevent recurrences.

. It should be emphasized that the Laboratory provides this service only on cluster specimens from bona fide outbreaks. Phage typing on single specimens are of no value to the physician or hospital and do not justify the time, competence and funds expended by the Laboratory.

COLLECTION AND SHIPMENT OF SPECIMENS

It is recommended that the hospital laboratory subculture all coagulase positive staphylococci isolated and store them in the refrigerator (or at room temperature) for 4-6 weeks. If no outbreaks have occurred, the subcultures may be discarded. If, however, something suspicious does occur, then the appropriate subcultures may be submitted to the Laboratory for phage typing. Specimens for staphylococcal phage typing should be shipped in an appropriate container (see section on shipment of bacterial subcultures, page 14) with the proper data slip completed (Figure 2-13). Only coagulase-positive cultures should be submitted for

REPORTING OF LABORATORY RESULTS

The phage type will be reported on the appropriate data slip as illustrated below (Figure 2-13). The phage type in this particular instance was a 52/80/81 at routine test dilution.

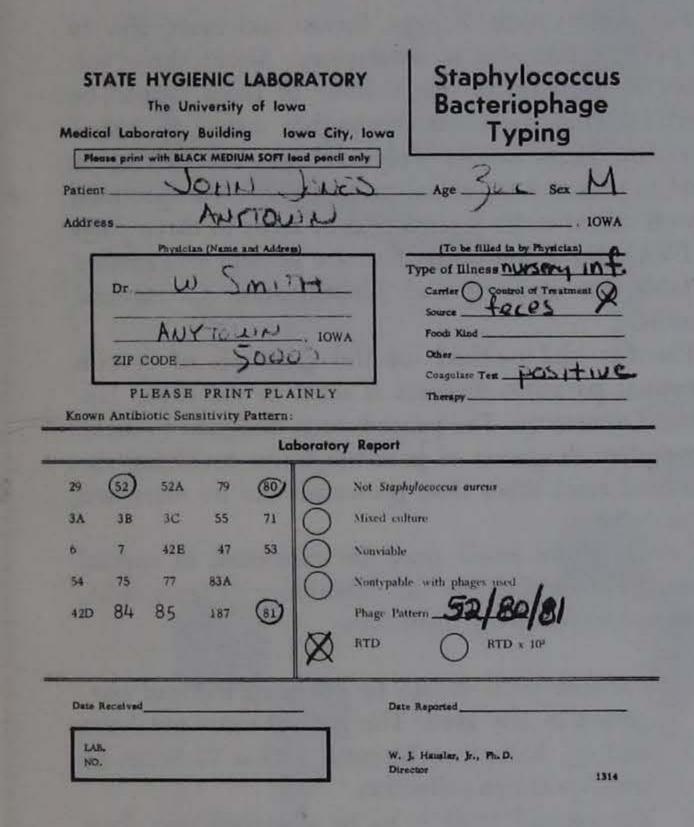


Figure 2-13 Bacteriophage typing report form

INTRODUCTION

Accurate clinical diagnosis of intestinal parasite infections is difficult, and laboratory confirmation is usually necessary. Demonstration of the diagnostic stage or stages by direct examination of specimens is the most reliable method of establishing a diagnosis for the majority of parasitic infections, although indirect (serologic) methods are available in a few cases such as trichinosis, echinococcosis, chronic schistosomiasis, or extraintestinal amebiasis, where the organism is not readily demonstrated.

This unit is concerned with the direct laboratory procedures used in recovery and identification of intestinal and blood parasites. Competent direct examinations are dependent on several factors such as: 1) personnel trained in the examination of specimens and the accurate identification of organisms, 2) adequate laboratory facilities, including a good microscope and 3) satisfactory specimens for examination. It is understood that any laboratory procedure employed for the direct demonstration of parasites is only as reliable as the microscopist who examines the specimen.

COLLECTION AND SHIPMENT OF SPECIMENS

The importance of properly collected specimens for diagnosis cannot be overemphasized. Inadequate, old or improperly preserved samples are usually of little or no value in establishing a diagnosis and may lead to erroneous conclusions. Feces is the most common type of material submitted for parasitologic examination, but other body exudates, such as sputum and urine, may be utilized in certain cases. Sera or fluids may be obtained for immunologic diagnosis.

Collection of Fecal Specimens

Fecal specimens should be collected in clean containers or on clean paper and transferred to the specimen bottles supplied in the P & O specimen kit provided by the Laboratory (Figure 3-1). Feces deposited on the soil do not provide a satisfactory specimen since free-living larvae and other contaminants from the soil cause confusion in diagnosis. Feces obtained from toilet bowls are unsatisfactory because there is

danger of contamination by organisms, and water will destroy trophozoites.

Specimens should not be allowed to freeze since freezing and thawing may destroy protozoan cysts and trophozoites. Since protozoan trophozoites do not multiply or encyst outside of the body, they are destined to die and degenerate unless properly preserved. Adequate and proper preservation of the fecal sample is essential.

For preservation of eggs, larvae and cysts, five to 10 percent formalin is satisfactory. About three volumes of formalin to one volume of feces should be used and the specimen thoroughly mixed to insure complete preservation. Polyvinyl alcohol (PVA) is used to preserve intestinal protozoa. After a specimen is well mixed in PVA (one part of feces to three parts of PVA), the trophozoites that are present will remain suitable for staining and identification for several months.

The two-vial method for the diagnosis of certain intestinal parasitic diseases is used in the State Hygienic Laboratory. The procedure is designed to make a complete diagnosis of parasitic forms involving the intestinal tract other than pinworms (to be discussed separately).

Two separate stools must be submitted in special containers furnished by this laboratory (Figure 3-1). Observe the following directions:

- a. The first stool should be obtained without purgation of any kind. The patient must not have had oil, barium or bismuth within 72 hours of stool specimen collection.
- b. The second stool is to be obtained two days after the first.

Portions of each stool (a and b) are to be examined by both the formalin and PVA (polyvinyl alcohol) technics. The directions given below must be followed exactly in obtaining and preparing these specimens.

The vial labeled formalin solution is designed primarily to preserve hardy parasitic forms such as ova, cysts, larvae, etc. Add one part stool to nine parts formalin solution, screw cap on tightly and mix thoroughly.

The vial labeled PVA (polyvinyl alcohol) is designed to preserve the motile forms of amoebae, ciliates and flagellates which normally disintegrate shortly after leaving the body. When the stool is diarrheal, add

^{*}Most of the instructions provided in this section have been taken from various manuals published by the Center for Disease Control, DHEW, Atlanta.



Figure 3-1 Parasites and ova specimen collection kit

equal parts of stool and PVA, screw cap on tightly and mix thoroughly. If the stool is formed or mushy, add one part stool to three parts PVA and mix thoroughly. Stool must be free of urine.

It is imperative that the stool when passed be *immediately* placed in the solutions in the *ratios* mentioned above. Failure to adhere to these directions may lead to unsatisfactory end results.

The administration of antibiotics or other antiparasitic drugs *seriously interferes* with the demonstration of amoebae, ciliates, flagellates and other parasitic forms.

Use one applicator for adding stool to formalin and one for PVA. Be sure there is no cross mixture. Mix each thoroughly.

Containers are distributed for immediate use only due to the perishable nature of PVA. Pack the two vials in the styrofoam shipping container, enclose the data form with the requested information and send to the State Hygienic Laboratory in the pre-addressed chipboard mailing box.

Adult worms or proglottids of tapeworms frequently are passed with or without fecal material. These can be picked out of feces and placed in a vial of 70 percent alcohol or 10 percent formalin.

Collection of Multiple Specimens With and Without Catharsis

Because of the intermittent passing of certain parasites from the host and the limitations of diagnostic techniques available, the possibility of finding organisms is increased by the examination of multiple specimens. In general, nematode species such as Ascaris, hookworm and Trichuris shed eggs more or less constantly and may be detected daily in feces. However, other parasite species, especially the protozoa, are passed irregularly. The production of eggs in certain of the helminth infections is also irregular, particularly with the schistosomes and Diphyllobothrium latum. Proglottids of Taenia spp. may be passed at intervals also. From these observations, it would appear preferable to distribute the collection of specimens over several days, or perhaps a few weeks, rather than to obtain them on successive days.

Collection of Specimens Other than Feces

Certain of the intestinal parasites pass diagnostic stages in material other than feces. Sputum specimens should be collected in suspected cases of paragonimiasis. Paragonimus eggs are commonly found in sputum. In about 40 percent of the cases they may be present in the stools, and, for this reason, fecal speci-

mens as well as sputum specimens should be examined. Pulmonary amebiasis and echinococcosis may also be detected by examination of sputum specimens. In cases of amebiasis, sputum should be examined immediately for trophozoites or else preserved in PVA-fixative for subsequent staining. Sputum for diagnosis of paragonimiasis or echinococcosis may be preserved in 10 percent formalin if delayed examination is necessary.

Urine specimens are utilized in the diagnosis of Trichomonas vaginalis and Schistosoma haematobium infections. Recently, it has been shown that the optimum urine specimen for revealing eggs of S. haematobium is the one passed about, or shortly after, noon. The specimen for S. haematobium can be preserved in five or 10 percent formalin.

In examinations for *Trichomonas vaginalis*, fresh urine specimens, preferably the first portion of voided urine, should be used.

Anal swabs are the usual means of detecting Enterobius vermicularis infections since the female worm ordinarily deposits the eggs in the perianal region rather than within the intestinal tract. Two types of preparations are commonly in use: the cellulose tape slide preparation and the vaseline-paraffin swab. Because of the peculiar migratory habits of the female, these swabs are best collected either between the hours of 9:00 p.m. and midnight or early in the morning before defecation or bathing. Since migration of the females may not occur every day, a single examination will miss a significant percentage of the pinworm infections and repeated collections should be made.

A cellulose tape slide kit is available from the State Hygienic Laboratory for the diagnosis of pinworm infections (Figure 3-2). The kit consists of a microscope slide covered with cellophane tape, gummed edge down, a tongue depressor, illustrated instructions, the appropriate data slip, a heavy cardboard mailer and an envelope. To collect the specimen, hold the slide against the tongue depressor one inch from the end and lift the long portion of the tape from the slide. Loop the tape over the end of the depressor to expose gummed surface. Hold the tape and slide against the tongue depressor. Press gummed surfaces against several areas of the perianal region. Replace tape onto the slide and smooth with cotton or gauze.

In infections with Strongyloides stercoralis and Giardia lamblia, duodenal drainage often reveals organisms when the stool specimens are negative and should be collected in suspected cases when diagnosis cannot be established by fecal examinations. The specimen should be placed in five or 10 percent formalin for preservation.



Figure 3-2 Pinworm specimen collection kit

Collecting and Handling Blood Specimens for Parasitic Serologic Tests

Hemolysed, chylous or bacterially contaminated specimens cannot be satisfactorily examined; for this reason the following precautions should be exercised in securing and shipping specimens:

- 1. At least five ml of perfectly clear serum should be submitted. This will require that about 10 ml of whole blood be drawn. (Serum specimens are preferred. When this is impossible, suitable precautions against hemolysis and bacterial contamination of whole blood specimens should be taken. Whole blood should be shipped so as not to arrive on a weekend.)
- Blood should be drawn before breakfast or at least three hours after the last meal in order to avoid chylous specimens.
- Blood should be drawn before or at least 24 hours after the application of intradermal tests.
- All apparatus for collecting specimens should be washed free of alkali and acid before sterilization. Only sterile dry apparatus should be used throughout.
- A dry needle and syringe should be used to draw the blood.
- 6. The blood should be allowed to clot at room temperature (one to two hours), then placed in the refrigerator to retract the clot. Serum should be drawn off the clot with a sterile pipette and rendered perfectly free of red cells by centrifugation in a sterile dry tube.
- 7. 0.02 ml of one percent aqueous solution of borated merthiolate per ml of serum may be added to prevent bacterial growth. Indicate on the tube label if merthiolate has been added. (1 gm powdered Merthiolate Lilly, 1.4 gm borax, 100 ml distilled water. Make fresh solution every 30 days. Store in refrigerator.)
- 8. Serum should not be heated (inactivated).
- 9. Tubes containing serum for shipment should be properly sealed, either with rubber stoppers or screw tops, to prevent leakage, should be properly labeled for identification, and should be carefully wrapped to prevent breakage.
- 10. A copy of the covering letter or report form should always be enclosed with each serum specimen. When possible, a brief statement of the patient's illness should be included.

REPORTING OF LABORATORY RESULTS

The laboratory results will be reported on the Para-

| STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Examination for Parasites and Ova |
|--|--|
| Please print with BLACK MEDIUM SOFT lead pancil only MARY SMITH Address ANTICALLY Physician (Name and Address) Dr. F. Jones ANTICAL J. Jowa Zip Code SGOOD | Age 19 Sex F , 10WA (To be filled in by Physician) Purpose for which specimen taken: Aid in Diagnosis Release O Source: Feces O Other |
| PLEASE PRINT PLAINLY Laboratory Re Parasites Found | Specimen: 1st 2nd 0 |
| Entance ba con | <u>li</u> |
| Date Received | Date Reported |
| LAR, NO, | W. J. Hausler, Jr., Ph.D. Director 1312 |

Figure 3-3 Parasite and ova report form

sites and Ova data slip as illustrated (Figure 3-3). Both pathogens and nonpathogens will be reported.

BLOOD PARASITES

INTRODUCTION

Many of the organisms included in the category of "blood parasites" commonly inhabit tissues other than blood. Even those parasites which are present in the blood may be demonstrable only in certain phases of their development or at certain stages of the disease

produced. For these reasons, the techniques used to demonstrate this group of blood parasites are varied and in many cases must be adapted to the specific parasite concerned.

Direct procedures which actually reveal the parasite are more widely used than the indirect or immunodiagnostic procedures for most of the blood parasites. In certain cases, however, an immunodiagnostic procedure is the most practical and reliable method.

It is necessary for the technologist or diagnostician to be familiar with the biology and life cycle of the organisms, their location in the host and the type of specimen to be collected as well as the techniques of handling and examination of the material.

COLLECTION AND SHIPMENT OF SPECIMENS

Direct methods of diagnosis depend on demonstration of the parasite or its diagnostic stage in some type of body material. Therefore, the collection and handling of the proper types of specimens for identification of blood parasites is of primary importance since inadequate or poor samples may lead to erroneous conclusions. Not all of the organisms grouped in the category of blood parasites are diagnosed from blood specimens, however; peritoneal fluid, spinal fluid, and aspirates and biopsies of various organs and tissues are used in many cases. The particular specimen to be obtained depends on the location of the parasite or its diagnostic stage within the body. Two types of blood samples are collected for diagnosis of those parasites whose diagnostic stages are found in peripheral blood: dried blood films for staining and whole blood samples. This applies primarily to diagnosis of malaria, trypanosomiasis and filariasis, with the exception of onchocerciasis.

Blood Films

Stained blood films are the most common preparations used for diagnosis and usually are prepared from either finger pricks or ear puncture, although blood obtained by venipuncture is satisfactory if the smear is made immediately after collection. It should be noted, however, that citrated, oxalated or heparinized blood will frequently not adhere to the slide and may interfere with staining.

Blood films may be either "thin films" with the blood spread over an area of the slide in a thin layer, or "thick films" with the blood concentrated in a relatively small area. For routine diagnosis, the thick film is preferred since it permits a fairly rapid examination of a large amount of blood and often reveals light infections missed by the thin film method. However,

parasite morphology may be more distinct and typical in thin films. For this reason, a thick and thin film combination on the same slide is the recommended preparation.

The area to be pricked should be thoroughly cleaned with gauze soaked in 70 percent alcohol. Cotton pads should not be used since they often leave fibers which are confusing in a stained film. Alcohol will "fix" the blood and interfere with dehemoglobination of thick films, so the cleaned area should be wiped dry or allowed to air dry before being punctured. It is essential that the puncture be deep enough to give sufficient blood for preparation of satisfactory films. A rather large, single drop or several smaller drops will be necessary for a good thick film. Disposable blood lancets have been recommended in place of reusable ones. These sterile, individually packaged lancets are available at low cost and provide a safe and satisfactory puncture for preparation of one or two combination slides.

Correctly prepared blood films are essential for accurate and reliable diagnosis and proper preparation cannot be overemphasized.

The thin blood film is prepared in the usual manner by placing a small drop of blood near one end of the slide and smearing it with a second slide held at an angle. For diagnosis of blood parasites, this film should be of a single red cell layer around the edges and at the terminal end. If the preparation is thicker than this, parasite and cellular morphology may be obscured. Ideally, the thin film should cover about two-thirds of the slide and end in a rounded edge.

The thick film should be located at one end of the slide and should be about the size of a dime and of such a density that small newsprint can just be read through the center portion when the blood is wet. The drop of blood used is about twice or perhaps three times the size of the drop used for thin films. The thick smear should contain from 10 to 20 times as much blood as the thin film. An average of 10 leucocytes per oil immersion field has been suggested as an ideal density. A well-prepared thick film should be several erythrocyte layers thick in the center and somewhat thinner, preferably a single layer, around the outer edges. The thick film may be prepared in either of two ways: 1) touch the undersurface of the slide to a fresh large drop of blood on the finger, taking care not to touch the skin, and rotate the slide while in contact with the blood to form a film of the appropriate size and density; 2) several drops may be placed closely together on the slide and "puddled" with the corner of a slide, an applicator stick, toothpick or needle to form the thick film. Sometimes there is distortion of the white blood cells and parasites in such "puddled" films and the first or "touch" method

of preparation is preferred.

The blood films should be allowed to air dry in a horizontal position or on a flat surface to insure an even distribution of blood. If tilted, the blood will collect along one edge and subsequently peel or flake off. A fan may be used to hasten drying, but excessive heat should be avoided since the blood will be heatfixed which will interfere with the staining process. The slides should be protected from dust, debris and insects, particularly flies and cockroaches, while drying. This can be accomplished by covering them with a petri dish, or placing them upside down in a slide box, taking care to keep the box vertical so the films will dry evenly. Thick films dried in this upside-down position will generally have the thicker center and thinner edges desirable in a good preparation.

Thin films will dry quickly, but thick films require eight to 12 hours or overnight to dry thoroughly. Thick smears stained before this time may not adhere to the slide or may exhibit a meshlike, fibrinous background and lack the sharpness and clarity of a well dried film.

Blood films should be labeled completely and distinctly as with other types of specimens. If a thin film is present, the identifying information can be written in the thicker portion of the thin smear with an ordinary lead pencil. If only a thick film is present, the information should be written on the end of the slide with a glass marking pencil or a tape or paper label can be used. No writing should be done in the thick film as the entire area should be left free for examination.

Time of Collection

The hour or time of day may be important in collection of specimens for examination for blood parasites, especially in malaria or certain filaria infections. It is suggested that the optimum time for taking blood smears for diagnosis of malaria is about midway between the chills when the developing parasites will be more easily identified.

Collection of Body Fluids, Aspirates and Biopsies

In visceral leishmaniasis (Leishmania donovani in-

fections), the most common method of demonstration of the organism is in bone marrow obtained by puncture of the sternum or iliac crest. Occasionally, biopsy of liver, spleen or lymph nodes may be employed.

In some cases of onchocerciasis, tissue biopsies from shoulders, cheeks, calves, thighs, etc., as well as the eyes, may reveal microfilariae when examination of skin from nodules is negative. Time of collection in these instances is not especially critical.

Shipping of Specimens

Blood or tissue impression smears should be thoroughly dried before being packed for shipping. Dried films may be placed in slide boxes or wrapped in small packages. If slide boxes are used, tissue should be placed between and over the slides to prevent their being jarred or broken. Toilet tissue makes a satisfactory and convenient packing material for this purpose. Cardboard slide holders for one or two slides may be used.

Whole blood specimens or tissues should be put in tubes or vials fitted with screw-caps or tight-fitting cork stoppers, and wrapped in several layers of packing material. Identifying information should be included.

Storage of Specimens

Blood specimens obtained for demonstration of parasites, whether dried films or whole blood, should be examined as soon as possible after collection. Blood films lose their affinity for stain within a few days and should be stained within two to three days after collection. Thick films, especially, may become partially fixed by age or heat, and will not dehemoglobinize properly.

REPORTING OF LABORATORY RESULTS

The blood smears for malaria are reported on the slide examination data slips as shown in Figure 3-4. The malaria Indirect Fluorescent Antibody examinations and other blood parasite results are reported on miscellaneous examination slips as shown in Figure 3-5.

| STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Slide Examination GONORRHEA Vincent's Malaria (Check desired exam) | STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Miscellaneous Examination |
|--|---|--|--|
| Please print with BLACK MEDIUM SOFT lead pencil only DHW JONES ANYTOUN IN J. SMITH ANYTOUN LINE SOODS PLEASE PRINT PLAINLY | Age 23 Sex M (To be filled in by Physician) Purpose for which specimen taken: Aid in Diagnosis Treatment Control Release Source of Specimen: Cervix Urethra Prostate Blood | Please print with BLACK MEDIUM SOFT lead pencil only Patient UIII AM DHRION Address ANTOWN Physician (Name and Address) Dr. T. GREEL IOWA Zip Code SUCCIONAL IOWA PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS | Age 35 Sex M Reamination Desired: O Primary Culture O Subculture Source of Specimen: |
| Possesti Many O Lew O None Clinia attidategrain negative diplococci FOUND Clinia italia grain negative diplococci FOUND Clinia negative negative diplococci FOUND Clinia negative | NOT FOUND O | Laboratory Report Friend: 11.1.5 Fried: 1 | |
| TAN . | Date Reported | LAB NO. | Date Reported |

Figure 3-4 Positive slide report for malaria

1 The State of the

ALL DE LA PRINCIPAL DE LA PRIN

Figure 3-5 Positive IFA report for malaria

. IOWA

INTRODUCTION

The State Hygienic Laboratory provides diagnostic and reference services as well as drug susceptibility tests for specimens submitted for mycobacteria examination. Physicians having questions regarding the clinical management of patients with mycobacteriosis are urged to consult Dr. John E. Kasik, Oakdale Hospital, 319/353-3526.

COLLECTION AND SHIPMENT OF SPECIMENS

Specimens for mycobacteria examination can be grouped into two categories: primary cultures and subcultures. Two mailing kits for specimens are available free upon request. Please use the proper kit when submitting specimens to the State Hygienic Laboratory.

- 1. TB examination kit contains: a. "sputum" bottle with no preservative, b. "whirl-pak" bag, c. TB data slip, d. cotton, e. styrofoam mailing container, f. return addressed mailing box (Figure 4-1).
- 2. TB-gastric examination kit contains: a. "sputum" bottle with 10 ml trisodium phosphatezephiran solution, b. "whirl-pak" bag, c. TB data slip, d. cotton, e. styrofoam shipping container, f. return addressed mailing box. This kit is used only for gastric washings and urine specimens (Figure 4-2).
- For the submission of TB subcultures please refer to shipping instructions as outlined in the section on Reference Bacteriology

Species of the genus *Mycobacterium* can be isolated from any anatomical site on the human body as well as from the environment. Experience has shown sputum, nebulized sputum, bronchial washings, pleural fluid, gastric washings, urine, cerebral spinal fluid, lung tissues and lymph nodes to be the best types of specimens for the isolation of these bacteria. Specimens should be collected aseptically.

The following information is required on the TB data slip (Figure 4-3) that accompanies a specimen for mycobacteria examination.

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Mycobacteria Examination |
|--|---------------------------------------|
| Please print with BLACK MEDIUM SOFT lead pencil a | nly |
| Patient M. OISON | Age 65 Sex F |
| Physician (Name and Ambrest) | , 1011 |
| Dr. T. BROWN ANY-DUIL TOWA | SOURCE OF SPECIMEN: Sputum Subculture |
| Zip Code 50000 | Other 11141 PAS |
| PLEASE PRINT PLAINLY | Streptemy Cin |
| MICROSCOPIC FINDINGS | CULTURAL FINDINGS |
| ACID-FAST BACILLI not found O | ACID-FAST BACILLI not found O |
| Next report within weeks | |
| Specimen was unsatisfactory for | Next report withinweeks O |
| culture because: | FINAL REPORT |
| Date Received | |
| LAB | W. J. Hausler Jr., Ph.D. Director |

Figure 4-3 Completed TB data slip

Sputum

- A series of three to five early-morning, expectorated sputum specimens should be taken one per day. These should not be sinus excretions or saliva.
- Five to 10 ml of specimen will be enough for a proper examination. There is no advantage in collecting a larger volume.
- No preservative should be added.
- 4. All specimens should be refrigerated until they are mailed. Specimens pooled over a 24-, 48- and 72-hour period are unsatisfactory due to the relatively high amount of contamination present when compared with a single, early-morning expectorate.

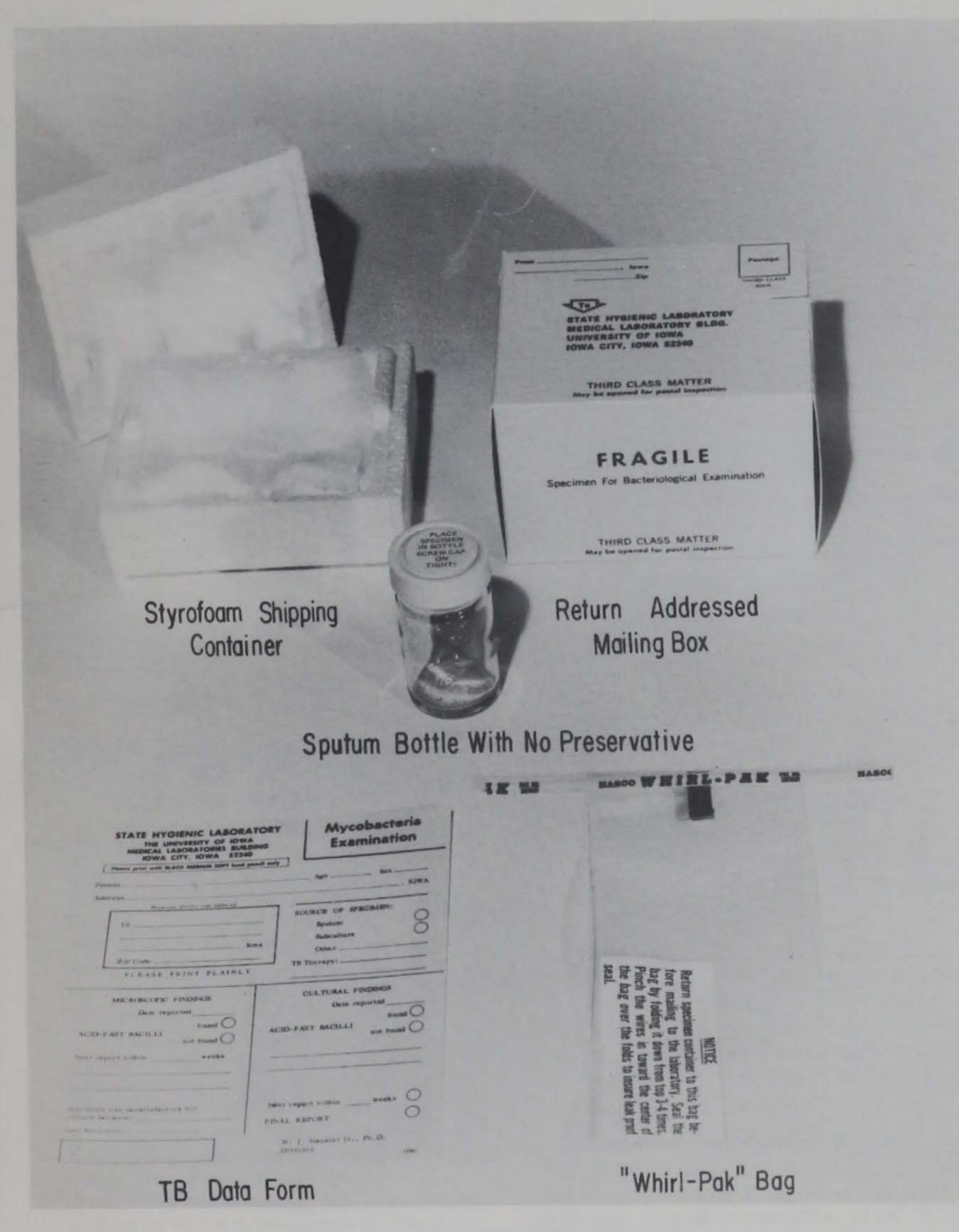


Figure 4-1 TB specimen collection kit

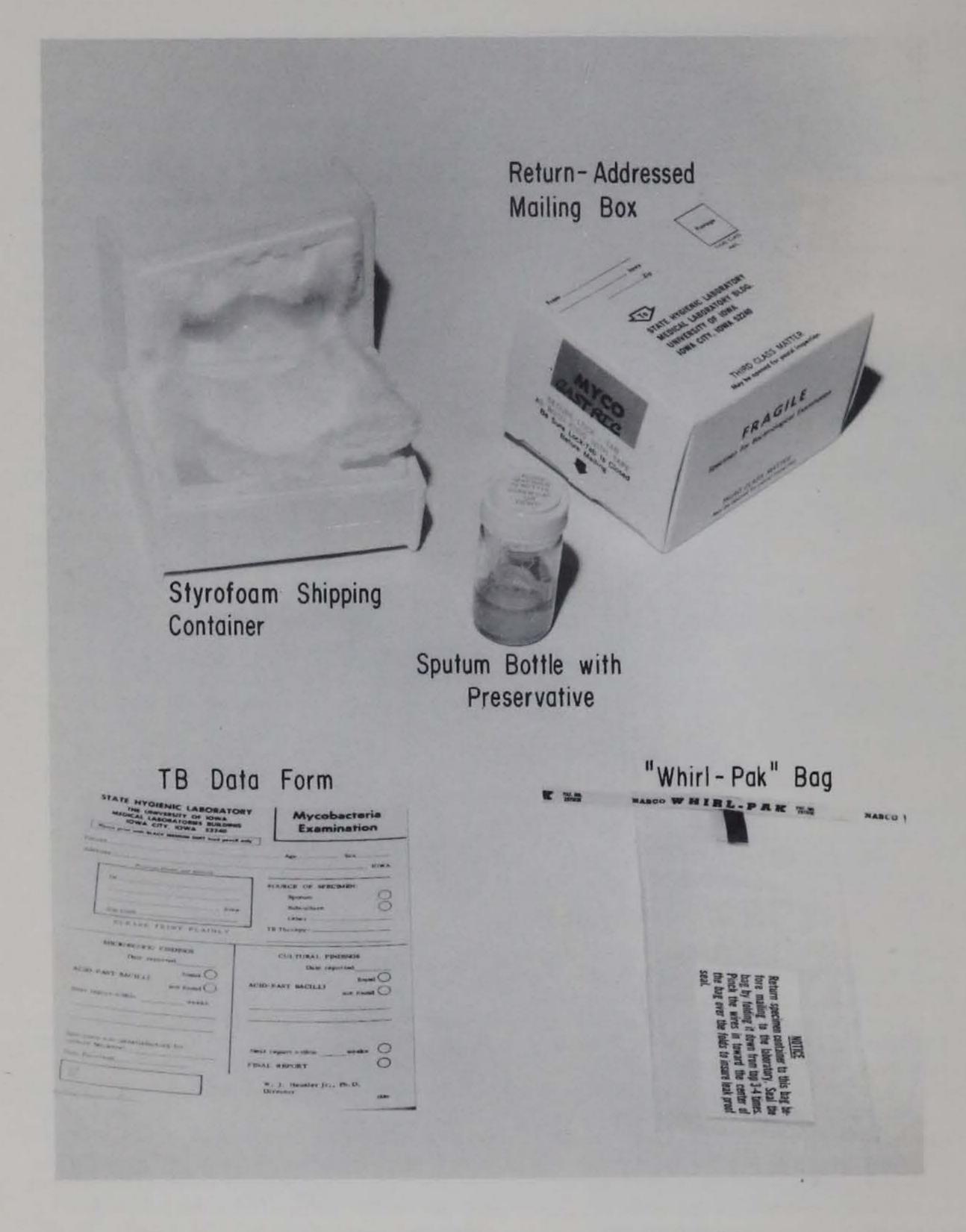


Figure 4-2 TB-Gastric specimen collection kit

Nebulized Sputum

1. Follow procedure for submission of sputum specimens.

2. Be sure to label nebulized sputum specimens in the following manner.

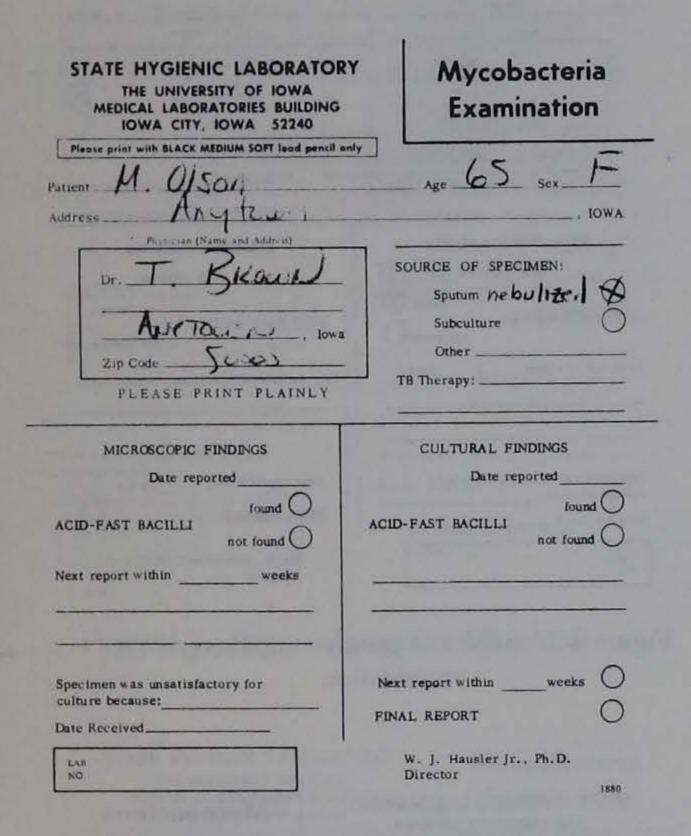


Figure 4-4 Identification of nebulized sputum

Gastric Washings or Lavage

- Submit a series of two to four specimens collected daily. These must be taken on a fasting stomach.
- 2. Add no more than 10 ml of specimen to a TB gastric outfit. If more than 10 ml is collected, use multiple kits, though there is no advantage to submitting more than three specimens.

The washings or lavage should be put in the preservative (digesting) solution as soon as the specimen is collected.

4. The unused specimen kits and those being held prior to mailing must be kept at room temperature. Gastric specimens not submitted in the proper manner will not be accepted.

Because Mycobacterium sp. "tap water" can be isolated in tap water, we recommend that sterile distilled water be used in the lavage.

Urine

1. Collect a series of two to four single, midstream specimens voided early in the morning.

2. No more than 10 ml of specimen should be added to the TB gastric outfit.

3. Follow the procedure for gastric specimens.

 Urine specimens pooled over 24-, 48- and 72hour periods are unsatisfactory.

Pleural Fluid

1. Submit at least four ml of specimen.

2. This specimen should be collected and mailed in a sterile bottle with no preservative added.

3. It should be refrigerated until mailed to the State Hygienic Laboratory.

NOTE: If less than four ml of specimen is received, a microscopic examination will not be performed.

Cerebral Spinal Fluid (CSF)

 The specimen should be submitted in a sterile bottle with no preservative added.

2. If possible, CSF should be centrifuged for 15 minutes at 3,000 rpm.

The supernatant should be sent in a sterile bottle with no preservative added.

4. Streak the sediment directly onto several tubes of Lowenstein-Jensen medium.

NOTE: If less than four ml of specimen is submitted, a microscopic examination will not be performed.

Lung Lesions or Lymph Node Tissues

Mail these specimens in the sterile bottle with chloramphenicol added. Chloramphenicol powder is available from the State Hygienic Laboratory.

Preparation of Stock Solution

Suspend 20 mg chloramphenicol in 10 ml of 95 percent alcohol. Add 90 ml of distilled water. If necessary, heat gently to complete solution. This is a stable solution.

Use

Add 1.0 ml of the stock solution to each sterile "sputum" bottle. This amount is ample for inhibition of contaminant in one to 10 ml of specimen. If the solution dries in the bottle before use, its effectiveness is unimpaired. The concentration desired is approximately 0.2 mg of chloramphenicol per ml.

REPORTING OF LABORATORY RESULTS

An acid-fast culture and, if possible, an acid-fast stain are performed on all specimens submitted. The microscopic examination can often aid the physician in the diagnosis of tuberculosis. If acid-fast bacilli are not found on a smear, it does not rule out the possibility of tuberculosis, as this is found frequently. On the other hand, a patient can have acid-fast bacilli in his or her sputum and not have tuberculosis, but instead have saprophytic *Mycobacteria* sp. The microscopic examination is reported within several days after the specimen is received.

Negative cultures are reported after an eight-week incubation period.

Positive cultures for acid-fast bacilli are reported as soon as sufficient growth appears. These can be either saprophytic or pathogenic.

Presumptive identification is based on the growth on Dubos-Middlebrook 7H10 or 7H11 agar (10-21 days).

The final identification is based on biochemical tests which usually take four to six weeks to complete after the presumptive identification is made.

Table 4-1 presents the groups of mycobacteria which have been shown on a statistical basis to be clinically "always or sometimes significant" or clinically "never or rarely significant." The implication is that some species are statistically more likely not to be related to disease. Not every acid-fast bacillus isolated can be clearly defined as pathogen or saprophyte.

Table 4-1 Potential clinical significance of various mycobacteria

| Runyon Group | Always or sometimes significant | Never or rarely significant |
|-----------------|--|---|
| | M. tuberculosis M. bovis | |
| I. | M. kansasii M. marinum* | |
| II. | M. scrofulaceum** | M. sp. tap water M. flavescens |
| III. | M. avium - M. intracellulare M. x ² nopi | M. terrae complex M. gastri M. triviale |
| IV. | M. fortuitum | Runyon Group IV- not M. fortuitum |

[°]M. marinum is pathogenic when isolated from the skin. such as swimming pool granuloma.

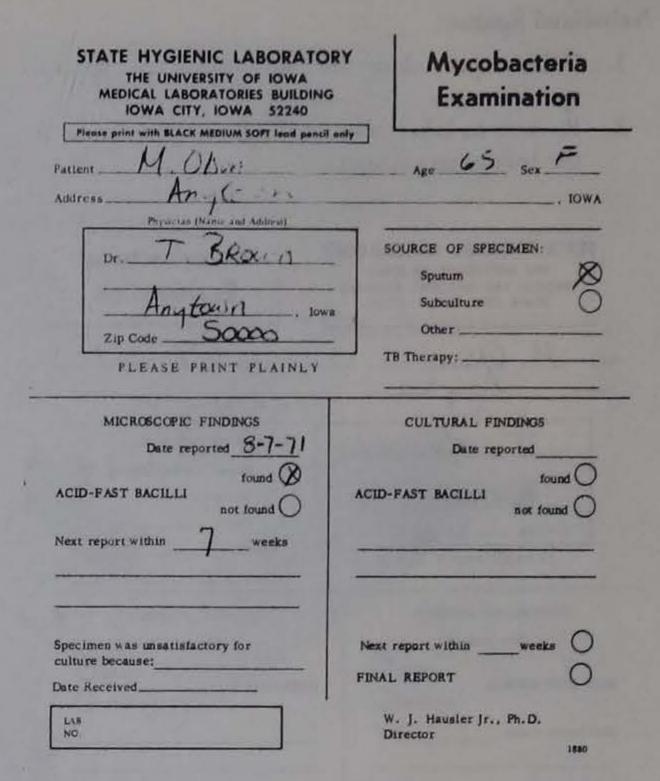


Figure 4-5 Report of positive microscopic TB examination

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDIN IOWA CITY, IOWA 52240 | Mycobaciena |
|---|---|
| Patient M. Olion Address Angli | Age 65 Sex F |
| Dr. T. BR lov Zip Code San PLEASE PRINT PLAINLY | Source of specimen: Sputum Subculture Other TB Therapy: |
| MICROSCOPIC FINDINGS Date reported | CULTURAL FINDINGS Date reported 6-7- found O acid-fast bacilli not found O |
| Specimen was unsatisfactory for culture because: | Next report withinweeks O |
| LAB NO | W. J. Hausler Jr., Ph.D. Director |

Figure 4-6 Report of negative microscopic TB examination

[°] M. scrofulaceum is pathogenic when isolated from lymph nodes.

| ation | MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | ZXdiiiiidii |
|---------|--|--|
| | Please print with BLACK MEDIUM SOFT lead pencil | only LE _ |
| | Address Physician (Numerand Address) | Age Sex For IOWA |
| EN: | ZIP CODE SULLI PLAINLY | SOURCE OF SPECIMEN: Sputum Subculture Other TB Therapy: |
| round O | MICROSCOPIC FINDINGS Date reported found O ACID-FAST BACILLI not found O Next report within weeks | CULTURAL FINDINGS Date reported 2-7-71 found S ACID-FAST BACILLI not found |
| weeks O | Specimen was unsatisfactory for culture because: Date Received | Next report within 4 weeks FINAL REPORT W. J. Hausler Jr., Ph.D. Director |
| - N e | NGS sal & 7-7/ found O ot found P weeks O | Patient M. Uis no Address Physician (N time and Address) Dr. T. BROWN Dr. T. BROWN Dr. T. BROWN Dr. T. BROWN PLEASE PRINT PLAINLY MICROSCOPIC FINDINGS Date reported found O ACID-FAST BACILLI No Specimen was unsatisfactory for culture because; Date Received |

| STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Mycobacteria Examination | STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52242 | Mycobacteria Identification |
|---|--------------------------------------|--|---|
| Please print with BLACK MEDIUM SOFT lead pencil only | | Please print with BLACK MEDIUM SOFT lead pencil ent | The second second second |
| Patient M. Olsen | Age 65 Sex = | Patient M O SON | Age 65 Sex F |
| | | A | , IOWA |
| Address | , 10WA | | , 1977 |
| Phytician (Name and Address) | | Physician (Name and Address) | SOURCE OF SPECIMEN |
| Dr. T. Bewin | SOURCE OF SPECIMEN: Sputum | Dr. T. BROWN | Sputum Subculture |
| Analism | Subculture | ANTONN IOWA | Other |
| Thy living, lowa | | Zip Code 50000 | Date Received |
| Zip Code | Other | Zip Code | |
| PLEASE PRINT PLAINLY | TB Therapy: | PLEASE PRINT PLAINLY | Date Reported |
| MICROSCOPIC FINDINGS | CULTURAL FINDINGS | Final Biochemical Id | dentification |
| Date reported | Date reported & 7-7/ | , | |
| | | M. tuberculosis | Runyon Group I - Photochromogen |
| found O | found 🏵 | M. bovis M. kansasii | ☐ Runyon Group II — Scotochromogen ☐ tap water subgroup |
| ACID-FAST BACILLI | ACID-FAST BACILLI | M. kansasii M. marinum | Runyon Group III - non-photo chromogen |
| not iound | 0 | M. scrofulaceum | ☐ M. terrae complex |
| Next report within weeks | thesumptive | O M. avium - M. intracellulare | □ M. gastri |
| | M tuberculosis | complex | D M. triviale |
| | 11. 2001/2010313 | M. xenopi M. fortuitum complex | □ Runyon Group IV - Rapid Grower |
| | | INTERPRETAT | TIONS - |
| | 1. 0 | ☐ Strictly Pathogenic | ☐ Probably Pathogenic |
| Specimen was unsatisfactory for culture because: | Next report within weeks | ☐ Potentially Pathogenic | □ Rarely Pathogenic |
| Date Received | FINAL REPORT | | W. J. Hausler, Jr., Ph.D. |
| Take Hoveling | | LAB NO. | Director |
| LAB NO | W. J. Hauster Jr., Ph.D. Director | | 2101 |

Figure 4-9 Presumptive TB identification report

Figure 4-10 Final report

Drug Susceptibility Profile

These tests are performed only on mycobacteria usually associated with disease. This is indicated on the final identification data slips.

Mycobacteria usually not associated with disease are generally resistant to the primary TB drugs (INH, PAS, Streptomycin and Ethambutol). Drug susceptibility profiles will be done on the saprophytic mycobacteria when a disease process is indicated. This should be requested by the physician. All drug tests are reported as either susceptible (S) or resistant (R) to a given concentration of a particular drug. These tests usually take four to six weeks to complete.

Sensitivity patterns to INH, PAS, Streptomycin,

Ethambutol, Kanamycin, Viomycin, Ethionamide, Capreomycin and Rifampin are performed routinely.

Pyrazinamide and Cycloserine sensitivity patterns are not performed routinely, due to the inherent instability and special environmental (and pH) requirements of these two compounds.

Drug susceptibility patterns will be performed according to the recommendations of the Center for Disease Control and the American Thoracic Society. These recommended concentrations and interpretations, based on the number of colonies growing in each concentration, are being utilized in order to attain some degree of standardized anti-tubercle susceptibility testing.

DRUG SUSCEPTIBILITY PROFILE

| Drug | μg/ml | S/R* | Drug | μg/ml | S/R* |
|--------------|--------|------|--|-------|----------------------------|
| INH | 0.2 | | Kanamycin | 5.0 | |
| INH | 1.0 | | Viomycin | 5.0 | No country |
| INH | 5.0 | | Pyrazinamide | 50.0 | A PERSON NAMED IN COLUMN 1 |
| PAS | 2.0 | | Ethionamide | 5.0 | |
| PAS | 10.0 | | Cycloserine | 20.0 | |
| Streptomycin | 2.0 | | Rifampin | 1.0 | |
| Streptomycin | 10.0 | | Capreomycin | 2.0 | |
| Ethambutal | 6.0 | | Capreomycin | 10.0 | |
| | | | | | |
| | | | The state of the s | | |
| *S = Susce | ptible | | *R = Resist | ant | |

Figure 4-11 TB drug susceptibility profile

INTRODUCTION

The State Hygienic Laboratory provides a diagnostic medical mycology laboratory as well as a reference service.

Among the fungi and actinomycetes there are more than 20 species which cause systemic or fatal diseases; 35 which cause less severe systemic disease or severe localized cutaneous, subcutaneous or lymphatic infections; and 45 which cause superficial infections of the skin. Under certain conditions, saprophytic fungi may become pathogenic and invade human tissue. These conditions may occur in patients under prolonged chemotherapy and in patients with lowered resistance due to cancer, diabetes and other diseases. Some of the fungi that invade tissue in this manner may be described as provisional pathogens.

Saprophytic fungi pose a major problem in hospital laboratories. They are ubiquitous, ranging from simple air-borne contaminants to isolates from pathological specimens. Often they are confused with pathogenic fungi. Saprophytes possess two common charac-

teristics:

 They grow very well at room temperature (25°C) on most mycological media and poorly or not at all at 37°C.

2. They are generally highly pigmented and produce abundant characteristic repro-

ductive structures.

With few exceptions, the systemic, lymphatic and subcutaneous mycoses are caused by fungi which are essentially free-living parasites in nature. These mycoses are not contagious, and infection in man usually follows inhalation of these fungi from decaying vegetation, humus, bird or animal excreta, soil, or soil enriched with bird excreta.

COLLECTION AND SHIPMENT OF SPECIMENS

The processing of specimens for mycologic culture mandates maximal precautions on the part of the microbiologist to protect himself as well as specific competency requirements to grow and identify the pathogenic fungi. For these reasons, specimens for mycologic culture must be collected, handled and shipped by the prescribed procedures detailed in the following pages. Anyone having questions about what to collect or how to handle the specimen shipment,

etc., should contact the Mycology Division prior to submission of specimens.

Mycologic specimens will be processed by one of three procedural methods:

1. Nocardia specific system,

2. Histoplasma specific system,

 Routine mycologic system (any specimen not specifically requesting histoplasma or nocardia culture).

Specimens for *Histoplasma capsulatum* culture will be rejected unless one of the following procedures is employed:

Freeze the specimen (dry ice or −20°C freezer)
 immediately upon collection and mail or ship it
 to the laboratory in a frozen condition;

Perform initial culture procedures in the hospital laboratory and submit subcultures for further study;

Inoculate animals with the specimen material and ship or transport the animals for further study.

Any specimen for *Histoplasma capsulatum* culture that has not been collected and transported as listed above will be considered a routine mycology specimen and treated accordingly. If doubt arose as to the culture system desired, the physician would be notified immediately.

Special Procedures

Small-volume specimens collected aseptically (CSF, pleural fluids, etc.) should be processed locally in the hospital laboratory and subcultures submitted for further examination. If body fluids are collected in large volumes (greater than 5 ml) these can be frozen immediately and shipped frozen to the laboratory for further examination. Call the Mycology Laboratory—353-5278—if any questions exist over proper handling of body fluids for mycologic examination.

A mailing container is available free upon request. It is preferred that the correct kit be used when submitting specimens to the State Hygienic Laboratory.

The Mycology examination outfit (Figure 5-1) contains:

- a. "sputum" bottle with no preservative
- b. "whirl-pak" bag
- c. mycology data form
- d. cotton
- e. styrofoam shipping container
- f. return-addressed mailing box

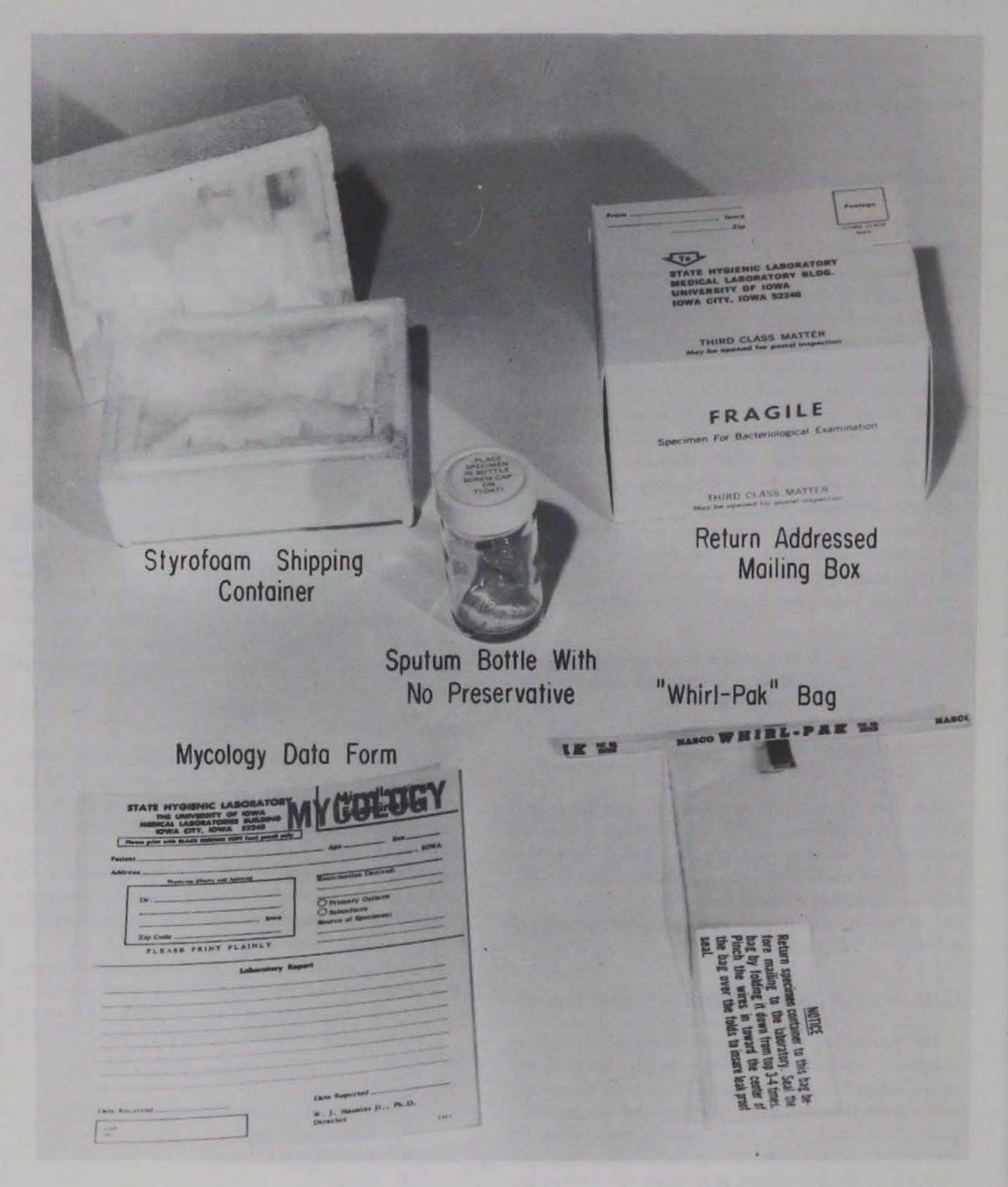


Figure 5-1 Mycology specimen collection kit

For submission of a mycology subculture, please refer to the shipping instructions as outlined in the section on Miscellaneous Bacteriology.

The following information (Figure 5-2) is required on all specimens submitted for mycological examination:

- Patient's name, age, sex, address, occupation (if known).
- 2. Source of specimen and site of infection.
- 3. Clinical diagnosis and/or brief clinical history of this particular infection.
- 4. Physician's or laboratory's name and address.
- If a subculture is submitted, please include copy of laboratory work performed.

Pathogenic fungi can be isolated from any human anatomical site as well as from the environment. All specimens must be collected aseptically. Skin, nail and hair specimens should be placed in a sputum bottle with no preservative added, and mailed. Other specimens, especially sputum, urine and exudates from lesions are of little value after shipment by mail, as saprophytic fungi and bacteria multiply faster than the pathogenic fungi, making it difficult to isolate the etiological agent. In these cases the specimens should

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52242 | Mycology Examination |
|--|-------------------------|
| Please print with BLACK MEDIUM SOFT lead pencil only | |
| tent U. JONES | Age 51 Sex |
| FAIOTOLINI | , IOWA |
| Physician (Name and Address) | |
| M. GIBBS | Examination Desired: |
| | Primary Culture |
| FARTOWN , IOWA | OSubculture |
| Zip Code | Source of Specimen: |
| alp cour | HAIR |
| PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS RINGWORN | 1 |
| 5 | |
| CLINICAL DIAGNOSIS RINGWORN | |
| CLINICAL DIAGNOSIS RINGWORN | |
| CLINICAL DIAGNOSIS RINGWORN Laboratory Re | port |
| CLINICAL DIAGNOSIS RINGWORN | |
| CLINICAL DIAGNOSIS RINGWORN Laboratory Re | port |

Figure 5-2 Completed mycology data form

be placed on culture media before they are mailed (Table 5-1).

The selection of the proper material is very important since it is difficult to know prior to microscopic examination of materials whether the fungus is in the sample selected. Sufficient material is necessary for both direct examination and culture. If the microscopic examination is negative, this does not rule out the possibility that the fungus may have been missed in sampling.

Laboratories using Sabouraud's (dextrose) agar with the antibiotics chloramphenicol (chloromycetin) and cycloheximide (actidione) added (commercially available as "Mycobiotic" or "Mycosel" agar) for a primary isolation medium must also use Sabouraud's (dextrose) agar without antibiotics in parallel. Cycloheximide reduces the rate of growth of many saprophytic fungi, but inhibits the growth of Cryptococcus neoformans, some Candida sp., Allescheria boydii, as well as the yeast phase of some systemic fungi. Chloramphenicol, on the other hand, inhibits the growth of Nocardia sp. and other actinomycetes.

When working with fungi, one must be constantly concerned with safety. All mycology cultures should be assumed to be pathogenic until proven otherwise. All subcultures submitted should be on tubed media because of the extreme hazards of working with systemic fungi, as well as the fact that media in petri dishes dry out rapidly.

From our experience, the best types of specimens, for mycological examination are: skin scrapings, nails, hair, scraping from ulcers, pus or exudates, spinal and other body fluids, urine, sputum, blood, bone marrow, stools, bronchial washings, biopsies and soil samples.

The antibiotics that are in Sabouraud's dextrose agar are cycloheximide (actidione), 10 cc of a one percent solution per liter of media, and chloramphenicol (chloromycetin), one gm per liter of media.

Skin Scrapings

- Wash the affected area or lesion with a nonmedicated soap and rinse thoroughly with sterile distilled water.
- Rinse the skin with 70 percent alcohol to completely remove dirt and any medication.
- 3. Scrape with a sterile scalpel, preferably from the active border areas of the lesions.
- Place the scales in a sterile bottle with no preservative added and mail with the data slip to the State Hygienic Laboratory.

Nails

1. Rinse the affected nails with 70 percent alcohol.

- Scrape or clip the nail, especially near the bed of the nail.
- Place the nail in a sterile bottle with no preservative added.
- 4. Mail with the data slip to the State Hygienic Laboratory.

Hair

- 1. A Wood's light (ultraviolet light rays of 3,660 Angstrom units) is useful in collecting infected hairs in scalp ringworm caused by Microsporum audouinii and M. canis. Trichophyton sp. and M. gypsum hair infections do not fluoresce.
- 2. Rinse the infected hairs with 70 percent alcohol.
- Pluck out the basal portion of the hairs or hair stubs with sterile tweezers.
- Place the hair in a sterile bottle with no preservative added and mail with the data slip to the State Hygienic Laboratory.

Scraping From Ulcers

- 1. Collect specimens aseptically.
- If possible streak onto Sabouraud's dextrose agar with and without antibiotics (Table 5-1), and incubate at 25° C.
- Place the scrapings in a sterile bottle with no preservative added and mail with the data slip to the State Hygienic Laboratory.

Pus or Exudates

- If possible, streak onto Sabouraud's dextrose agar with and without antibiotics (Table 5-1), and incubate at 25°C.
- Mail the swab in a sterile bottle with no preservative added and the data slip to the State Hygienic Laboratory.

Spinal and Other Body Fluids

- If possible, the body fluid should be centrifuged for 15 minutes at 3,000 rpm (except specimens for histoplasmosis).
- Streak the sediment directly onto two tubes of Sabouraud's dextrose agar (one with and one without antibiotics, grown at 25°C), and one blood agar tube grown at 37°C.
- Mail the supernatant in a sterile bottle with no preservative added and the data slip to the State Hygienic Laboratory.

Urine

 Use for the specimen a series of two to four single midstream specimens voided early in the morning.

- 2. If possible, the urine should be centrifuged for 15 minutes at 3,000 rpm.
- 3. These specimens must be streaked directly onto Sabouraud's dextrose agar without antibiotics to give a proper clinical picture. The cultures should be grown at 25°C.
- Urine specimens pooled over 24-, 48- and 72hour periods are unsatisfactory.
- Mail the subculture and data slip to the State Hygienic Laboratory.

Sputum

- Collect a series of three to five early-morning, expectorated specimens taken one per day. These should not be sinus excretions or saliva.
- Five to 10 ml of specimen are needed for proper examination. There is no advantage in collecting a larger volume.
- One ml of a chloramphenicol solution should be added as a preservative.
 Chloramphenicol powder is available from the State Hygienic Laboratory.

Preparation of Stock Solution

Suspend 20 mg chloramphenicol in 10 ml 95 percent alcohol. Add 90 ml of distilled water. If necessary, heat gently to complete solution. This is a stable solution.

Use

Add 1.0 ml of the stock solution to each sterile "sputum" bottle. This amount is ample for inhibition of contaminant in one to 10 ml of specimen. If the solution dries in the bottle before use, its effectiveness is unimpaired. The concentration desired is approximately 0.2 mg of chloramphenicol per ml.

- 4. These specimens must be refrigerated until they are mailed. Sputum specimens pooled over 24-, 48- and 72-hour intervals are unsatisfactory due to the high amount of contamination compared with a single, early-morning expectorate.
- Sputum specimens for isolation of Histoplasma capsulatum are not satisfactory as the fungus dies rapidly in the mail (freeze upon collection and ship frozen).
- 6. Place specimen in the sterile container provided in the outfit and mail with the data slip to the State Hygienic Laboratory.

Nebulized Sputum or Bronchial Washings

1. Follow procedure for submitting sputum specimens.

2. Be sure to label nebulized sputum specimens as shown in Figure 5-3.

Blood

Blood specimens for mycological examination are subgrouped into two types: serum for complement fixation, and blood clots for culture. The State Hygienic Laboratory upon request will examine serum submitted from cases of suspected blastomycosis, coccidioidomycosis and histoplasmosis. All blood specimens received for fungal examinations are routinely tested by the complement fixation test for the presence of antibody against a battery of these three mycotic diseases. The acute phase or first blood specimen should be collected as early as possible in the course of illness and sent to the laboratory. A convalescent or second blood specimen should be collected two to three weeks after the date of the first specimen and shipped without delay. Use the color-coded mailers for fungal serology as shown in Figure 5-4.

Complete one data slip on each specimen with particular emphasis on information concerning skin testing, antigen used and the reaction observed. Fungal examinations may need to be continued for a long period of time to detect change in antibody

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52242 | Mycology Examination |
|--|------------------------------|
| Please print with BLACK MEDIUM SOFT lead pencil only | |
| atient J. SmiTH | Age 32 Sex M_ |
| ddress ANYTOWN | , IOWA |
| Physician (Name and Address) Dr. J. ONES | Examination Desired: |
| ANYTOWN, IOWA | Primary Culture O Subculture |
| Zip Code 50000 | SATUM: NEBULIZED |
| PLEASE PRINT PLAINLY | - |
| CLINICAL DIAGNOSIS HISTOPLASMO | |

Figure 5-3 Identification of nebulized mycology sputum specimen

levels. The effect of antibody levels as a result of skin testing should be carefully considered prior to requesting serological studies. For detailed procedure on submitting serum specimens for complement fixation (CF) tests, refer to the section of this manual entitled "Laboratory Diagnostic Services for Viral Examination."

The blood specimen for culture should be centrifuged at 3,000 rpm for 15 minutes if possible. Otherwise, five to six ml of whole blood should be submitted for mycological examination. The clot should be digested with Sputolysin and streaked directly onto Sabouraud's dextrose agar with and without antibiotics grown at 25°C, and an enriched medium with blood grown at 37°C, or the clot should be mailed in a sterile container with no preservative added. Mail specimen and data slip to the State Hygienic Laboratory.

Bone Marrow

Place the specimen in a sterile bottle with no, preservative added and mail with the data slip to the State Hygienic Laboratory.

Stool

 Use series of two to four specimens voided early in the morning.

2. These specimens must be inoculated directly onto Sabouraud's dextrose agar with and without antibiotics. These cultures should be grown at 25°C.

Biopsies

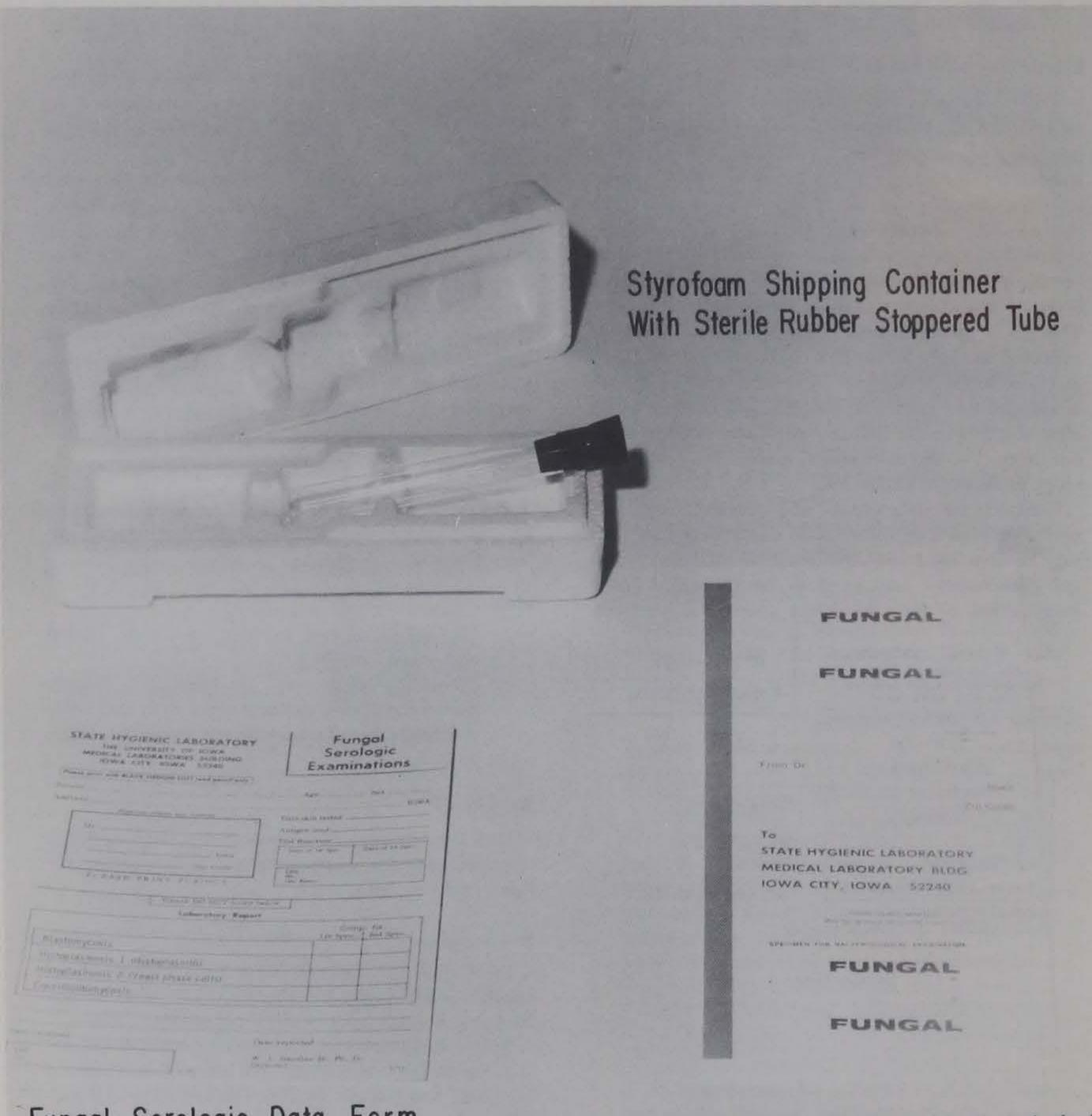
Mail the specimen in a sterile bottle with chloramphenicol added as a preservative. Follow directions listed under sputum.

Soil Samples

From time to time, the State Health Department and the State Hygienic Laboratory are asked to isolate and identify *Histoplasma capsulatum* from soil samples. Physicians and laboratories are asked to contact Dr. Stanley Hendricks, Division of Preventable Diseases, Iowa State Health Department, 515/281-5643, prior to submitting specimens.

REPORTING OF LABORATORY RESULTS

Since the identification of mycological cultures involves observing characteristic reproductive structures, or determining the nutritional requirements, or passage through animals, the length of time needed can be several months. Routine mycologic and *Nocardia sp.* cultures will be maintained for four weeks before



Fungal Serologic Data Form

Adhesive - backed Mailing Label

Figure 5-4 Fungal serology specimen collection kit

being discarded as negative. Cultures for *Histoplasma* capsulatum will be held ten weeks before being discarded as negative (Figure 5-5).

An example of a positive culture for a ringworm infection caused by *Trichophyton mentagrophytes* is shown in Figure 5-6.

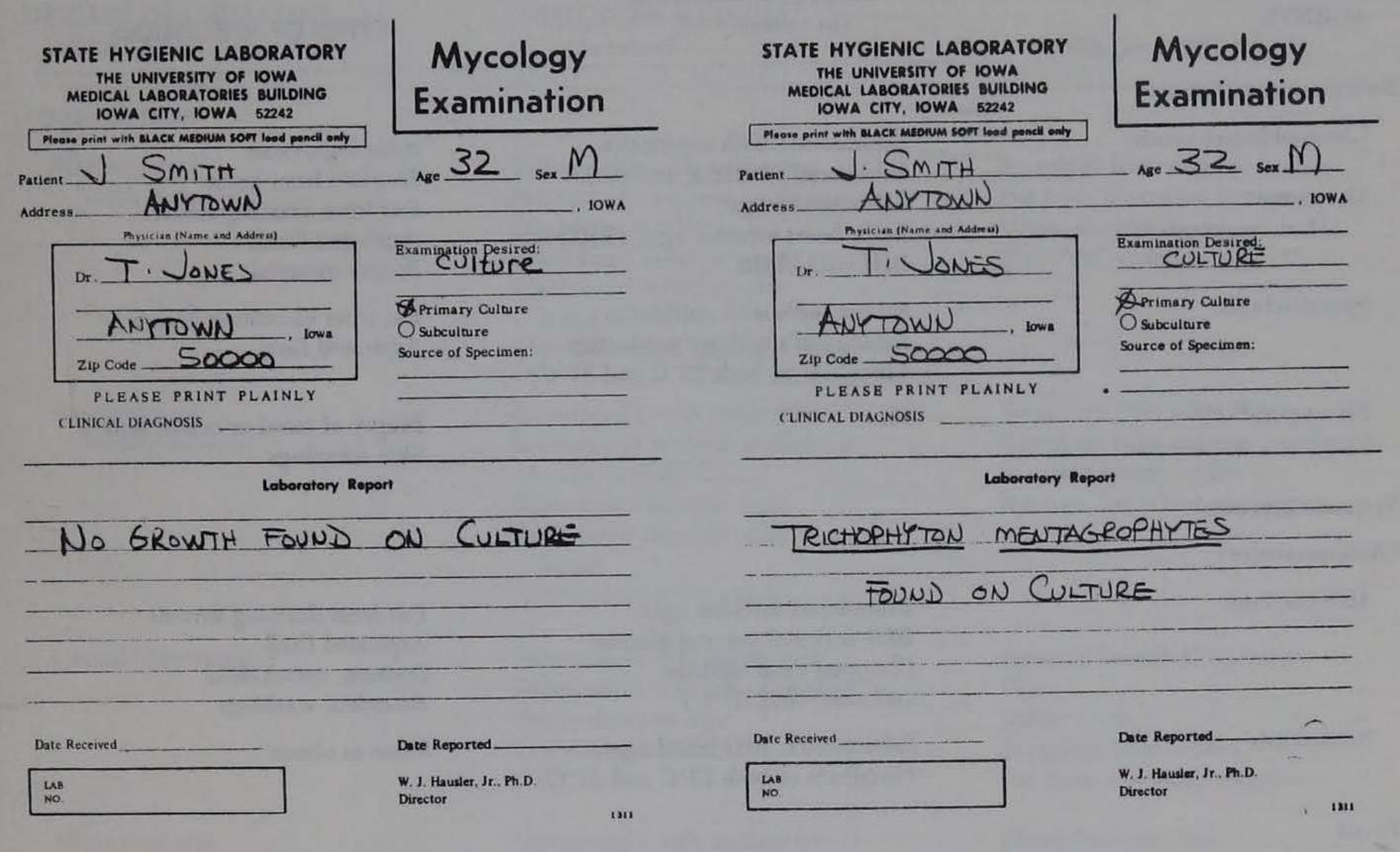


Figure 5-5 Negative mycology culture report

Figure 5-6 Positive mycology culture report

Table 5-1 Types of specimen and suggested media for isolation of the fungi-caused mycoses

| DISEASE OR CAUSATIVE AGENTS | CULTURE MEDIUM (in tubes) | TYPES OF SPECIMENS |
|--------------------------------|---|--|
| Superficial Mycoses | | |
| Tinea versicolor | None | Skin scrapings |
| Tinea nigra | Sabouraud's with antibiotics* Sabouraud's without antibiotics | Skin scrapings |
| Piedra | Sabouraud's agar | Cut hair |
| Cutaneous Mycoses | | |
| Tinea capitis | Dermatophyte Test Medium | Epilated hair |
| Tinea corporis, etc. | Dermatophyte Test Medium | Skin scrapings |
| Tinea unguium (Onychomycosis) | Dermatophyte Test Medium | Nail scrapings |
| Candidiasis | Sabouraud's with antibiotics | Skin scrapings |
| | Sabouraud's without antibiotics | Mucocutaneous scrapings Vaginal scrapings |

| DISEASE OR CAUSATIVE AGENTS | CULTURE MEDIUM (in tubes) | TYPES OF SPECIMENS |
|---|--|---|
| Subcutaneous Mycoses | | |
| Chromoblastomycosis Mycetoma (Maduromycosis) | Sabouraud's with antibiotics Sabouraud's without antibiotics Sabouraud's agar Brain-heart infusion agar (BHI) BHI with blood | Scrapings, crust Exudate from lesion Pus from draining sinuses Aspirated fluids Biopsy material |
| Sporotrichosis | Sabouraud's with antibiotics Sabouraud's without antibiotics (Incubate at both 25°C and 37°C) | Pus from ulcerating lesions Aspirated fluid |
| Rhinosporidiosis | None | Biopsy of nasal or ocular polyps Skin scrapings |
| Systemic Mycoses | | |
| (Actinomycetes) | | |
| Actinomycosis | Brain-heart infusion agar BHI with 0.2 percent glucose Chopped meat medium (all anaerobic, 37°C) | Pus from draining sinuses Aspirated fluid Sputum, spinal fluid Bronchial washings |
| Nocardiosis | Sabouraud's, BHI blood agar (Incubate at both 25°C and 37°C) | Same as above |
| Yeasts | | |
| Candidiasis | Sabouraud's with antibiotics Sabouraud's without antibiotics | Sputum, bronchial washings Spinal fluid Urine, stools |
| Cryptococcosis | Sabouraud's without antibiotics Christensen urea agar (Incubate at 25°C and 37°C) | Spinal fluid, sputum Pus from abscesses, sinus tracts Scrapings from skin lesions Urine |
| Geotrichosis | Sabouraud's with antibiotics Sabouraud's without antibiotics | Sputum, bronchial washings Stools |

| DISEASE OR CAUSATIVE AGENTS | CULTURE MEDIUM (in tubes) | TYPES OF SPECIMENS |
|---------------------------------|--|--|
| (Diphasie fungi) | | |
| Blastomycosis North American | Sabouraud's with antibiotics Sabouraud's without antibiotics (Incubate at 25°C) | Scrapings from edge of lesions Pus from abscesses, sinus tracts Urine, sputum Bronchial washings |
| | Brain-heart infusion agar Brain-heart infusion with blood (Incubate at 37°C only) | Bronchar washings |
| | (Incubate at 37 Conly) | |
| Blastomycosis South American | Sabouraud's with antibiotics Sabouraud's without antibiotics (Incubate at 25°C) Brain-heart infusion agar Brain-heart infusion with blood (Incubate at 37°C only) | Scrapings from edge of lesions Scrapings from mucous membranes Biopsied lymph nodes Sputum, bronchial washings |
| | (mediate at 57 5 only) | |
| Coccidioidomycosis | Sabouraud's with antibiotics Sabouraud's without antibiotics Potato dextrose agar (Incubate at 25°C) | Sputum, bronchial washings Urine Spinal fluid Scrapings from lesions Pus from abscesses, sinuses |
| Histoplasmosis | Sabouraud's with antibiotics Sabouraud's without antibiotics (Incubate at 25°C), Brain-heart infusion agar Brain-heart infusion with blood (Incubate at 37°C only) | Blood, bone marrow Sputum, bronchial washings Spinal fluid Pus from sinus tracts or ulcers Skin scrapings from lesions |
| | (Incubate at 37 Comy) | |
| Miscellaneous Mycoses | | |
| Aspergillosis | Sabouraud's with antibiotics Sabouraud's without antibiotics | Sputum, bronchial washings |
| Mucormycosis (Phycomycosis) | Sabouraud's with antibiotics Sabouraud's without antibiotics | Sputum, bronchial washings Biopsy material |
| Penicilliosis | Sabouraud's with antibiotics Sabouraud's without antibiotics | Sputum, bronchial washings Nail scrapings |
| External Otitis (Otomycosis) | Sabouraud's with antibiotics Sabouraud's without antibiotics | Epithelial scales and detritus |

[°]Antibiotics

- Cycloheximide (actidione)—10 cc of a one percent solution per liter of medium.
 Cycloheximide is available from Nutritional Biochemical Co., Cleveland, Ohio.
- Chloramphenicol (chloromycetin)—one gm per liter of medium.

LIST OF PRINCIPAL PATHOGENIC FUNGI THAT CAUSE DISEASE IN MAN AND ANIMALS

Actinomycetes

Actinomyces bovis

A. israelii

Nocardia asteroides

N. brasiliensis

Streptomyces madurae

S. pelletierii S. somaliensis Phycomycetes

> Absidia corymbifera Basidiobolus ranarum Rhizopus arrhizus

R. oyrzac

Deuteromycetes (Fungi Imperfecti)

Aspergillus fumigatus Blastomyces dermatitidis Candida albicans

Cephalosporium falciforme Cladosporium bantianum

C. carrionii C. werneckii

Coccidioides immitis Cryptococcus naoformans Epidermophyton floccosum

Fonsecaea compactum

F. pedrosoi

Geotrichium candidum Histoplasma capsulatum H. capsulatum var. duboisii Keratinomyces ajelloi

Madurella grisea

Deuteromycetes (continued)

M. mycetomii Malassezia furfur Microsporum audouinii

M. canis M. distorum M. ferrugineum M. gypseum M. nanum

M. vanbrzuseghenii

Paracoccidioid s brasiliensis

P. loboi

Phialophora jeanselemi

P. verrucosa Pyrenochaeta romeroi Rhionosporidium seeberii

Trichophyton concentricum

T. equinum T. gallinae T. gourvillii T. megninii T. mentagrophytes T. rubrum

T. schoenleinii T. soudanense T. tonsurans T. verrucosum T. violaceum

T. yaoundei Trichosporon cutaneum Ascomycetes

Allescheria boydii Leptosphaeria senegalensis

Piedraia hortai

LIST OF COMMONLY ENCOUNTERED SAPROPHYTIC FUNGI

Penicillium sp. Aspergillus sp. Scopulariopsis sp. Sporotrichium (Sporotrix) schenckii Cephalosporium sp.

Geotrichium (Oospora) sp.

Cladosporium sp. Nigrospora sp. Rhizopus sp. Rhodotorula sp. Trichoderma sp. Paecilomyces sp. Gliocladium sp. Fusarium sp. Alternaria sp.

Helminthosporium sp.

Mucor sp.

Syncephalastrum sp. Streptomyces sp.

INTRODUCTION

The Veterinary Medical Diagnostic Laboratory at Ames and the State Hygienic Laboratory at Iowa City are the only two laboratories in Iowa that routinely examine animal specimens for the presence of rabies. Both laboratories utilize the same laboratory procedures in the examination of each specimen and frequently submit reference specimens to each other for control purposes.

COLLECTION AND SHIPMENT OF SPECIMENS

To insure expediency and accuracy in the examination for the presence of rabies, please adhere to the following suggestions:

- Don't shoot or club the suspect specimen in the head. The animal's brain must be intact for proper laboratory examination.
- Don't send the whole carcass to the laboratory. The intact head is all that is needed for examination. The State Hygienic Laboratory in particular does not have suitable facilities for the disposal of larger animal carcasses.
- Pack the head in ice immediately. Brain tissue rapidly decomposes if not properly refrigerated, particularly in summer months.
- Don't freeze the specimen. Freezing alters brain tissue and makes it difficult to provide a proper diagnosis.
- Include with the specimen information concerning the exposure, name of person bitten and the name of the physician or veterinarian to whom the laboratory report is to be submitted.
- 6. Don't use the Iowa Highway Patrol as a delivery service unless it is an absolute and constituted emergency. An emergency consists of a person having been bitten by an animal likely to have rabies. Since mice and other rodents rarely, if ever, are infected with rabies, persons bitten by these animals do not constitute emergencies. Biting means the penetration of the skin by the teeth of the suspected animal. Bites about the head and neck, multiple and/or extensive bites elsewhere also constitute an emergency. Salivary exposure without a bite is not an emergency.

- 7. Don't submit live animals for examination. The laboratories do not have facilities for this service. Consult with your physician or veterinarian who will advise you on proper confinement procedures. If the animal is alive and appears healthy 10 to 14 days after the biting, this is proof that rabies was not present in the infectious stage at the time of biting.
- Don't kill the animal unless confinement is impossible. Observation of the animal during confinement is superior in most instances to the application of the most advanced laboratory techniques.
- Don't send specimens to the laboratory by mail. The U.S. Post Office refuses to handle rabies specimens. They may be shipped by Railway Express, United Parcel Service or Iowa Parcel Service. Bus or air shipment also may be used. Preferably, the persons involved should deliver the specimen directly to the laboratory themselves. Determine the time required for transportation to the laboratory and make certain that sufficient ice is used to provide refrigeration during shipment. Specimens destined for the State Hygienic Laboratory must be delivered to the Laboratory (Room 270, Medical Laboratories Building, The University of Iowa, Iowa City) and not just to Iowa City where the Laboratory is located. Originators of specimens destined for Iowa City who have not made prior arrangements for pickup and delivery directly to the Laboratory will be charged for this pickup service at the following rates: between 8:00 a.m. and 5:00 p.m. Monday through Friday, the submittor will be charged \$5.00, and during all other hours not specified above, the charge will be \$10.00. Bus transportation may be used in submitting specimens to the Veterinary Medical Diagnostic Laboratory at Ames.
- Don't send specimens to the Laboratory COD.
 They will not be accepted.
- 11. Include with the specimen, when available, a complete history of the animal including vaccinations administered, clinical signs, duration of illness and any post-mortem findings.

Both laboratories are interested in providing the best and most rapid rabies diagnosis. They will be helped immeasurably if the above directions are followed. If you need further assistance in submitting specimens to the laboratories, their telephone numbers are as follows:

Veterinary Medical Diagnostic Laboratory, 515/294-1950 State Hygienic Laboratory, 319/353-5990

REPORTING OF LABORATORY RESULTS

Results of rabies examinations are reported only to the physician or veterinarian responsible for submission of the specimen. Fluorescent antibody results (FRA) are reported on the day the specimen is received. A 15- and a 30-day report are also given subsequent to mouse inoculation of the specimen. Mouse inoculation is only done when there is human exposure and the FRA is negative. When there has been no human exposure, only the fluorescent antibody result is reported. Positive results are always reported immediately by telephone.

Differ market the wife of the special property is

THE RESERVE AND THE PARTY OF TH

INTRODUCTION

The syphilis serology unit of the Serology Division is responsible for performing serologic tests for syphilis. This unit is also charged with conducting the Laboratory Evaluation Program in syphilis serology.

The serologic evaluation program for the diagnosis of syphilis has been established by the State Department of Health as a means for approval of laboratories to perform the prenatal and premarital tests required by law. Any laboratory wishing approval may request application forms from:

State Department of Health Venereal Disease Control Division Lucas State Office Building Des Moines, Iowa 50319

The nontreponemal (VDRL) and the treponemal (FTA-ABS) tests are the methods used for the sero-diagnosis of syphilis. The VDRL test is used to screen all blood and spinal fluid specimens received. Any specimen which shows some degree of reactivity is quantitated until an end point is reached. A change in reactivity is useful for evaluating therapy.

The Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test is the confirmatory method used. It is highly specific and sensitive. Once this test is positive, it will remain so for the life of the patient. The FTA-ABS test is performed only on serum specimens which:

Show reactivity or weak reactivity by the VDRL screening test,

Are cases under treatment or contact cases (indicated by the physicians on the data forms),

3. Have had two previously nonreactive VDRLs but syphilis is still clinically suspected, and

 Have been sent to us by one of the "approved" laboratories.

This test climinates the necessity for requesting a Treponema Pallidum Immobilization test (TPI) which is only done at the Center for Disease Control (CDC) in Atlanta, Georgia.

COLLECTION AND SHIPMENT OF SPECIMENS

The following precautions must be taken when collecting specimens:

1. Specimens can only be collected in a tube which contains *no* anticoagulant.

If wet syringes are used, they must be rinsed with normal saline before using.

 Hemolyzed specimens are not suitable for testing. Hemolysis often occurs in very warm or cold weather. During these periods of temperature extremes, the serum should be removed from the clot prior to shipment.

Follow these instructions for the collection and shipment of specimens for syphilis serology:

1. Following the procedure shown in Figure 7-1, collect approximately three to five ml of blood in the serology tube provided in the serology kit (Figure 7-2).

2. Secure the rubber stopper.

3. Fill out the serologic examination data form (Figure 7-3).

 Wrap data slip around serology tube and place in mailing container.

5. Attach adhesive-backed mailing label and mail.

Referral Specimens

An approved laboratory may perform the VDRL on all serum specimens and submit specimens to the State Hygienic Laboratory for confirmation only. The referring laboratory *must* state on the data form if the serum being submitted has been heated for 30 minutes at 56°C. Either heated or unheated serum may be sent.

Premaritals for Out-of-State Marriages

If the submitted blood specimen is for an out-ofistate marriage, the state in which the person is going to be married must be put on the data form. The proper premarital health certificate for that state will then be issued.

The approved laboratory may perform the VDRL test for all premaritals. Out-of-state marriage forms (Figure 7-4) may be obtained from the State Hygienic Laboratory upon request. This form should be completed by the referring laboratory and sent to the State Hygienic Laboratory. DO NOT SEND THE SERUM. The proper marriage certificate for the state requested will be sent to the laboratory by return mail. Be sure to affix your approval stamp to each form to insure a rapid reply.

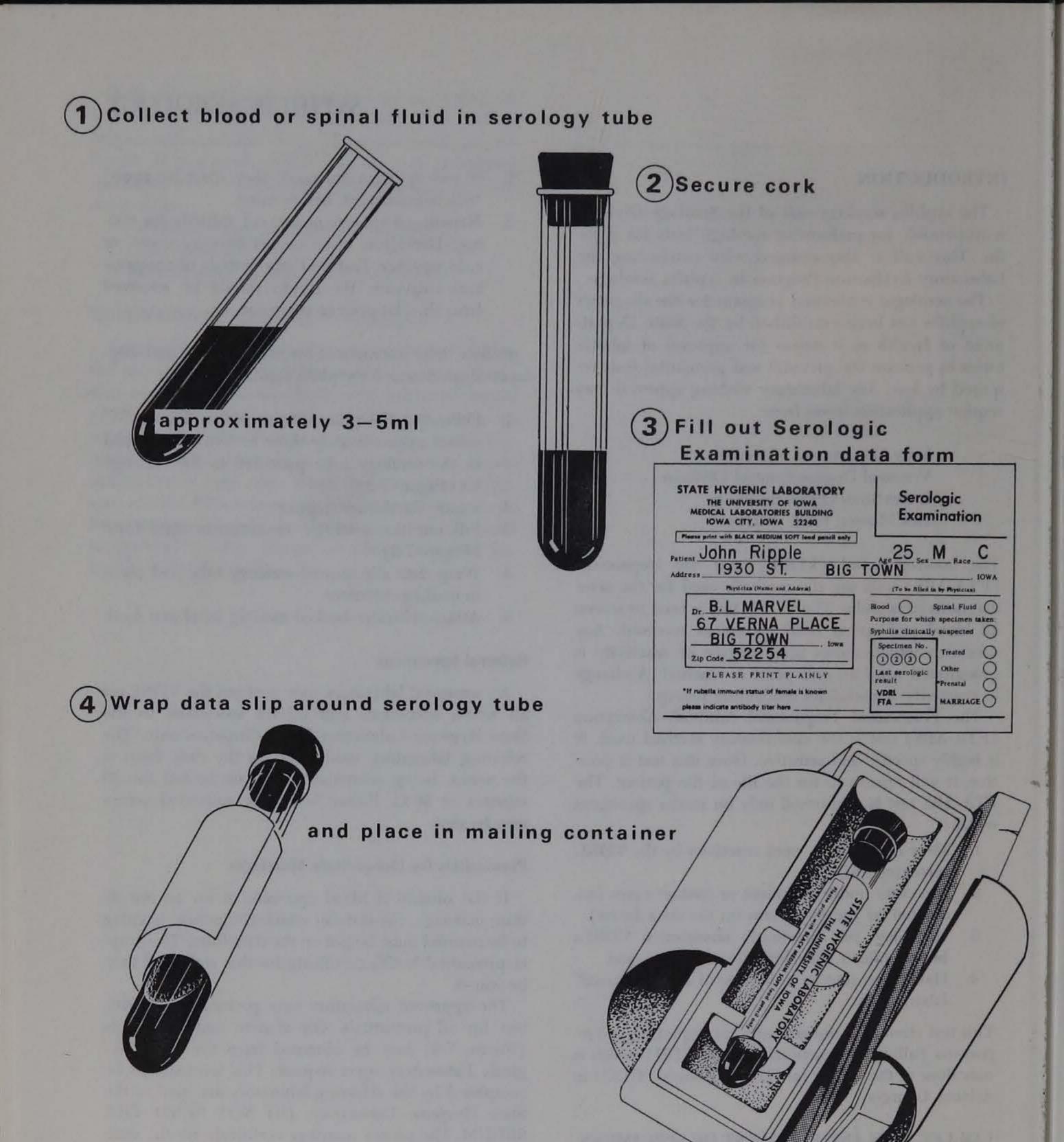


Figure 7-1 Procedure for obtaining syphilis serology blood specimen

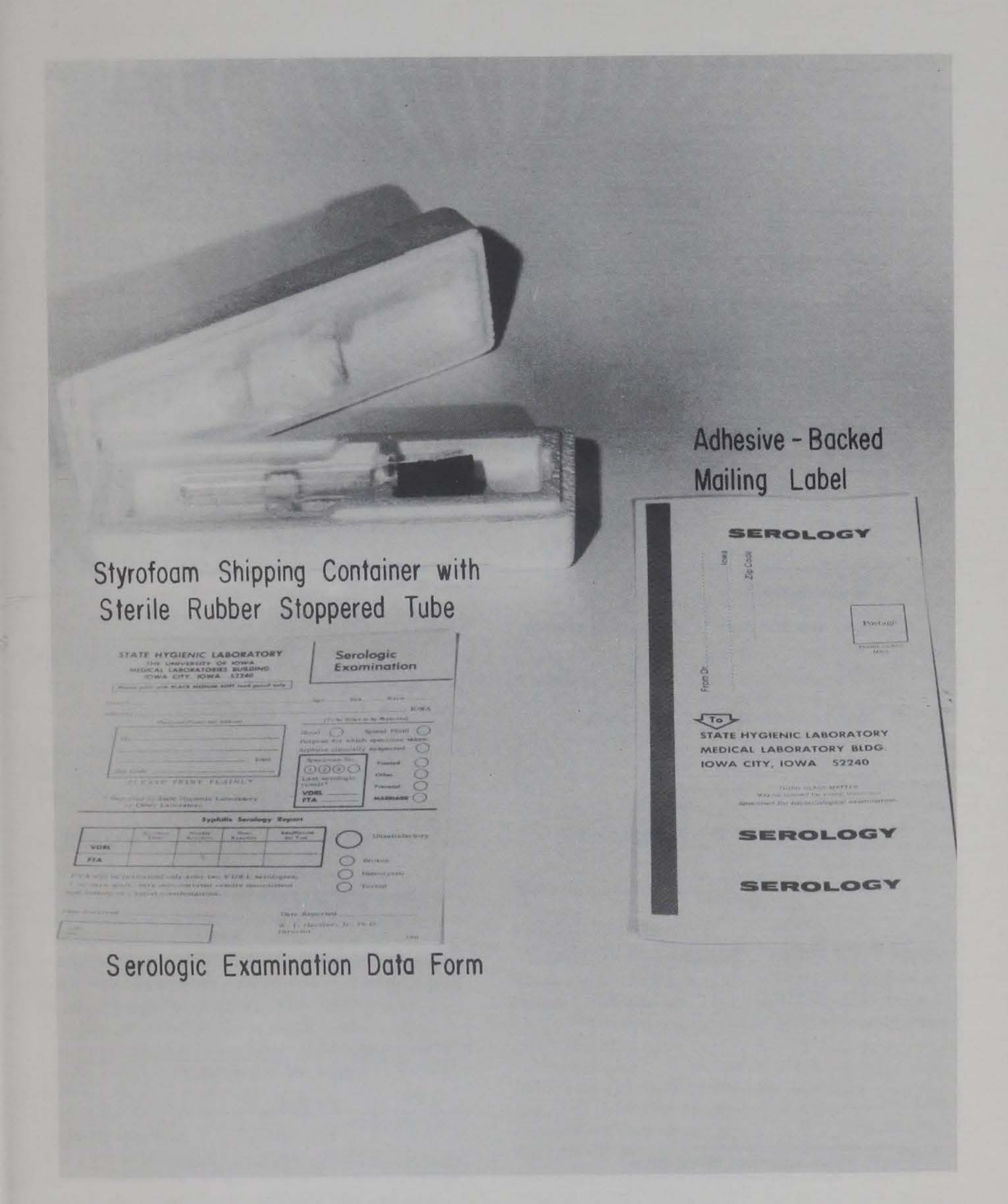


Figure 7-2 Syphilis serology specimen collection kit

INFORMATION NEEDED BY THE STATE HYGIENIC LABORATORY IN COMPLETING REQUIREMENTS FOR OUT OF STATE MARRIAGE

| I) Name | e and address of phy | sician drawing the b | lood and who will sign the certifica |
|------------|------------------------|-----------------------|--------------------------------------|
| | Richard | W Smithson | |
| | 402 Beau | (name) mont Street | |
| | | (street address) | |
| - | Any town | | |
| | | (city) | |
| Poll | Name and address o | f marriage applicant | |
| | | | 1 a la company |
| - | Peter (first) | Raymond (middle) | Johnson (last) |
| | (11131) | (| |
| - | 492 1st Ave | | |
| | | (street address) | |
| | Any town, low | a | |
| | | (city) | |
| λ Λ ~ ~ | sex and race of ma | rriage applicant | |
| n Age, | sex and race of ma | mage appricam. | |
| Age_ | 22 | SexM | Race C |
| Nam. | e of serologic test a | nd result. | |
| i, i talli | c or serving to rost a | | |
| | VDRL | | Nonreactive |
| | (test) | | (result) |
|) Date | specimen drawn an | d examined. | |
| | | | |
| | 4-7-71 | | 4-7-71 (examined) |
| | (drawn) | | (examined) |
| State | in which marriage | is to take place. | |
| | Texas | | |
| | | | |
| | | | Mercy Hospital |
| | | | (Name of Approved Laboratory) |
| | | | (Street address) |
| | | | |
| lease pl | ace approval stamp | here. | (City) |

Figure 7-4 Out-of-state marriage form

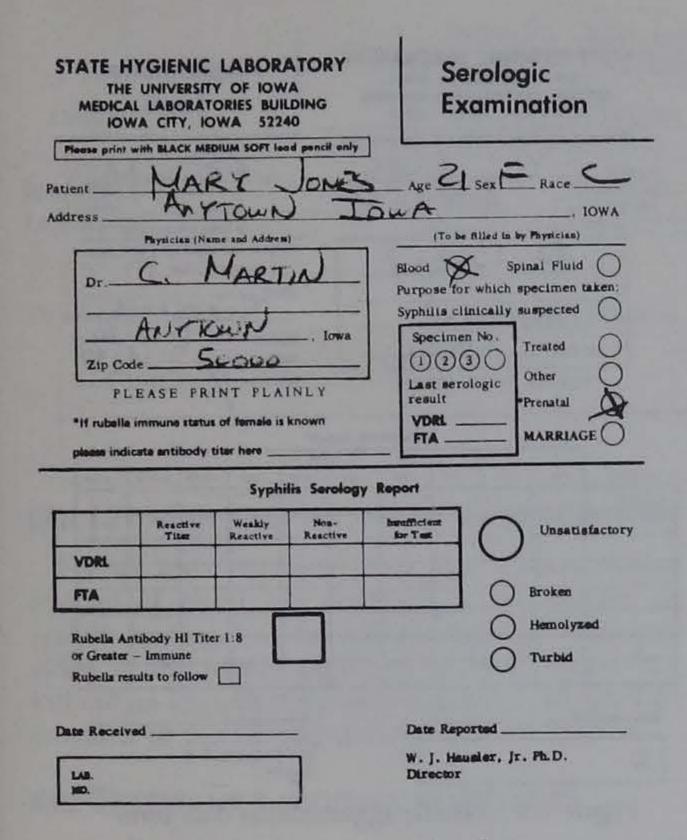


Figure 7-3 Serologic data form

Prenatal and Rubella Test

The State Hygienic Laboratory has expanded its rubella program to include all prenatal sera which are sent to this laboratory for VDRL testing. These sera will be screened for Rubella Hemaglutination Inhibition Antibody (HI). Details are given in the section on services for viral and rickettsial diseases.

REPORTING OF LABORATORY RESULTS

Results of the VDRL and/or FTA-ABS tests are reported to the physician or laboratory submitting the specimen on a copy of the serologic examination form (Figure 7-3). The VDRL will be reported as either reactive (titer given in the reactive titer column), weakly reactive or nonreactive (a check mark is made in the appropriate column). The results of the FTA-ABS test are reported by a check mark in either the reactive or nonreactive column. A check mark in the weakly reactive column indicates that the fluorescence is on the borderline of reactivity and another specimen should be submitted in seven to 10 days. Prenatal rubella HI results of 1:8 or greater are recorded on the serologic examination form by checking the large square below the VDRL and FTA results (Figure 7-5). If the patient has an antibody titer less than 1:8, "rubella results to follow" is checked, and the result

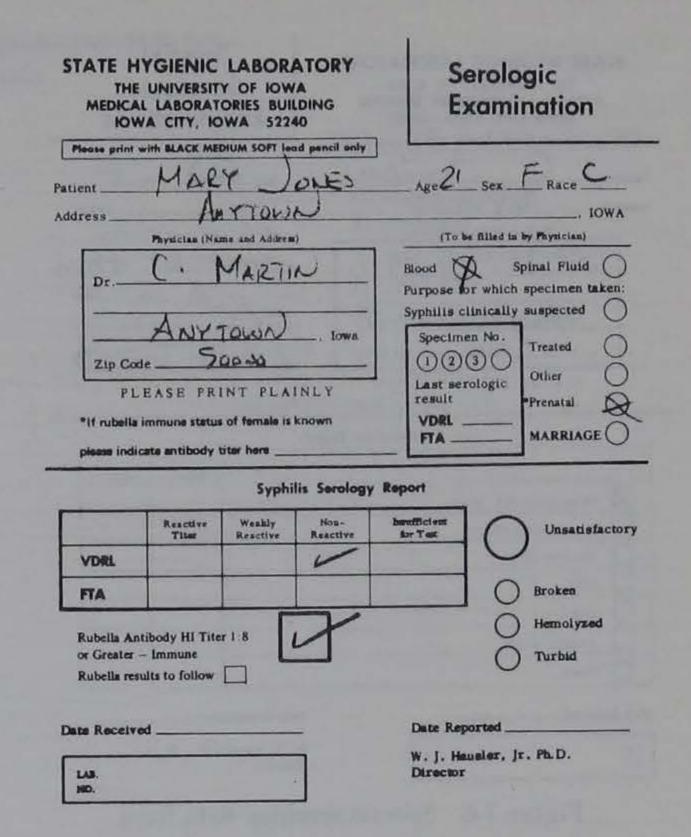


Figure 7-5 Report of serologic results

is sent on a rubella HI test form along with the "Explanation of Rubella Hemaglutination Inhibition Test and Reporting" and a self-addressed reply card to be used after the patient has delivered. Completion of these data will permit an assessment of the value of the HI program. If the rubella-immune status of the female is known, be sure to indicate the antibody titer as requested on the serologic examination form.

SPECIAL SEROLOGY

INTRODUCTION

Special serologic procedures for the sero-diagnosis of infectious mononucleosis, trichinosis, rheumatoid arthritis, antistreptolysin "O," Anti D Nase B (ADB), CRP and toxoplasmosis are routinely performed in this unit.

The Davidsohn presumptive test is routinely performed on all sera submitted for infectious mononucleosis to determine the titer of the total heterophile antibody content of the serum. If a significant antibody titer is detected in the presumptive test, Davidsohn's differential test is carried out to differentiate infectious mononucleosis from Forsmann and serum sickness antibodies.

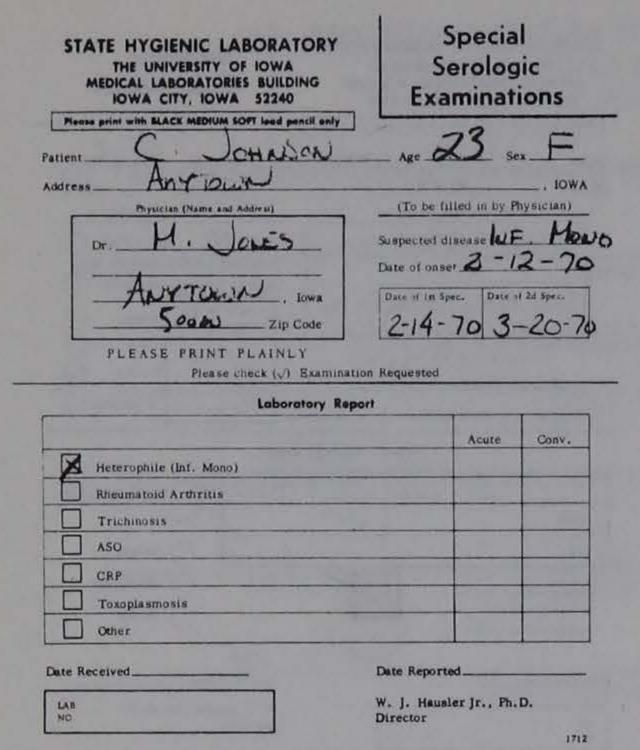


Figure 7-6 Special serology data form

A rapid slide screening test is used for the detection of trichinosis antibodies, rheumatoid arthritis, and C Reactive Protein (CRP). Reactive results are not titered.

Sera submitted for an ASO test are also tested for ADB antibodies. The combined use of these two tests may increase the detection rate of Group A streptococcus infections to 95%, as opposed to an 80% detection rate when screening for the ASO antibody only.

The Indirect Hemagglutination (IHA) test is used as a screening test for toxoplasmosis; all sera showing a positive IHA test are then titered by the Indirect Fluorescent Antibody test.

COLLECTION AND SHIPMENT OF SPECIMENS

For all tests requested from this section, submit five ml of blood (as described in the section entitled "Syphilis Serology") in the rubber stoppered tube provided in the serology kit (Figure 7-2). Fill out a special serologic examination data form (Figure 7-6) and indicate the test desired.

REPORTING OF LABORATORY RESULTS

Results on any of the special serologic examinations are reported only to the physician or laboratory sub-

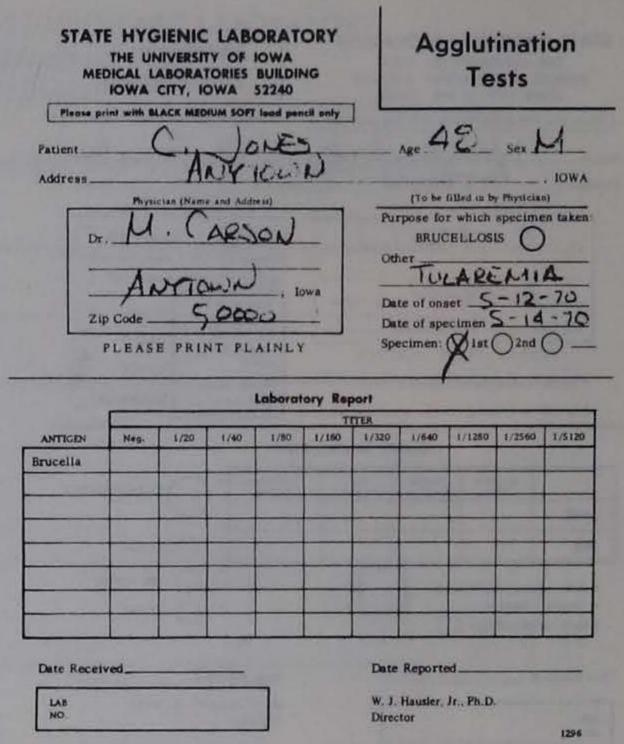


Figure 7-8 Febrile agglutination data form

mitting the specimen. Heterophile antibodies are reported as nonreactive or reactive with the titer given. Rheumatoid arthritis and trichinosis results are reported as either reactive or nonreactive. ASO and ADB results are reported as nonreactive or reactive, given in Todd units; with every reactive result, the explanation is given that single titrations yielding a titer of 170 units or less may be considered as evidence against rheumatic fever. CRP results are reported as nonreactive or reactive, reactivity being expressed from 1+ to 4+, determined in mm of precipitate in the column (1+=1 mm). The results for toxoplasmosis are reported as nonreactive, or if positive, as the highest titer that shows fluorescence.

FEBRILE AGGLUTINATION

INTRODUCTION

Agglutination tests for some febrile diseases are performed in this unit. These include tests for brucellosis, tularemia and leptospirosis. The test for brucellosis is performed on all sera for which any of the three febrile agglutination tests have been requested.

Table 7-1 Battery of agglutination tests for febrile diseases

| Group | Diseases | Antigens |
|------------|---------------|---------------------------------------|
| Brucella | Brucellosis | Brucella abortus |
| Leptospira | Leptospirosis | L. pomona L. icterohaemorrhagiae |
| Tularemia | Tularemia | L. canicola Pasteurella tularensis |

COLLECTION AND SHIPMENT OF SPECIMENS

For all tests requested from this section, submit five ml of blood (as described in the section on syphilis serology) in the rubber stoppered tube provided in the febrile agglutination kit (Figure 7-7). Fill out an agglutination tests data form (Figure 7-8) provided in the kit and indicate the test desired.

REPORTING OF LABORATORY RESULTS

Brucellosis and tularemia are reported as negative if the titer is less than 1:20. Reactive results are reported as the highest dilution which gives 50 percent or more agglutination, and this titer is written on the data slip. Leptospirosis is reported as negative when the titer is less than 1:10. If reactive, the titer is recorded on the data slip as the highest dilution that gives 50 percent or greater agglutination. Results are reported only to physicians or laboratories submitting the specimen.

Styrofoam Shipping Container With Rubber Stoppered Tube Agglutination Porpose for which specime
MADCHALOMS Specimen: O set O 200 O _ PLEASE PRINT PLAINLY Laboratory Report W. J. Hicoster, Jr., Ph. D.

Agglutination Data Form

AGGLUT STATE HYGIENIC LABORATORY MEDICAL LABORATORY BLDG. IOWA CITY, IOWA 52240 THERE CLASE MATTER
May be served for postal imposition
times for becoming of accountation AGGLUT AGGLUT

> Adhesive-Backed Mailing Label

Febrile agglutination specimen collection kit

INTRODUCTION

The most important function of a public health viral diagnostic laboratory is to establish the etiologic agent(s) responsible for outbreaks of disease. Laboratory results are useful in aiding physicians in their diagnosis of illness that cannot be differentiated clinically. Similar symptoms have been produced by a variety of viruses, and, conversely, a virus may be capable of producing a variety of symptoms. There are still many diseases for which the etiology is presently unknown. Information resulting from virologic examination of clinical specimens is also important for epidemiologic investigations.

There are three avenues of approach to the laboratory diagnosis of viral infections. Tissues may be examined microscopically for pathological changes or for the presence of viral material. Serological tests can be performed to demonstrate the appearance or rise in titer of specific antibodies during the course of illness. Finally, viruses can be isolated and identified from clinical materials.

COLLECTION AND SHIPMENT OF SPECIMENS

Table 8-1 (page 63) has been prepared as a guide to ensure that specimens from the proper source will be submitted. Current tests available are also listed in this table. A summary of this table has been included for your convenience in requesting services from the virology laboratory (Tables 8-2 and 8-3).

The virus laboratory can best serve the physicians of Iowa if the following procedure is used:

- Collect the specimens listed in Table 8-1 for a particular disease.
- The acute blood specimen should be taken at onset of illness. Acute blood specimens obtained one week later are of little diagnostic value.
- Convalescent blood specimens should be collected at least two weeks after onset of illness.
 It is not necessary to wait for the convalescent blood before shipping clinical materials.
- 4. Please fill in the patient history form (Figure 8-1) and the data form (Figure 8-2). Complete one data form for each specimen submitted, but

- only one history form need be completed for each patient.
- A clinical diagnosis should be made on all patients for such conditions as aseptic meningitis, herpangina, pleurodynia, etc. Do not write "Coxsackie infection" or "virus studies."

Please observe instructions for the proper collection and shipment of specimens for virus isolation and the instructions for submitting blood specimens for viral and rickettsial serology. It is important to remember that specimens for virus isolation must be kept refrigerated or frozen promptly if they cannot be transported immediately to the Laboratory because most viruses are rapidly inactivated when separated from their host.

Specimens For Viral Isolation

- Fill in one patient history form for each patient. Complete one data form for each specimen submitted and identify each specimen with: name, kind of specimen, date collected.
- 2. Collect feces from patients as soon after onset

| THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 | Virus Examination (Complete one data card for each specimen submitted) |
|--|---|
| Please print with BLACK MEDIUM SOFT lead pencil only atient ANYTOWN Physician (Name and Address) Dr. M. JONES ALLYTOWN, lowa Zip Code Scroop PLEASE PRINT PLAINLY Furnish clinical history with provisional diag | Age 3C Sex M [To be filled in by physician] Suspected disease ASEPTIC MELBAGITIS Date of onset Z-12-71 Type of specimen CSF |
| test(s) to be performed. Laboratory Re | |
| test(s) to be performed. | |

Figure 8-2 Virus & rickettsial data form

STATE HYGIENIC LABORATORY University of Iowa Medical Laboratory Building Iowa City, Iowa 52240

PATIENT HISTORY
Complete one history for each patient

| Number | | Ago | Sav | |
|--------------------------|--|--|--|--|
| Patient | | Age | Sex | |
| Address | | City | | |
| Date of Onset | SONAL DIACNOS | | pation | |
| CLINICAL OR PROVI | SIONAL DIAGNOS | 13 | | |
| | | | | |
| VIRAL IMMUNIZATIO | ONS & DATES | | | |
| Polio: Salk# | | Measles | | |
| Oral # | | | Mumps | |
| Rubella (German Measles) | | Smallpox | | |
| SIGNS AND SYMPTO | OMS | | | |
| Fever: | 1110 | O Duration | | |
| | Sweating | | ain/Myalgia | |
| Rash: Type | | the state of the s | s membrane lesions | |
| Respiratory: | Rhinits | Pharyn | and the same of th | |
| Kespiratory. | Cough | | adenopathy | |
| | Chest pain | | | |
| Gastrointestinal: | The state of the s | Consti | pation | |
| | Abdominal pain | Vomiti | | |
| | Appetite | Nause | | |
| Cardiovascular: | Mycocarditis | Perica | rditis | |
| Central Nervous | Headache | Optic | | |
| System: | Balance | | weakness | |
| | Leghargy | Nucha | I rigidity | |
| Other: | 9 | - Line | | |
| | | 0.00 | | |
| EPIDEMIOLOGICAL | DATA | | | |
| Recent Travel (Lo | ocation) | | A STATE OF THE STATE OF | |
| Family Contacts_ | | | the section of the se | |
| Community Cont | | | The state of the s | |
| Animal and/or a | | | | |
| Group Activities | | | | |
| Food Outbreaks_ | | | | |
| | NICODALATIONI DI | | of this form | |
| OTHER PERTINENT I | NFORMATION - PI | ease use back | or this form. | |
| | | Dr | | |
| | | | THE RESERVE OF STREET | |
| | | - | | |
| | | Zip Code | | |
| | | Zip Code | | |

Figure 8-1 Virus & rickettsial patient history form

of illness as possible. Place walnut-size specimen in a one-oz, screw-cap bottle, secure cap firmly to prevent leakage, label and store in freezer. DO NOT USE PLASTIC CONTAINERS. The details of this procedure are shown in Figure 8-3.

3. Spinal fluids for virus isolation should be collected in sterile tubes and frozen immediately.

DO NOT USE PLASTIC TUBES. Figure 8-4 illustrates the procedure for collection and

shipping of spinal fluid.

4. Throat washings are collected by asking the patient to gargle with approximately 10 ml of sterile broth. After vigorous gargling, the broth is expectorated into a clean cup. Also expectorate into the cup any sputum that is coughed up. Repeat the gargling with broth once or twice and pour the total yield into a glass container, tighten screw-cap and freeze immediately.

oropharynx vigorously with two sterile swabs. The specimen should include material from the posterior pharynx, the tonsils and the faucial pillars. Transfer the swabs immediately to a test tube containing four to five ml of sterile nutrient broth and agitate vigorously. DISCARD THE SWABS after squeezing dry by pressing against the side of the tube. Freeze immediately. For a detailed procedure on obtaining throat swabs for viral isolation, see Figure 8-5.

6. Vesicular fluids and cellular material from the base of lesions must be collected during the first three days after the eruption appears. Wash vesicle with sterile saline and aspirate the fluid using a 26-27 gauge needle attached to a tuberculin syringe or use a capillary pipette. Either instrument used should be first moistened with broth by drawing broth into the syringe or capillary pipette and expelling it. Dilute the fluid immediately into sterile broth to prevent clotting. Alternately use sterile swab to absorb fluid from vesicles and then agitate swab vigorously in sterile broth. Discard swab, tighten screw-cap on tube and freeze.

7. Blood samples are sometimes submitted for virus isolation if collected in the very early stage of illness. In this case, allow blood to clot for at least ½ hour in a refrigerator. Free the clot from the wall of tube with the aid of sterile applicator stick or capillary pipette. Centrifuge and transfer the clear serum to a sterile tube. After the serum has been separated from the clot, freeze both immediately.

8. When shipping, isolate each specimen individually by placing in a plastic "whirl-pak" bag before putting specimens in the styrofoam container. Pack about 10 pounds of dry ice on top of the specimen(s), close lid and seal the cardboard shipping carton by fastening the buckles of the nylon straps. This styrofoam shipping container and its double cardboard cartons are reusable so please do not discard or dismantle. The shipping unit is shown in Figure 8-6. Use the pre-addressed label provided and send by fastest means of transportation to:

State Hygienic Laboratory
The University of Iowa
Medical Laboratory Building
Iowa City, Iowa 52242

Usually, Iowa Parcel Service, United Parcel Service or Parcel Post Special Delivery have been satisfactory. To avoid delay enroute, DO NOT SHIP ON WEEKENDS.

 If for any reason, you have questions concerning collection or shipment of specimens, please call the virus laboratory, 319/353-5990.

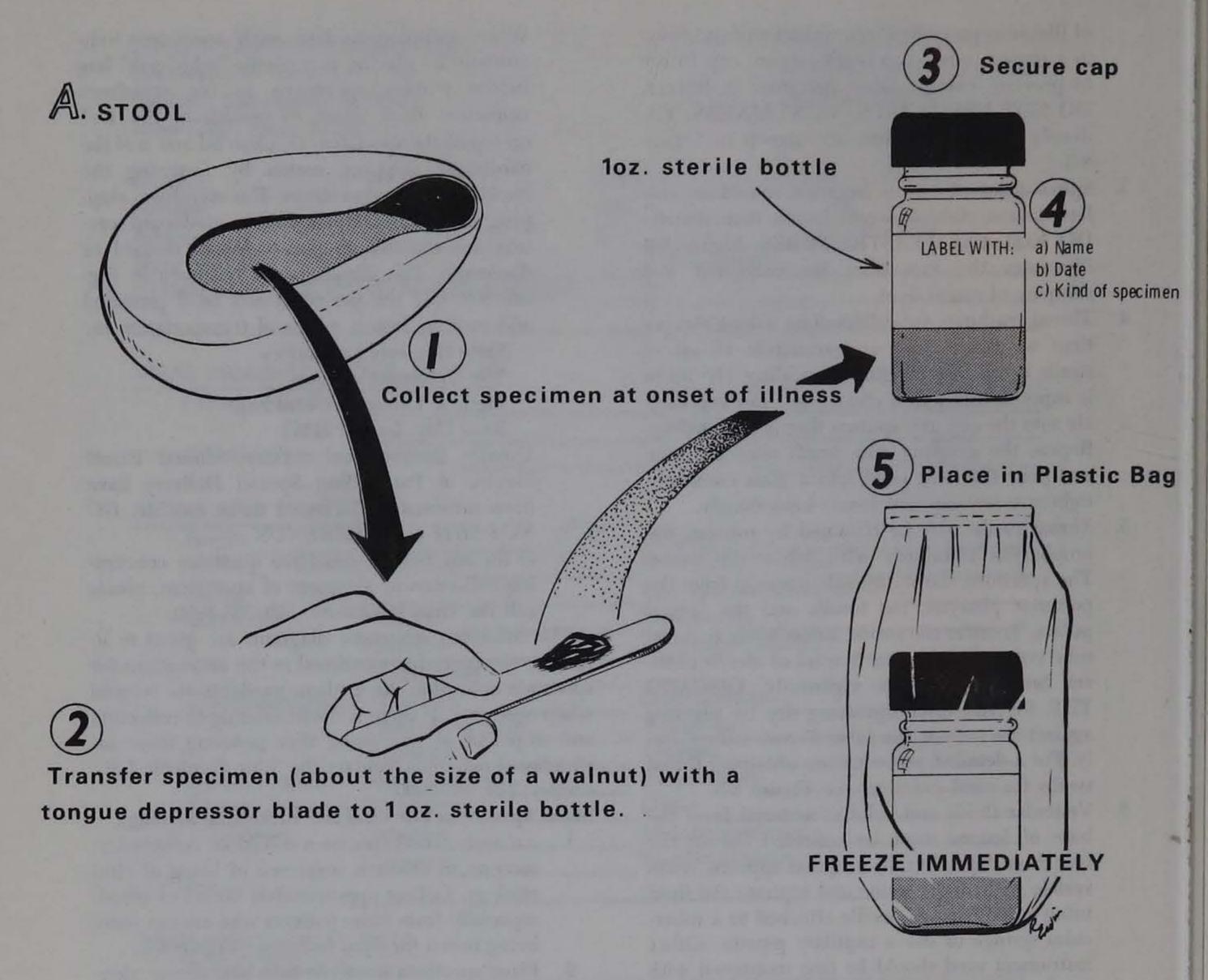
The following schematic diagrams are given to illustrate the procedures outlined in the instructions for specimen collection. Keep them available for referral when necessary. If there is doubt relating to collection and shipment of specimens after perusing these instructional materials, contact the State Hygienic Laboratory, 319/353-5990.

Blood Specimens For Viral and Rickettsial Serology

1. An acute blood specimen should be collected as soon as an illness is suspected of being of viral etiology. Collect approximately six ml of blood, especially from those patients who are currently being tested for virus isolation (Figure 8-7).

Place specimen in sterile tube and secure stopper. DO NOT FREEZE whole blood specimens.

 There are areas in Iowa where it is impossible to obtain dry ice. Under these circumstances, the proper use of cans of frozen water or other coolants will keep the specimens chilled during transit. These "freezer paks," "sno-gels" or "freezer cans" are sold in grocery and hardware stores. When using these types of coolants, it is important to keep them in the freezer until just before the frozen specimen is packed in the shipping container. The shipping container should be sent to the bus depot, airport or post office shortly before scheduled departure. If precautions are taken so that specimens are not more than 18 hours in transit from the time the frozen coolant is removed from the freezer, the specimens should arrive in the laboratory in satisfactory condition.



6 FILL OUT data card for EACH SPECIMEN:
Complete only that portion of data card shown below

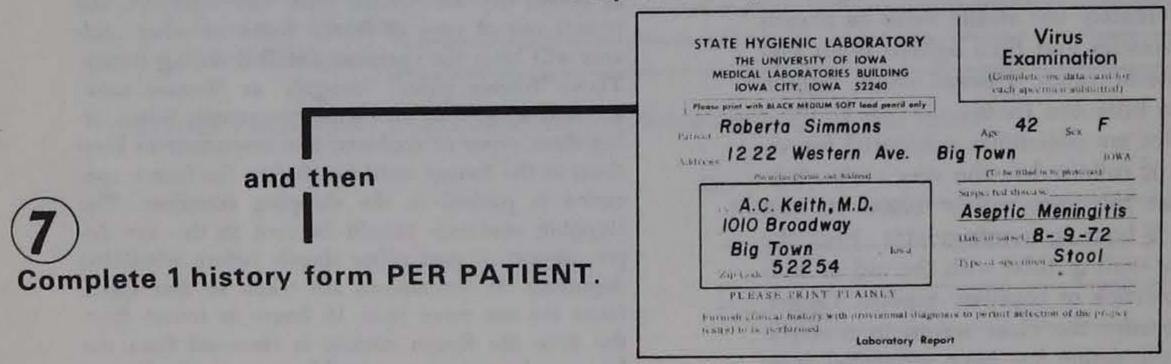


Figure 8-3 Procedure for collection of V & R fecal specimen

SPECIMENS FOR VIRUS ISOLATION

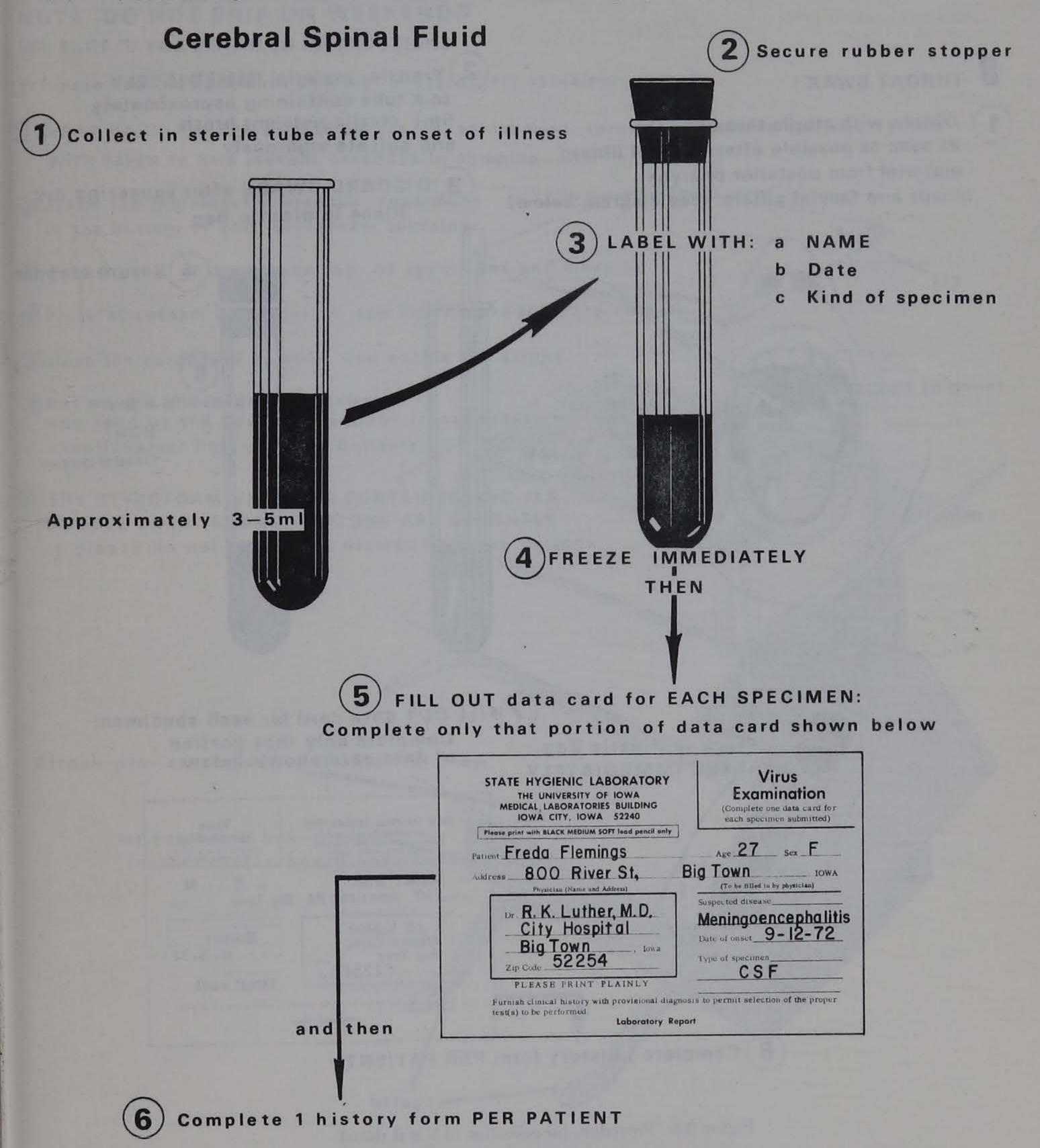
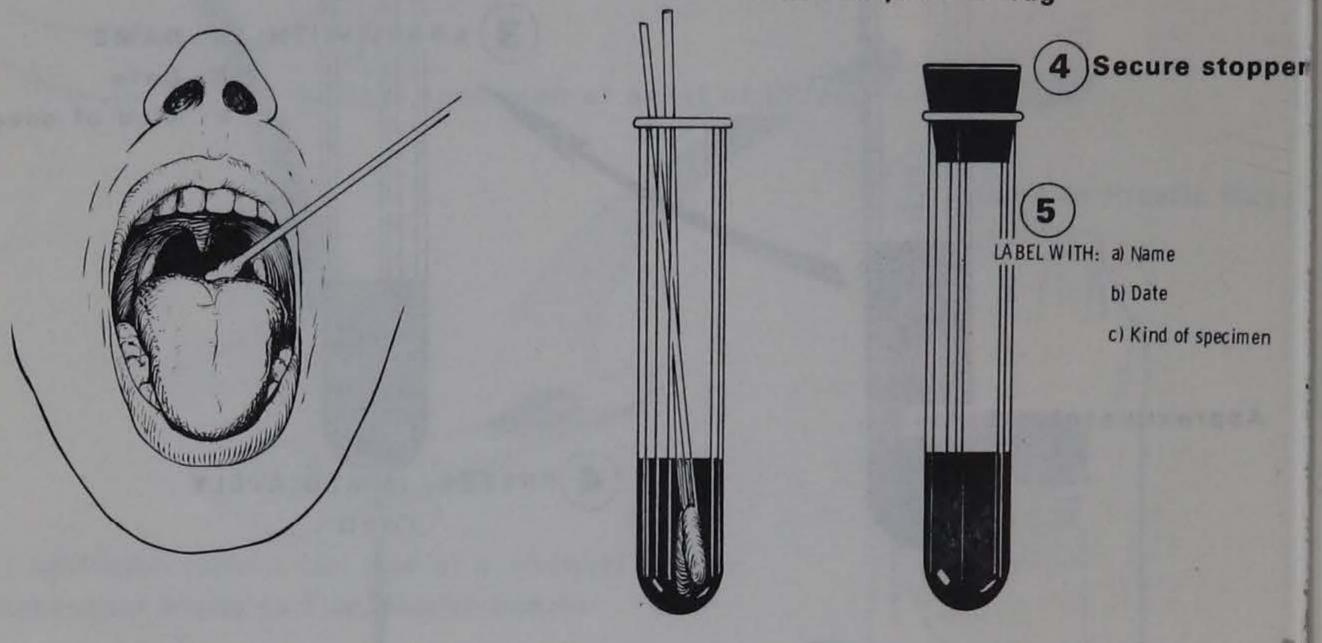


Figure 8-4 Procedure for collection of V & R CSF specimen

Specimens for VIRUS ISOLATION

- B THROAT SWAB
- 1 Obtain with sterile throat swabs, as soon as possible after onset of illness material from posterior pharynx, tonsils and faucial pillars. (see diagram below)
- 2 Transfer material IMMEDIATELY to a tube containing approximately 5ml sterile nutrient broth and agitate vigorously
 - 3 DISCARD SWABS after squeezing dry
 Place in plastic bag



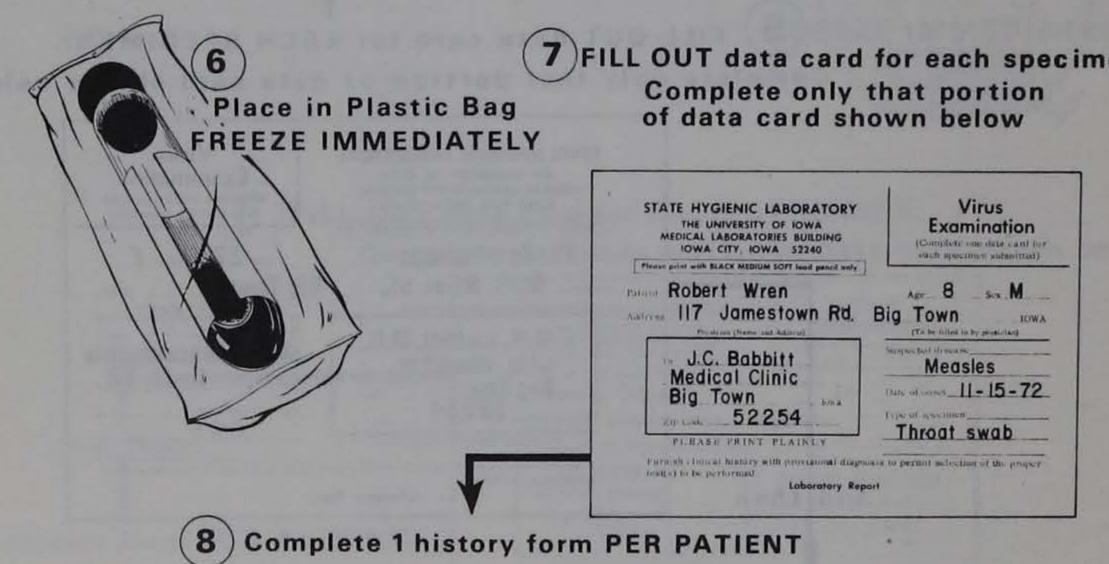


Figure 8-5 Procedure for collection of V & R throat specimen

SHIPPING INSTRUCTIONS: NOTE: DO NOT SHIP ON WEEKENDS

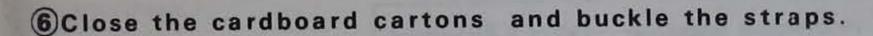
SEE PAGE 57 FOR DETAILED INSTRUCTIONS

- 1) Freeze specimens before packing in styrofoam container.
- ② WRAP EACH FROZEN SPECIMEN (feces, spinal fluid, throat swab, blood clot and serum)
 WITH PAPER to help prevent breakage in shipping.

Lid

Wrapped in paper

- 3 Place the individually wrapped specimens in the bottom of the styrofoam container.
- 4 Pack 10 lbs. of Dry ice on top of specimens and close lid
- 5 Place styrofoam container in the 2 cardboard shipping cartons.

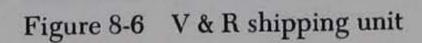


Put on the pre-addressed label and send by the fastest means of transportation usually Parcel Post-Special Delivery

8 THE STYROFOAM SHIPPING CONTAINER AND ITS DOUBLE CARD- BOARD CARTONS ARE REUSABLE so please do not discard or dismantle these cartons.

Attach pre-addressed label on outer flap

1st cardboard box-placed inside 2nd cardboard box



Straps

VIRAL and RICKETTSIAL SEROLOGIC EXAMINATION

Collecting and shipping of specimens

Collection of Acute Blood specimen: Collect blood as soon as possible after onset of illness(use aseptic technique). FILL OUT data card for EACH SPECIMEN: Complete only that portion of data card shown below Approximately 6ml of blood Virus & Rickettsial STATE HYGIENIC LABORATORY THE UNIVERSITY OF IOWA MEDICAL LABORATORIES BUILDING IOWA CITY, IOWA 52240 Serologic Examinations Sterile tube Flance print with BLACK MEDIUM SOFT lend pentil only Patron Walter Fox Address 827 Rush St. Big Town Influenza Date of onset 3-15-70 R.T. Abel, M.D. 123 Professional Bldg Big Town 3-17-70 4-1-70 Zip Code Secure stopper CNS Respiratory Other (Please Specify) Please DO NOT write he line Laboratory Report Wrap data card around test tube containing the whole blood and place in a special styrofoam mailer .-Kettsial Wrap V&Rself-adhesive shipping label around mailer to seal it. 6)MAIL IMMEDIATELY (same day the blood is collected)

Figure 8-7 Procedure for obtaining V & R blood specimen

DO NOT FREEZE WHOLE BLOOD

to the STATE HYGIENIC LABORATORY

- 3. Complete one patient history form for each patient. Fill out one data card for each specimen. Complete the data card shown in Figure 8-2.
- 4. Wrap the data card around the test tube containing the whole blood specimen and place in the special styrofoam mailer with the patient history form. Then wrap the "V and R" coded label around the mailer.
- 5. Mail immediately to the:
 State Hygienic Laboratory
 The University of Iowa
 Medical Laboratory Building
 Iowa City, Iowa 52242

Collect convalescent blood specimens 14-28 days after onset of illness. Then follow the same procedure for collection and shipment as you would for an acute specimen.

REPORTING OF LABORATORY RESULTS

The virus laboratory does not make a diagnosis. This is the sole responsibility of the physician. The laboratory report may confirm or serve to support clinical observations.

Virus Isolation. The failure to isolate a virus from a specimen does not indicate that the suspected agent is absent or that a particular diagnosis can be eliminated, since successful isolations are dependent on the time of collection and shipment of the specimens as well as preparation for inoculation and host system employed. Virus isolations usually indicate a recent infection, but caution must be exercised since the isolate may have caused an inapparent infection while not having produced the clinical symptoms observed. Therefore, it is extremely important to correlate the virus isolated with serologic results as well as clinical and epidemiologic data.

Serologic Results. A four-fold rise in antibody titer in the convalescent serum that has been tested in parallel with an acute phase serum is of significance in determining the importance of a virus isolated with the current illness of the patient. Similarly, only paired sera are of diagnostic value when tested against other viral or rickettsial antigens. A single serum is of little importance since many individuals may have antibodies against certain viruses from previous exposure. Acute phase serum must be taken as early as possible in the illness to help establish the correct base line for evaluating serologic results.

Sometimes, in cases of mumps, it is possible to arrive at a presumptive diagnosis with an acute serum. This is accomplished by the use of two specific mumps antigens, the viral (V) antigen and the soluble (S)

antigen. Antibody for the S antigen rises frequently before antibody against the V antigen, but the V antibody persists for longer periods of time. Therefore, the presence of a significant anti-S titer and a low or no anti-V titer is very suggestive of a current mumps infection. A convalescent serum should still be examined in such a case.

Results will be reported on the data card and returned to the sender.

RUBELLA HI TEST

INTRODUCTION

The State Hygienic Laboratory screens prenatal blood specimens received in the Iowa City Laboratory for rubella antibody by the "CDC Standard Rubella Hemagglutination Inhibition Test." A report indicating the presence or absence of HI antibody will be sent to the physician submitting the specimen. The State Hygienic Laboratory will also screen prenatal blood specimens from laboratories approved to perform prenatal serology tests for syphilis but who do not perform the rubella HI test.

COLLECTION AND SHIPMENT OF SPECIMENS

- Collect approximately six ml of blood. (Use aseptic technique.) DO NOT FREEZE WHOLE BLOOD.
- 2. Place specimen in the sterile tube and secure the stopper.
- 3. Fill out a data card for EACH SPECIMEN. Complete only that portion of data card shown in Figure 8-8.
- 4. Wrap the data card around the tube containing the whole blood and place in the special styrofoam mailer which is provided in the rubella HI specimen kit (Figure 8-9). Wrap the rubella-coded, adhesive-backed label around the mailer.
- 5. MAIL IMMEDIATELY (same day the blood is collected) to:

State Hygienic Laboratory
The University of Iowa
Medical Laboratory Building
Iowa City, Iowa 52242

REPORTING OF LABORATORY RESULTS

A 1:8 dilution is the lowest dilution of serum tested in this program. Therefore, a titer of 1:8 or greater indicates detectable antibody or immunity to rubella. The lack of HI antibody in a dilution of 1:8 should

be considered as susceptibility to rubella. If a female is found to have rubella HI antibody, no risk from rubella exists for the fetus she is presently carrying or in any future pregnancy. The fact that she has detectable antibody should be recorded in her medical history and the rubella HI test not requested in the future. If she is susceptible, a rubella risk does exist during the present pregnancy; however, no attempt should be made to alter her immune status until after delivery. Soon after she is delivered she may be immunized against rubella.

Prenatal serum specimens containing rubella HI antibody will be reported on the serologic examination form (Figure 7-3) with an indication that a rubella HI titer of 1:8 or greater was found. Specimens showing no detectable rubella HI antibody will be reported on a rubella HI test form (Figure 8-8) indicating a titer of less than 1:8. Accompanying this report will be an information sheet and a reply card. The reply card is to inform the State Health Department of the course of action chosen for the susceptible patient. The physician is to complete this card and send it to the State Department of Health at his or her earliest convenience.

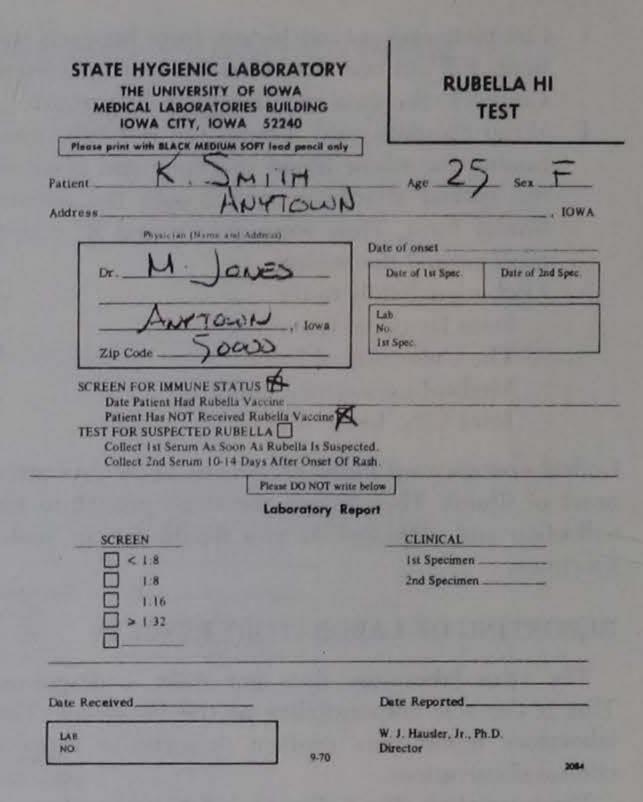


Figure 8-8 Rubella HI data form

Will Street on the second second second

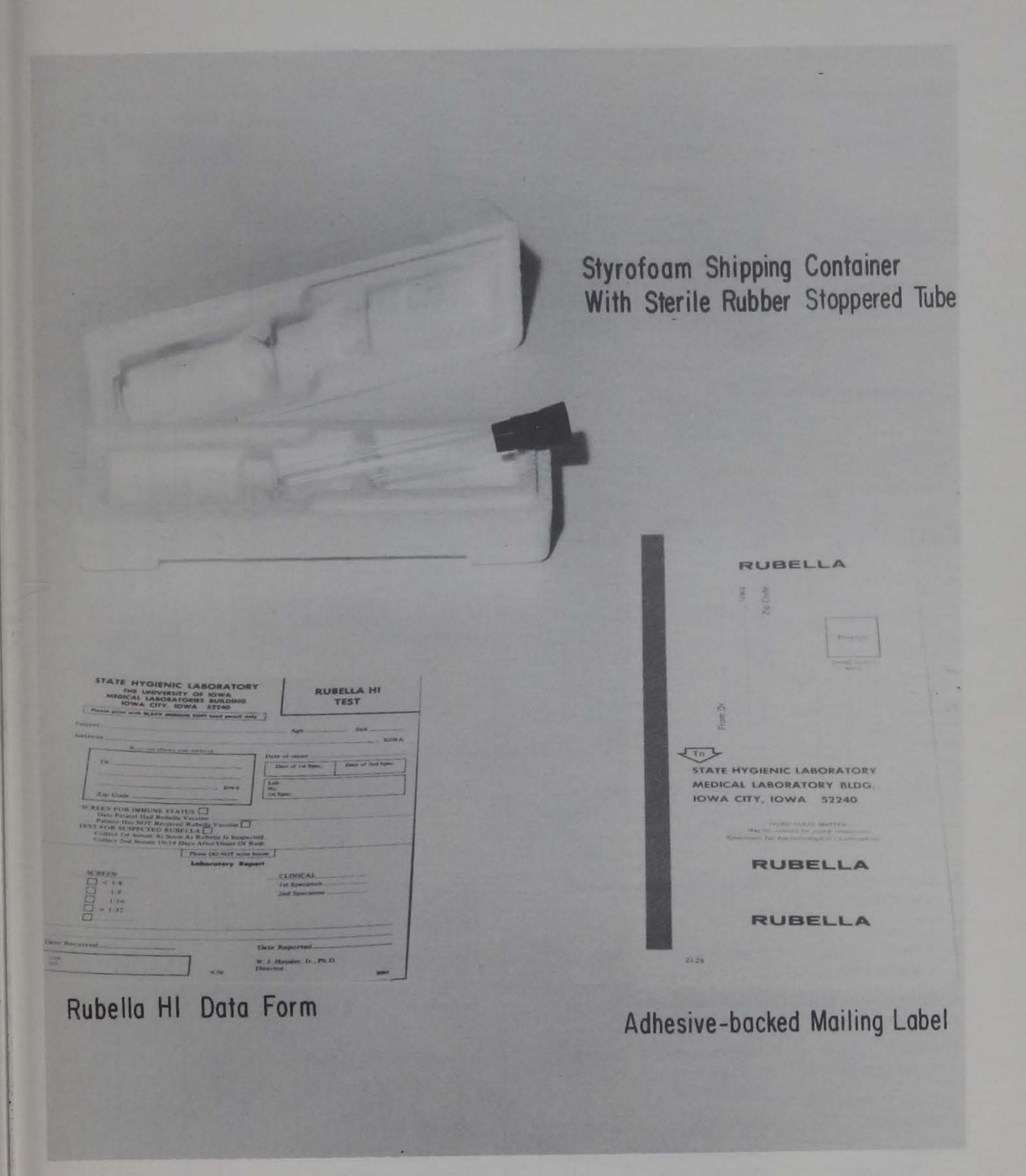


Figure 8-9 Rubella HI Collection Kit

Table 8-1 Tests available for viral and rickettsial diagnosis

| DISEASE | | SPECIMENS FOR ISOLATION Clinical Post Mortem | | AGENTS | | SEROLOGY TESTS AVAILABLE* CF FA HI Neut Other | | | |
|---------|---|---|---|--|-----------------------|---|------------------|------------------|------|
| | | | | | CF | FA | HI | Neu | Othe |
| | | C | ENTRAL NERV | OUS SYSTEM | | | | | |
| 1. 4 | | Throat washings, spinal fluid, stool | Brain cortex, blood, spinal cord and fluid, feces, segment of colon | Polioviruses Coxsackie viruses ECHO viruses Herpesvirus Lymphocytic choriomeningitis virus Mumps virus | X X X | X | X | X O O X | |
| 2. | 0 | Same as 1., also acute phase clotted blood | Same as 1. | Eastern Encephalitis virus Western Encephalitis virus California Encephalitis virus St. Louis Encephalitis virus Measles virus Mumps virus Rubella virus | X X X X X | | X X X X | | |
| 3. | Paralytic disease | Same as 1. | Same as 1. | Polioviruses Coxsackie viruses ECHO viruses | X | | | X O O | |
| 4. | Guillian-Barré | Same as 1. | Same as 1. | Coxsackie Group A viruses ECHO viruses | | | | 0 | |
| | | | RESPIRATOR | Y SYSTEM | | | | | |
| 1. | Influenza | Throat washings (freeze immediately) | Tracheo- bronchial swab, lung tissue | Influenza viruses | X | X | X | | |
| 2. | Acute respiratory infection | Throat washings | Same as 1. | Adenoviruses Parainfluenza viruses Respiratory Syncytial virus | X X X | | x | 0 | |
| 3. | "Croup" laryngo- tracheobronchitis | Same as 2. | Same as 1. | Parainfluenza viruses | X | | X | 0 | |
| 4. | Pleurodynia (Bornholm disease) | Throat washings and stool | Same as 1. | Coxsackie Group B viruses | | | | 0 | 0 |
| 5. | Primary atypical pneumonia | Throat washings and acute phase clotted blood | Same as 1. | Eaton Agent (PPLO) | X | | | | X |
| 6. | Psittaeosis | Same as 5. | Same as 1. | Psittacosis Agent | X | | | - 1 | |
| 7. | Others: Herpangina Stomatitis Pharyngitis | Throat washings, stool and swab of oral lesions | Same as 1. | Adenoviruses, Coxsackie viruses or Herpesvirus | X | x | | 0 0 X | 0 |

TABLE 8-1 (Cont.)

| | SPECIMENS FOR ISOLATION | | LEGIS HAT BEEN AND ADDRESS OF | SE | SEROLOGY TESTS AVAILABLE* | | | |
|---------------------------------|--|---|--|----|---------------------------|---|---|-------|
| DISEASE | Clinical | Post Mortem | AGENTS | CF | | | - | Other |
| | | EXANTH | EMS | | | | | |
| 1. Rubella | Throat washings, urine, clotted blood | Liver and spleen | Rubella virus | X | | x | | |
| 2. Measles | Same as 1. | Same as 1. | Rubeola virus | X | | | | |
| 3. Smallpox | Throat washings, vesicle fluid, crusts | | | | | | | |
| 4. Vaccinia | Same as 3. | Same as 1. | Vaccinia virus | | 1 | | | |
| 5. Chickenpox | Same as 3. | Same as 1. | Varicella virus | X | | | | 0 |
| 6. Other | Throat washings, stool and vesicle fluid | Same as 1., also segment of colon | Coxsackie viruses, ECHO viruses or Herpesvirus | | X | | 0 | 0 |
| **** | | RICKETT | SIAL | | | | | |
| 1. Q fever | Blood and sputum | | Rickettsia burneti | X | | | | |
| 2. Rickettsialpox | Blood | | R. akari | X | | | | |
| 3. Rocky Mountain spotted fever | Blood | | R. rickettsi | X | | | | |
| 4. Typhus, murine | Blood | | R. mooseri | X | | | | |
| 5. Typhus, epidemic | Blood | | R. prowazeki | X | | | | |

TABLE 8-1 (Cont.)

| | SPECIMENS F | OR ISOLATION | | SEROLOGY TESTS | | | | |
|----------------------------------|--|--|-----------------------------------|----------------|----|--------------|------|-------|
| DISEASE | SEASE Clinical Post Mortem AGENTS | | AGENTS | - Cur | | CF FA HI Neu | | |
| | I was a second | 1 | | LCF | FA | HI | Neut | Other |
| | | MISCELLAN | NEOUS | | | | | |
| 1. Mumps | Saliva and clotted blood | | Mumps virus | X | | | | |
| 2. Myocarditis | Throat washings and stool | Heart muscle | Coxsackie Group B viruses | | | | O | 0 |
| 3. Pericarditis | Same as 2, and pleural fluids | Same as 2., also pleural fluids | Coxsackie Group B viruses | | | | 0 | 0 |
| 4. Epidemic keratoconjunctivitis | Conjunctival washings or scrapings | | Adenovirus Type 8 | X | | | 0 | |
| 5. Ocular herpes | Same as 4., | | Herpesvirus | X | X | | X | |
| 6. Hepatitis | Serum | | Australian Antigen | | | | | x |
| 7. Lymphogranuloma venereum | Bubo pus, tissue, spinal fluid | | Lymphogranuloma Venereum Agent | X | | | | |
| 8. SSPE | Serum, spinal fluid | | Rubeola | X | | | | |
| 9. Infantile diarrhea | Stool | | Coxsackie and ECHO viruses | | | | 0 | 0 |
| 10. Cytomegalic inclusion | Saliva, urine | The state of the s | Cytomegalovirus | x | x | 1 | 1 | |

^{*}CF = Complement Fixation; FA = Fluorescent Antibody; HI = Hemagglutination-Inhibition; Neut = Neutralization; and Other = Developmental.

X = Tests available on routine basis.

O = Tests performed only when a virus is isolated from clinical materials or with virus known to be prevalent. Specimens must be accompanied by clinical history.

Acute blood specimens are to be collected at onset and convalescent blood specimens two to four weeks later. Paired sera are mandatory to demonstrate whether antibodies have appeared or increased in titer during illness. Do not freeze clotted blood.

Table 8-2 Summary of specimens to collect for laboratory diagnosis of viral and rickettsial infection

| | Paired Sera 10-21-day Interval | Feces | Cerebro- Spinal Fluid | Throat Swab | Other |
|------------------------|--------------------------------------|--------|--------------------------|----------------|--|
| Aseptic meningitis | + | + | + | + | |
| Meningo-encephalitis | + | + | + | + | |
| Paralytic disease | + | + | + | + | |
| Carditis | + | + | + | + | ALL HARMAN |
| Congenital anomalies | | H P SE | | | |
| Cytomegalovirus | + | | | + | Urine |
| Rubella | + | | + | + | Urine |
| Respiratory | + | | | + | |
| Stomatitis | + | + | | + | |
| Eye lesions | + | | | | Scrapings of conjunctiva or cornea |
| Exanthems | + | + | | + | Vesicle material |
| Serum hepatitis | + | | | | |
| Rickettsial infections | + | | | | |

Table 8-3 Summary of viral and rickettsial tests available

| available | | 7 11 1 | | |
|--|-----------|----------|---------|--|
| Visco - Infantion | Isolation | Serology | Adeno | Adenovirus |
| Virus or Infection | Isolation | Serology | CEV | California encephalitis virus |
| Adenovirus | R | R | CF | Complement fixation |
| Arbovirus | ST | R | Cox | Coxsackie virus Group A or B |
| Coxsackie virus | R | ST | Eaton's | Eaton agent (Mycoplasma pneumoniae) |
| Cytomegalovirus | R | R | ЕСНО | ECHO virus (Enteric Cytopathogenic |
| Eaton Agent (PPLO) | R | R | Lerio | Human Orphan virus) |
| ECHO virus | R | ST | DEE | |
| Herpes simplex | R | R | EEE | Eastern equine encephalitis |
| Influenza A and B | R | R | HI | Hemagglutination inhibition |
| Mumps | R | R | Herpes | Herpes simplex |
| Rubeola (measles) | R | R | Infl A | Influenza type A |
| Parainfluenza virus | R | R | Infl B | Influenza type B |
| Poliovirus | R | ST | LCM | Lymphocytic choriomeningitis |
| Poxvirus | R | ST | 30 30 | |
| Psittacosis - LGV Agents | ST | R | LGV | Lymphogranuloma venereum |
| Rabies (Post Immun. Titer) | | ST | Neut | Neutralization |
| Reovirus | R | ST | Psitt | Psittacosis |
| Respiratory syncytial virus | R | R | SLE | St. Louis encephalitis |
| Rickettsial infection | ST | R | WEE | Western equine encephalitis |
| Rubella | ST | R | | Less than |
| Serum hepatitis | - | R | < | The state of the s |
| Varicella - Zoster | ST | R | > | Greater than |
| The second secon | | | 4 | Less than or equal to |

Table 8-4 Abbreviations and symbols

R = Routine Test ST = Special Test

ENVIRONMENTAL SCIENCE SERVICES

The State Hygienic Laboratory offers a wide range of services designed to prevent and monitor the causes of environmental pollution, including programs for air and water pollution, radiological health, pesticides and industrial hygiene. Many of these services are performed on a contractual basis for various state agencies, but most can be done at the request of a private party if scheduling is possible. Unlike the diagnostic services offered by the Laboratory, the majority of these divisions do not have specific sampling kits available, due to the complexity of the equipment and personnel necessary for obtaining environmental samples.

Charges will be assessed according to time involved. In most cases arrangements for scheduling, charges and other details for these services may be made by

contacting the Office of the Director.

The testing services offered for municipal and private water supplies are the exceptions in the Environmental Science Services insofar as mechanics of sampling is concerned. For these services specific kits are available and are described in the following sections.

All of the Divisions in Environmental Science Services, with again the exception of the Water Analysis Division, report their findings in the form of a multipage report, which may be available upon request to the party or agency who initiated the survey or investigation. For municipal and private water analyses, appropriate data slips are used for reporting results.

AIR POLLUTION SURVEILLANCE

INTRODUCTION

The objective of the Air Pollution Surveillance Division is to provide the Iowa Air Quality Commission and the Department of Environmental Quality with data on the quality of air within the state. These data are used to determine whether or not air quality standards are being met as well as to determine the adequacy of those standards. Services to other organizations may be available depending on availability of staff time and equipment.

COLLECTION AND SHIPMENT OF SPECIMENS

Sampling of air contaminants is a complex problem requiring specialized equipment and procedures. Most sampling will require our Laboratory staff to operate the equipment. Therefore, any determinations of air contaminants will be handled on an individual basis after discussions on the scope of individual problems and the equipment capabilities of the Laboratory have been considered. All requests for the services of Air Pollution Surveillance are to be made by contacting:

Director, State Hygienic Laboratory Medical Laboratories Building The University of Iowa Iowa City, Iowa 52242 Telephone: 319/353-5990

Types of services available include measurement of:

- 1. Dustfall
- 2. Coefficient of Haze
- 3. Sulfur dioxide
- 4. Carbon monoxide
- 5. Suspended particulates
- 6. Ozone
- 7. Hydrocarbons
- 8. Fluorides
- 9. Hydrogen sulfide
- 10. Ammonia
- 11. Nitrogen oxides
- 12. Particulate emission
- 13. Gas flow rates in stacks
- Meteorological data (wind speed, direction, barometic pressure, temperature, humidity)
- 15. Particle identification
- 16. Soil analysis for selected pollutants
- 17. Crop analysis for selected pollutants

(Other determinations may be available depending on availability of staff time and equipment.)

REPORTING OF LABORATORY RESULTS

Reports of all findings are submitted in writing to the Iowa Air Quality Commission as well as to other interested parties.

Findings are checked for compliance with state and federal regulations.

INDUSTRIAL HYGIENE

INTRODUCTION

The objective of the Industrial Hygiene Division is to recognize, evaluate and control those environmental factors or stresses arising in or from the work place, which may cause sickness, impaired health and well-being, or significant discomfort and inefficiency among workers or among the citizens of the state. Services are performed primarily for sponsoring state agencies. However, services are available to the public when needed.

All requests for the Industrial Hygiene Division's services are to be made to:

Director, State Hygienic Laboratory Medical Laboratories Building The University of Iowa Iowa City, Iowa 52242 Telephone: 319/353-5990

COLLECTION AND SHIPMENT OF SPECIMENS

Every sample submitted should have adequate descriptions for our records. Please include the following information:

- 1. Name of town or city
- 2. Source of sample
- 3. Specific location where sample was collected
- 4. Date collected
- 5. Collector's name
- 6. Bottle number (unless identification is included on the container)
- What determination is requested
- 8. Any other pertinent information about the sample, conditions of sampling or reason for sampling

The cost will be quoted at the time arrangements are made for analysis. Tests may be run for physicians (e.g., company physicians) even if theirs is not a state problem.

The Des Moines Branch of the State Hygienic Lab-

oratory will run most tests. A preliminary contact with the Laboratory should be made to set a testing date so the samples can be run on the date received. THE SAMPLES ARE PERISHABLE.

Lead in Blood

- 1. Specimens should be drawn under the auspices of a physician.
- 2. A minimum of 10 ml of blood is required per test.
- Thoroughly mix the blood and anticoagulant immediately after drawing.
- 4. Use 10 ml vacutainer specimen tubes with ACG or heparin anticoagulants. If a substitution is required, verify its suitability with the Laboratory as several available anticoagulants interfere with the determination.
- Include one unused tube from the same manufacturer's lot number of specimen tubes as a control with each series of tests.
- 6. Keep the samples iced and deliver to the Laboratory within 24 hours.

In addition to date and personal identification, information should be included if the individual is taking medication of the sequestering type (e.g., EDTA's) as these interfere with the determination. The analysis is reported as $\mu g/100$ ml (micrograms of lead in 100 ml of blood).

This report method is considered similar to the $\mu g/100$ g reports. Correlations with coproporphyrin levels and stipple cell counts are available to the physician through medical literature. The determination of lead in urine, particularly where samples are not 24-hour samples, has been more variable and is therefore not currently used. More convenient techniques will be adopted when they are made available and verified. At present, however, blood lead determinations provide a proven valid reference. The test will measure to the nearest $\mu l/100$ ml (standard deviation = 2). This is more than adequate to discriminate between background levels and actual exposures.

The laboratory determination is made on a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer. Guides are available for corrective action on the part of industry. It must be recognized that a physician should be consulted concerning significance of lead level, mode of application and treatment of patients. The above guide applies only to INOR-GANIC lead exposure. Organic forms, such as tetraethyl lead used in gasoline, require additional clinical tests.

Lead in Paint Chips, Flakes or Scrapings

- 1. Submit at least one gram of paint specimen, preferably at least a 10 square centimeter area, in as few pieces as possible for qualitative emission spectrographic analysis for lead or quantitative atomic absorption analysis for lead.
- 2. Preferably, layers of paint which have flaked free from underlying plaster or paper should be submitted. If paint free of these cannot be located, the Laboratory will attempt a separation by soaking the specimen in water before analysis. If reasonably sized pieces are submitted for quantitative analysis, a concentration of lead per area can be reported as well as concentration per weight.

Mercury in Urine

- Collect a 24-hour composite sample of urine in a nitric acid rinsed glass container. A nonmetal closure is required.
- Keep the urine specimen cool during and after collection and mail to the Laboratory within 24 hours after collection.

Both lead and mercury analyses are done by atomic absorption. The sensitivity of the lead determination is about 0.01 mg/1, detection is about 0.002 mg/1 and the sensitivity of the mercury determination is about 0.002 mg/1, with detection of about 0.0005 mg/1.

Qualitative Metals Determination

- Submit at least one gram of solid sample or approximately 10 ml or more of liquid sample with a request for emission spectrographic analysis.
 The request should state the metals of prime interest.
- Samples for emission spectrographic analysis should be submitted in glass vials or bottles with nonmetal closures, but may be submitted in plastic containers, or even wrapped in paper if solid.

Sensitivity and detection limits vary, depending on the specific metal and/or the nature of the sample. In general, detections down to low parts per million (dry weight) may be possible, but are not quantitative under our routine method of analysis. Atomic absorption is used for quantitation where required. Atomic absorption analysis can be used for samples submitted for emission spectrographic analysis, but sample amounts should be at least 10 times greater than the minimum amounts stated for emission spectrographic analysis.

Miscellaneous Determinations

The following tests are also available upon request. Please contact the State Hygienic Laboratory, Medical Laboratories Building, The University of Iowa, Iowa City, Iowa 52242 for arrangements, sampling, and scheduling concerning these tests.

 Identification and determination of concentrations of both organic and inorganic solid contaminants, e.g., lime, silica, and metals.

 Identification and determination of concentrations of air contaminants such as acid mist, carbon monoxide, oil mist, solvents, and metals in welding fumes.

 Measurement of ventilation as necessary to define a problem. Such measurements include direction, velocity, and static pressure.

Determination of flash point of combustible liquids by Tag closed cup, Pensky-Martens closed cup, and Cleveland open cup methods.

5. Determination of heat stress.

- Light studies for intensity and glare determinations.
- Noise measurement including octave band an alysis.
- Measurement of levels of explosive vapors in the air.
- Measurement of oxygen levels in work atmospheres.

REPORTING OF LABORATORY RESULTS

The Laboratory will forward data to the company management or the requesting physician. Where a state agency has requested that a problem be defined, the reports will be forwarded through that agency. Reports of all findings are submitted in writing. Values obtained are checked for compliance with state and federal standards.

RADIOLOGICAL HEALTH

INTRODUCTION

The objective of the Radiological Health Division is the detection and prevention of radiological hazards to the citizens of Iowa from X-rays, tests of nuclear devices for military and peaceful applications, nuclear power generating stations, industrial and medical uses of radionuclides, and other sources of ionizing and nonionizing radiation. The Division provides technical assistance and engages in environmental surveillance programs for collecting and assessing data on all forms

of public radiation exposure throughout the state. Services are primarily performed for sponsoring state agencies.

COLLECTION AND SHIPMENT OF SPECIMENS

The collection of specimens for analysis by the Radiological Health Division is a complex process requiring specialized equipment and procedures. Most sampling will require our laboratory staff to operate the equipment. Therefore, any determinations of radiological health hazards will be handled on an individual basis after discussions on the scope of individual problems and the equipment capabilities of the Laboratory have been considered. All requests for the services of the Radiological Health Division are to be made by contacting:

Director, State Hygienic Laboratory Medical Laboratories Building The University of Iowa Iowa City, Iowa 52242 Telephone: 319/353-5990

Tests available:

- 1. Direct dose rate measurements for Beta, Gamma and X radiation to determine human occupancy radiation levels
- Direct measurement of non-ionizing radiation emitted from electronic products
- 3. Personal surveys of X-ray facilities
- 4. Gross Beta-, Gamma- and Alpha-radiation analyses of environmental samples
- Radioanalyses of milk for Iodine-131, Barium-140, Cesium-137, Potassium-40 and Strontium-90
- Radioanalyses of surface and ground water for Radium-226, Strontium-90 and Tritium, and other specific radionuclides as required
- 7. Upon request, specific radionuclides in other media such as fish, vegetation, soil, etc.
- 8. Leak testing of sealed radiation sources

REPORTING OF LABORATORY RESULTS

Reports of all findings are submitted in writing. X-ray survey reports are provided as shown in Figures 11-1, 11-2, 11-3, 11-4, 11-5 and 11-6.

- X-ray facilities are surveyed for compliance with the recommendations of the National Council on Radiation Protection and Measurements as listed in NCRP Reports 33, 34 and 35.
- Electronic products are surveyed for compliance with the Radiation Control for Health and Safety Act of 1968, Public Law 90-602.

Facility

| Name of Facility | / | | | A BANK BANK | - |
|------------------------------------|----------|--|--|-------------------------|--|
| Administrator_ | | | | | |
| Location | (1) | | | | |
| | | | Surveyors A. | В | |
| | 2000000 | | | | |
| Person in Charge | e of X- | ray FacilityName | | Title | |
| Person Interview | ved | | 38 | | The state of the s |
| | | Name | | Position | |
| Type of Facility | r: | THE PARTY OF THE P | | CONTRACTOR OF STREET | The state of the s |
| | | | | 0.1 | |
| Pr | ivate C | office | Hospital | Other: | |
| М | obile | | Clinic | | |
| | | | | | |
| Previous Radiat | ion Su | | | Date | |
| | | Name | | Date | |
| NCRP Report No. 33 Reference | | | ITEM | | YES NO |
| Reference | | Total Number commetionally expected | | | |
| | A. B. | Total Number occupationally exposed Total Number of operators | | | THE RESERVE |
| 7.3.1 | C. | Total Number monitored | | | |
| 7.3 | D. | Monitoring system employed. | | | |
| 7.3.2 | E. | Monitoring system adequate. | | | |
| 7.3.3 | F. | Monitoring records satisfactory. | | | |
| | G. | Patients radiographed (Ave. per week) | | | |
| | H. | Patients fluoroscoped (Ave. per week) | | | |
| | I. | Total number of machines at this facility | | | |
| | J. | Total number of units (tubes) | | | |
| | K. | Brand and type of X-ray film used | | | |
| | L. | Type of intensifying screen | the same of the sa | The same of the same of | |
| 12.02 | M. | Darkroom light tight, | | | |
| 2.4.7 | N. | Type of developer | | | C. Calledon |
| 2.4.7 | O. P. | Automatic process. How often changed | | weeks. | |
| 2.4.7 | Q. | How often replenished | | | |
| 2.4.7 | R. | Thermometer present. | | | |
| 2,4.7 | S. | Thermostatically controlled. | | | |
| 2,4,7 | T. | Temperature indicated | | °F. | |
| 2.4.7 | U. | Timer present. | | | The state of the s |
| 2.4.7 | V. | Time in developer | | minutes. | I de la constitución de la const |
| | W. | Radium ever present. | | | |
| | X. | Other isotopes ever present. | | | |
| 6.1.7 | Y. | Adequate warning devices | | | |
| REASON FOR | SUP | /FV· | | | |
| Initial | SUR | | low-up | Resurvey | |
| | | | acotomic and a second a second and a second | * | |
| Request | | Con | nplaint | Other | |
| COMMENTS: | | | | | |

Figure 11-1 X-ray protection survey report - facility

Radiographic

| Name of Facil | ity | | | |
|---------------|------------|---|------------------|----------------|
| Administrator | <u> </u> | | | |
| Location | | | | |
| Date of Surve | y | Surveyors | АВ | |
| | | ay Facility | | |
| | | Name | Title | |
| Person Interv | iewed | Name | Position | |
| Control Panel | Manufac | turer | | |
| Tube Head M | anufactu | er and Model | | |
| Tube Head M | anuractu | ci and model | Max. kVp (rated) | Max. mA(rated) |
| TYPE: | | | | |
| Fixed Ra | diographi | Mobile | | |
| Photofluo | orographi | Other | | |
| | | | | |
| NCRP | | | | |
| Reference | 33 | ITEM | | YES NO |
| 3.2.1 (d) | A. | Filtration, total mm. aluminum equivalent | | |
| 3.2.1(b) 3.2 | | Source-film distance 72 inches, beam size within limits. | | |
| 3.2.1(b) 3.2. | | Source-film distance 40 inches, beam size within limits. | | - |
| 3.2.1 (f) | D. | Exposure switch, adequate location. | | |
| 3.2.1 (e) | E. | Timer, adequate. | | |
| | F. | Equipment electrically grounded. | | - |
| 3.2.3 (b) | G. | Gonadal shield used. | | |
| 3.2.3 (e) | Н. | Operator protection adequate. | | |
| | I. | Primary and secondary barriers adequate. | | - |
| 3.2.1 (a) | J. | Diagnostic-type tube housing | | |
| 2.2 | K. | Radiation exposure to occupational personnel, within limits | | |
| 2.2 | L. | Radiation exposure to nonoccupational personnel, within lir | nits. | |
| EXPOSUR | E RATE | MEASUREMENTS Taken atkVp | mA | |
| | | tion barrier | mR/hr. | |
| A. Ben | ina prote | don barner | | |
| B. Out | side prote | ction barrier | mR/hr. | |
| C. Beh | ind lead g | lass | mR/hr. | |
| D. Beh | nind wall | of chest board | mR/hr. | |
| E. Oth | ner measu | rements | | |

COMMENTS:

Figure 11-2 X-ray protection survey report - radiographic

COMMENTS:

X-RAY PROTECTION SURVEY REPORT

Fluoroscopic

| Name of Facility | y | | | | | | |
|------------------------------------|----------|---|-------------|-----------|--------|-------------|----------|
| Administrator_ | | | | | | | |
| Location | | | | | | | 1 |
| Date of Survey | | | Surveyors A | | B | | 999 |
| Person in Charg | e of X | -ray Facility | | | | | |
| TOTAL DI CHAIL | | Name | | Title | | Tear of the | |
| Person Interview | wed _ | Name | | Position | | | |
| Control Panel M | Manufa | | | | | | |
| T. 1. 11 - 116 - | | | | | | | |
| Tube Head Man | nuractu | irer and Model | | | - | - | |
| | | | | | | | |
| NCRP Report No. 33 Reference | | | ITEM | | 177307 | | YES NO |
| 3.1.3 (k) | A. | Leaded gloves used. | | | | | |
| 3.1.3 (j) | В. | Leaded apron used | | | | | |
| 3.1.1 (g) | C. | Bucky slot covered. | | | | | |
| 3.1.1 (h) | D. | Leaded drapes around screen. | | | | | |
| | E. | Fluoroscope and console electrically grounded. | | | | | |
| 3.1.1 (d) | F. | Shutters function properly. | | | | | The same |
| 3.1.2 (d) | G. | Useful beam limited to screen. | * | | | | |
| 3.1.1 (d) | H. | Fluoroscopic screen ganged to X-ray tube. | | | | | |
| 3.1.1 (b) | I. | Target to panel | inches. | | | | |
| 3.1.3 (a) | J. | Useful beam at panel surface | | R/minute. | | | |
| 3.1.1 (c) | K. | (The recommended limit is 10 R/minute.) Filtration, total mm. aluminum equivalent | | | | | |
| 3.1.1 (e) | L. | Deadman type exposure switch. | | | | - | |
| 3.1.1 (i) | M. | Cumulative timing device. | | | | | |
| 3.1.2 (c) | N. | Leaded glass satisfactory. | | | | 11020 | 100 |
| EXPOSURE R | ATF N | MEASUREMENTS Taken at | kVp | | mA | | |
| LAI OBOKE K | | | | | | | |
| Exposure rate | above | the fluoroscopic screen | _mR/hr. | | | | |
| Exposure rate | to the | fluoroscopist | mR/hr. | | | | |
| Exposure rate | to the | assistant | _mR/hr. | | | | |
| Other | | massyramants | | | | | |
| Other exposur | e rate i | measurements: | | | 30 | | |

Figure 11-3 X-ray protection survey report - fluoroscope

Facility-Dental

| Name o | f User(s) | | |
|----------|---------------|---|--|
| Locatio | n · | | |
| Date of | Survey | Surveyors A. | В |
| Person I | Interviewed_ | name | position |
| Type of | Practice: | | |
| | | | |
| | | Pedodontic | |
| | | | Other (specify) |
| Orth | iodontic | Prosthodontic | |
| Previou | s Radiation S | name | date |
| NCRP | | | |
| Report | No. 35 | | |
| Referen | ice | ITEM | YES NO |
| | A. | Total number occupationally exposed | |
| | B. | Total number of operators | |
| 6.3.1 | C. | Total number monitored | |
| 6.3 | D. | Monitoring system employed.(Type) | |
| 6.3.1 | E. | Monitoring system adequate. | THE RESERVE OF THE PERSON OF T |
| 6.3.2 | F. | Monitoring records satisfactory. | The state of the s |
| | G. | Patients radiographed (Ave. per week) | |
| | H. | Total number of machines at this facility | |
| 711.1754 | I. | | |
| 4.9 | J. | Brand and type of X-ray film used | |
| 4.11 | K. | Darkroom light tight. | |
| 4.11 | L. | Type of developer | |
| 4.11 | M. | Automatic process. | |
| 4.11 | N. | How often changed weeks. | |
| 4.11 | O. | How often replenished days. | |
| 4.11 | P. | Thermometer present. | |
| 4.11 | Q. | Thermostatically controlled. Temperature indicated OF. | |
| 4.11 | R. S. | Zonipolation middless | |
| 4.11 | T. | Timer present. Time in developer minu | tes. |
| | | | |
| | ON FOR SUP | | |
| | Initial | Follow-up | Resurvey |
| | | | |
| | Request | Complaint | Other |
| | | | |

COMMENTS:

Figure 11-4 X-ray protection survey report - facility, dental

Radiographic-Dental

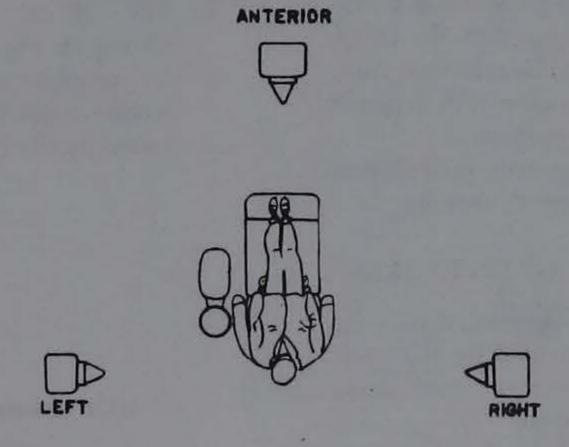
| Name of User | (s) | | | | |
|----------------|-----------|---|---------------------|---------------------------|-----------------|
| Location | | | | | - |
| Date of Surve | v | Surveyors A. | | R | |
| Date of Surve | , | Survey of s A. | | | |
| Person Intervi | iewed | name | | position | |
| Control Panel | Manufac | cturer | | Serial No | |
| | | | | | |
| | | rer and Model | | | |
| Maximum kV | p (rated) | | Maximum mA (r | ated) | |
| | | | | | |
| Pointer Cone | : | | | | |
| | None | Sho | rt Open | Long Open | C. S. J. (1950) |
| | | pinted Lon | | | |
| NCRP | - | | | | |
| Report No. 3 | 15 | | | | |
| Reference | | ITEM | | | YES NO |
| 2.1.2a | A. | Field diameter at end of pointer cone | inches. | | |
| 2.1.3 | В. | Filtration, total mm. aluminum equivalen | | | |
| | C. | Roentgen output at end of pointer cone | R/sec. | | |
| 2.1.5b | D. | Exposure switch, adequate location. | | | |
| 2.1.4 | E. | Timer, adequate. | | | |
| | F. | X-ray unit grounded. | | | 1 |
| 2.1.2f | G. | Stability of tube head, adequate. | | | |
| 2.1.2d | H. | Cone attenuation, adequate. | | | - |
| 2.1.1a | I. | Tube housing radiation leakage within sta | ndards. | | |
| 5.1 | J. | Primary and secondary barriers, adequate | | | 13-1- |
| 4.8.2 | K. | Gonad shielding used. | | | |
| 4.2 4.8. | 1 L. | Film holders used. | | | - |
| | M. | Estimated Number of films per week on t | his unit | | |
| | N. | Average exposure time per film | seckVp | mA. | |
| EXPOSURE | | IEASUREMENTS Taken atk | | | |
| A. | | tewing Exposure 1. Dentistm | | | |
| В. | | r Upper Periapical Exposure 1. Dentist | | | |
| C. | Right B | itewing Exposure 1. Dentist | mR/hr. 2. Assistant | mR/hr. 3. Other (specify) | mR/hr. |
| D. | Other n | neasurements-See attached detail. | | THE WARRENCE WITH THE | |

COMMENTS:

Figure 11-5 X-ray protection survey report - radiographic, dental

Scatter Radiation Measurements

Radiation measurements preceded by the letters L, R and A designate exposure rates to personnel at the locations indicated during Left, Right and Anterior projections.



Exposure Rate to Pelvic Region (unshielded) _____mR/hr.

Figure 11-6 X-ray protection survey report - scatter radiation measurements

3. Environmental levels of radioactivity are checked for compliance with standards established by the Iowa Water Quality Commission, United States Public Health Service and guidelines established by the Federal Radiation Council.

DRINKING WATER

INTRODUCTION

The Iowa City Laboratory offers a service whereby a single sample may be tested for coliform bacteria, nitrates, iron, hardness and iron bacteria in drinking water. This service is available to private individuals as well as municipalities throughout the state.

Regular bacteriological sampling programs for municipalities throughout Iowa are required by the DEQ. The minimum number of samples to be collected from the distribution system and tested each month depends upon the population served by the system.

Individuals using water from a private well should ideally have their water tested every six months.

COLLECTION AND SHIPMENT OF SPECIMENS

The bacteriological tests will be performed only on samples collected in containers provided by the Laboratory. If you wish to have your water tested, a request for a sample kit should be sent to:

> State Hygienic Laboratory Medical Laboratories Building Iowa City, Iowa 52242

After receiving your request, the Laboratory will promptly send a kit (Figure 12-1) consisting of a sterile brown glass bottle with a white cap for unchlorinated water supplies, a styrofoam shipper, a return addressed mailing box, a set of sampling instructions and a data slip. If your water supply is chlorinated or treated with iodine or bromine, indicate this in your original request for a kit. For chlorinated supplies, the sample bottle has a black cap indicating that it contains a small quantity of sodium thiosulfate to neutralize the residual chlorine so that the bacteriological examination will indicate the true bacterial content at the time of sampling. Use the white-capped bottles only for sampling of unchlorinated water supplies and the black-capped bottles only for sampling of chlorinated water supplies or those treated with iodine or bromine.

However, if a bacteriological and nitrate test are desired for a chlorinated water supply, a black-capped

bottle must be used for the bacteriological test and a white-capped bottle must be used for the nitrate test. The reason for this is that the dechlorinating agent (sodium thiosulfate) present in the black-capped bottle interferes with the nitrate test.

To collect the sample:

- Take water samples only from a cold water faucet.
- 2. Thoroughly flame the exterior of the water faucet from which the sample is to be taken.
- 3. Run water for two to three minutes.
- 4. Uncap the bottle without touching the inside of the cap or lip of the bottle. Do not put the cap down while filling the bottle.
- 5. Fill the bottle to within about ½ inch of the top.
- 6. Recap the bottle tightly.
- 7. Fill out only that portion of the data slip shown in Figure 12-2. The information must be complete and accurate. If the date of collection is not indicated, the sample will not be tested by the Laboratory.

STATE HYGIENIC LABORATORY-WATER ANALYSIS

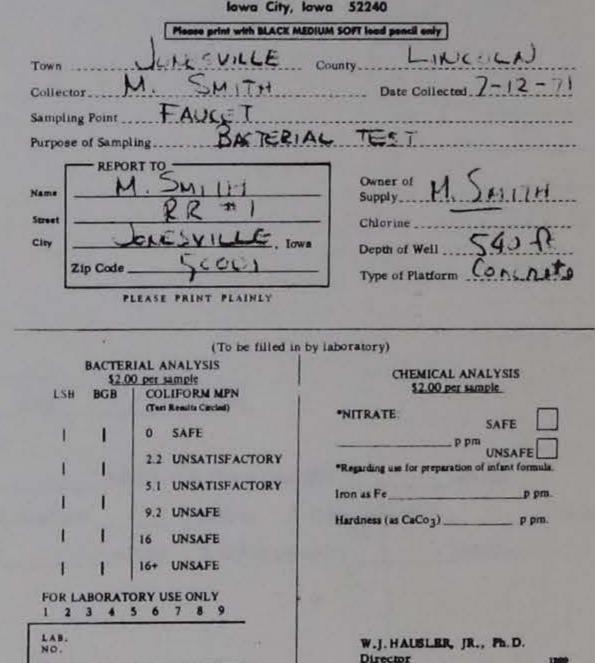


Figure 12-2 Water analysis data slip

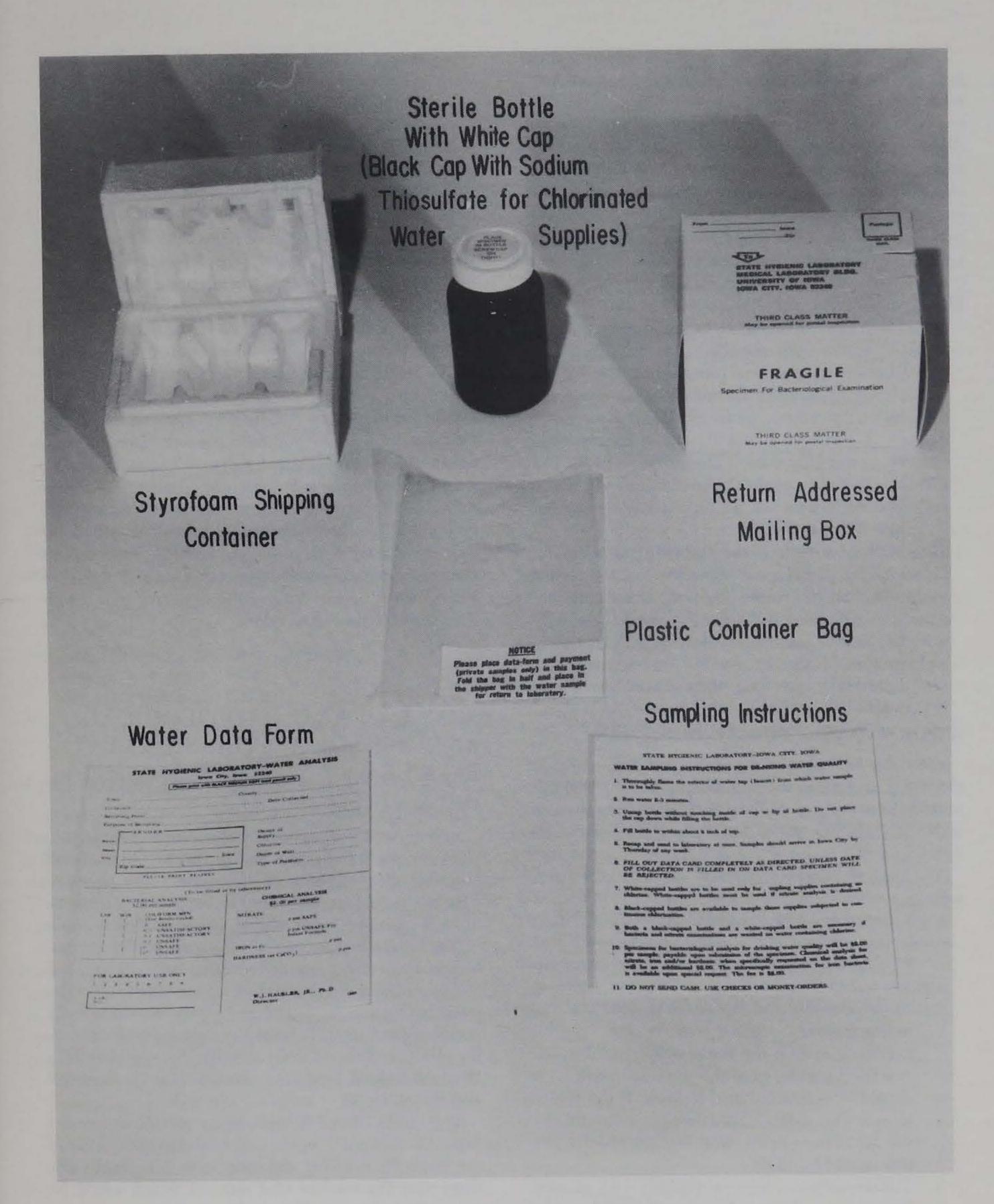


Figure 12-1 Drinking water collection kit

The "purpose of sampling" should designate which tests you wish to have done. Indicate if you wish only a bacterial test. The laboratory charge for a "bacterial test only" is \$3.00. If you wish chemical analyses, indicate iron, hardness, nitrate or all of these. An additional \$3.00 fee is charged whether one or all of the chemical analyses are requested. If you desire an iron bacteria examination, also indicate this under "purpose of sampling." The iron bacteria examination fee is \$5.00. The total cost of an examination for total coliforms, nitrate, iron, hardness and iron bacteria is \$11.00 per sample.

8. Place the filled and tightly capped bottles in the mailer with the data slip enclosed in the plastic bag which is provided in the kit. Include payment for the examination of private water specimens with the data slip. Use a check or money order payable to the State Hygienic Laboratory. Do not send cash.

 Place mailer inside the pre-addressed mailing box and secure the mailing lock-tabs. Add ap-

propriate postage.

10. Mail the sample to the State Hygienic Laboratory by parcel post immediately after collection. Samples received more than two days after collection will not be accepted for analysis. Sample collection and shipping should be performed so that the samples arrive in the Laboratory no later than Thursday of any week.

REPORTING OF LABORATORY RESULTS

After the laboratory tests are completed, copies of the report are sent to the individual (or city) who submitted the sample as well as to the State Department of Health or DEQ, depending upon the source of the sample.

The bacteriological (coliform) interpretation is as follows:

- Satisfactory-coliform bacteria (MPN) 0 to less than 2.2
 This indicates that the water is bacteriologically safe to drink.
- Unsatisfactory—coliform bacteria (MPN) 2.2
 to less than 9.2
 Contamination of the water supply is indicated.
 A second sample from the same sampling point should be submitted as a recheck. If the second sample also shows contamination, a careful survey for defects in the well and distribution system should be made.
- Unsafe—coliform bacteria (MPN) 9.2 or more This indicates bacterial contamination. A careful

survey for defects in the well and distribution system should be made. If no defects are found a second sample should be submitted for a recheck.

If your well is determined by testing to be bacteriologically unsafe, instructions and advice for correcting the condition can be obtained from your local health department or the State Department of Health.

A nitrate concentration of more than 45 ppm is unacceptable when the water is used for preparing "formula" or other beverages for infants under one year of age. Humans older than this are generally unaffected by nitrate cyanosis. Should the nitrate content be near the upper limit of 45 ppm, monthly retesting of the water is advisable if the water is to be used for infants.

Iron, hardness and iron bacteria have no health significance but are of aesthetic concern. Iron, if present in concentrations greater than 0.3 ppm, can cause staining of plumbing fixtures and clothing. Hardness indicates the soap consuming capacity of water. The greater the hardness, the more difficult it will be to develop a lather with soap. Iron bacteria, if present in the water distribution system, can cause plugging of filters, water lines, water softeners, and may cause objectionable tastes and odors.

MINERAL AND RADIOLOGICAL ANALYSIS OF WATER SUPPLY SAMPLES

INTRODUCTION

The mineral analysis of a water supply sample provides precise information on significant physical and chemical characteristics of the water. The chemical parameters include not only the usual anions and cations present in a water supply, but an extensive study of the heavy trace metals which could be detrimental to human consumption or industrial use.

The radiological analysis of water supply samples consists of gross alpha and gross beta-gamma activity counts. Those samples having picocurie levels exceeding United States Public Health Service Drinking Water Standard limits are analyzed for Radium 226 and Strontium 90.

The results of the mineral, heavy metals, and radiological analyses can be used to evaluate the quality of the water for drinking and other uses. The results also determine the method of treatment which might be necessary to improve the quality of the water supply. The tests performed are listed below:

Alkalinity Sodium Phenolphthalein Solids Total Dissolved Bicarbonate Total Sulfate Calcium Carbonate Trace Metals Chloride Arsenic Conductance Barium Cadmium Fluoride Chromium Hardness Copper Iron Soluble Lead Total Mercury Magnesium

Manganese Radiological Activity

Zine

Nitrate Gross alpha

Potassium Gross beta-gamma

Silica

This analytical service is available on request to municipalities, state agencies, and private individuals. This service is provided by the Des Moines Branch, and all requests for sample kits should be directed to:

State Hygienic Laboratory Des Moines Branch East 7th and Court Street, Room 405 Des Moines, Iowa 50309 Telephone: 515/281-5371

A fee is charged to cover the analytical expense.

COLLECTION AND SHIPMENT OF SPECIMENS

Upon receiving a request for a mineral and radiological analysis, the Des Moines Branch Laboratory will ship three different size plastic bottles and a data card for each sample to be analyzed (Figure 12-3). The bottles supplied consist of a one-gallon plastic bottle for radiological analysis, a half-gallon plastic bottle for general mineral analysis, and a pint plastic bottle containing a small amount of nitric acid as a preservative for trace metals. The pint bottle is sealed in a plastic bag as a precautionary measure against acid leakage. Please use the bottles supplied and not bottles of your own choice.

- 1. Pump well to waste for at least 30 minutes before sampling so that the collected sample is representative of the well water. The pumping time and rate in gallons per minute (if known) should be recorded on the data card.
- Fill the one-gallon and half-gallon bottles to the neck and tightly cap them. Remove the pint bottle

from the plastic bag and fill to the neck without spilling the nitric acid or overflowing the bottle. Replace the pint bottle in the plastic bag and reseal before shipment.

Fill a separate clear glass container with water and observe whether or not there is any turbidity (cloudiness) present in the water. Record this observation in the appropriate location on the mineral data card.

Supply the information requested on the data card including town (if applicable) and county in which the well is located, the collector's name, owner of well, collection date, well name or number, well depth, construction date of well, and the name and address of the person to whom the report should be sent. Also indicate whether or not polyphosphates are being used in the treatment process and make certain that all three sample bottle numbers appear on the data card.

If possible, it is desirable to have a record of water temperature, alkalinity and pH at the time of collection; however, this is not mandatory.

The water samples for mineral and radiological analysis, accompanied by the data card, should be shipped to the Des Moines Branch Laboratory on the day of collection. Both sample collection and shipping should be arranged so that the sample arrives in Des Moines no later than Thursday.

For optimum accuracy no more than three days should elapse between collection time and arrival

of the sample at the Laboratory.

REPORTING OF LABORATORY RESULTS

Results of the analysis will be mailed within approximately four weeks of receiving the sample. The parameters are reported in parts per million (equivalent to milligrams per liter) with the exception of specific conductance, mercury, and pH. Nitrate concentrations are reported as nitrate and not nitrate-nitrogens. Copies of all mineral reports are sent to the Iowa Department of Environmental Quality.

Inquiries concerning the meaning of the reported values and their relationship to drinking water quality should be directed to the Iowa Department of Environmental Quality.

STATE HYGIENIC LABORATORY The University of Iowa Medical Laboratory Building Iowa City, Iowa 52242 319-353-5990

| COLLECTOR'S NAME_ | | COUNTY | AL ANAI | YSIS | Repor Sampl | al No ted ed ved | |
|--|---------------------|--|------------------------|--------------------------|--|--|----------|
| OWNER OF SUPPLYCOLLECTOR'S NAME | | COUNTY | | | Repor Sampl | teded | |
| OWNER OF SUPPLYCOLLECTOR'S NAME | | COUNTY | | | Sampl | ed | |
| OWNER OF SUPPLY COLLECTOR'S NAME REPORT TO: | | | | | Sampl | ed | |
| AND THE PARTY OF T | | | | | | | |
| REPORT TO: | | | | | Receiv | ved | |
| REPORT TO: | | | | | | | 20 |
| | | | | | | | |
| | | | | | | | |
| | | | - | | | | |
| | | | | | | | |
| | | FIELD DA | ATA | 1000 | WELI | L DATA | |
| SOURCE | | | | ***** | | | |
| SAMPLING POINT | | | | NAME O | | The state of the s | - |
| | | | | DEPTH | UCTION | DATE | - |
| | | | | |) | HRS AT | GPM |
| WAS SAMPLE FREE OF | TURBIDIT | Y WHEN COL | LECTED | | | | |
| TEMPERATURE °C | | KALINITY (m | | | T | pH | |
| IS A POLYPHOSPHATE | | 2, 2 | DAT | E OF PR | EVIOUS | SAMPLES | |
| | | | | | - | | = |
| | | Y ANALYSIS | | | | 0.1 | |
| Specific Conductance at | t 25°F | | omhos | | ilica (Si | .02) | - Albert |
| Total Residue | - | | e Iron (I Iron (Fe) | The second second | | | |
| Filtrable Residue | - | T T | pH | | Hs | | |
| Alkalinity as CaCO ₃ P Hardness as CaCO ₃ | _ | mg/1_ | | | The same of the sa | y Index | |
| | ANIONS | | TRACE I | The second second second | u L | RADIOACTI | VITY |
| | (NO ₃ =) | | As | | | (picocuries/ | L) |
| (Na+) | (F ⁻) - | | Ва | | | Gross Alpha | |
| (Ca++) | (C1-) | | Cd | | | 226 Radium | |
| | (SO ₄ =) | STREET, STREET | Cr | | | Gross Beta | |
| (Mn++) | (HCO3) | 10 mm | Cu | - | - | 90 Strontiu | n |
| | (CO ₃ =) | District of the | Pb | | | Other | |
| | (CN-) | William Strategy | Hg | dillen de | - | Organics | |
| | (PO4=) | | Se | | - | CCE_ | |
| | | | Ag | - | - | CAE _m _ | 100 |
| PESTICIDES (mg/L) | | | Zn | | _ | MBAS | |
| Chlorinated Hydrocarbo | 200 | Organophosph | nato & Ca | rhamates | THE RES | Herbicides | Coyalla. |
| Chiorinated Hydrocarbo | ons | Organophospi | iace a oc | i baila co. | Tellips | | |
| | | - | - | | 1 4 4 1 3 | - | |
| | | - | - | | · LOSSIE | | |
| 1 | _ | | | | | - | |
| The state of the s | | - | | | | - | |

Figure 12-3 Mineral analysis data slip

FLUORIDE ANALYSIS AND PROFICIENCY TESTING

INTRODUCTION

Fluoride analysis proficiency testing is a service provided by the Laboratory to each fluoridated public water supply to meet requirements of the Iowa Department of Environmental Quality. The purpose of this program is to ensure that water plant operators are accurately measuring the fluoride content of the fluoridated drinking water. This is necessary if fluoride concentrations are to be maintained at the optimum level to prevent dental caries.

When fluoridation equipment is installed in a municipal water plant, the plant operator will be provided with a six months' supply of fluoride sample kits. Thereafter, the operator is expected to request an additional supply from the Des Moines Branch Laboratory. We suggest this be done when the original supply is diminished to two kits.

COLLECTION AND SHIPMENT OF SPECIMENS

Each fluoride sample kit (Figure 12-4) consists of a styrofoam mailer, a yellow, adhesive-backed label for return mailing, a 100 milliliter brown glass bottle with a black cap marked FLUORIDE, and a small plastic bag with a data slip inside. The following steps should be observed when collecting the sample:

1. Use a black lead, medium soft pencil and not blue ink for completing the data slip.

2. The collector should complete the top half of the data slip, being sure he has recorded his analytical result. "Collector's Remarks" should include anything unusual about plant operation at the time of sampling as well as any interferences with the fluoride analysis which are known to be present. The collector can indicate a need for additional sample kits under "Collector's Remarks."

3. After filling the bottle, the cap should be screwed on tightly to prevent leakage. The bottle and data slip (in the plastic bag) can then be placed in the shipper and both halves of the shipper put together. Peeling the backing layer from the yellow label exposes the adhesive surface. The label can then be wrapped around the two halves of the kit to bind them together.

4. After placing sufficient postage on the label the sample is ready to be mailed to:

State Hygienic Laboratory
Des Moines Branch
East 7th and Court Street, Room 405
Des Moines, Iowa 50309

5. IMPORTANT: No portion of the fluoride sample kit should be interchanged with the bacteriological sample kit which has a similar styrofoam shipper and brown sample bottle.

The second part of this program includes sending a set of three samples, each containing an unknown fluoride concentration, to every public water supply system which fluoridates. The person responsible for the fluoride analysis at each facility is asked to analyze the three samples and send his results to the Des Moines Branch Laboratory. This provides an additional check on each analyst's ability to perform the fluoride analysis.

Training courses for fluoride analysis are held in the Des Moines Branch Laboratory for new water plant operators or operators of plants which are scheduled to have fluoridation equipment installed. These one-day training sessions are scheduled whenever there is a sufficient number of trainees to make a class.

FLUORIDE ANALYSIS FOR PRIVATE WELLS

Individuals wishing to have their private wells tested for fluoride content may do so by requesting a sampling kit from the Des Moines Branch Laboratory. A fee of \$8.00 is charged for each analysis.

REPORTING OF LABORATORY RESULTS

Although plant operators of fluoridated supplies analyze the fluoride content on a daily basis, they are required monthly to submit a duplicate sample to the Des Moines Branch Laboratory for a check analysis. If the deviation between the operator's result and the Des Moines Branch Laboratory's result is greater than \pm 0.2 parts per million, a request is sent back to the operator for a recheck sample. If the recheck still indicates a discrepancy in the operator's analysis, the operator is contacted by telephone or through correspondence to determine the possible cause of the problem. If the plant operator's analytical problem cannot be resolved by this route, the operator is called in for additional training in fluoride analysis.

Reports on monthly check samples, recheck samples and fluoride unknowns are sent to the Iowa Department of Environmental Quality. Distribution of reports to the municipalities is handled by the above department.

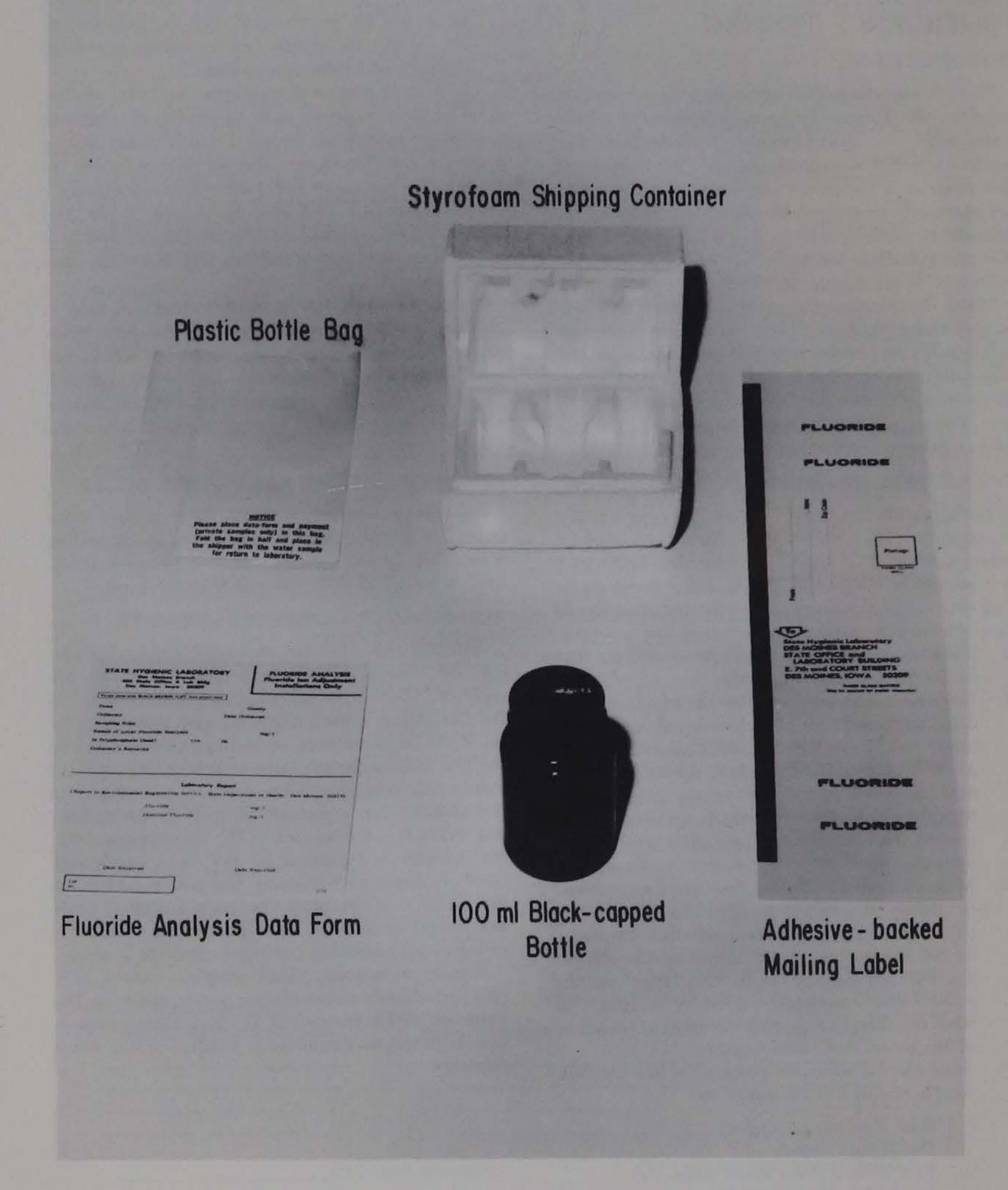


Figure 12-4 Fluoride analysis collection kit

SURFACE WATER AND WASTEWATER ANALYSIS

INTRODUCTION

The first priority of the State Hygienic Laboratory concerning water and wastewater analysis is to provide analytical support to the Iowa Department of Environmental Quality and the Iowa Geological Survey as well as the Laboratory's Limnology Division which conducts water pollution surveillance studies for the Iowa Water Quality Commission. Consistent with time and manpower available after fulfilling these analytical needs, samples will be accepted from municipalities, local government, industry, private individuals or other non-governmental groups. Regardless of the sample source, the request for analytical service should be made at least one week and preferably two weeks in advance of sampling. Many of the tests which are performed on water and wastewater samples must be done within a relatively short time after collection to obtain valid results. This necessitates prescheduling of all samples received by the Laboratory. If your sample has not been scheduled, the work load may not permit it to be accepted for analysis.

Table 12-1 lists tests available for surface water and wastewater samples. Analytical methods used by this Laboratory are those recommended by the current edition of Standard Methods for the Examination of Water and Wastewater.

Arrangements for analytical work on surface water and wastewater samples can be made by contacting:

State Hygienic Laboratory
Des Moines Branch
East 7th and Court Street, Room 405
Des Moines, Iowa 50309
Telephone: 515/281-5371

A laboratory fee is charged for these services.

COLLECTION AND SHIPMENT OF SPECIMENS

Many of the parameters listed in the following table require special methods of preservation if accurate

Table 12-1 Tests available, surface water and wastewater

| Acidity | |
|------------|------------------------|
| Alkalinity | |
| Phenoly | ohthalein |
| Total | |
| Biochemi | cal Oxygen Demand (BOD |
| Calcium | |
| CCEm* | |
| CAEm° | |
| Chemical | Oxygen Demand (COD) |
| Chloride | |
| Color | |
| Conducti | vitv |
| Cvanide | The second second |
| Detergen | ts |
| | iform Bacteria |
| Fluoride | |
| Hardness | |
| Herbicide | es° |
| Iron | |
| MBAS | |
| Magnesiu | m |
| Mangane | |

* Intial contact should be made with:

State Hygienic Laboratory

Medical Laboratories Building

The University of Iowa

Iowa City, Iowa 52242

Metals Aluminum Arsenic Barium Cadmium Chromium Copper Lead Mercury Selenium Silver Zine Nitrogen Organic Ammonia Nitrate Nitrite Oil and Grease pH pHs Phenol Phosphorus Soluble Total

Potassium Radionuclides* Gross beta-gamma Gross alpha Radium 226 Strontium 90 Tritium Sodium Specific conductance Silica Solids, settleable Solids Total-fixed and volatile Dissolved-fixed and volatile Suspended-fixed and volatile Stability Index Sulfate Sulfide Turbidity Volatile acids (total organic acids) analytical results are to be obtained. Also, the volume of sample required and sampling procedures are dependent to a large extent upon the kinds of analyses which are to be performed. When initial contact is made with the Laboratory to arrange for testing, specific details concerning (1) sampling procedures, (2) sample volume, (3) preservatives, and (4) sample shipment will be discussed.

REPORTING OF LABORATORY RESULTS

After the analytical work has been completed, the results pertinent to each sample will be reported by mail to the individual or agency which submitted the sample. Copies of all reports are also sent to the Iowa Department of Environmental Quality. Unless otherwise indicated, concentrations of chemical constituents are reported as milligrams per liter (equivalent to parts per million).

SPECIAL ANALYTICAL SERVICES

Occasionally special water supply problems are encountered such as contamination by pesticides, herbicides, metals, gasoline, oil or visible forms of biological life. The State Hygienic Laboratory is equipped to provide analytical assistance for these and other problems on an individual request basis. Before collecting and shipping a sample, however, either the Des Moines Branch Laboratory or the Iowa City Laboratory should be contacted to discuss the nature of the problem, the type of analysis which may be pertinent, volume of sample required and preservatives used.

EFFLUENT QUALITY ANALYSIS PROGRAM

INTRODUCTION

The EQAP (Effluent Quality Analysis Program) service is provided by the Des Moines Branch Laboratory to the Waste Water Section of DEQ to enable it to monitor the performance of all municipal and selected industrial waste treatment plants throughout Iowa. Participation in this program by municipalities and industries is required by the DEQ.

Every month a sampling kit is automatically sent by the Laboratory to the operator of each waste treatment facility. This kit consists of a screw-cap fiberboard shipper, a pint plastic bottle containing a small quantity of preservative, instructions specifying how and when to collect the sample, a data slip and a return mailing label (Figure 12-5).

COLLECTION AND SHIPMENT OF SPECIMENS

Complete instructions for collecting the sample are included in a letter which accompanies each sampling kit; however, there are five important points worthy of repeating:

- Collect the original effluent sample in a larger container (one quart minimum) and mix thoroughly before filling the pint plastic sample bottle.
- Fill the pint plastic sample bottle only to the mark on the shoulder of the bottle. Do not allow the bottle to overflow or the preservative will be lost.
- Cap the bottle tightly so no leakage will occur during shipment.
- Record any abnormalities in plant operation at the time of sampling on the data slip.
- 5. After collection, the sample should be sent immediately to the Des Moines Branch Laboratory by parcel post. Mail to:

State Hygienic Laboratory
Des Moines Branch,
East 7th and Court Ave., Room 405
Des Moines, Iowa 50309

REPORTING OF LABORATORY RESULTS

Upon receipt in the Des Moines Branch Laboratory the samples from each waste treatment facility are analyzed for Biochemical Oxygen Demand (BOD) and selected samples are also tested for their ammonia and phosphorus content.

The results of these tests are reported to the DEQ and copies of the results are also mailed to each respective waste treatment facility.

Any inquiries regarding this program should be directed to the Des Moines Branch Laboratory.

WATER POLLUTION SURVEILLANCE

INTRODUCTION

The Limnology Division of the State Hygienic Laboratory conducts detailed water pollution investigations on surface waters throughout Iowa to evaluate the effects of waste discharges and to determine

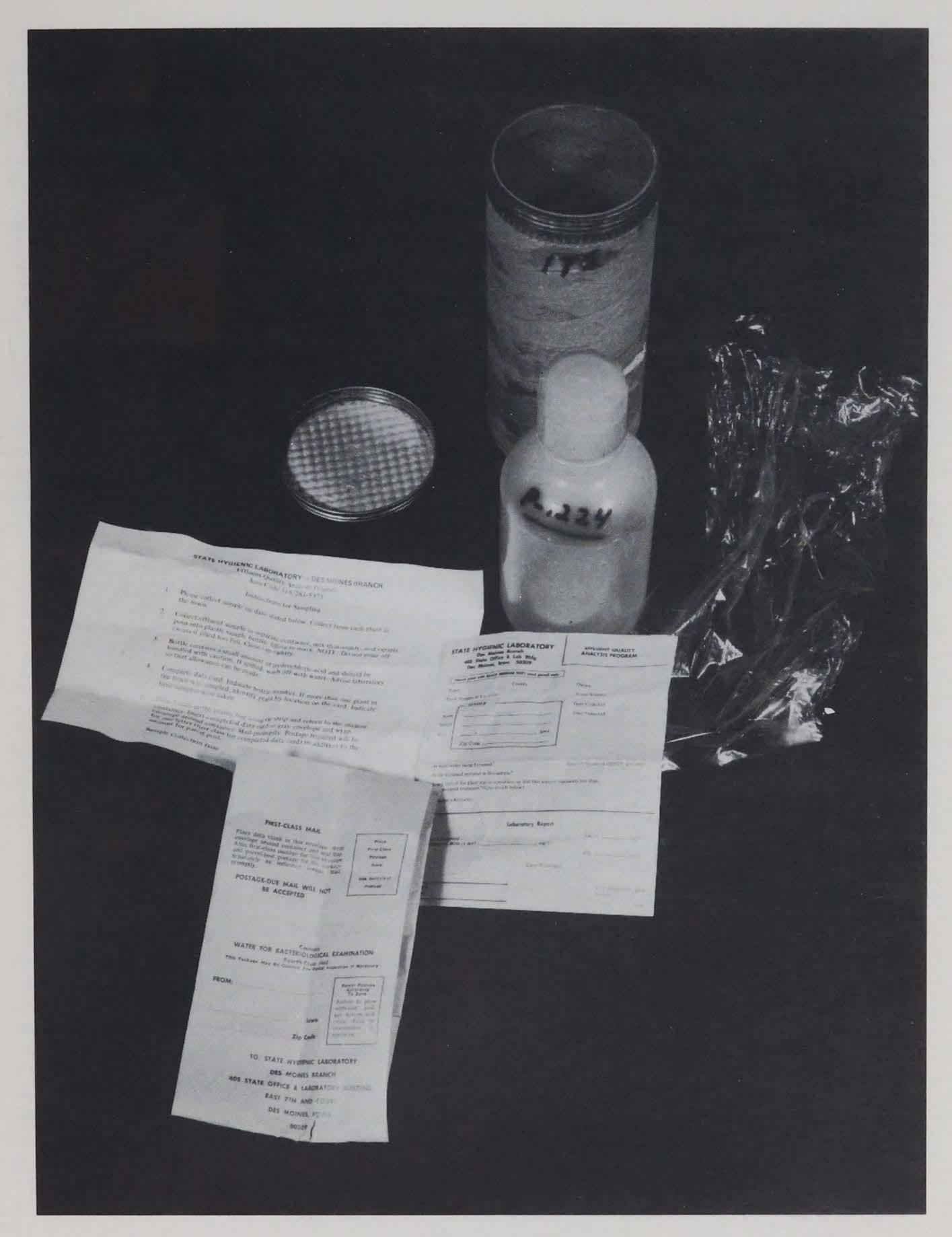


Figure 12-5 EQAP analysis kit

if Iowa Water Quality Standards are being met. The techniques used in these studies include complete water chemistry analysis, diurnal dissolved oxygen measurement, fecal coliform enumeration and the use of benthic organisms as water quality indicators.

COLLECTION AND SHIPMENT OF SPECIMENS

Water pollution surveys on rivers, lakes or ponds are not conducted by the Limnology Division at the request of an individual. The appropriate procedure for initiating such a survey is the submission of a written petition to the Iowa Water Quality Commission. According to the Iowa Water Pollution Control Law of 1966, the written petition must originate from:

(1) the governing body of any city or town, (2) the local board of health, (3) the supervisors of any county, (4) 25 residents of the state or (5) any state agency.

REPORTING OF LABORATORY RESULTS

Written reports of the studies with subsequent recommendations for appropriate action are submitted to the Iowa Water Quality Commission. Copies of the final reports are available to the general public upon request.

SWIMMING POOLS

INTRODUCTION

The State Hygienic Laboratory at Iowa City examines swimming pool samples for bacterial quality according to the EPA drinking water standards. Samples from outdoor public pools should be submitted to the State Hygienic Laboratory at Iowa City during the summer months while the pools are in operation. This service is provided in conjunction with the Iowa State Department of Health.

A month's supply of the regular black-capped (for chlorinated supplies) sterile sample bottles is sent to public pools prior to the opening season (Figure 12-

Replacement sample containers are automatically shipped to pool operators on a monthly basis. Indoor public pool sampling schedules are included in the municipal water mailing list from the State Hygienic Laboratory at Iowa City.

Private agencies and individuals who wish their swimming pool water examined must contact the State Hygienic Laboratory at Iowa City and request the proper sample container to be sent to them. The fee for this service is \$3.00 per sample. Payment should be enclosed with the specimen.

COLLECTION AND SHIPMENT OF SPECIMENS

Collect two samples, one at the shallow end and one at the deep end. Samples will be rejected if received in the Laboratory more than two days after the date of collection.

- 1. Grasp the sample bottle near the bottom and remove the cap; be careful not to lay the cap down. Hold the bottle at a 45° angle.
- 2. Fill the bottle in one full sweep down through the water with the mouth of the bottle always ahead of the hand.
- 3. Carefully recap bottle tightly.
- Time of sampling should coincide with the peak load.
- Fill out the data form completely as directed.
 Unless the date of collection is filled in on the data form, the specimen will be rejected.
- 6. Place the completed data form in the plastic bag provided; place the plastic bag and filled sample bottle in the styrofoam shipping container. Mail at once, using the return-addressed mailing box provided in the kit, to:

State Hygienic Laboratory Medical Laboratories Building The University of Iowa Iowa City, Iowa 52242

REPORTING OF LABORATORY RESULTS

On completion of the sample examination, a copy of the original data sheet with the laboratory findings is reported by mail to the agency or individual submitting the sample, and to the State Department of Health.

MILK QUALITY

INTRODUCTION

The program for examining milk and other dairy products is set up on a contractual basis. Only samples submitted by the Johnson County Board of Health, an area private dairy or the University of Iowa Environmental Health Services are included in the program.

The ultimate aim of the control program is to provide a product in which the original nutritive qualities, flavor, and appearance have been preserved and no harmful organisms or substances are present to affect the consumer. Specimens are examined according to the latest edition of Standard Methods for the Examination of Dairy Products.

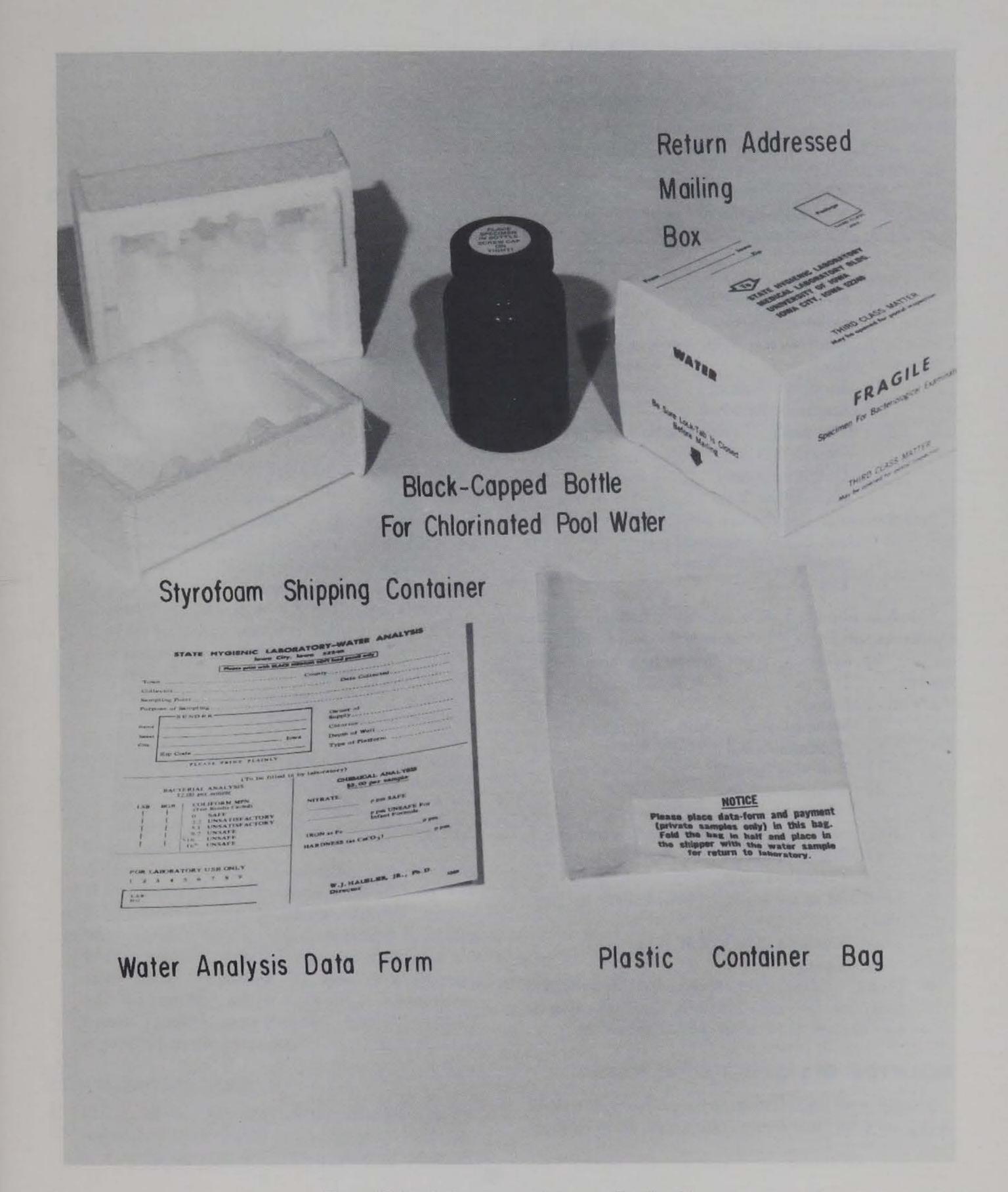


Figure 12-6 Swimming pool water collection kit

COLLECTION AND SHIPMENT OF SPECIMENS

Samples examined for the Johnson County Board of Health are submitted by the milk sanitarian at regular intervals.

Types of samples and examinations include:

- 1. Raw milk (producer samples)
 - a) Standard Plate Count
 - b) Antibiotic Residual
 - c) Microscopic Cell Count (mastitis control)
- 2. Processed milks
 - a) Standard Plate Count
 - b) Coliform Count
 - c) Phosphatase Test (proper pasteurization)
 - d) Butterfat Content
 - e) Antibiotic Residual
- 3. Other dairy products (cheeses, butter, dips)
 - a) Standard Plate Count
 - b) Coliform Count
 - c) Phosphatase Test

Processed milk samples are received daily from an area private dairy and are examined for:

- a) Standard Plate Count
- b) Coliform Count

Samples submitted on a weekly schedule for the University of Iowa Environmental Health Services include the following types of samples and examinations:

- 1. Processed milks
 - a) Standard Plate Count
 - b) Coliform Count
 - e) Butterfat Content
 - d) Phosphatase Test
 - e) Antibiotic Residual
- 2. Other dairy products (cheeses, ice creams, dips)
 - a) Standard Plate Count
 - b) Coliform Count
- Adult and infant formulas from University Hospital
 - a) Standard Plate Count
 - b) Coliform Count
- Utensil swab samples (University cafeterias, sorority and fraternity kitchens, University dormitory kitchens): Standard Plate Count only.

REPORTING OF LABORATORY RESULTS

Results of all examinations are reported to the proper agencies for their consideration and interpretation.

PEDIATRIC LEAD POISONING PROGRAM

INTRODUCTION

Laboratory analytical services related to the problem of plumbism in children 1-6 years of age are available in the State Hygienic Laboratory, Des Moines Branch.

The analysis consists of a screening test procedure for Erythrocyte Protoporphyrin (EP) as the primary indicator of toxicity due to body burden of lead or iron deficiency anemia. A confirmatory micro-blood lead analysis will be requested in those situations as indicated by the EP result.

COLLECTION AND SHIPMENT OF SPECIMENS

- A. Erythrocyte Protoporphyrin
 - All supplies necessary to the collection and shipment of these specimens will be provided by the Des Moines Branch Laboratory.
 - 2. A minimum of twenty microliters (20 μ l) of blood is taken from a finger prick source.
 - The collection capillary tube is capped and immediately placed in an opaque carrier tube which has been identified by the patient's name and case number. This information should also be placed on the accompanying data form.
 - Return sample to the laboratory in the mailer provided (Figure 13-1). NOTE: As mentioned in item 1, mailers will be provided for either single or multiple sample submissions.
- B. Blood Lead Analysis

All supplies necessary to collection and shipment of specimens for micro-blood lead analysis will be provided by the Des Moines Branch Laboratory. NOTE: Very specific, controlled sampling techniques are required for the collection of micro-blood specimens for lead analysis and will be supplied, along with other information, to new program areas that have had no previous experience in this procedure.

REPORTING RESULTS

 Results of the EP test will be reported on the respective data forms as equivalents of free erythrocyte porphyrin (FEP) μg/100 ml of whole

- blood by the method of Piomelli (J. Lab. Clin. Med., 81:932, 1973).
- The results of the micro-blood lead analysis will be reported on the respective data forms as μg of lead/100 ml of whole blood per the CDC modification of the micro Delves cup-AA procedure.

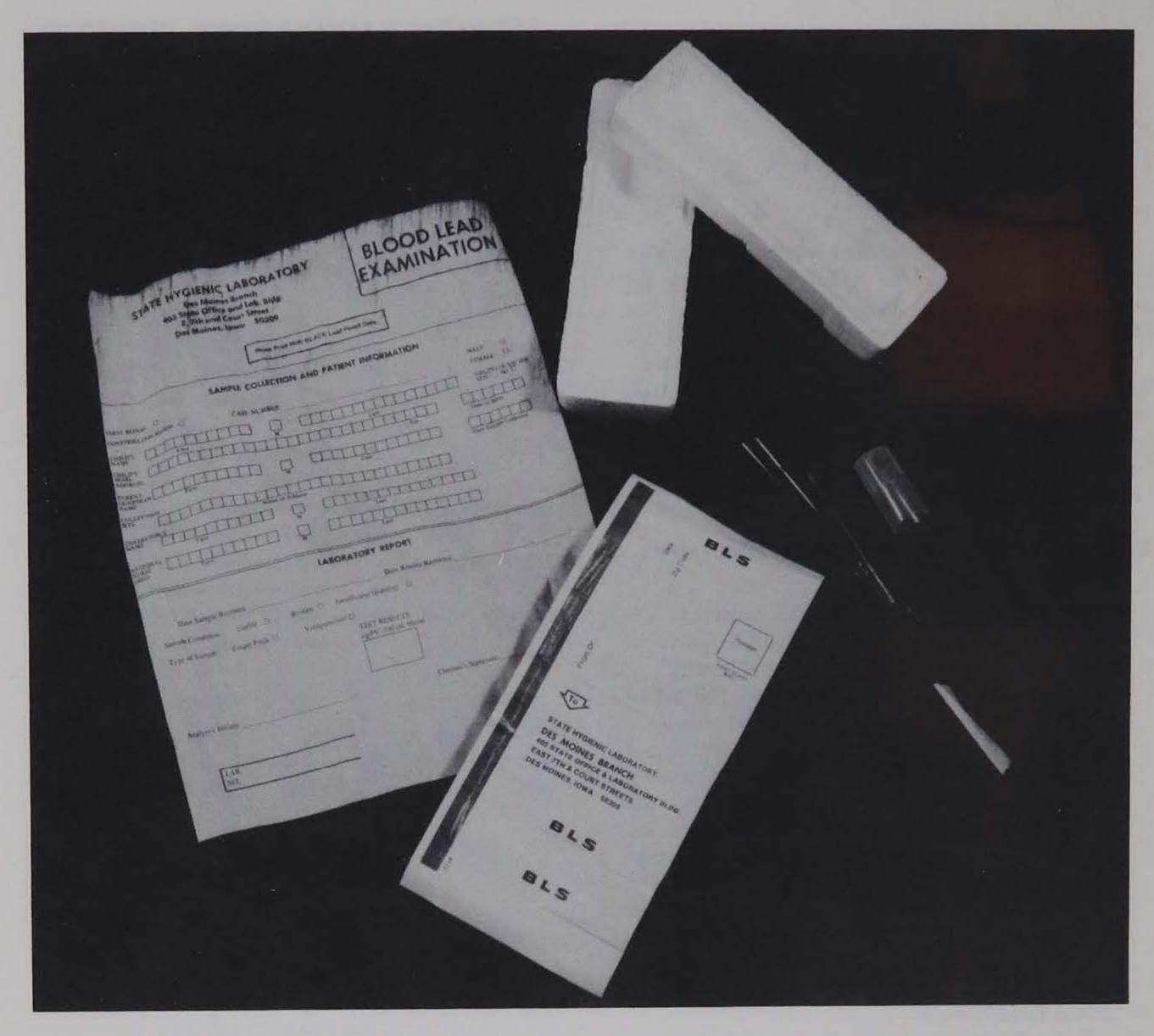


Figure 13-1 Blood lead collection kit

INTRODUCTION

The Laboratory Consultation and Development Division offers services which benefit all Iowans, lay and professional persons alike. Its function is to improve the performance of clinical laboratories in Iowa. This laboratory improvement is effected by utilizing three principal areas of activity.

- A voluntary laboratory performance evaluation program is conducted which determines competency of personnel, and suitability and reliability of procedures in local laboratories. Performance evaluation is available in five laboratory specialties: bacteriology, clinical chemistry, special serology, parasitology and phenylketonuria (PKU). Those laboratories which perform the VDRL test for syphilis are required to participate in the performance evaluation program for this specialty.
- 2. Depending upon needs of local laboratories demonstrated in the performance evaluation surveys, and upon the needs evidenced by requests for assistance to the State Hygienic Laboratory, training courses in various laboratory disciplines are presented both at the State Hygienic Laboratory in Iowa City and at various field locations as requested. Examples of such courses conducted in recent years include: laboratory management, instrumentation in clinical chemistry, fluorescence microscopy, bacteriology, parasitology and hematology.
- The Laboratory Consultation and Development Division assists the State Health Department in certification of local laboratories for participation in Medicare. This function involves periodic on-site inspections of hospitals and independent laboratories.

COLLECTION AND SHIPMENT OF SPECIMENS

The only activity of the Laboratory Consultation and Development Division which involves the collection of specimens is the Phenylketonuria (PKU) Screening and Confirmatory Testing Service. THE STATE HYGIENIC LABORATORY WILL CONFIRM WITHOUT CHARGE ALL PRESUMPTIVE POSITIVE TESTS (4mg% or greater) (EITHER GUTHRIE OR QUANTITATIVE) FOR ANY IOWA LABORATORY OR HOSPITAL. THE STATE HY-

GIENIC LABORATORY DOES NOT PERFORM THE PKU TESTS ROUTINELY FOR ANY IOWA HOSPITAL except on newborn infants delivered at the University of Iowa Hospitals.

The Guthrie test for phenylalanine is performed on dried blood specimens submitted to the State Hygienic Laboratory on a special PKU blood test card complete with microlance and instructions for collecting the blood sample (Figure 14-1). The PKU blood test kit is available from Difco.

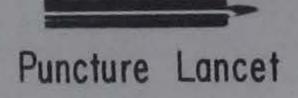
Specimens which show a screening value suggestive of phenylketonuria are examined further by a quantitative procedure. One ml of serum (not whole blood) is required for this procedure and should be submitted to the PKU laboratory, State Hygienic Laboratory. The serum for quantitative examination may be shipped as described for blood specimens in the section on virology in this manual.

The PKU tests are performed in compliance with

| Patient Age Sex | , IOW |
|--|-------|
| Physician (Name and Address) Dr. 7. H. AR. MEANDRIBE HOST AL Brimmation Desired: PRO Primary Culture O Subculture Source of Specimen: PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| Dr. 7. H. AR. Beammation Desired: Meandrine Hose at PRO Primary Culture Source of Specimen: PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Examination Desired: PRO Primary Culture Source of Specimen: Laboratory Report | |
| Dr. Mentales Host at Oprimary Culture Bloide D. Jowa Zip Code Social Source of Specimen: PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| MEANDRIBLE HOSE ALL OPRIMARY Culture BIGIOLIA , Iowa Zip Code SOCCI PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| Zip Code Social Source of Specimen: PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| PLEASE PRINT PLAINLY CLINICAL DIAGNOSIS Laboratory Report | |
| CLINICAL DIAGNOSIS | - |
| | |
| 12may 21. [1] | |
| <2mg/s ptorplateanx | |
| | |
| | |
| | 1 |
| | |
| Date Reported | |
| Date Reported | |
| LAB W. J. Hausler, Jr., Ph.D. | |
| NO. Director | |

Figure 14-2 PKU report form





PKU Blood Test Card

PKU BLOOD TEST

Fill in all information with pencil only:

| Baby's | Varne | | | | |
|----------------------|----------|------------|----------|--------------|---|
| baoy's | value | | | | |
| Date of | birth | | | | |
| | feeding | | - | | |
| Bottle [|); B | reast []; | Both | | |
| Date of | | | -01000 | | |
| Prematur Baby's D | | Yes []; | No [|] | |
| octor's | Address: | | | | |
| | FILL 3 | CIRCLES V | WITH BLO | ood uah.) | |
| | (Be sur | e blood at | | | |
| 1 | 1 | | 1 | | 1 |
| (|) | (|) | |) |
| 1 | | | | - | |

Sampling Instructions

INSTRUCTIONS FOR COLLECTING BLOOD SAMPLE

After the skin is sterilized, the infant's heel is punctured with a sterile disposable lancet. If bleeding is slow, it is helpful to hold the limb dependent for a short period before spotting the blood on the filter paper. To insure an adequate test, each circle on the filter paper must be filled completely with a large drop of blood which has soaked through the paper. This can best be done by placing this side of the filter paper against the baby's heel and watching for the blood to appear on the front side of the paper and completely fill the circle.

The blank spaces are filled in with pencil (ball point pen or regular ink blurs and becomes illegible in the autoclaving that precedes the testing).

the Iowa law recommending testing of infants for phenylketonuria. The PKU Screening and Confirmatory Testing Service is a significant part of a program designed to prevent mental retardation.

REPORTING OF LABORATORY RESULTS

In the case of PKU specimens with values of four mg percent of phenylalanine or greater, the result is telephoned to the physician or laboratory which submitted the specimen. Results on all specimens are reported on a miscellaneous examination form (Figure 14-2) to the physician or laboratory submitting the specimens. All specimens with an elevated (> four mg percent) phenylalanine value are reported to the director, Child Development Clinic, University of Iowa Hospitals and Clinics, Iowa City, and by him to the director, Maternal and Child Health Division, Iowa State Department of Health, Des Moines.

DRUG ANALYSIS

INTRODUCTION

The laboratory for analysis of controlled substances within the State Hygienic Laboratory (Des Moines Branch) will provide analytical service on clinical specimens (urine) for diagnostic and treatment monitoring purposes. Specimens will be accepted from any hospital, mental health institute, drug abuse treatment authority, private clinic or other agencies having legal basis for submission of specimens and need for laboratory identification. A fee of \$7.00 will be charged for each urine specimen.

COLLECTION AND SHIPMENT OF SPECIMENS

Submission of fluid urine specimens to the laboratory will be made in State Hygienic Laboratory prepared containers.

Fluid urine specimens collected in these containers which have a special preservative added may be shipped directly to:

State Hygienic Laboratory
Des Moines Branch
405 State Office & Lab Bldg.
East 7th & Court
Des Moines, Iowa 50309

The urine specimen should be at least 50 ml in volume but the container should not be overflowed or rinsed out prior to sampling. The appropriate information should be legibly entered on the data slip accompanying the specimen.

Containers are available on request from the Des

Moines Branch Laboratory.

REPORTING OF LABORATORY RESULTS

The result of laboratory analysis will be entered on the laboratory report form. These reports will be forwarded directly to the referring physician, approved drug abuse program with copies to the Drug Abuse Authority where they will be used for statistical purposes. Reports of all findings are submitted in writing.

3 1723 02075 1319