state hygienic laboratory manual of services

prepared for practicing physicians and public agencies of iowa

State Hygienic Laboratory

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (cont'd)

PREFACE

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.

It is hoped that this manual will be a useful and frequently consulted reference. It has been designed to accommodate any additions or deletions which may be needed to keep it updated. The State Hygienic Laboratory will notify all concerned of any such changes and provide the materials necessary, if any, to make these changes. In this way the manual will not become obsolete.

Editor

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.

1. 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.*
3. 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 attainable.

1. 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, remain there for a few seconds and then deftly withdraw it.
3. 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 membrane 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.

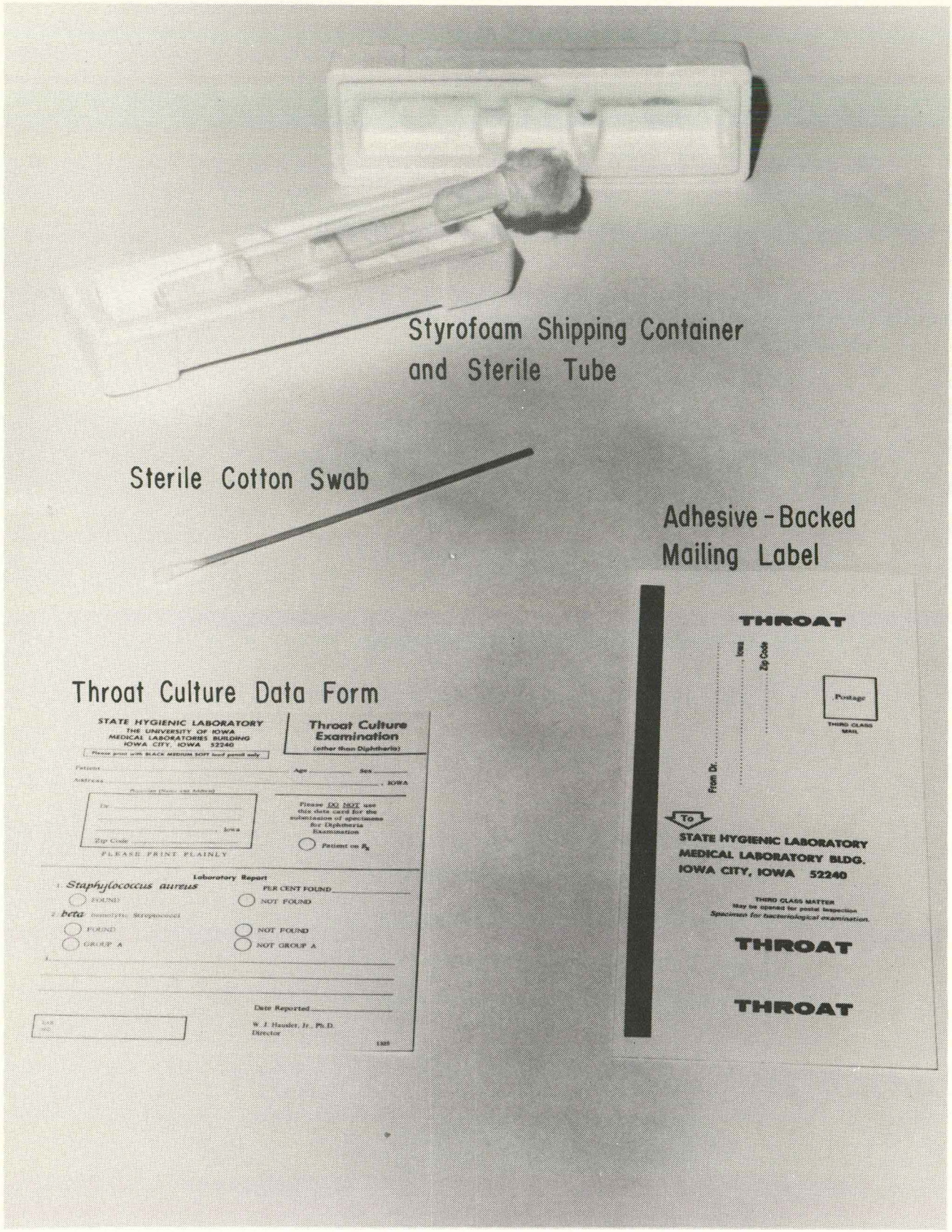
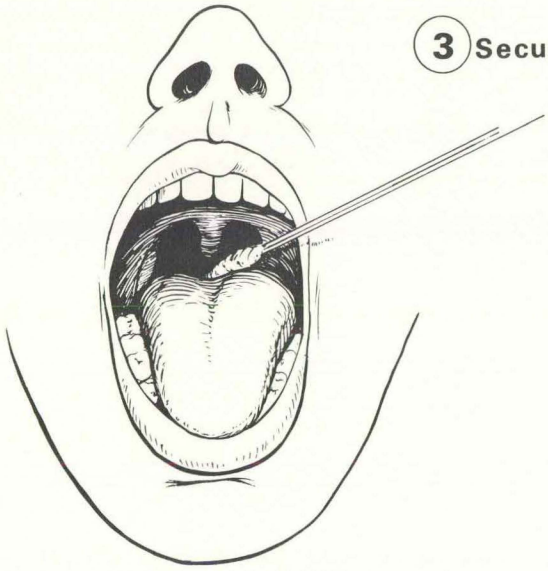


Figure 2-1 Throat specimen collection kit

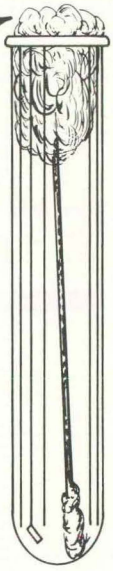
1 Obtain with sterile throat swab as soon as possible after onset of illness, material from posterior pharynx, tonsils and faucial pillars.

(see diagram below)



2 Place swab in tube DO NOT touch any exterior surface of tube.

3 Secure cotton



4 DO NOT BREAK SWAB

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

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Throat Culture Examination
(other than Diphtheria)

Patient Marvin Kellogg Age 19 Sex M
Address 1037 Kent St. Big Town, IOWA

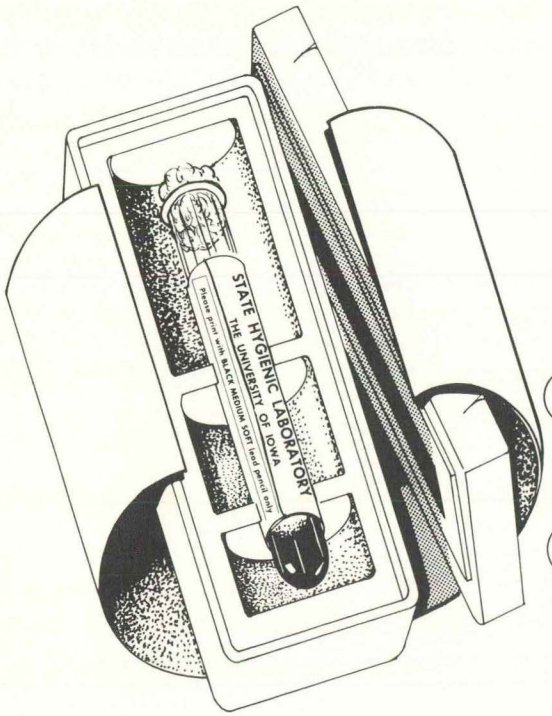
Physician (Name and Address)

Dr. Daniel Lyon
Medical Clinic
Big Town, Iowa
Zip Code 52254

PLEASE PRINT PLAINLY

Please DO NOT use this data card for the submission of specimens for Diphtheria Examination

Patient on R_x



6 Place in mailing container

7 Wrap adhesive label around container and mail

Figure 2-2 Procedure for obtaining throat swab

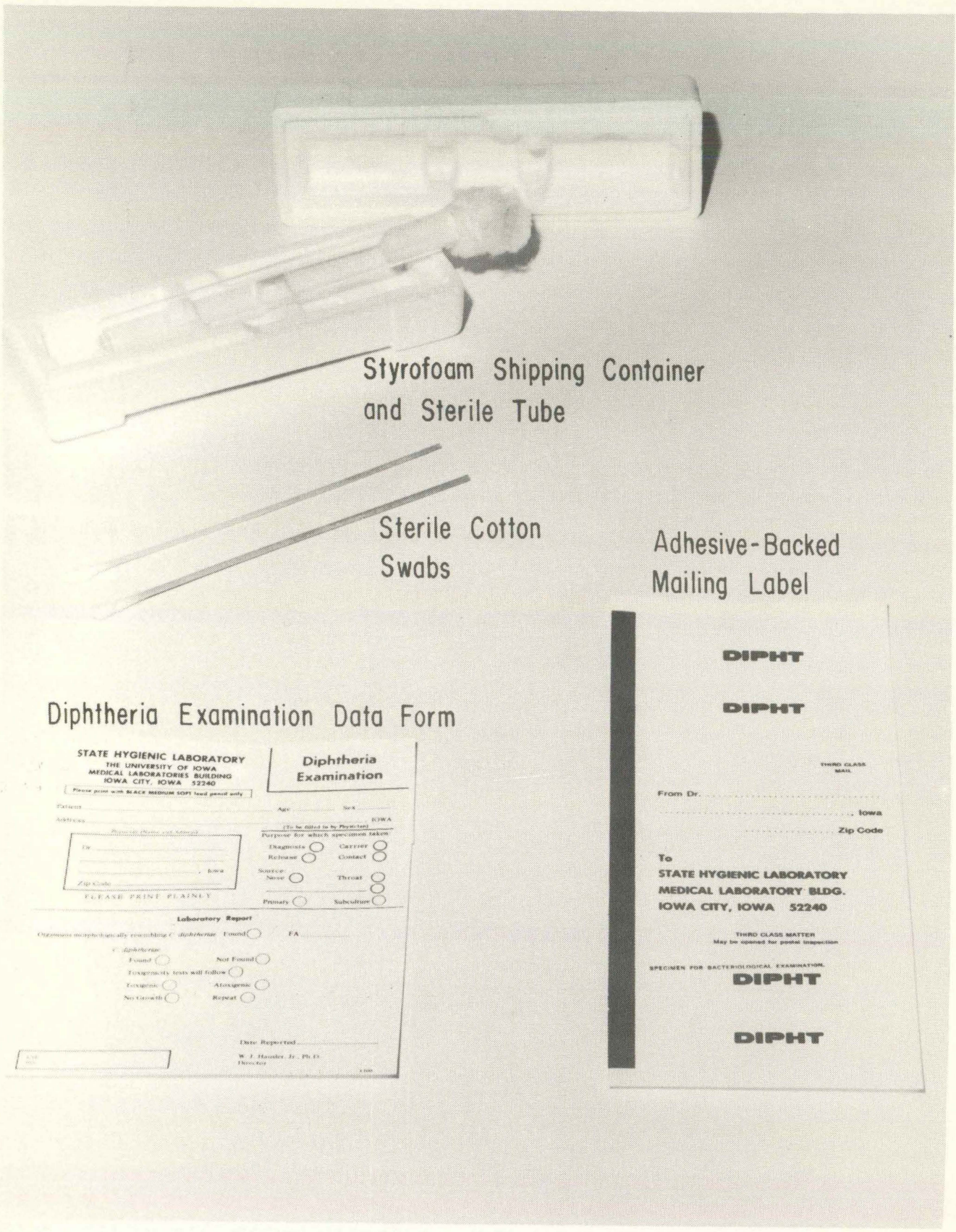


Figure 2-3 Diphtheria specimen collection kit

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

1. 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.
3. 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.
4. Complete data form supplied in the kit by giving all requested information.
5. 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.

Remember:

1. Don't ship a specimen without using a preservative.
2. Don't collect an excessive volume of stool specimen.
3. Be sure to secure screw-cap tightly.
4. Place the specimen bottle in the plastic bag, roll and bend wire tabs before putting it in the styrofoam mailer.

Submission of Subcultures by the Local Laboratory

The State Hygienic Laboratory serves as a reference laboratory for all of the local laboratories throughout Iowa. Only pure subculture isolates should be submitted for confirmation, identification, serogrouping and sero-typing as shown in Figure 2-6.

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 2-8), 3) slide examination kit for *Neisseria gonorrhoeae* (Figure 2-7). The Laboratory will examine, subculture and identify organisms isolated from commercial blood culture bottles and other commercial media such as Thayer-Martin or Transgrow for the detection of gonorrhea in the asymptomatic female. We cannot, however, provide commercial blood culture bottles or special media such as Thayer-Martin or Transgrow. These are available commercially from most laboratory supply houses.

Identification, confirmation and toxigenicity testing services are also provided on pure culture isolates submitted by laboratories within the state.

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

COLLECTION OF ENTERIC SPECIMENS

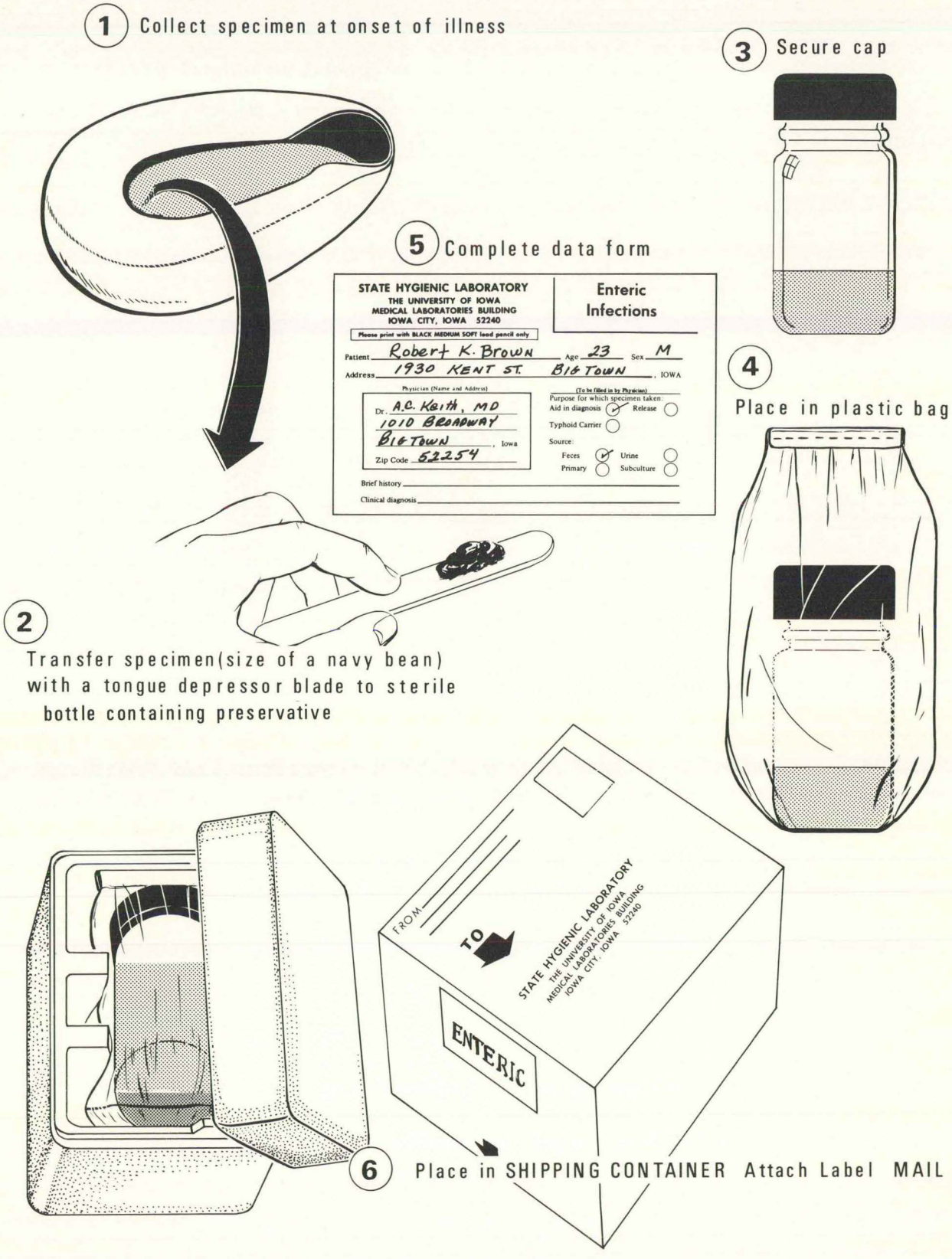


Figure 2-4 Procedure for collection of enteric specimen

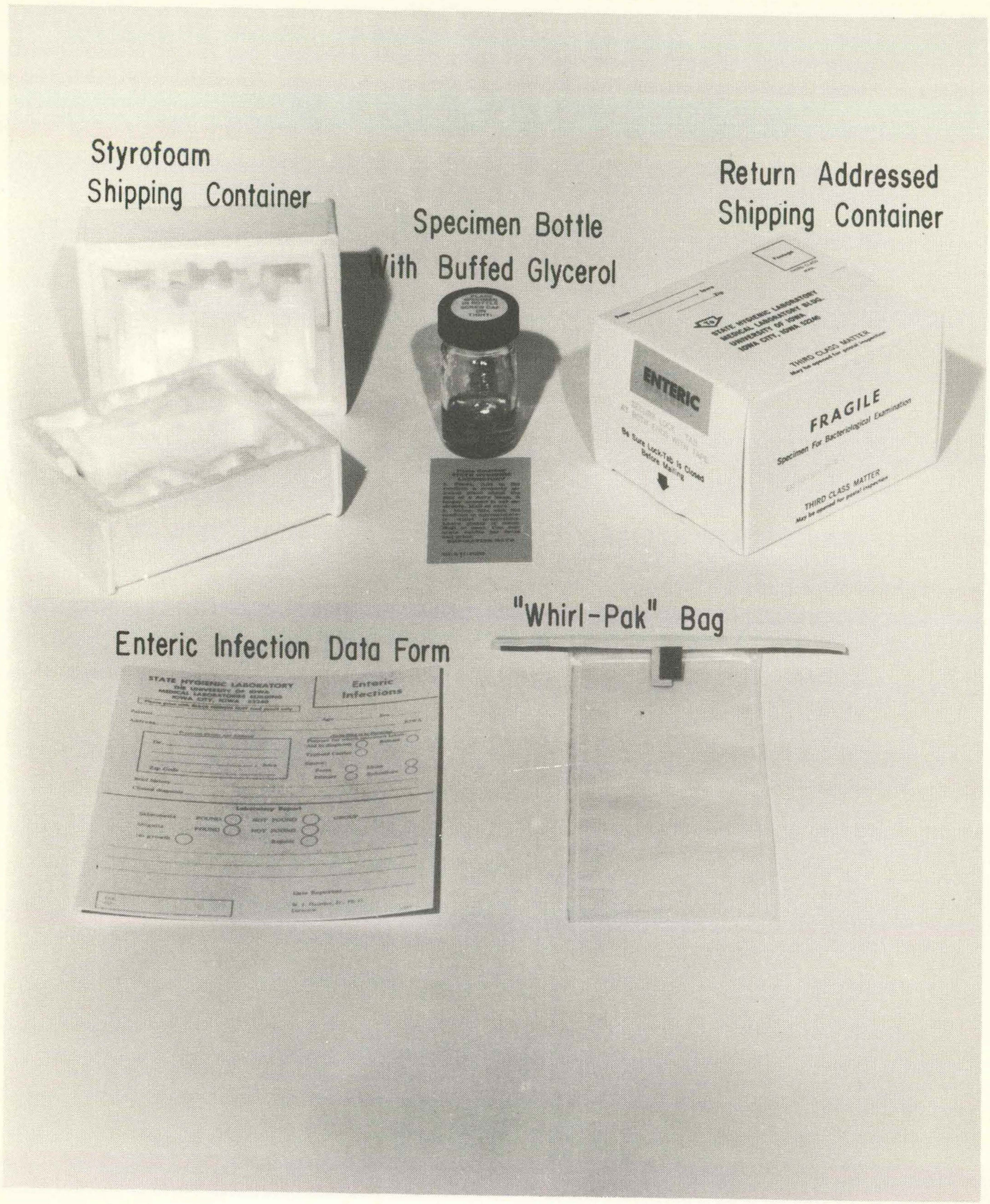


Figure 2-5 Enteric specimen collection kit

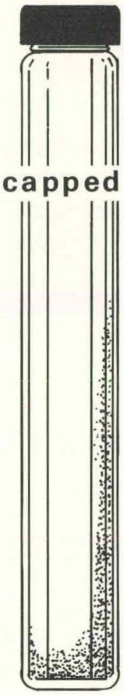
1

Streak specimen on appropriate plating media

petri dish

2

Pick single colony isolate to agar slant



screw capped tube

Agar Slant

3 **Incubate overnight at 37°C**

4 **Identify organism as far as possible**

5 **Complete data form**

STATE HYGIENIC LABORATORY
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MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

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Enteric Infections

Patient Ronald Fox Age 26 Sex M

Address 2233 23 St Big Town, IOWA

Physician (Name and Address)

Dr. B.L. Marvel
67 Verna Pl
Big Town, Iowa
Zip Code 52245

(To be filled in by Physician)

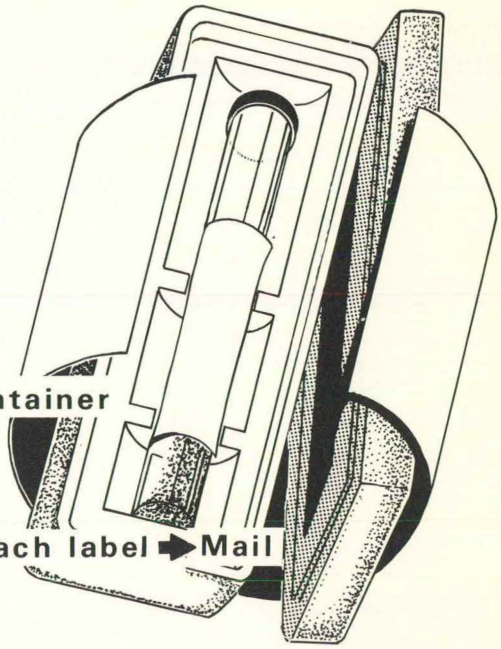
Purpose for which specimen taken:
Aid in diagnosis ◯ Release ◯
Typhoid Carrier ◯

Source:
Feces ◯ Urine ◯
Primary ◯ Subculture ◯

Brief history

Clinical diagnosis

Place in mailing container



Attach label ➡ Mail

Figure 2-6 Procedure for enteric subculture

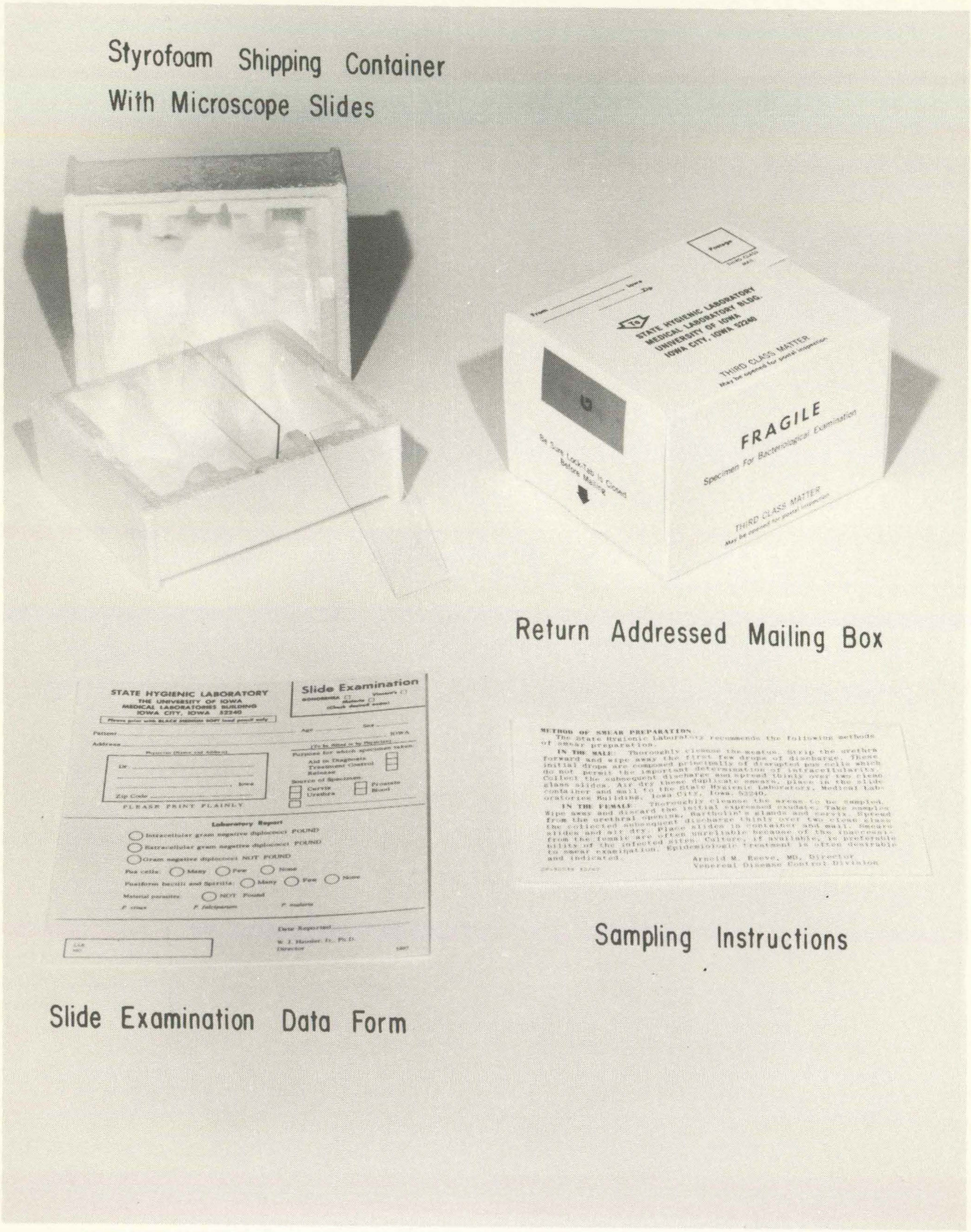
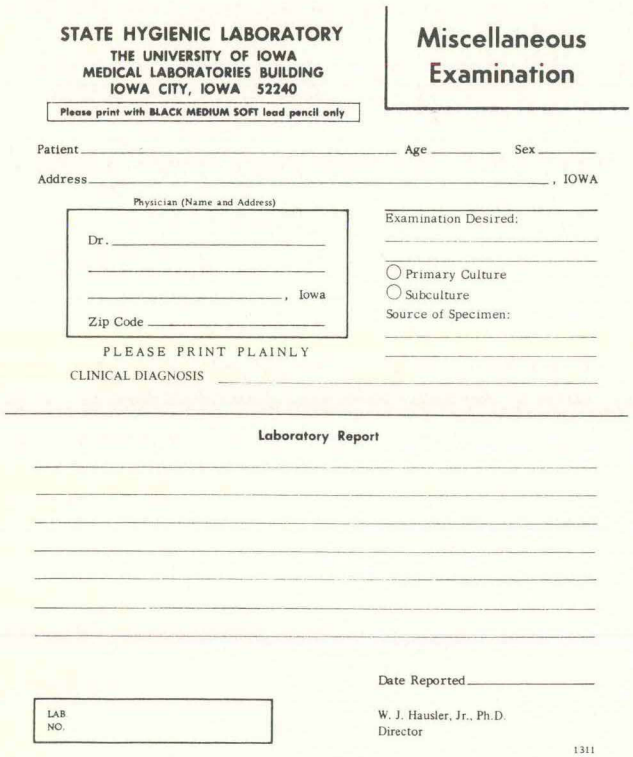


Figure 2-7 Slide examination kit

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 organisms. 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.



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Miscellaneous Examination

Patient _____ Age _____ Sex _____

Address _____, IOWA

Physician (Name and Address)

Dr. _____

_____

_____, Iowa

Zip Code _____

Examination Desired:

_____

_____

○ Primary Culture
○ Subculture

Source of Specimen:

_____

_____

PLEASE PRINT PLAINLY

CLINICAL DIAGNOSIS _____

Laboratory Report

_____

_____

_____

_____

_____

_____

_____

Date Reported _____

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 2-8 Miscellaneous data form

Miscellaneous culture kit

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. Contact the Office of the Director for additional information.

Slide and Culture Kits for the Diagnosis of Gonorrhea

Gonorrhea, unlike many other diseases, affects the male and female in a totally different manner. 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 female, however, is usually asymptomatic and the smear technique is almost valueless. Female patients must be cultured on special gonococcal media such as Thayer-Martin (TM), Transgrow (TG) or, in some instances, plain chocolate agar.

NOTE: We are unable to supply Thayer-Martin or Transgrow media. These are available commercially from most laboratory supply houses. Preliminary evaluation indicates that storage life of the commercial culture medium stored at room temperature may be in excess of three months, but the use of fresh medium will probably assure more reliable results.

Validity of culture results depends on proper techniques for obtaining, inoculating and handling specimens.

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 2-7). 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 medium. 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. If one of the Transgrow-type media is employed, be sure to follow the directions of the manufacturer to insure that no CO_2 escapes from the bottle (*hold upright at all times, never neck down as the CO_2 will escape*). 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, then the culture plate is incubated in an inverted position in a candle jar. A wide-mouth pickle or mayonnaise jar with any type of candle in it will provide the proper CO_2 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 gonococci 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 staining of direct smears.

For any miscellaneous examination, it is important that the laboratory request slip (Figure 2-8) 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.

Subcultures

Subcultures should be accompanied by the submitting laboratory's results of completed work on the specimen. Cultures should be purified before shipment.

Aerobic organisms are best submitted on agar slants of a medium suitable to support growth of the organism but without any fermentable carbohydrate. (Chocolate agar slants with CO_2 or carbohydrate-free CTA media are favorable for the shipment of *Neisseria* cultures.) The culture tubes should then be sealed with rubber or paraffin-treated corks taped on, or screw-caps tightened securely, and sealed with paraffin or waterproof tape. Each tube should also have identification of the culture—laboratory number, patient's name, sender and transport medium—on white tape or on the label on the outside of the tube along one side (behind the agar slant and not obstructing view of the culture). The culture should then be placed in the inner container and packed well with cotton. The miscellaneous data slip containing information about the culture should be placed between the two walls of a double container mailer.

Anaerobic cultures can be shipped in tubes of liquid or semisolid media. Plates or slants are not satisfactory. Cultures can best be shipped in a carbohydrate-free medium containing 0.3 to one percent agar such as motility medium or carbohydrate-free CTA medium. The medium should be freshly prepared and tubed two to three inches deep in screw-cap tubes. This method may also be used for shipment of aerobic organisms. *Clostridium* cultures in plain chopped meat medium or cultures of the nonsporeformers in thioglycollate medium can also be shipped. Prior to shipment, a ¾-inch to one-inch overlay of melted paraffin or five

percent agar should be added to actively growing cultures in either semisolid or liquid media. Screw-caps should be tightened, sealed with waterproof tape and packaged as above. Again these tubes should have identification (as above) that does not block view of the culture.

Since accuracy and reliability of laboratory findings are adversely affected by delay in transit, it is best that the sender hold the specimens over weekends and holidays and maintain them until they can be shipped to arrive during Laboratory working hours on weekdays.

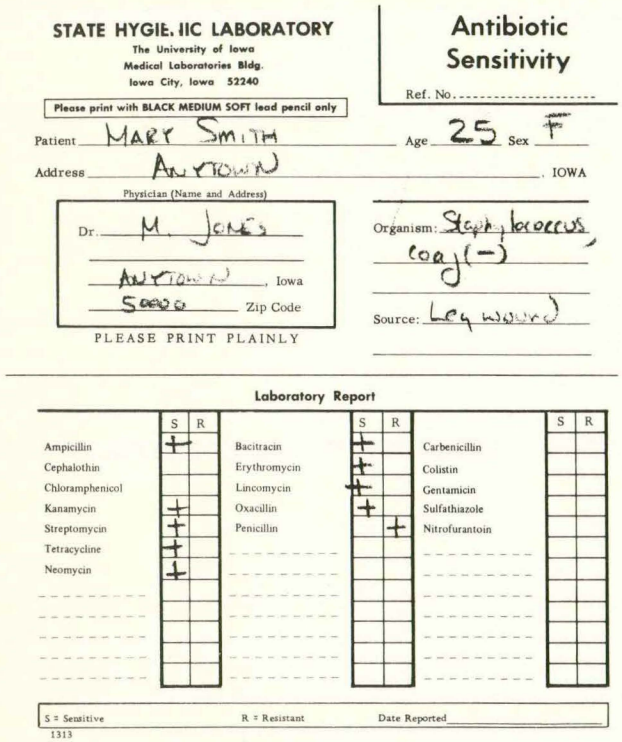
REPORTING OF LABORATORY RESULTS

Primary Specimens

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 2-9). 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 2-10). 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.

Subcultures

Results of subcultures are handled as above with the exception that antibiotic susceptibility tests are not performed. The report is accompanied by a profile of the organism's characteristics as determined by the State Hygienic Laboratory. This profile can be used by the laboratory referring the specimen as a quality-control check on their procedures, reagents and media.


STATE HYGIENIC LABORATORY
The University of Iowa
Medical Laboratories Bldg.
Iowa City, Iowa 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Antibiotic Sensitivity

Ref. No.

Patient MARY SMITH Age 25 Sex F

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M. JONES

ANYTOWN, Iowa

50000 Zip Code

PLEASE PRINT PLAINLY

Organism: Staphylococcus, coag (-)

Source: Leg wound

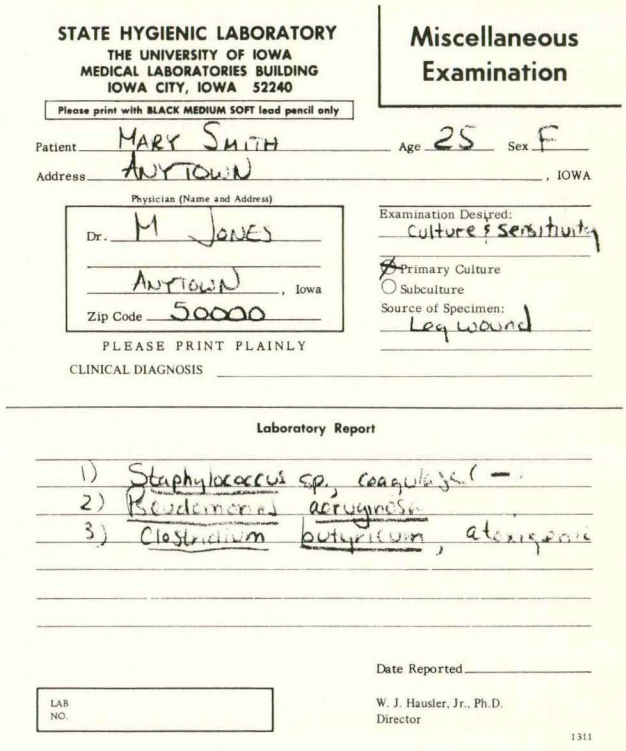
Laboratory Report

	S	R		S	R		S	R
Ampicillin	+		Bacitracin	+		Carbenicillin		
Cephalothin			Erythromycin	+		Colistin		
Chloramphenicol			Lincomycin	+		Gentamicin		
Kanamycin	+		Oxacillin	+		Sulfathiazole		
Streptomycin	+		Penicillin		+	Nitrofurantoin		
Tetracycline	+							
Neomycin	+							

S = Sensitive R = Resistant Date Reported

1313

Figure 2-9 Antibiotic sensitivity report form


STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Miscellaneous Examination

Patient MARY SMITH Age 25 Sex F

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M JONES

ANYTOWN, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

Examination Desired: Culture & Sensitivity

Primary Culture

Subculture

Source of Specimen: Leg wound

CLINICAL DIAGNOSIS

Laboratory Report

1) Staphylococcus sp., coagulase (-)
2) Pseudomonas aeruginosa
3) Clostridium butyricum, ateriogenic

Date Reported

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 2-10 Miscellaneous examination report form

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

1. 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.
2. 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
3. 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.
4. Food samples showing any decomposition or other evidence of mishandling subsequent to collection cannot be accepted for microbiological analysis by the Laboratory.
5. 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.
6. 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.

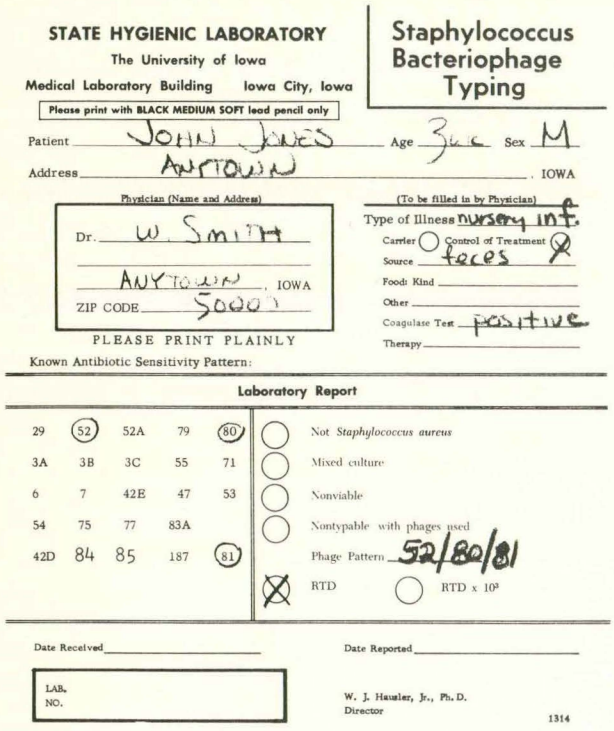
COLLECTION AND SHIPMENT OF SPECIMENS

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 2-11). Only coagulase-positive cultures should be submitted for phage typing as studies have shown that coagulase-negative isolates rarely if ever phage type. It should be emphasized that this is a *special service* and neither required nor recommended to be used on a routine basis.

REPORTING OF LABORATORY RESULTS

The phage type will be reported on the appropriate data slip as illustrated below (Figure 2-11). The phage type in this particular instance was a 52/80/81 at routine test dilution.



STATE HYGIENIC LABORATORY
The University of Iowa
Medical Laboratory Building Iowa City, Iowa
Please print with BLACK MEDIUM SOFT lead pencil only

Staphylococcus Bacteriophage Typing

Patient JOHN JONES Age 3yrs Sex M
Address ANYTOWN, IOWA

Physician (Name and Address)
Dr. W. SMITH
ANYTOWN, IOWA
ZIP CODE 50001
PLEASE PRINT PLAINLY

(To be filled in by Physician)
Type of Illness nursery inf.
Carrier ◯ Control of Treatment ⊗
Source feces
Food: Kind
Other
Coagulase Test positive
Therapy

Known Antibiotic Sensitivity Pattern:

Laboratory Report

29	(52)	52A	79	(80)
3A	3B	3C	55	71
6	7	42E	47	53
54	75	77	83A	
42D	84	85	187	(81)

◯ Not *Staphylococcus aureus*
◯ Mixed culture
◯ Nonviable
◯ Nontypable with phages used
Phage Pattern 52/80/81
⊗ RTD ◯ RTD x 10^3

Date Received
Date Reported
LAB. NO.
W. J. Hausler, Jr., Ph. D.
Director
1314

Figure 2-11 Bacteriophage typing report form

PARASITES AND OVA*

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. This stool should be a *purged stool.*

   Preferably on arising in the morning on an empty stomach, give patient two oz of a saturated solution of sodium sulphate followed by three glasses of water. Collect the specimen from the latter part of the purging.

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

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

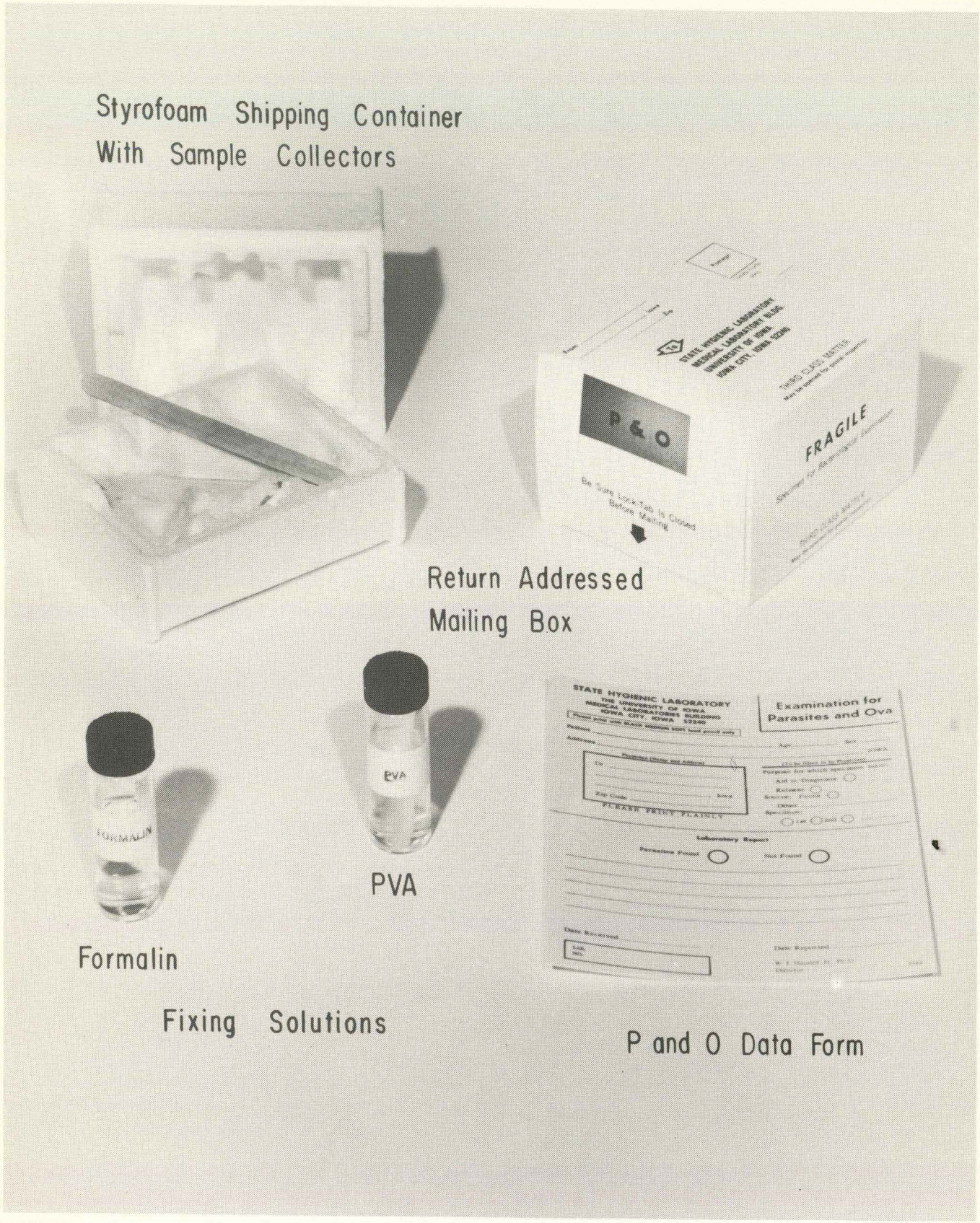


Figure 3-1 Parasites and ova specimen collection kit

formalin solution, screw cap on tightly and mix thoroughly.

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

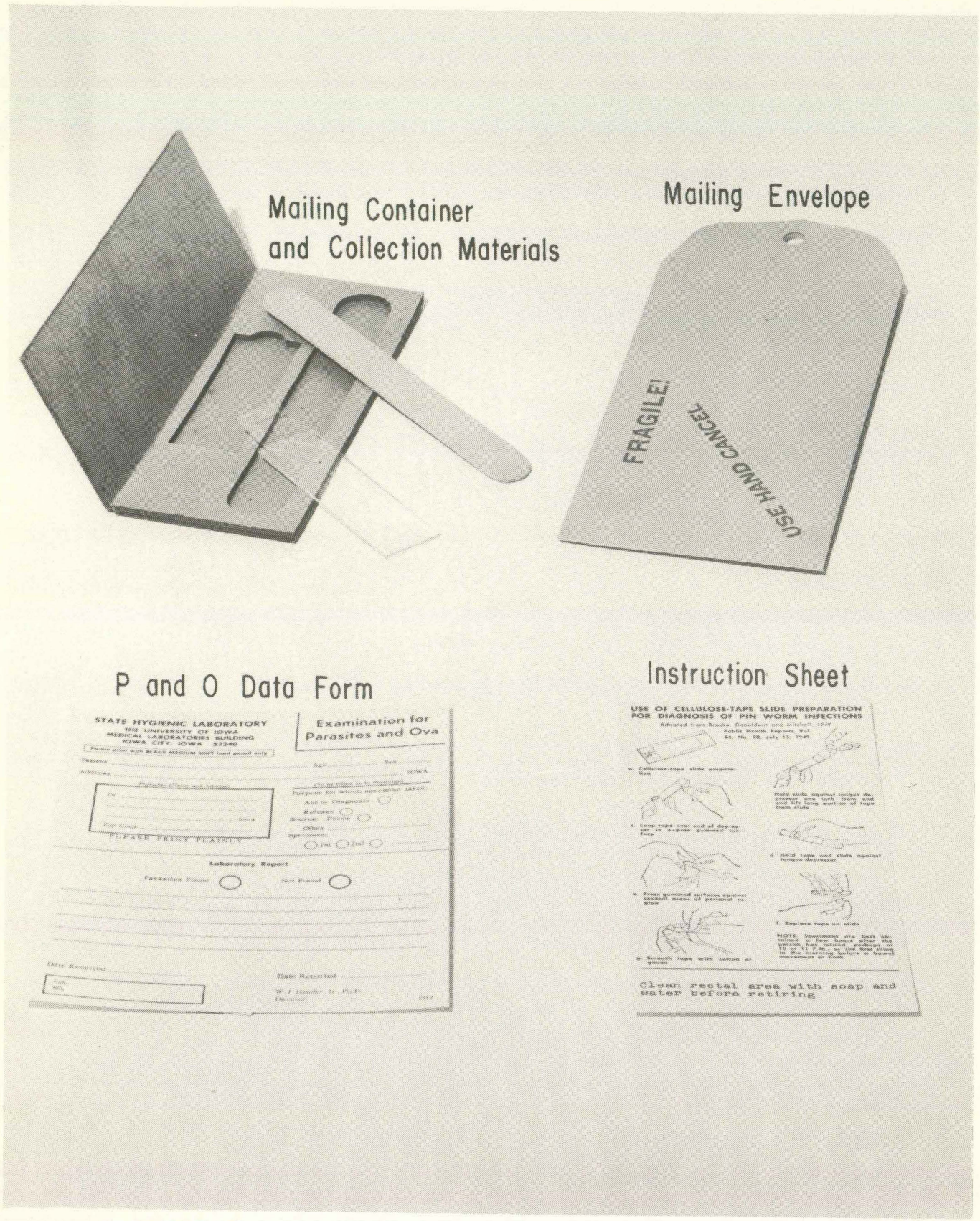


Figure 3-2 Pinworm specimen collection kit

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.

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.)
2. Blood should be drawn before breakfast or at least three hours after the last meal in order to avoid chylous specimens.
3. Blood should be drawn before or at least 24 hours after the application of intradermal tests.
4. All apparatus for collecting specimens should be washed free of alkali and acid before sterilization. Only sterile dry apparatus should be used throughout.
5. 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.

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 pencil only

Patient MARY SMITH Age 19 Sex F

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. F. JONES
ANYTOWN, Iowa
Zip Code 50000
PLEASE PRINT PLAINLY

(To be filled in by Physician)

Purpose for which specimen taken:
Aid in Diagnosis ⊘
Release ○
Source: Feces ⊘
Other
Specimen:
⊘ 1st ○ 2nd ○

Laboratory Report

Parasites Found ⊘ Not Found ○

Entamoeba coli

Date Received

Date Reported

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

1312

Figure 3-3 Parasite and ova report form

REPORTING OF LABORATORY RESULTS

The laboratory results will be reported on the Parasites 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 heat-fixed 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* infections), 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

Please print with BLACK MEDIUM SOFT lead pencil only

Slide Examination

GONORRHEA ☐ Vincent's ☐ Malaria ☒ (Check desired exam)

Patient JOHN JONES Age 23 Sex M

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr J. SMITH

ANYTOWN, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

(To be filled in by Physician)

Purpose for which specimen taken:

Aid in Diagnosis ☒
Treatment Control ☐
Release ☐

Source of Specimen:

☐ Cervix ☒ Prostate
☐ Urethra ☒ Blood
☐ _____

Pus cells: Many ◯ Few ◯ None ◯

Intracellular gram negative diplococci: FOUND ◯ NOT FOUND ◯

Extracellular gram negative diplococci: FOUND ◯ NOT FOUND ◯

Gram negative diplococci: NOT FOUND ◯

Vincents (Fusiform Spirilla): FOUND ◯ NOT FOUND ◯

Malarial parasites: FOUND ◯

NOT FOUND ☒

Date Reported _____

LAB NO.

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Director

1297

Figure 3-4 Positive slide report for malaria

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Miscellaneous Examination

Patient WILLIAM JOHNSON Age 35 Sex M

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. T. GREEN

ANYTOWN, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

Examination Desired: Malaria IFA

◯ Primary Culture
◯ Subculture

Source of Specimen: Serum

CLINICAL DIAGNOSIS _____

Laboratory Report

Malaria IFA
P. vivax: 1:320
P. falciparum: Negative
P. malariae: Negative
P. ovale: Not performed

Date Reported _____

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 3-5 Positive IFA report for malaria

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 either Dr. Elizabeth Procter, Iowa State Health Department, 515/281-5445, or 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. "whirlpack" 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 phosphate-zephiran solution, b. "whirlpack" 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).
3. For the submission of TB subcultures please refer to shipping instructions as outlined in the section on Miscellaneous 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.

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. OLSON Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. BROWN

ANYTOWN, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊘
Subculture ○
Other

TB Therapy: INH PAS Streptomycin

MICROSCOPIC FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ○
Next report within ____ weeks
Specimen was unsatisfactory for culture because:
Date Received

CULTURAL FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ○
Next report within ____ weeks ○
FINAL REPORT ○

LAB NO.

W. J. Hausler Jr., Ph.D.
Director

1880

Figure 4-3
Completed TB data slip

Sputum

1. 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.
2. *Five to 10 ml of specimen will be enough for a proper examination.* There is no advantage in collecting a larger volume.
3. 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.

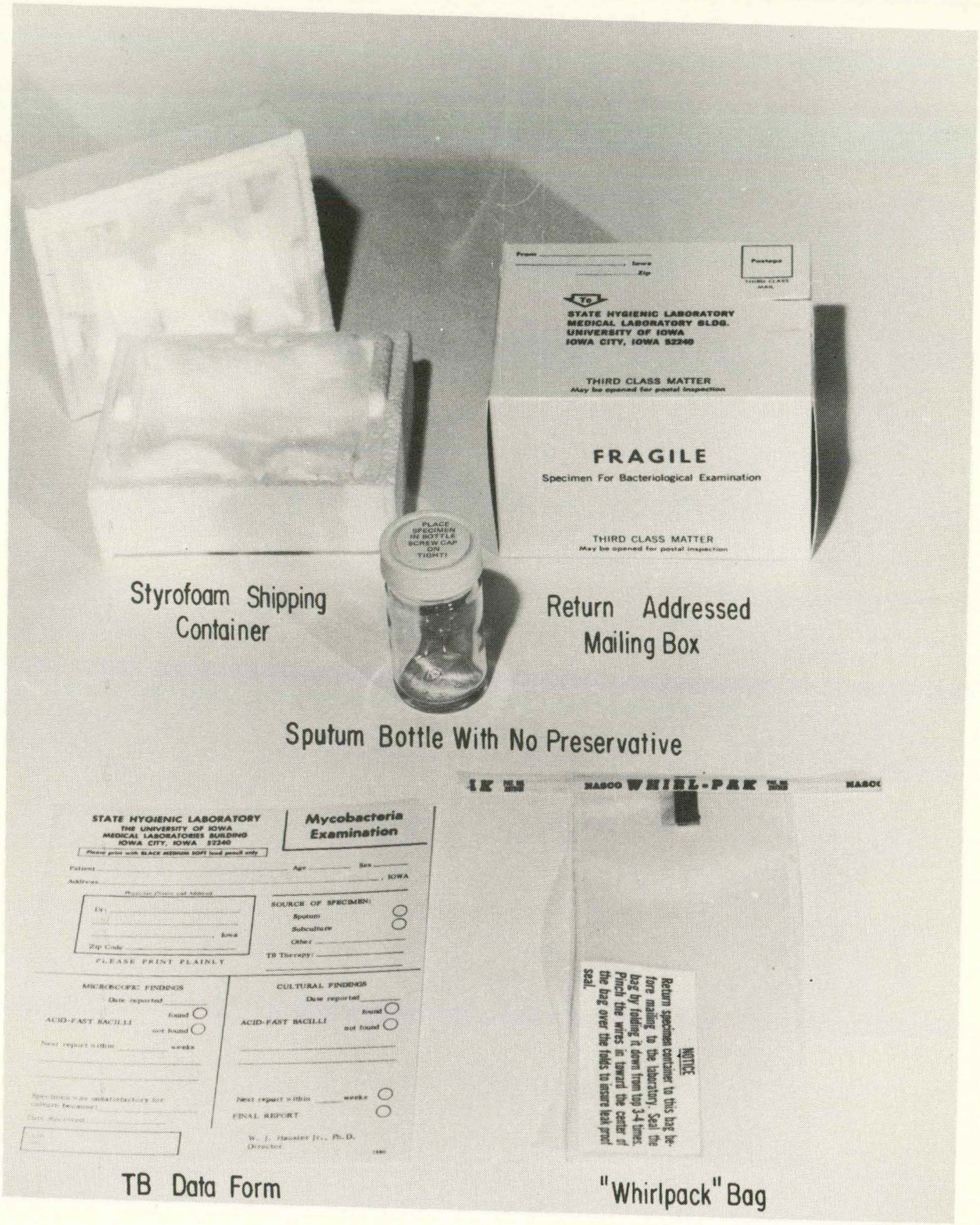

Figure 4-1 TB specimen collection kit

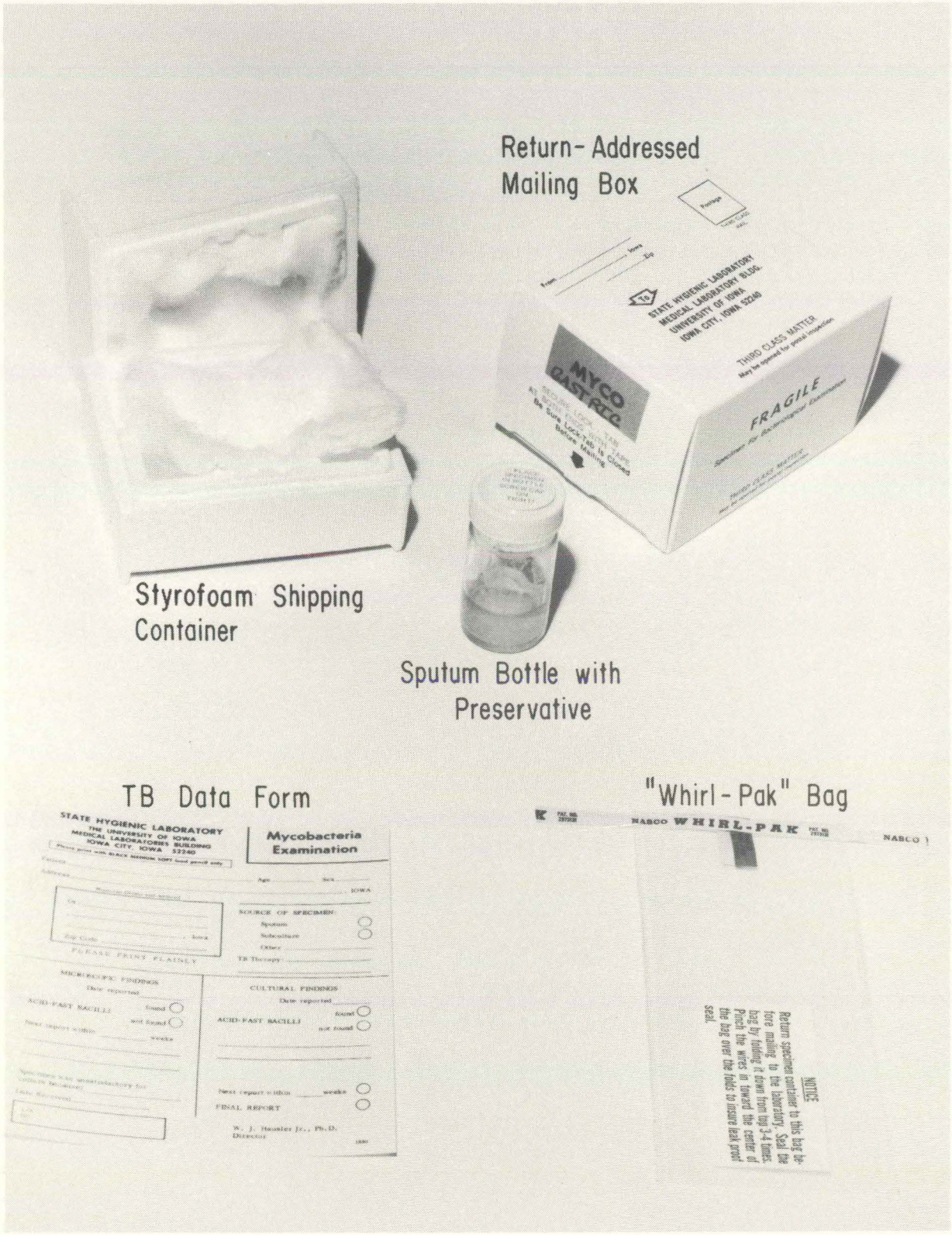

Figure 4-2 TB-Gastric specimen collection kit

Nebulized Sputum

1. Follow procedure for submission of sputum specimens.
2. Be sure to label nebulized sputum specimens in the following manner.

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olson Age 65 Sex F
Address Anytown, IOWA

Physician (Name and Address)
Dr. T. Brown
Anytown, Iowa
Zip Code 50000
PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum nebulized ⊗
Subculture ○
Other
TB Therapy:

MICROSCOPIC FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ○
Next report within weeks

CULTURAL FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ○

Specimen was unsatisfactory for culture because:
Date Received
Next report within weeks ○
FINAL REPORT ○

LAB NO.

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Director
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Figure 4-4 Identification of nebulized sputum

Gastric Washings or Lavage

1. 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.
3. 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.
5. 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.
4. Urine specimens pooled over 24-, 48- and 72-hour 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)

1. 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.
3. 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.

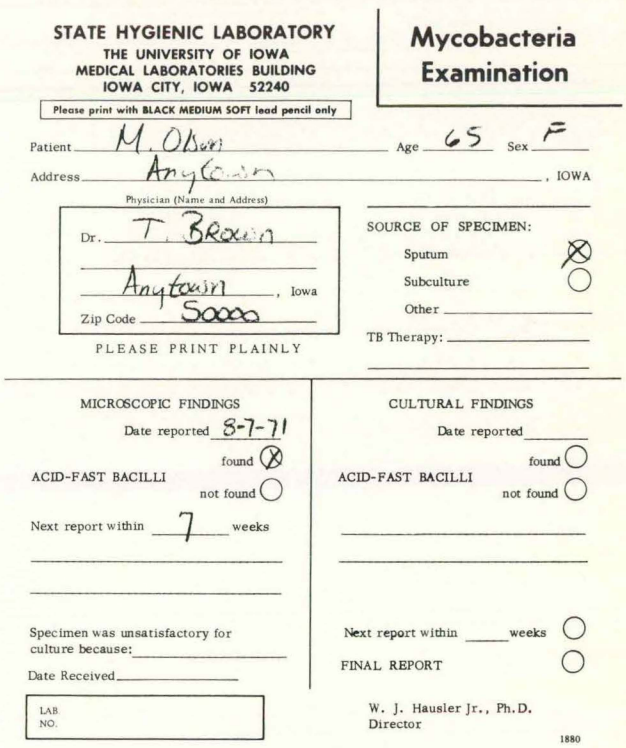
Negative cultures are reported after a seven-week incubation period.

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.


STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊗
Subculture ○
Other
TB Therapy:

MICROSCOPIC FINDINGS
Date reported 8-7-71
ACID-FAST BACILLI found ⊗ not found ○
Next report within 7 weeks
Specimen was unsatisfactory for culture because:
Date Received
LAB NO.

CULTURAL FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ○
Next report within weeks ○
FINAL REPORT ○

W. J. Hausler Jr., Ph.D.
Director

1880

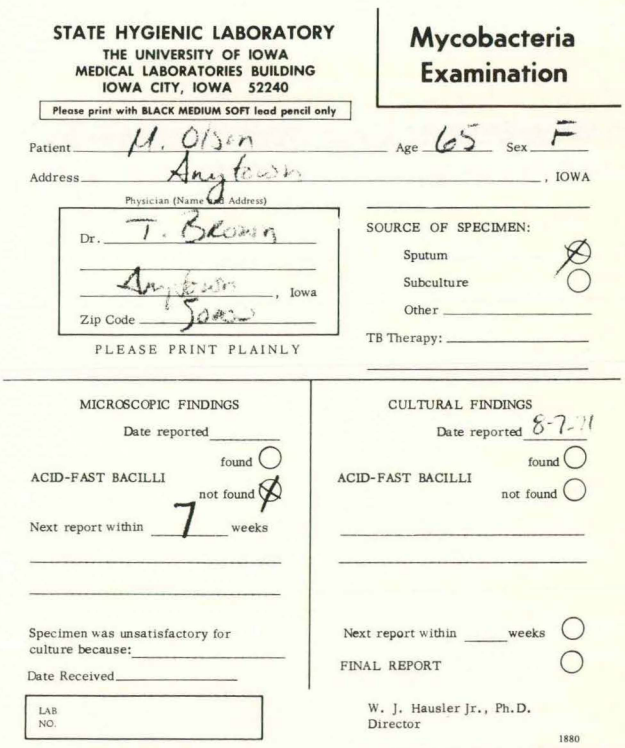
Figure 4-5 Report of positive microscopic TB examination

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. xenopi*	*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.

***M. scrofulaceum* is pathogenic when isolated from lymph nodes.


STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊗
Subculture ○
Other
TB Therapy:

MICROSCOPIC FINDINGS
Date reported
ACID-FAST BACILLI found ○ not found ⊗
Next report within 7 weeks
Specimen was unsatisfactory for culture because:
Date Received
LAB NO.

CULTURAL FINDINGS
Date reported 8-7-71
ACID-FAST BACILLI found ○ not found ○
Next report within weeks ○
FINAL REPORT ○

W. J. Hausler Jr., Ph.D.
Director

1880

Figure 4-6 Report of negative microscopic TB examination

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊘
Subculture ○
Other _____
TB Therapy: _____

MICROSCOPIC FINDINGS
Date reported _____
ACID-FAST BACILLI found ○ not found ○
Next report within _____ weeks
Specimen was unsatisfactory for culture because: _____
Date Received _____

CULTURAL FINDINGS
Date reported 8-7-71
ACID-FAST BACILLI found ○ not found ⊘
Next report within _____ weeks ○
FINAL REPORT ⊘

LAB NO.

W. J. Hausler Jr., Ph.D.
Director

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Figure 4-7 Report of negative TB culture

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊘
Subculture ○
Other _____
TB Therapy: _____

MICROSCOPIC FINDINGS
Date reported _____
ACID-FAST BACILLI found ○ not found ○
Next report within _____ weeks
Specimen was unsatisfactory for culture because: _____
Date Received _____

CULTURAL FINDINGS
Date reported 8-7-71
ACID-FAST BACILLI found ⊘ not found ○
Next report within 4 weeks ⊘
FINAL REPORT ○

LAB NO.

W. J. Hausler Jr., Ph.D.
Director

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Figure 4-8 Report of positive TB culture

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Examination

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN:
Sputum ⊘
Subculture ○
Other _____
TB Therapy: _____

MICROSCOPIC FINDINGS
Date reported _____
ACID-FAST BACILLI found ○ not found ○
Next report within _____ weeks
Specimen was unsatisfactory for culture because: _____
Date Received _____

CULTURAL FINDINGS
Date reported 8-7-71
ACID-FAST BACILLI found ⊘ not found ○
Presumptive
M. tuberculosis
Next report within 6 weeks ⊘
FINAL REPORT ○

LAB NO.

W. J. Hausler Jr., Ph.D.
Director

1880

Figure 4-9 Presumptive TB identification report

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Identification

Patient M. Olsen Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN
Sputum ⊘
Subculture ○
Other _____
Date Received _____

Final Biochemical Identification

Date Reported 8-7-71

Usually Pathogenic	Usually Saprophytic
☒ *M. tuberculosis*	☐ Runyon Group I – Photochromogen
☐ *M. bovis*	☐ Runyon Group II – Scotochromogen
☐ *M. kansasii*	☐ tap water subgroup
☐ *M. marinum*	☐ Runyon Group III – non-photo chromogen
☐ *M. scrofulaceum*	☐ *M. terrae* complex
☐ *M. avium – M. intracellulare* complex	☐ *M. gastri*
☐ *M. xenopi*	☐ *M. triviale*
☐ *M. fortuitum*	☐ Runyon Group IV – Rapid Grower
☒ Drug Susceptibility Not Completed Report Will Follow	☐ Drug Susceptibility Not Performed

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

2101

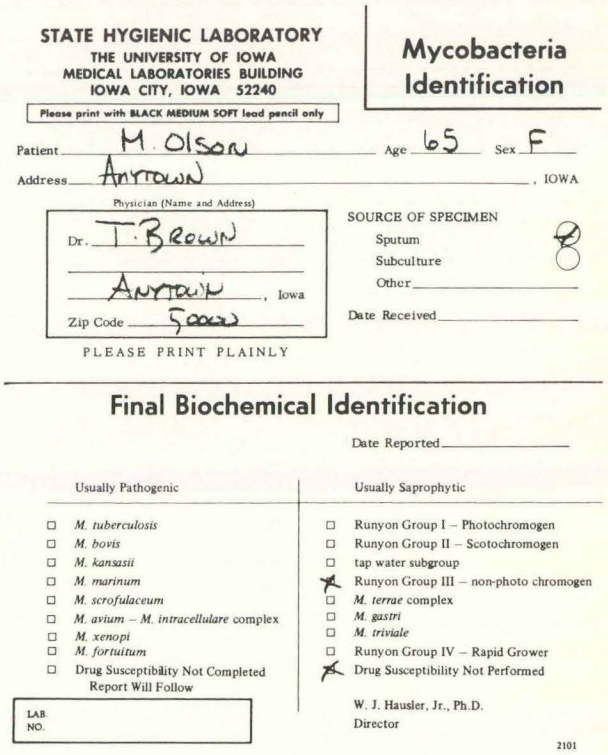
Figure 4-10 Report of TB drug susceptibility to be performed

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 and Ethambutol are performed routinely. Kanamycin, Viomycin, Pyrazinamide, Ethionamide, Cycloserine, Capreomycin and Rifampin are done on request.



STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Mycobacteria Identification

Patient M. Olson Age 65 Sex F

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Brown

Anytown, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

SOURCE OF SPECIMEN
Sputum ✓
Subculture 8
Other

Date Received

Final Biochemical Identification

Date Reported

Usually Pathogenic	Usually Saprophytic
☐ *M. tuberculosis*	☐ Runyon Group I – Photochromogen
☐ *M. bovis*	☐ Runyon Group II – Scotochromogen
☐ *M. kansasii*	☐ tap water subgroup
☐ *M. marinum*	☒ Runyon Group III – non-photo chromogen
☐ *M. scrofulaceum*	☐ *M. terrae* complex
☐ *M. avium – M. intracellulare* complex	☐ *M. gastri*
☐ *M. xenopi*	☐ *M. triviale*
☐ *M. fortuitum*	☐ Runyon Group IV – Rapid Grower
☐ Drug Susceptibility Not Completed Report Will Follow	☒ Drug Susceptibility Not Performed

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

2101

Figure 4-11 Report of TB drug susceptibility not to be performed

DRUG SUSCEPTIBILITY PROFILE Date Reported

Drug	ug/ml	Drug	ug/ml	Drug	ug/ml
INH	0.2	Kanamycin	5.0	Cycloserine	20.0
INH	1.0	Kanamycin	20.0	Cycloserine	40.0
INH	5.0	Kanamycin	100.0	Cycloserine	100.0
PAS	2.5	Viomycin	5.0	Rifampin	1.0
PAS	10.0	Viomycin	20.0	Rifampin	5.0
PAS	25.0	Viomycin	100.0	Rifampin	10.0
Streptomycin	5.0	Pyrazinamide	10.0	Capreomycin	5.0
Streptomycin	20.0	Pyrazinamide	50.0	Capreomycin	20.0
Streptomycin	100.0	Pyrazinamide	100.0	Capreomycin	100.0
Ethambutol	1.0	Ethionamide	2.5		
Ethambutol	5.0	Ethionamide	10.0		
Ethambutol	10.0	Ethionamide	25.0		

S = Susceptible R = Resistant

Figure 4-12 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 characteristics:

1. 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 reproductive 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

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. "whirlpack" bag
c. mycology data form
d. cotton
e. styrofoam shipping container
f. return-addressed mailing box

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:

1. 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.
5. 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

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Miscellaneous
MYCOLOGY
Examination

Patient J. JONES Age 51 Sex M

Address FAIRTOWN, IOWA

Physician (Name and Address)

Dr. M. GIBBS
FAIRTOWN, Iowa
Zip Code 50000

Examination Desired:
culture

● Primary Culture
○ Subculture

Source of Specimen:
hair

PLEASE PRINT PLAINLY

CLINICAL DIAGNOSIS ringworm

Laboratory Report

Date Reported

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 5-2
Completed mycology data form

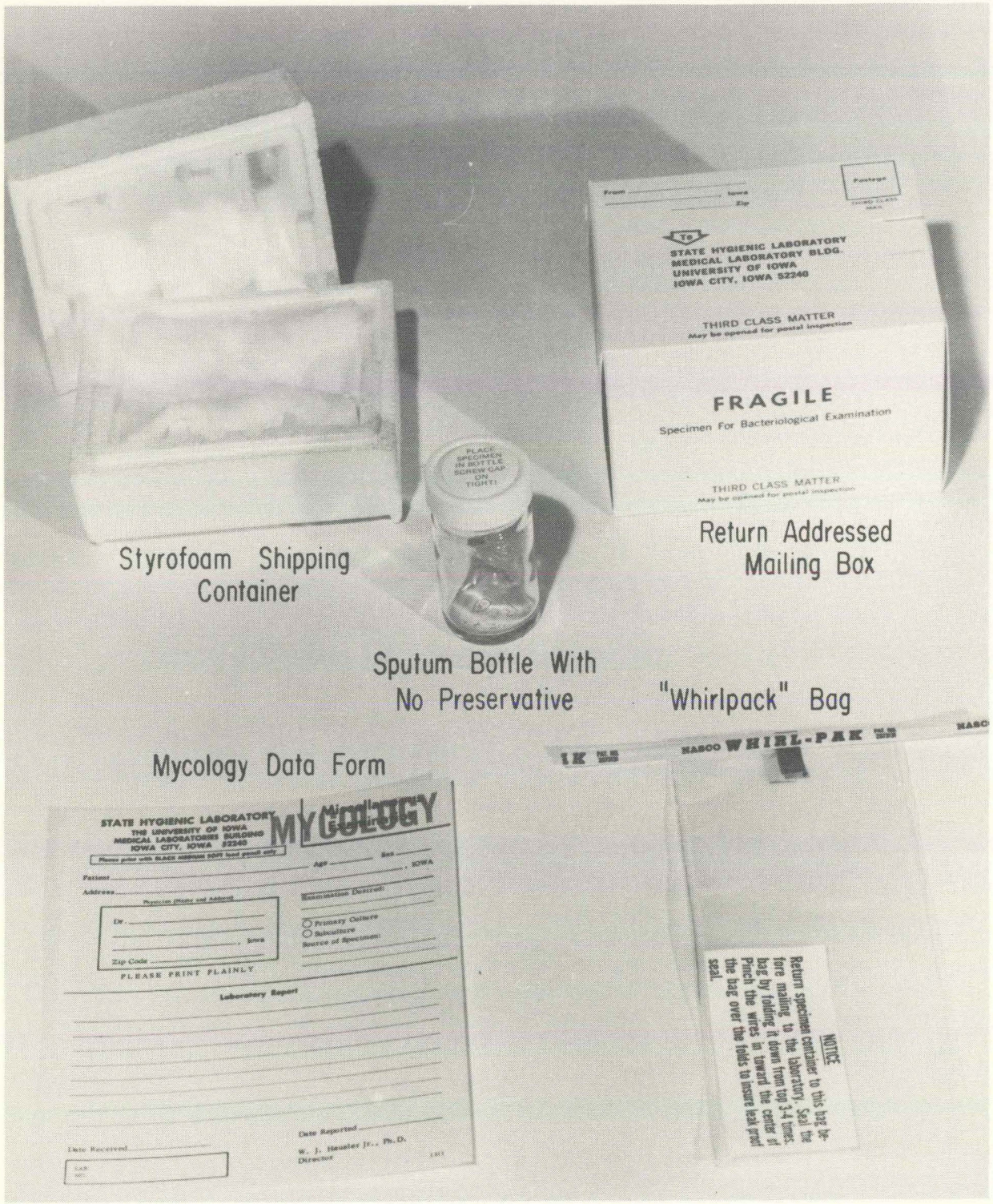


Figure 5-1 Mycology specimen collection kit

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 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

1. Wash the affected area or lesion with a non-medicated soap and rinse thoroughly with sterile distilled water.
2. 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.
4. 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.
2. Scrape or clip the nail, especially near the bed of the nail.
3. 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.
3. Pluck out the basal portion of the hairs or hair stubs with sterile tweezers.
4. 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.
2. If possible streak onto Sabouraud's dextrose agar with and without antibiotics (Table 5-1), and incubate at 25° C.
3. 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

1. If possible, streak onto Sabouraud's dextrose agar with and without antibiotics (Table 5-1), and incubate at 25°C.
2. 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

1. If possible, the body fluid should be centrifuged for 15 minutes at 3,000 rpm.
2. 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.
3. Mail the supernatant in a sterile bottle with no preservative added and the data slip to the State Hygienic Laboratory.

Urine

1. 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.
4. Urine specimens pooled over 24-, 48- and 72-hour periods are unsatisfactory.
5. Mail the subculture and data slip to the State Hygienic Laboratory.

Sputum

1. Collect a series of three to five early-morning, expectorated specimens taken one per day. These should not be sinus excretions or saliva.
2. Five to 10 ml of specimen are needed for proper examination. There is no advantage in collecting a larger volume.
3. 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.
5. Sputum specimens for isolation of *Histoplasma capsulatum* are not satisfactory as the fungus dies rapidly in the mail.
6. Place specimen in the sterile container provided in the outfit and mail with the data slip to the State Hygienic Laboratory.

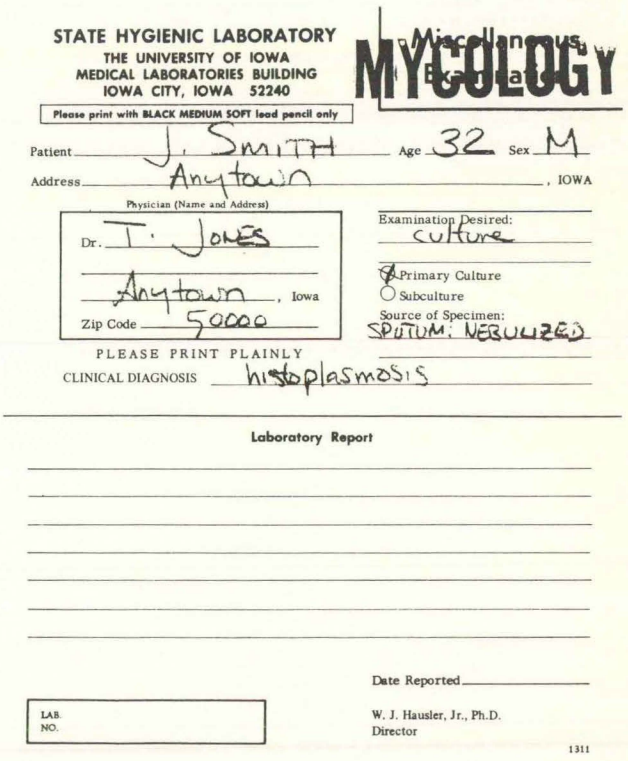
Nebulized Sputum or Bronchial Washings

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


STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Miscellaneous
MYCOLOGY

Please print with BLACK MEDIUM SOFT lead pencil only

Patient J. SMITH Age 32 Sex M

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. JONES
Anytown, Iowa
Zip Code 50000

Examination Desired: culture

☑ Primary Culture
○ Subculture

Source of Specimen: SPUTUM: NEBULIZED

PLEASE PRINT PLAINLY

CLINICAL DIAGNOSIS histoplasmosis

Laboratory Report

Date Reported

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 5-3 Identification of nebulized mycology sputum specimen

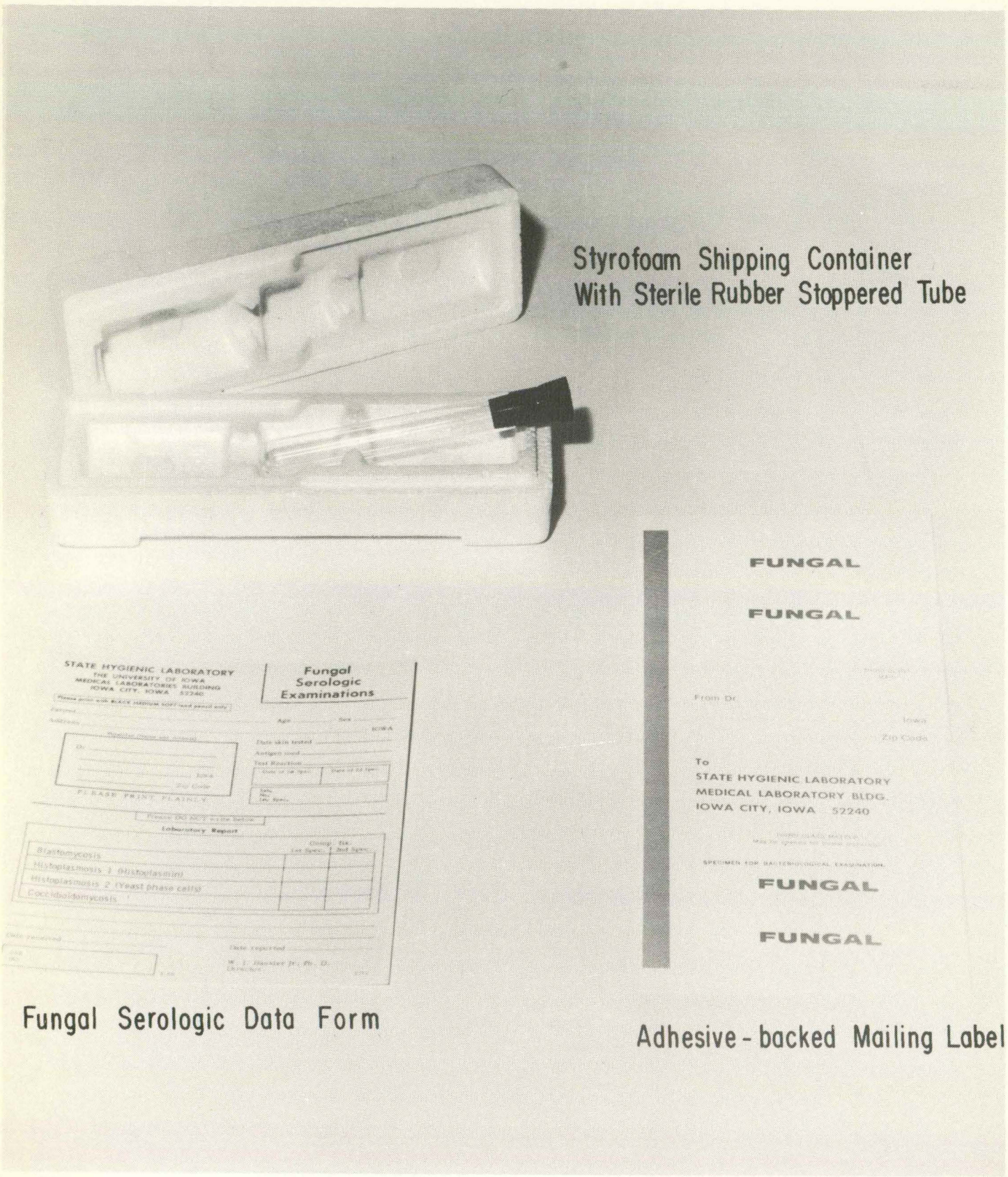


Figure 5-4 Fungal serology specimen collection kit

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

1. 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.

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. oyrzae*

Deuteromycetes (Fungi Imperfecti)
- *Aspergillus fumigatus*
- *Blastomyces dermatitidis*
- *Candida albicans*
- *Cephalosporium falciforme*
- *Cladosporium bantianum*
- *C. carrionii*
- *C. werneckii*
- *Coccidioides immitis*
- *Cryptococcus neoformans*
- *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. vanbreuseghenii*
- *Paracoccidioides brasiliensis*
- *P. loboi*
- *Phialophora jeanselemi*
- *P. verrucosa*
- *Pyrenochaeta romeroi*
- *Rhinosporidium seeberi*
- *Sporotrichium (Sporotrix) schenckii*
- *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*

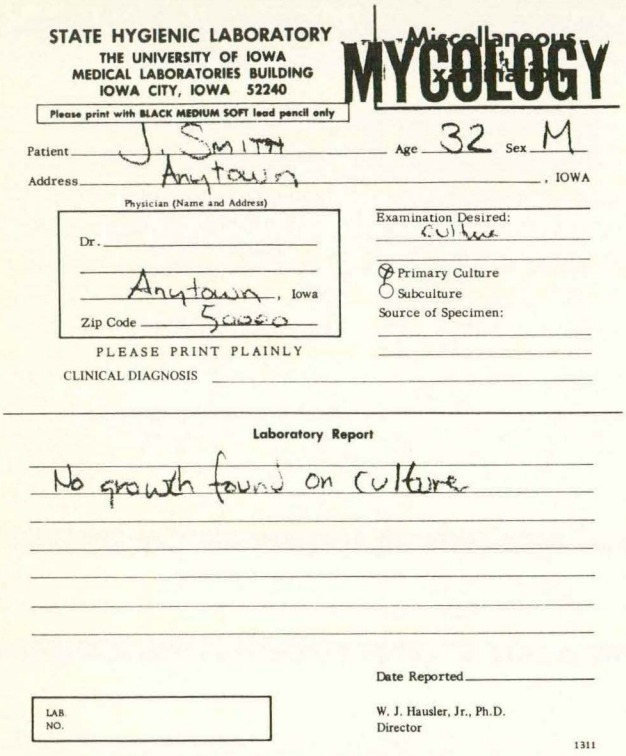
LIST OF COMMONLY ENCOUNTERED SAPROPHYTIC FUNGI

- *Penicillium* sp.
- *Aspergillus* sp.
- *Scopulariopsis* sp.
- *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.

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. Negative cultures are reported after at least four weeks incubation (Figure 5-5).

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


STATE HYGIENIC LABORATORY
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IOWA CITY, IOWA 52240

Miscellaneous Examination

MYCOLOGY

Please print with BLACK MEDIUM SOFT lead pencil only

Patient J. Smith Age 32 Sex M

Address Anytown, IOWA

Physician (Name and Address)

Dr.

Anytown, Iowa

Zip Code 50000

Examination Desired: Culture

Primary Culture

Subculture

Source of Specimen:

PLEASE PRINT PLAINLY

CLINICAL DIAGNOSIS

Laboratory Report

No growth found on culture.

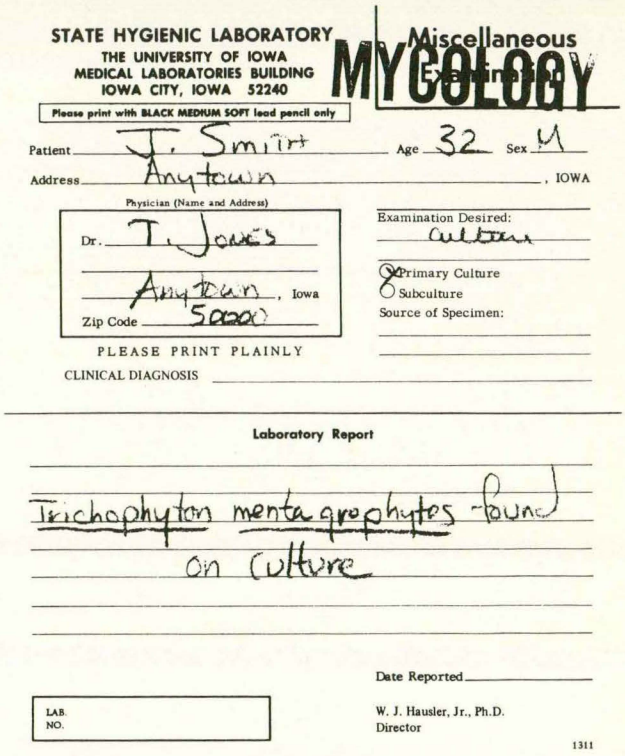
Date Reported

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 5-5 Negative mycology culture report


STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Miscellaneous Examination

MYCOLOGY

Please print with BLACK MEDIUM SOFT lead pencil only

Patient J. Smith Age 32 Sex M

Address Anytown, IOWA

Physician (Name and Address)

Dr. T. Jones

Anytown, Iowa

Zip Code 50000

Examination Desired: Culture

Primary Culture

Subculture

Source of Specimen:

PLEASE PRINT PLAINLY

CLINICAL DIAGNOSIS

Laboratory Report

Trichophyton mentagrophytes found on culture

Date Reported

LAB. NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

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 Sabouraud's without antibiotics	Skin scrapings Mucocutaneous scrapings Vaginal scrapings

Table 5-1 (Continued)

DISEASE OR CAUSATIVE AGENTS	CULTURE MEDIUM (in tubes)	TYPES OF SPECIMENS
Subcutaneous Mycoses		
Chromoblastomycosis	Sabouraud's with antibiotics Sabouraud's without antibiotics	Scrapings, crust Exudate from lesion
Mycetoma (Madduromycosis)	Sabouraud's agar Brain-heart infusion agar (BHI) BHI with blood	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

Table 5-1 (Continued)

DISEASE OR CAUSATIVE AGENTS	CULTURE MEDIUM (in tubes)	TYPES OF SPECIMENS
(Diphasic fungi)		
Blastomycosis North 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 Pus from abscesses, sinus tracts Urine, sputum Bronchial washings
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
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
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

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

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:

1. Don't shoot or club the suspect specimen in the head. The animal's brain must be intact for proper laboratory examination.
2. 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.
3. Pack the head in ice immediately. Brain tissue rapidly decomposes if not properly refrigerated, particularly in summer months.
4. Don't freeze the specimen. Freezing alters brain tissue and makes it difficult to provide a proper diagnosis.
5. 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.
8. 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.
9. 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 Laboatory must be delivered to the Laboratory (Room 223, 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.
10. 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.

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 serodiagnosis 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:

1. Show reactivity or weak reactivity by the VDRL screening test,
2. 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
4. Have been sent to us by one of the "approved" laboratories.

This test eliminates 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.
2. If wet syringes are used, they must be rinsed with normal saline before using.
3. 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).
4. Wrap data slip around serology tube and place in mailing container.
5. Attach adhesive-backed mailing label and mail.

Referral Specimens

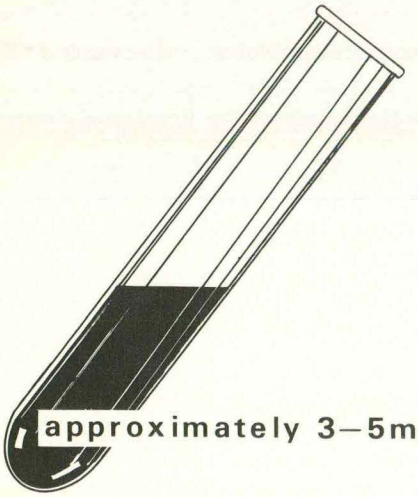
An approved laboratory may perform the VDRL on all serum specimens and submit specimens to the State Hygienic Laboratory for confirmation only. The referring laboratory *must* state on the data form if the serum being submitted has been heated for 30 minutes at 56°C. Either heated or unheated serum may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

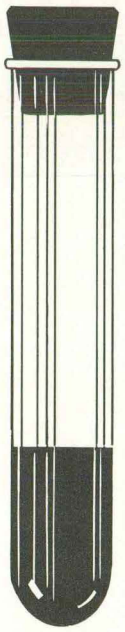
Premaritals for Out-of-State Marriages

If the submitted blood specimen is for an out-of-state 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.

1 Collect blood or spinal fluid in serology tube





2 Secure cork

3 Fill out Serologic Examination data form

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Serologic Examination

Patient John Ripple Age 25 Sex M Race C
Address 1930 ST. BIG TOWN, IOWA

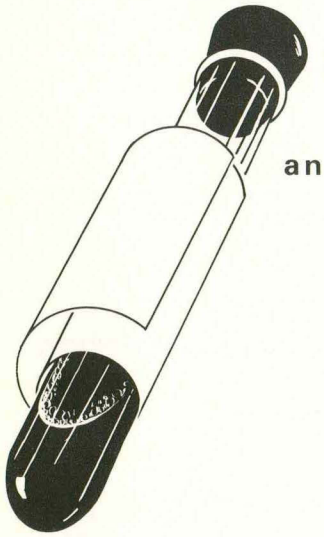
Physician (Name and Address)
Dr. B.L MARVEL
67 VERNA PLACE
BIG TOWN, Iowa
Zip Code 52254

PLEASE PRINT PLAINLY

*If rubella immune status of female is known
please indicate antibody titer here

(To be filled in by Physician)
Blood ◯ Spinal Fluid ◯
Purpose for which specimen taken:
Syphilis clinically suspected ◯
Specimen No. ① ② ③
Last serologic result
VDRL
FTA
Treated ◯
Other ◯
*Prenatal ◯
MARRIAGE ◯

4 Wrap data slip around serology tube



and place in mailing container

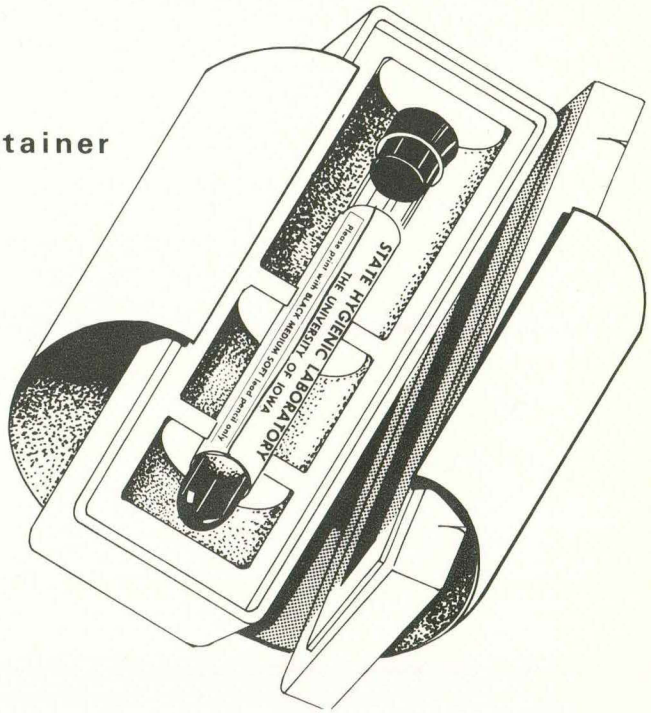


Figure 7-1 Procedure for obtaining syphilis serology blood specimen

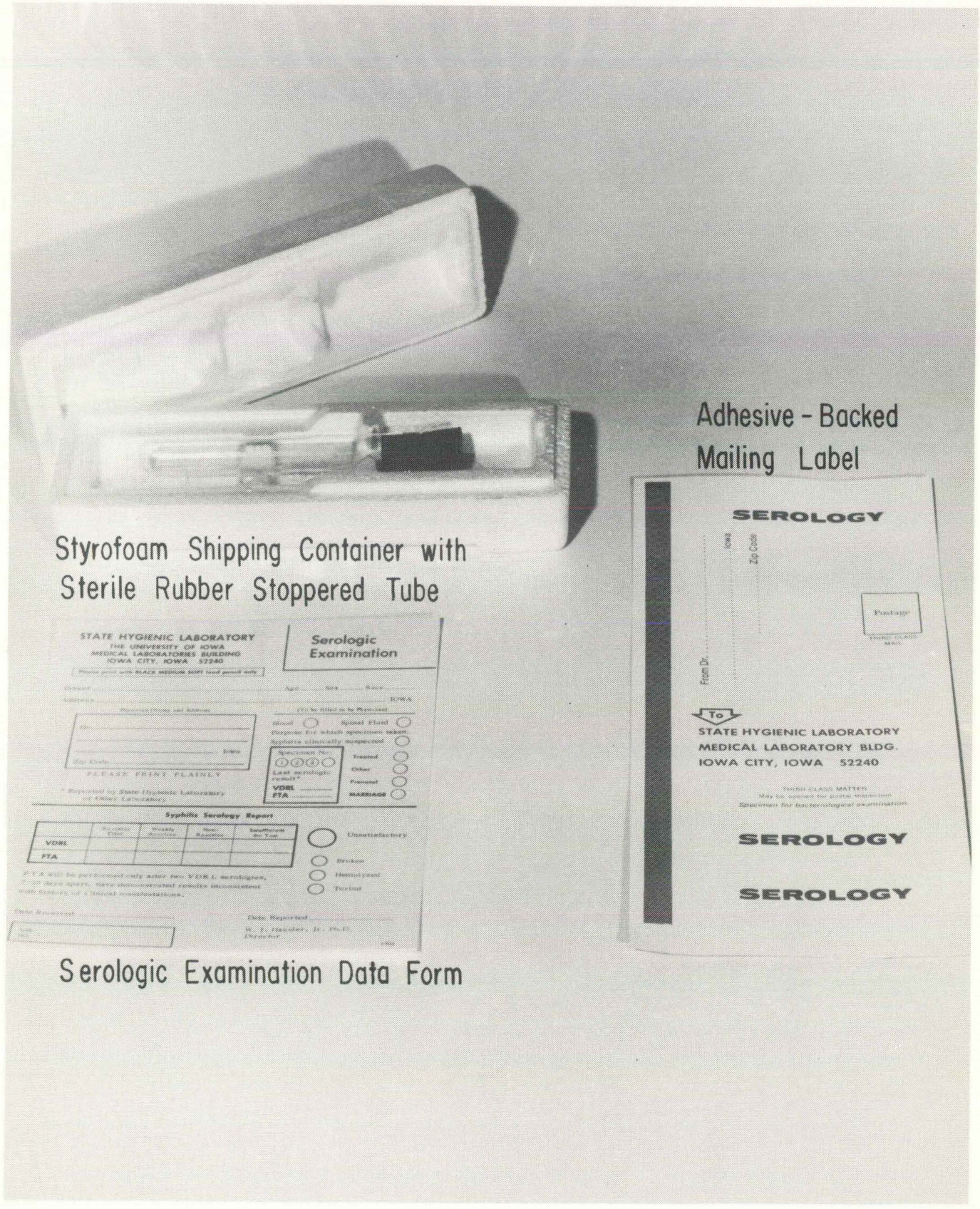


Figure 7-2 Syphilis serology specimen collection kit

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

(1) Name and address of physician drawing the blood and who will sign the certificate.

Richard W Smithson
(name)

402 Beaumont Street
(street address)

Any town, Iowa
(city)

(2) Full Name and address of marriage applicant.

Peter (first) Raymond (middle) Johnson (last)

492 1st Ave
(street address)

Any town, Iowa
(city)

(3) Age, sex and race of marriage applicant.

Age 22 Sex M Race C

(4) Name of serologic test and result.

VDRL (test) Nonreactive (result)

(5) Date specimen drawn and examined.

4-7-71 (drawn) 4-7-71 (examined)

(6) State in which marriage is to take place.

Texas

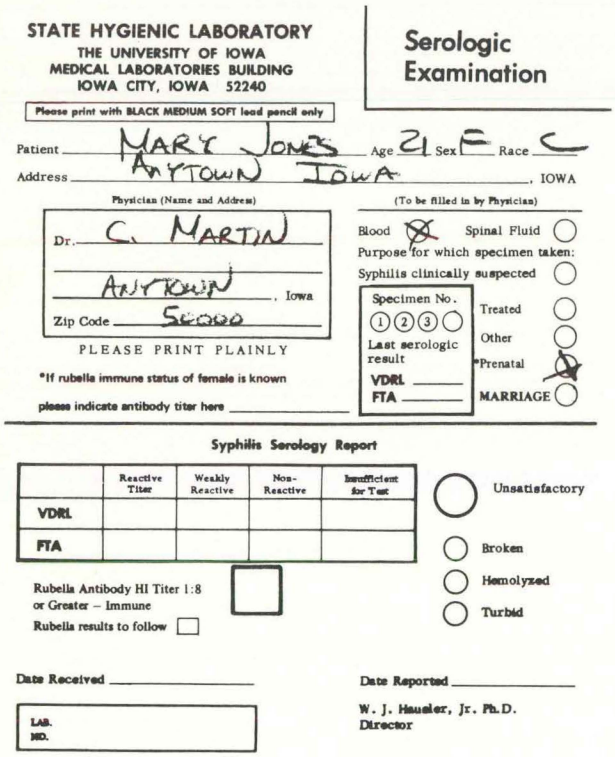
Mercy Hospital
(Name of Approved Laboratory)

1817 1st Ave N.W.
(Street address)

Cedar Rapids, Iowa
(City)

Please place approval stamp here.

Figure 7-4 Out-of-state marriage form



STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Serologic Examination

Please print with BLACK MEDIUM SOFT lead pencil only

Patient MARY JONES Age 21 Sex F Race C
Address ANYTOWN IOWA, IOWA

Physician (Name and Address)
Dr. C. MARTIN
ANYTOWN, Iowa
Zip Code 50000
PLEASE PRINT PLAINLY

(To be filled in by Physician)
Blood ⊗ Spinal Fluid ○
Purpose for which specimen taken:
Syphilis clinically suspected ○
Specimen No. ①②③○
Last serologic result
VDRL ____
FTA ____
Treated ○
Other ○
*Prenatal ⊗
MARRIAGE ○

*If rubella immune status of female is known please indicate antibody titer here ____

Syphilis Serology Report

	Reactive Titer	Weakly Reactive	Non-Reactive	Insufficient for Test
VDRL				
FTA				

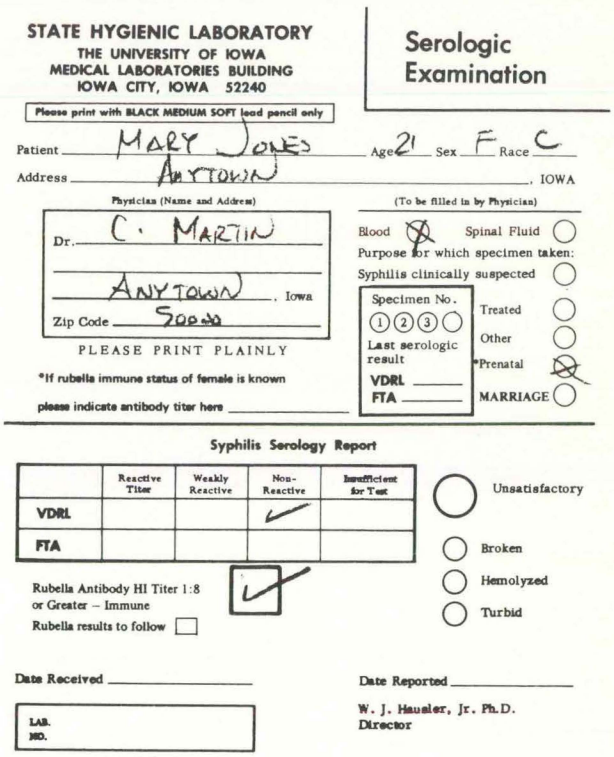
Unsatisfactory ○
Broken ○
Hemolyzed ○
Turbid ○

Rubella Antibody HI Titer 1:8 or Greater – Immune ☐
Rubella results to follow ☐

Date Received ____
Date Reported ____
W. J. Hausler, Jr. Ph.D.
Director

LAB.
MD.

Figure 7-3 Serologic data form



STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Serologic Examination

Please print with BLACK MEDIUM SOFT lead pencil only

Patient MARY JONES Age 21 Sex F Race C
Address ANYTOWN, IOWA

Physician (Name and Address)
Dr. C. MARTIN
ANYTOWN, Iowa
Zip Code 50000
PLEASE PRINT PLAINLY

(To be filled in by Physician)
Blood ⊗ Spinal Fluid ○
Purpose for which specimen taken:
Syphilis clinically suspected ○
Specimen No. ①②③○
Last serologic result
VDRL ____
FTA ____
Treated ○
Other ○
*Prenatal ⊗
MARRIAGE ○

*If rubella immune status of female is known please indicate antibody titer here ____

Syphilis Serology Report

	Reactive Titer	Weakly Reactive	Non-Reactive	Insufficient for Test
VDRL			✓	
FTA				

Unsatisfactory ○
Broken ○
Hemolyzed ○
Turbid ○

Rubella Antibody HI Titer 1:8 or Greater – Immune ✓
Rubella results to follow ☐

Date Received ____
Date Reported ____
W. J. Hausler, Jr. Ph.D.
Director

LAB.
MD.

Figure 7-5 Report of serologic results

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 (HAI). 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 HAI 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 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 HAI 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, ASO and CRP 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.

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Special Serologic Examinations

Patient C. JOHNSON Age 23 Sex F

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M. JONES

ANYTOWN, Iowa

50000 Zip Code

(To be filled in by Physician)

Suspected disease INF. MONO

Date of onset 2-12-70

Date of 1st Spec.	Date of 2d Spec.
2-14-70	3-20-70

PLEASE PRINT PLAINLY

Please check (√) Examination Requested

Laboratory Report

	Acute	Conv.
☒ Heterophile (Inf. Mono)		
☐ Rheumatoid Arthritis		
☐ Trichinosis		
☐ ASO		
☐ CRP		
☐ Toxoplasmosis		
☐ Other		

Date Received _____ Date Reported _____

LAB NO.

W. J. Hausler Jr., Ph.D.
Director

1712

Figure 7-6 Special serology data form

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Agglutination Tests

Patient C. JONES Age 48 Sex M

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M. CARSON

ANYTOWN, Iowa

Zip Code 50000

(To be filled in by Physician)

Purpose for which specimen taken:

BRUCELLOSIS ◯

Other TULAREMIA

Date of onset 5-12-70

Date of specimen 5-14-70

Specimen: ☒ 1st ◯ 2nd ◯ —

PLEASE PRINT PLAINLY

Laboratory Report

ANTIGEN	TITER Neg.	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Brucella										

Date Received _____ Date Reported _____

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

1296

Figure 7-8 Febrile agglutination data form

A rapid slide screening test is used for the detection of rheumatoid arthritis and trichinosis antibodies. Reactive results are *not* titered.

The antistreptolysin "O" (ASO) and the C-reactive protein (CRP) are performed as requested.

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 submitting the specimen. Heterophile antibodies are reported as nonreactive, weakly reactive (1:32) or reactive with the titer given. Rheumatoid arthritis and trichinosis results are reported as either reactive or nonreactive. ASO 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 166 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).

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.

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.

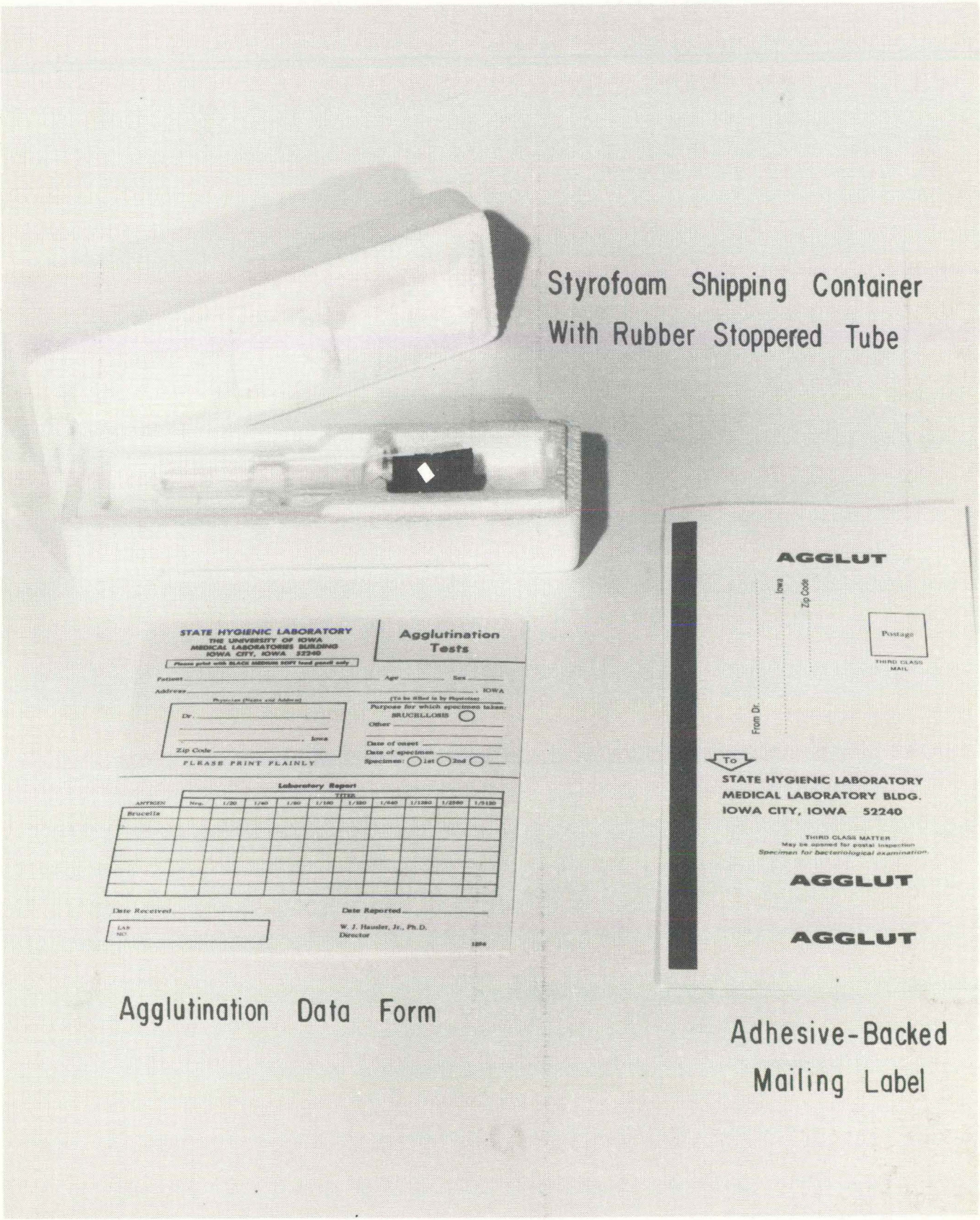


Figure 7-7 Febrile agglutination specimen collection kit

Table 7-1 Battery of agglutination tests for febrile diseases

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

VIRAL AND RICKETTSIAL DISEASES

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:

1. Collect the specimens listed in Table 8-1 for a particular disease.
2. The acute blood specimen should be taken at onset of illness. Acute blood specimens obtained one week later are of little diagnostic value.
3. 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.
5. 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

1. 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

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Virus Examination
(Complete one data card for each specimen submitted)

Patient V. MARTIN Age 3C Sex M

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M. JONES

ANYTOWN, Iowa

Zip Code 50000

PLEASE PRINT PLAINLY

(To be filled in by physician)

Suspected disease ASEPTIC MENINGITIS

Date of onset 2-12-71

Type of specimen CSF

Furnish clinical history with provisional diagnosis to permit selection of the proper test(s) to be performed.

Laboratory Report

Date Received _____

Date Reported _____

LAB. NO.

W. J. HAUSLER JR., Ph. D.
Director

1638

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 _____ Date _____
Patient _____ Age _____ Sex _____
Address _____ City _____ County _____
Date of Onset _____ Occupation _____
CLINICAL OR PROVISIONAL DIAGNOSIS _____
_____

VIRAL IMMUNIZATIONS & DATES

Polio: Salk # _____ Measles _____
Oral # _____ Mumps _____
Rubella (German Measles) _____ Smallpox _____

SIGNS AND SYMPTOMS

Fever: _____ F°/C° Duration _____
Chills/Sweating _____ Joint pain/Myalgia _____
Rash: Type _____ Mucous membrane lesions _____
Respiratory: Rhinits _____ Pharyngitis _____
Cough _____ Lymphadenopathy _____
Chest pain _____
Gastrointestinal: Diarrhea _____ Constipation _____
Abdominal pain _____ Vomiting _____
Appetite _____ Nausea _____
Cardiovascular: Mycocarditis _____ Pericarditis _____
Central Nervous System: Headache _____ Optic _____
Balance _____ Muscle weakness _____
Leghargy _____ Nuchal rigidity _____
Other: _____

EPIDEMIOLOGICAL DATA

Recent Travel (Location) _____
Family Contacts _____
Community Contacts _____
Animal and/or arthropod contact _____
Group Activities _____
Food Outbreaks _____

OTHER PERTINENT INFORMATION - Please use back of this form.

Dr _____
_____
_____
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.
5. Throat swabs may be obtained by rubbing the 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 pack 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 52240

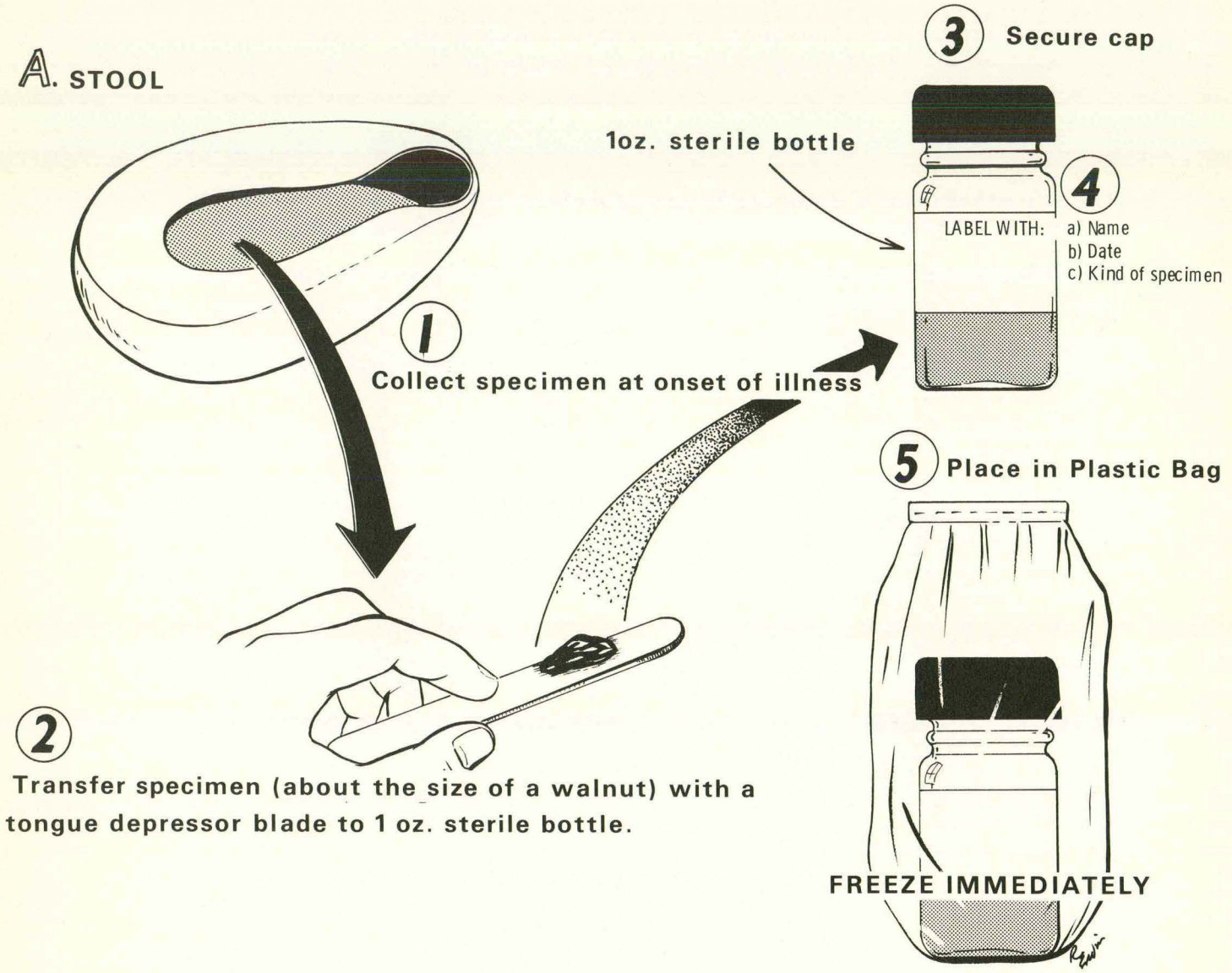
   Usually, Iowa Parcel Service, United Parcel Service or Parcel Post Special Delivery have been satisfactory. To avoid delay enroute, *DO NOT SHIP ON WEEKENDS.*
9. If for any reason, you have questions concerning collection or shipment of specimens, please call the virus laboratory, 319/353-5990.

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).
2. 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

and then

7 **Complete 1 history form PER PATIENT.**

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Virus Examination
(Complete one data card for each specimen submitted)

Patient Roberta Simmons Age 42 Sex F

Address 1222 Western Ave. Big Town, IOWA

Physician (Name and Address)

Dr. A.C. Keith, M.D.
1010 Broadway
Big Town, Iowa
Zip Code 52254

PLEASE PRINT PLAINLY

(To be filled in by physician)

Suspected disease Aseptic Meningitis

Date of onset 8-9-72

Type of specimen Stool

Furnish clinical history with provisional diagnosis to permit selection of the proper test(s) to be performed.

Laboratory Report

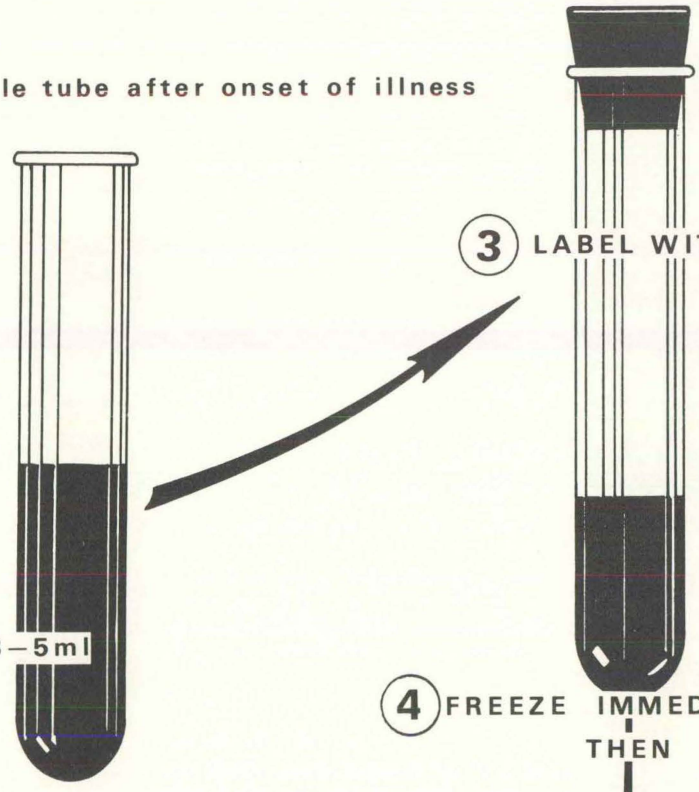
Figure 8-3 Procedure for collection of V & R fecal specimen

SPECIMENS FOR VIRUS ISOLATION

Cerebral Spinal Fluid

(2) Secure rubber stopper

(1) Collect in sterile tube after onset of illness



(3) LABEL WITH: a NAME
b Date
c Kind of specimen

Approximately 3–5ml

(4) FREEZE IMMEDIATELY

THEN

(5) FILL OUT data card for EACH SPECIMEN:

Complete only that portion of data card shown below

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Virus Examination
(Complete one data card for each specimen submitted)

Patient Freda Flemings Age 27 Sex F

Address 800 River St, Big Town, IOWA

Physician (Name and Address) (To be filled in by physician)

Dr. R. K. Luther, M.D.
City Hospital
Big Town, Iowa
Zip Code 52254

Suspected disease Meningoencephalitis

Date of onset 9-12-72

Type of specimen CSF

PLEASE PRINT PLAINLY

Furnish clinical history with provisional diagnosis to permit selection of the proper test(s) to be performed.

Laboratory Report

and then

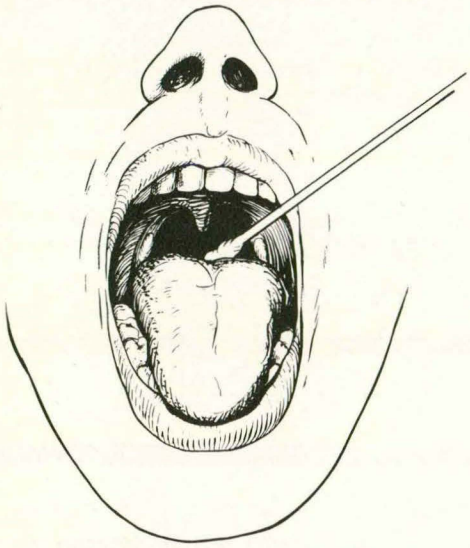
(6) Complete 1 history form PER PATIENT

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

Specimens for VIRUS ISOLATION

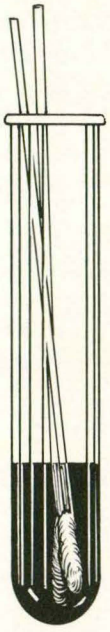
B THROAT SWAB

(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)

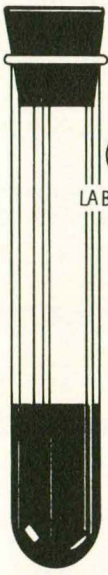


(2) Transfer material IMMEDIATELY to a tube containing approximately 5ml sterile nutrient broth and agitate vigorously

(3) DISCARD SWABS after squeezing dry Place in plastic bag



(4) Secure stopper



(5) LABEL WITH: a) Name
b) Date
c) Kind of specimen

(6) Place in Plastic Bag FREEZE IMMEDIATELY

(7) FILL OUT data card for each specimen: Complete only that portion of data card shown below

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Virus Examination
(Complete one data card for each specimen submitted)

Patient Robert Wren Age 8 Sex M
Address 117 Jamestown Rd. Big Town IOWA
Physician (Name and Address)
(To be filled in by physician)

Dr. J.C. Babbitt
Medical Clinic
Big Town, Iowa
Zip Code 52254
PLEASE PRINT PLAINLY

Suspected disease Measles
Date of onset 11-15-72
Type of specimen Throat swab

Furnish clinical history with provisional diagnosis to permit selection of the proper test(s) to be performed.

Laboratory Report

(8) Complete 1 history form PER PATIENT

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

SHIPPING INSTRUCTIONS:
NOTE: DO NOT SHIP ON WEEKENDS

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.

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 2cardboard shipping cartons.

⑥Close the cardboard cartons and buckle the straps.

⑦Put on the pre–addressed label and send by the fastest means of transportation usuallyParcel 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.

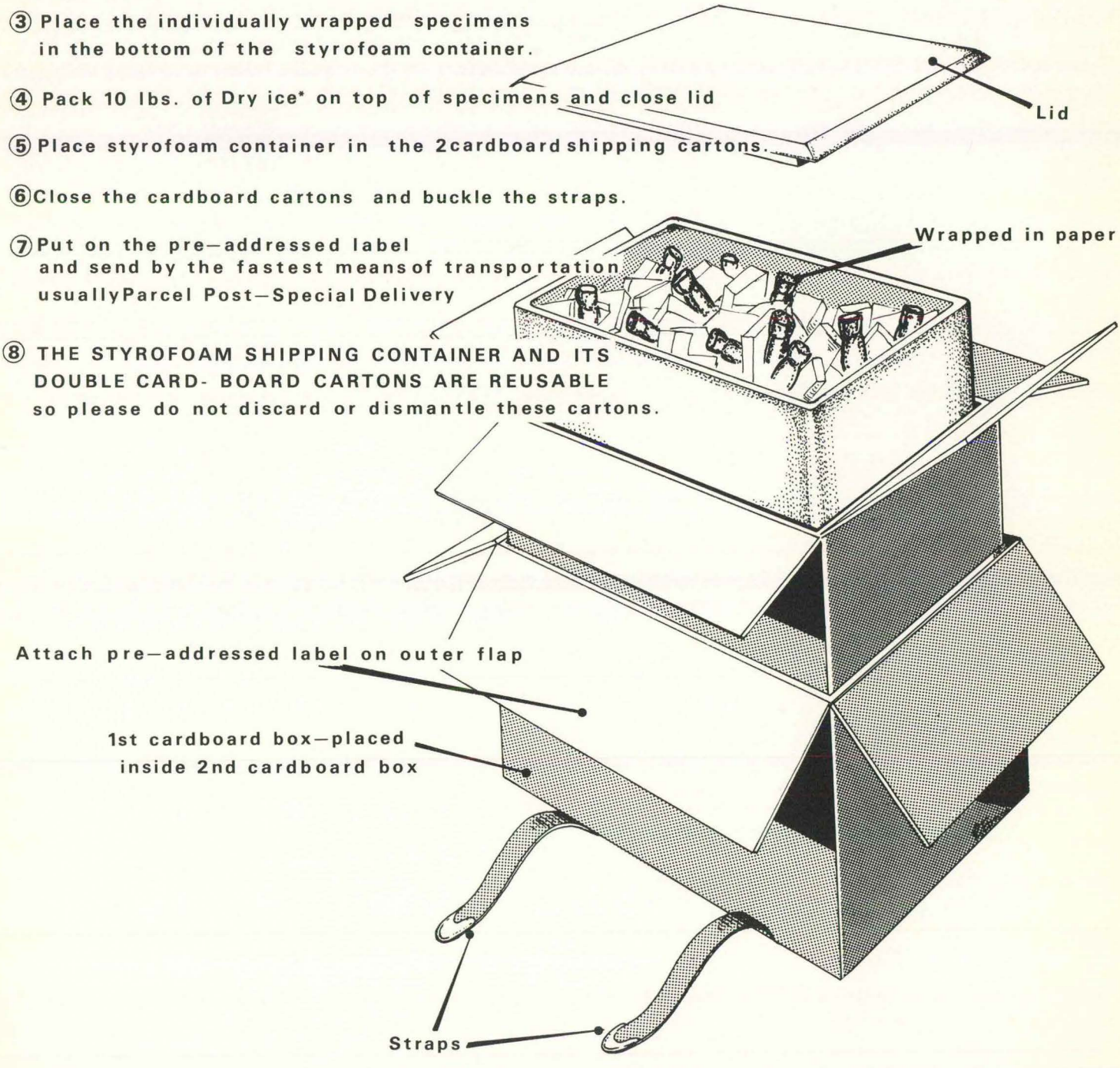
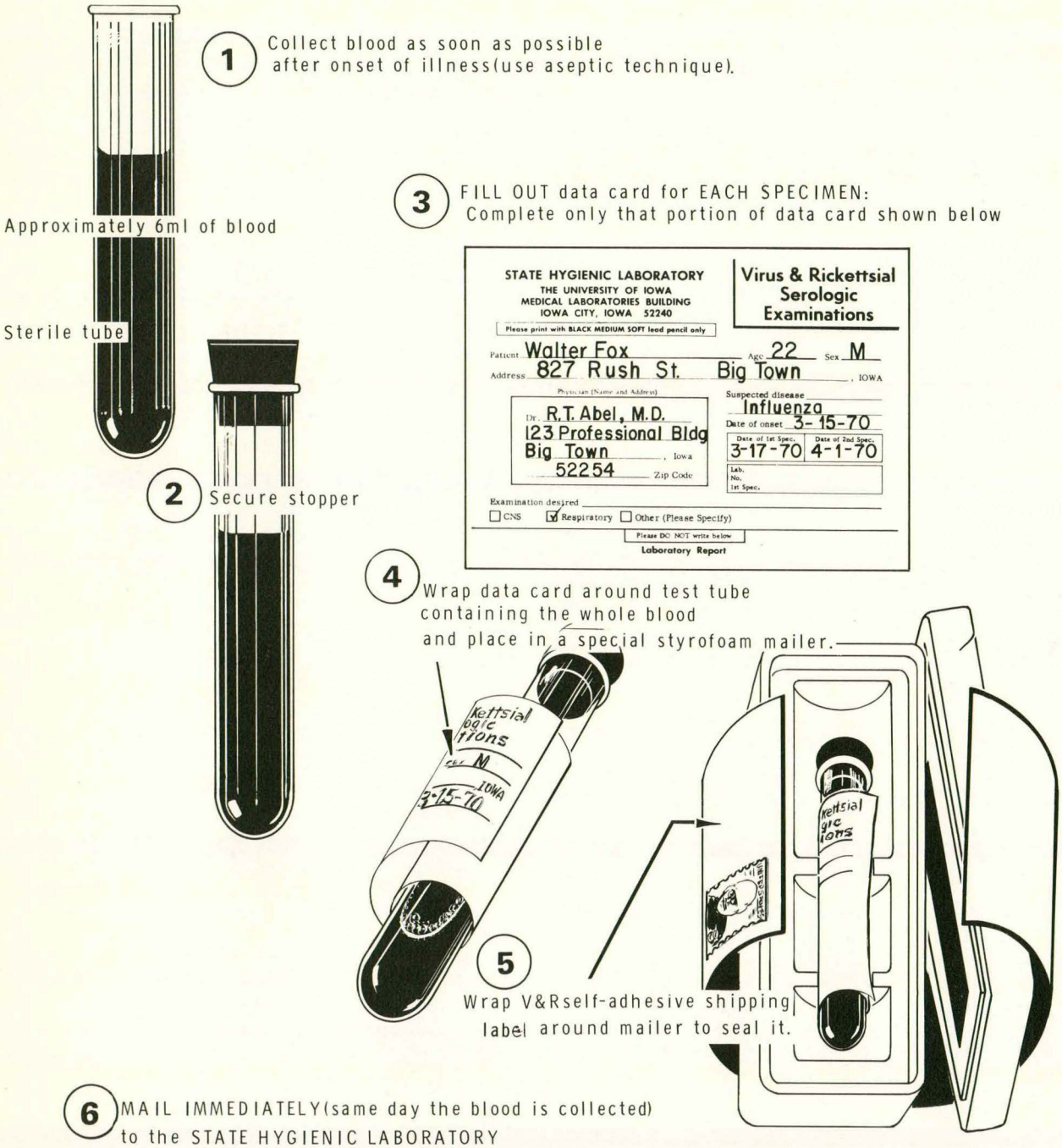


Figure 8-6 V & R shipping unit

VIRAL and RICKETTSIAL SEROLOGIC EXAMINATION

Collecting and shipping of specimens

Collection of **Acute Blood** specimen:



DO NOT FREEZE WHOLE BLOOD

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

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 52240

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

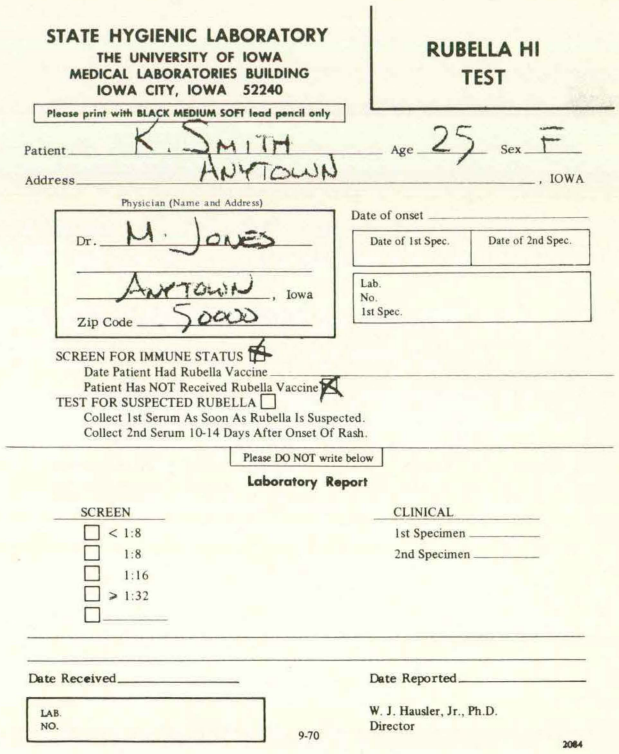
1. Collect approximately six ml of blood. (Use aseptic technique.) DO NOT FREEZE WHOLE BLOOD.
2. Place specimen in the sterile tube and secure the stopper.
3. Fill out a data card for EACH SPECIMEN. Complete only that portion of data card shown in Figure 8-8.
4. Wrap the data card around the tube containing 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 52240

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.



STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

RUBELLA HI TEST

Please print with BLACK MEDIUM SOFT lead pencil only

Patient K. SMITH Age 25 Sex F

Address ANYTOWN, IOWA

Physician (Name and Address)

Dr. M. JONES

ANYTOWN, Iowa

Zip Code 50000

Date of onset

Date of 1st Spec.	Date of 2nd Spec.

Lab. No. 1st Spec.

SCREEN FOR IMMUNE STATUS ☒

Date Patient Had Rubella Vaccine

Patient Has NOT Received Rubella Vaccine ☒

TEST FOR SUSPECTED RUBELLA ☐

Collect 1st Serum As Soon As Rubella Is Suspected.

Collect 2nd Serum 10-14 Days After Onset Of Rash.

Please DO NOT write below

Laboratory Report

SCREEN

- ☐ < 1:8
- ☐ 1:8
- ☐ 1:16
- ☐ > 1:32
- ☐ _____

CLINICAL

1st Specimen _____

2nd Specimen _____

Date Received _____

Date Reported _____

LAB. NO.

9-70

W. J. Hausler, Jr., Ph.D.
Director

2084

Figure 8-8 Rubella HI data form

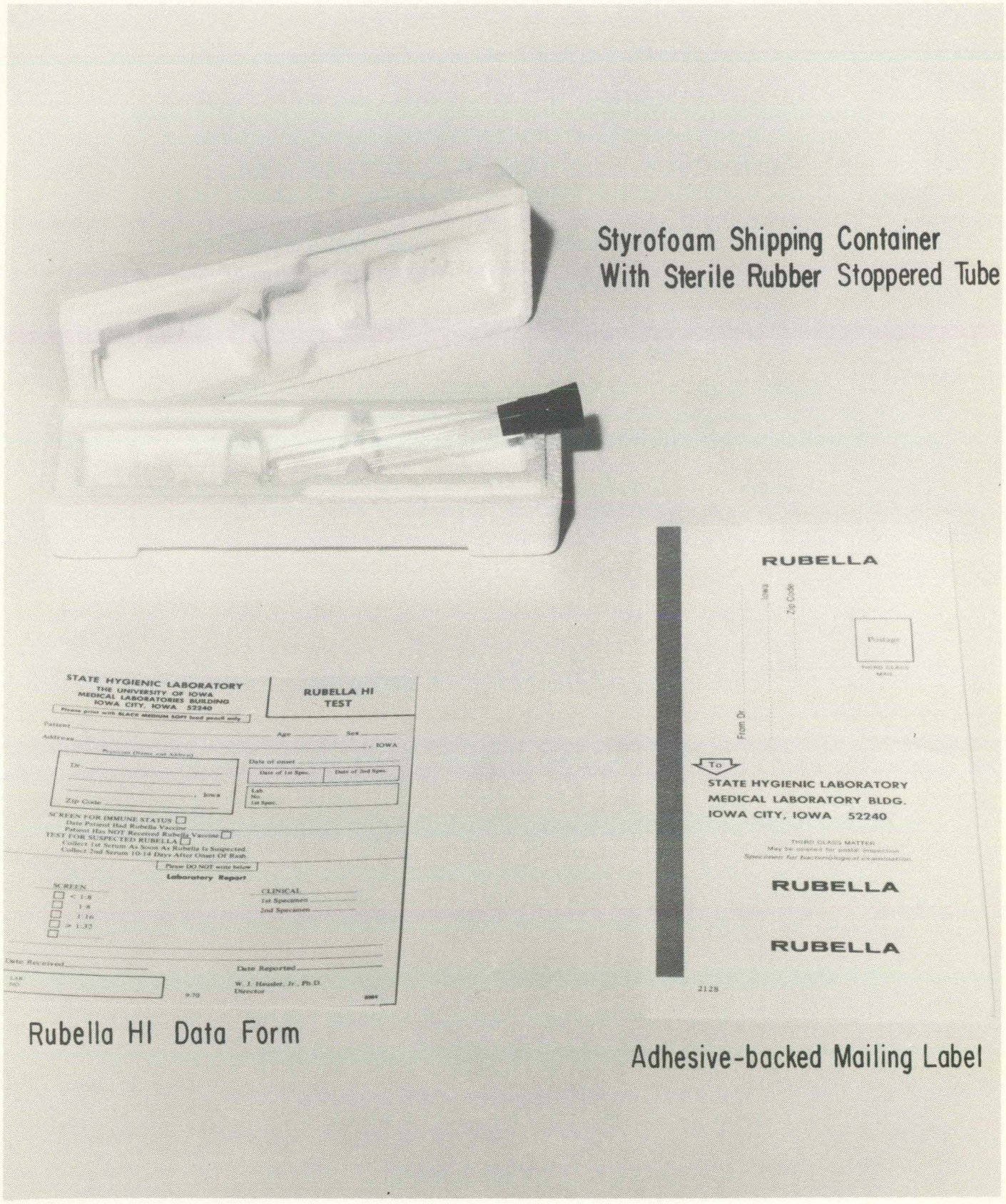


Figure 8-9 Rubella HI Collection Kit

Table 8-1 Tests available for viral and rickettsial diagnosis

DISEASE	SPECIMENS FOR ISOLATION		AGENTS	SEROLOGY TESTS AVAILABLE*				
	Clinical	*Post Mortem*		*CF*	*FA*	*HI*	*Neut*	*Other*
CENTRAL NERVOUS SYSTEM								
1. Aseptic meningitis	Throat washings, spinal fluid, stool	Brain cortex, blood, spinal cord and fluid, feces, segment of colon	Polioviruses	X			X	
			Coxsackie viruses				O	
			ECHO viruses				O	
			Herpesvirus	X	X		X	
			Lymphocytic choriomeningitis virus	X				
			Mumps virus	X		X		
2. Meningo-encephalitis	Same as 1., also acute phase clotted blood	Same as 1.	Eastern Encephalitis virus	X		X		
			Western Encephalitis virus	X		X		
			California Encephalitis virus	X		X		
			St. Louis Encephalitis virus	X		X		
			Measles virus	X				
			Mumps virus	X				
			Rubella virus			X		
3. Paralytic disease	Same as 1.	Same as 1.	Polioviruses	X			X	
			Coxsackie viruses				O	
			ECHO viruses				O	
4. Guillian-Barré	Same as 1.	Same as 1.	Coxsackie Group A viruses				O	
			ECHO viruses				O	
RESPIRATORY 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	X			O	
			Parainfluenza viruses	X		X	O	
			Respiratory Syncytial virus	X				
3. "Croup" laryngo-tracheobronchitis	Same as 2.	Same as 1.	Parainfluenza viruses	X		X	O	
4. Pleurodynia (Bornholm disease)	Throat washings and stool	Same as 1.	Coxsackie Group B viruses				O	O
5. Primary atypical pneumonia	Throat washings and acute phase clotted blood	Same as 1.	Eaton Agent (PPLO)	X				X
6. Psittacosis	Same as 5.	Same as 1.	Psittacosis Agent	X				
7. Others: Herpangina	Throat washings, stool and swab	Same as 1.	Adenoviruses,	X			O	
			Coxsackie viruses or				O	O
Stomatitis Pharyngitis	of oral lesions		Herpesvirus	X	X		X	

TABLE 8-1 (Cont.)

DISEASE	SPECIMENS FOR ISOLATION		AGENTS	SEROLOGY TESTS AVAILABLE*				
	Clinical	*Post Mortem*		*CF*	*FA*	*HI*	*Neut*	*Other*
EXANTHEMS								
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	Same as 1.	Variola virus	X				
4. Vaccinia	Same as 3.	Same as 1.	Vaccinia virus	X				
5. Chickenpox	Same as 3.	Same as 1.	Varicella virus	X				O
6. Other	Throat washings, stool and vesicle fluid	Same as 1., also segment of colon	Coxsackie viruses, ECHO viruses or Herpesvirus		X		O O	O
RICKETTSIAL								
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.)

DISEASE	SPECIMENS FOR ISOLATION		AGENTS	SEROLOGY TESTS AVAILABLE*				
	Clinical	*Post Mortem*		*CF*	*FA*	*HI*	*Neut*	*Other*
MISCELLANEOUS								
1. Mumps	Saliva and clotted blood		Mumps virus	X				
2. Myocarditis	Throat washings and stool	Heart muscle	Coxsackie Group B viruses				O	O
3. Pericarditis	Same as 2. and pleural fluids	Same as 2., also pleural fluids	Coxsackie Group B viruses				O	O
4. Epidemic keratoconjunctivitis	Conjunctival washings or scrapings		Adenovirus Type 8	X			O	
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				O	O
10. Cytomegalic inclusion	Saliva, urine		Cytomegalovirus	X	X			

*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	+	+	+	+	
Congenital anomalies					
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

Virus or Infection	Isolation	Serology
Adenovirus	R	R
Arbovirus	ST	R
Coxsackie virus	R	ST
Cytomegalovirus	R	R
Eaton Agent (PPLO)	R	R
ECHO virus	R	ST
Herpes simplex	R	R
Influenza A and B	R	R
Mumps	R	R
Measles	R	R
Parainfluenza virus	R	R
Poliovirus	R	ST
Poxvirus	R	ST
Psittacosis - LGV Agents	ST	R
Rabies (Post Immun. Titer)	–	ST
Reovirus	R	ST
Respiratory syncytial virus	R	R
Rickettsial infection	ST	R
Rubella	ST	R
Serum hepatitis	–	R
Varicella - Zoster	ST	R

R = Routine Test
ST = Special Test

Table 8-4 Abbreviations and symbols

Adeno	Adenovirus
CEV	California encephalitis virus
CF	Complement fixation
Cox	Coxsackie virus Group A or B
Eaton's	Eaton agent (Mycoplasma pneumoniae)
ECHO	ECHO virus (Enteric Cytopathogenic Human Orphan virus)
EEE	Eastern equine encephalitis
HI	Hemagglutination inhibition
Herpes	Herpes simplex
Infl A	Influenza type A
Infl B	Influenza type B
LCM	Lymphocytic choriomeningitis
LGV	Lymphogranuloma venereum
Neut	Neutralization
Psitt	Psittacosis
SLE	St. Louis encephalitis
WEE	Western equine encephalitis
$<$	Less than
$>$	Greater than
$\leqq$	Less than or equal to

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 Pollution Control Commission and the State Department of Health 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 52240
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. Fluorides
7. Hydrogen sulfide
8. Ammonia
9. Nitrogen oxides
10. Particulate emission
11. Gas flow rates in stacks
12. Meteorological data (wind speed, direction, barometric pressure, temperature, humidity)
13. Particle identification
14. Soil analysis for selected pollutants
15. 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 Pollution Control 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 52240
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)
7. 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.
3. 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.
5. 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 μg/100 ml (micrograms of lead in 100 ml of blood.)

This report method is considered similar to the μg/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 μl/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 INORGANIC 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

1. Collect a 24-hour composite sample of urine in a nitric acid rinsed glass container. A nonmetal closure is required.
2. 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

1. 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.
2. 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, Iowa City, Iowa 52240, for arrangements, sampling and scheduling concerning these tests.

1. Identification and determination of concentrations of contaminants such as lime, organic compounds, silica (crystalline and amorphous) and metals
2. Identification and determination of concentrations of toxic gases such as carbon monoxide, solvent fumes and selected decomposition products from ovens
3. Identification and determination of concentrations of contaminants and fumes such as metal from welding and foundry operations
4. Identification and determination of concentrations of contaminants such as acid and oil in mists
5. Measurement of ventilation as necessary to define a problem. Such measurements include direction, velocity and static pressure
6. Determination of flash point on combustible liquids by Tag closed cup, Pensky-Martens closed cup and Cleveland open cup
7. Determination of heat stress
8. Light studies for intensity and glare determinations
9. Noise measurement, including octave band analysis
10. Microscopic particle identification
11. Measurement of explosive levels in contaminated atmospheres
12. Measurement of 0_2 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 prevention and detection 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 isotopes, and other sources of ionizing and non-ionizing 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 52240
Telephone: 319/353-5990

Tests available:

1. Direct dose rate measurements for Beta, Gamma and X radiation to determine human occupancy radiation levels
2. 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
5. Radioanalyses of milk for Iodine-131, Barium-140, Cesium-137, Potassium-40 and Strontium-90
6. 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.

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

Facility

Name of Facility _____

Administrator _____

Location _____

Date of Survey _____ Surveyors A. _____ B. _____

Person in Charge of X-ray Facility _____
Name Title

Person Interviewed _____
Name Position

Type of Facility:

Private Office ☐ Hospital ☐ Other: ☐

Mobile ☐ Clinic ☐

Previous Radiation Survey _____
Name Date

NCRP Report No. 33 Reference		ITEM	YES	NO
	A.	Total Number occupationally exposed _____.		
	B.	Total Number of operators _____.		
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 _____.		
	M.	Darkroom light tight.	___	___
2.4.7	N.	Type of developer _____.		
2.4.7	O.	Automatic process.	___	___
2.4.7	P.	How often changed _____ weeks.		
2.4.7	Q.	How often replenished _____ days.		
2.4.7	R.	Thermometer present.	___	___
2.4.7	S.	Thermostatically controlled.	___	___
2.4.7	T.	Temperature indicated _____ ^{o}F.		
2.4.7	U.	Timer present.	___	___
2.4.7	V.	Time in developer _____ minutes.		
	W.	Radium ever present.	___	___
	X.	Other isotopes ever present.	___	___
6.1.7	Y.	Adequate warning devices	___	___

REASON FOR SURVEY:

Initial Follow-up Resurvey

Request Complaint Other

COMMENTS:

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

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

Radiographic

Name of Facility _____

Administrator _____

Location _____

Date of Survey _____ Surveyors A. _____ B. _____

Person in Charge of X-ray Facility _____
Name Title

Person Interviewed _____
Name Position

Control Panel Manufacturer _____

Tube Head Manufacturer and Model _____

TYPE: Max. kVp (rated) Max. mA(rated)

Fixed Radiographic _____ Mobile _____

Photofluorographic _____ Other _____

NCRP Report No. 33 Reference		ITEM	YES	NO
3.2.1 (d)	A.	Filtration, total mm. aluminum equivalent _____		
3.2.1(b) 3.2.2(b)	B.	Source–film distance 72 inches, beam size within limits.	____	____
3.2.1(b) 3.2.2(b)	C.	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)	H.	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 limits.	____	____

EXPOSURE RATE MEASUREMENTS Taken at _____ kVp _____ mA

A. Behind protection barrier _____ mR/hr.

B. Outside protection barrier _____ mR/hr.

C. Behind lead glass _____ mR/hr.

D. Behind wall of chest board _____ mR/hr.

E. Other measurements

COMMENTS:

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

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

Fluoroscopic

Name of Facility _____

Administrator _____

Location _____

Date of Survey _____ Surveyors A. _____ B. _____

Person in Charge of X-ray Facility _____
Name Title

Person Interviewed _____
Name Position

Control Panel Manufacturer _____

Tube Head Manufacturer and Model _____

NCRP Report No. 33 Reference		ITEM	YES	NO
3.1.3 (k)	A.	Leaded gloves used.	___	___
3.1.3 (j)	B.	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.	___	___
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. (The recommended limit is 10 R/minute.)		
3.1.1 (c)	K.	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.	___	___

EXPOSURE RATE MEASUREMENTS Taken at _____ kVp _____ mA

Exposure rate above the fluoroscopic screen _____ mR/hr.

Exposure rate to the fluoroscopist _____ mR/hr.

Exposure rate to the assistant _____ mR/hr.

Other exposure rate measurements:

COMMENTS:

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

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

Facility–Dental

Name of User(s)_____

Location_____

Date of Survey_____ Surveyors A._____ B._____

Person Interviewed_____
name *position*

Type of Practice:

General _____ Pedodontic _____ More than one Type _____
Oral Surgery _____ Periodontic _____ Other (specify) _____
Orthodontic _____ Prosthodontic _____

Previous Radiation Survey _____
name *date*

NCRP Report No. 35 Reference		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.	____	____
6.3.2	F.	Monitoring records satisfactory.	____	____
	G.	Patients radiographed (Ave. per week) _____.		
	H.	Total number of machines at this facility _____.		
	I.	Total number of units (tubes) _____.		
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.	____	____
4.11	R.	Temperature indicated _____ ^{o}F.		
4.11	S.	Timer present.	____	____
4.11	T.	Time in developer _____ minutes.		

REASON FOR SURVEY:

Initial Follow-up Resurvey

Request Complaint Other

COMMENTS:

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

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

Radiographic–Dental

Name of User(s) _____

Location _____

Date of Survey _____ Surveyors A. _____ B. _____

Person Interviewed _____
name *position*

Control Panel Manufacturer _____ Serial No. _____

Tube Head Manufacturer and Model _____ Serial No. _____

Maximum kVp (rated) _____ Maximum mA (rated) _____

Pointer Cone:

None _____ Short Open _____ Long Open _____
Short Pointed _____ Long Pointed _____ Other (specify) _____

NCRP Report No. 35 Reference			ITEM	YES	NO
2.1.2a		A.	Field diameter at end of pointer cone _____ inches.		
2.1.3		B.	Filtration, total mm. aluminum equivalent _____.		
		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.	____	____
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 standards.	____	____
5.1		J.	Primary and secondary barriers, adequate.	____	____
4.8.2		K.	Gonad shielding used.	____	____
4.2	4.8.1	L.	Film holders used.	____	____
		M.	Estimated Number of films per week on this unit _____.		
		N.	Average exposure time per film _____ sec. _____ kVp _____ mA.		

EXPOSURE RATE MEASUREMENTS Taken at _____ kVp _____ mA.

A. Left Bitewing Exposure 1. Dentist _____ mR/hr 2. Assistant _____ mR/hr 3. Other (specify) _____ mR/hr.

B. Anterior Upper Periapical Exposure 1. Dentist _____ mR/hr. 2. Assistant _____ mR/hr. 3. Other (specify) _____ mR/hr.

C. Right Bitewing Exposure 1. Dentist _____ mR/hr. 2. Assistant _____ mR/hr. 3. Other (specify) _____ mR/hr.

D. Other measurements–See attached detail.

COMMENTS:

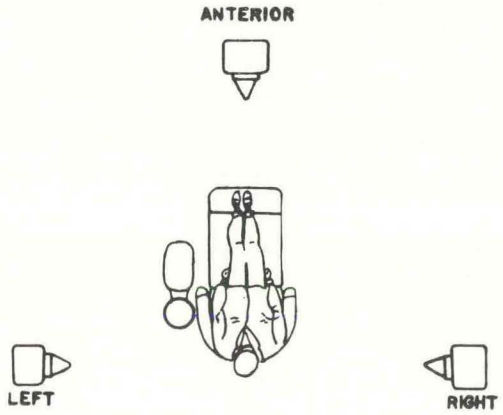
Figure 11-5 X-ray protection survey report - radiographic, dental

THE UNIVERSITY OF IOWA STATE HYGIENIC LABORATORY

X-RAY PROTECTION SURVEY REPORT

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

1. X-ray facilities are surveyed for compliance with the recommendations of the National Council on Radiation Protection and Measurements as listed in Reports 33, 34 and 35.
2. Electronic products are surveyed for compliance with the Radiation Control for Health and Safety Act of 1968, Public Law 90-602.
3. Environmental levels of radioactivity are checked for compliance with standards established by the Iowa Water Pollution Control 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 State Department of Health. 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 hould 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 Laboratory Building
Iowa City, Iowa 52240

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 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.

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:

1. 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
Iowa City, Iowa 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Town JONESVILLE County LINCOLN
Collector M. SMITH Date Collected 7-12-71
Sampling Point FAUCET
Purpose of Sampling BACTERIAL TEST

REPORT TO
Name M. SMITH
Street RR #1
City JONESVILLE, Iowa
Zip Code 50001

PLEASE PRINT PLAINLY

Owner of Supply M. SMITH
Chlorine
Depth of Well 540 ft
Type of Platform Concrete

(To be filled in by laboratory)

BACTERIAL ANALYSIS
$2.00 per sample

LSB	BGB	COLIFORM MPN (Test Results Circled)	
		0	SAFE
		2.2	UNSATISFACTORY
		5.1	UNSATISFACTORY
		9.2	UNSAFE
		16	UNSAFE
		16+	UNSAFE

FOR LABORATORY USE ONLY
1 2 3 4 5 6 7 8 9

LAB. NO.

CHEMICAL ANALYSIS
$2.00 per sample

*NITRATE: SAFE ☐
_____ p pm UNSAFE ☐
*Regarding use for preparation of infant formula.

Iron as Fe _____ p pm.
Hardness (as $CaCO_3$) _____ p pm.

W. J. HAUSLER, JR., Ph.D.
Director

Figure 12-2 Water analysis data slip

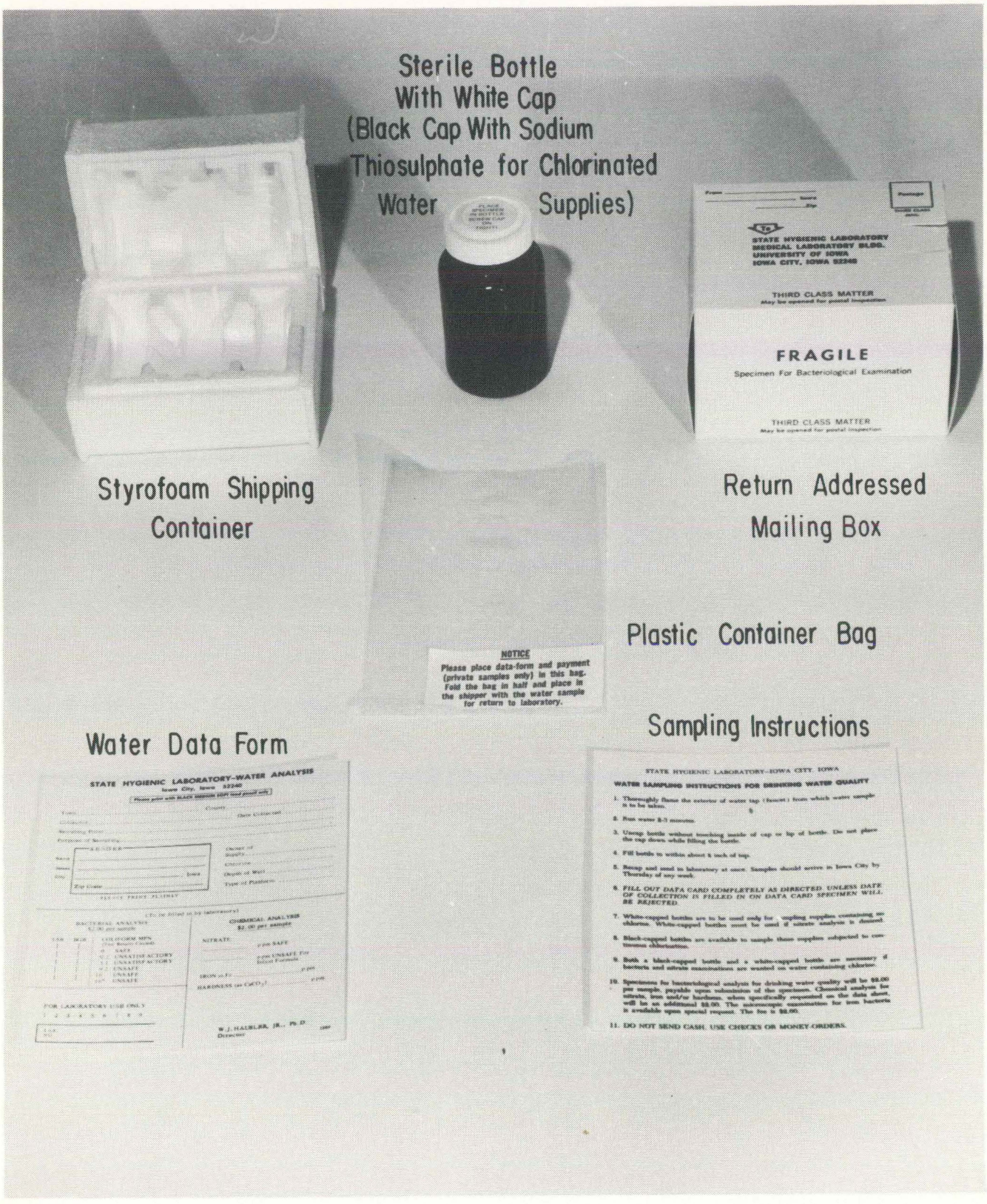


Figure 12-1 Drinking water collection kit

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 $2.00. If you wish chemical analyses, indicate iron, hardness, nitrate or all of these. An additional $2.00 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 $2.00. The total cost of an examination for total coliforms, nitrate, iron, hardness and iron bacteria is $6.00 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.
9. Place mailer inside the pre-addressed mailing box and secure the mailing lock-tabs. Add appropriate postage.
10. Mail the sample to the State Hygienic Laboratory by parcel post immediately after collection. Samples received more than three 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.

The bacteriological (coliform) interpretation is as follows:

1. Satisfactory—coliform bacteria (MPN) - 0 to less than 2.2
   This indicates that the water is bacteriologically safe to drink.
2. 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.
3. 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 ANALYSIS OF WATER SUPPLY SAMPLES

INTRODUCTION

The mineral analysis of water supply samples provides specific information on the significant physical and chemical characteristics of the water. The results of a mineral analysis can be used to evaluate the quality of water for drinking and to determine the method of treatment which might be necessary to improve the quality.

The tests performed in a mineral analysis are listed below:

- Alkalinity:
  - Phenolphthalein
  - Total
- Bicarbonate
- Calcium
- Carbonate
- Chloride
- Conductance
- Fluoride
- Hardness
- Iron:
  - Soluble
  - Total
- Magnesium
- Manganese
- Nitrate
- Potassium
- Silica
- Sodium
- Solids:
  - Dissolved
  - Total
- Sulfate

The mineral analysis service is available on request to municipalities, state agencies and private individuals. This analytical 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 Ave., Room 405
Des Moines, Iowa 50309

A fee is charged to cover the analytical expense.

COLLECTION AND SHIPMENT OF SPECIMENS

Upon receiving a request for a mineral analysis, the Des Moines Branch Lab will ship a one-half gallon plastic bottle and a data card for each sample to be analyzed (Figure 12-3). Please use our acid-cleaned bottles 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 truly representative of the well water. The pumping time and rate in gallons per minute (if known) should be recorded on the data card.
2. Fill the plastic bottle to the neck with a sample and cap the bottle.
3. 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.
4. 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 the number of the sample bottle appears 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.
5. The water sample for mineral 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 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, turbidity and pH. Nitrate concentrations are reported as nitrate and not nitrate nitrogen. Copies of all mineral reports are sent to the Water Supply Division of the Iowa State Department of Health.

Inquiries concerning the meaning of the reported values and their relationship to drinking water quality should be directed to the Water Supply Division of the State Department of Health.

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 State Department of Health. The purpose of this program is to insure 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. "Collector's Remarks" should include anything unusual about plant operation at the time of sampling as well as any interferences to the fluoride analysis which are known to be

STATE HYGIENIC LABORATORY, DES MOINES BRANCH

The University of Iowa
405 STATE OFFICE & LAB BLDG, E 7th & COURT
DES MOINES, IOWA 50309

LAB NO.

MINERAL ANALYSIS

MINERAL NO.

REPORTED

TOWN COUNTY
OWNER OF SUPPLY
COLLECTOR'S NAME
DATE COLLECTED
REPORT TO

FIELD DATA

SOURCE WELL DATA
SAMPLING POINT NAME OR NUMBER
CONSTRUCTION DATE DEPTH
PUMPED HRS AT GPM

WAS SAMPLE FREE OF TURBIDITY WHEN COLLECTED?
TEMPERATURE ° C ALKALINITY (mg/l $CaCO_3$)P T pH
IS A POLYPHOSPHATE BEING USED? DATE OF PREVIOUS SAMPLE

LABORATORY ANALYSIS
(MILLIGRAMS PER LITER)

SPECIFIC CONDUCTANCE AT 25° C micromhos SILICA (SiO_2)
TOTAL RESIDUE TOTAL IRON (Fe)
FILTRABLE RESIDUE SOLUBLE IRON (Fe)
ALKALINITY AS $CaCO_3$ P T pH DATE
HARDNESS AS $CaCO_3$ mg/l gpg

POSITIVE IONS		NEGATIVE IONS		TRACE METALS	
POTASSIUM (K^+)		NITRATE (NO_3^-)		ARSENIC	
SODIUM (Na^+)		FLUORIDE (F^-)		BARIUM	
CALCIUM (Ca^{++})		CHLORIDE (Cl^-)		CADMIUM	
MAGNESIUM (Mg^{++})		SULFATE (SO_4^{--})		CHROMIUM	
MANGANESE (Mn^{++})		BICARBONATE (HCO_3^-)		COPPER	
		CARBONATE (CO_3^{--})		LEAD	
				ZINC	

ANALYST

R. L. MORRIS, Ph.D.
ASSOCIATE DIRECTOR AND
PRINCIPAL CHEMIST

2155

Figure 12-3 Mineral analysis data slip

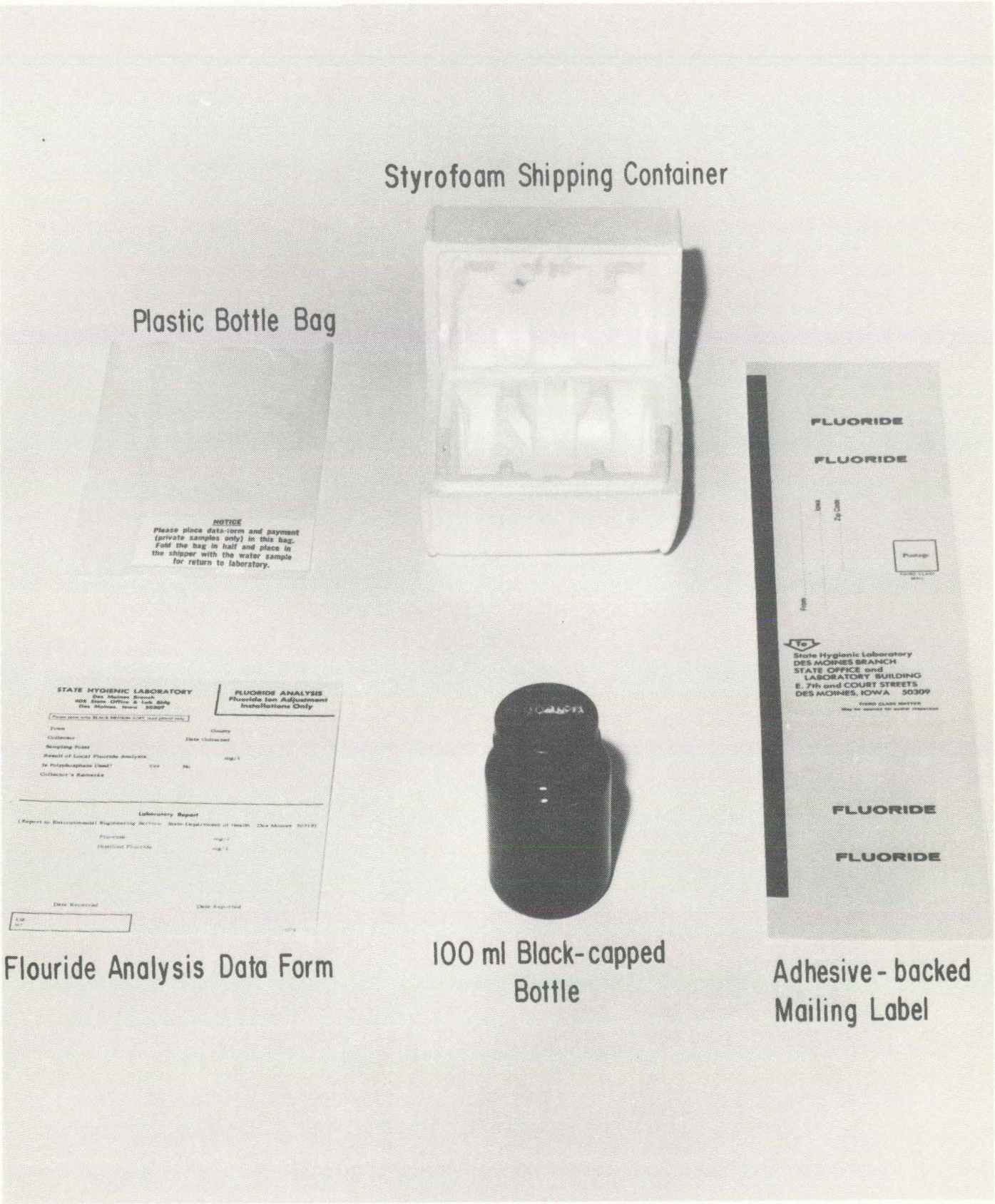


Figure 12-4 Fluoride analysis collection kit

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 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 Ave., 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 Lab. 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 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 ± 0.2 parts per million, a request is sent back to the operator for a recheck sample. If the rechecek still indicates a discrepancy in the operator's analysis, he or she 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, he or she is then called in for additional training in fluoride analysis.

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 State Department of Health, State Conservation Commission and Iowa Geological Survey as well as the Laboratory's Limnology Division which conducts water pollution surveillance studies for the Iowa Water Pollution Control 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 nongovernmental 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 *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 Ave., Room 405
Des Moines, Iowa 50309

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 ac-

curate 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 sample volume, preservatives, sampling procedures and 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 State Department of Health, Environmental Engineering Division. 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.

Table 12-1 Tests available, surface water and wastewater

- Acidity
- Alkalinity
  - Phenolphthalein
  - Total
- Biochemical Oxygen Demand (BOD)
- Calcium
- CCEm
- CAEm
- Chemical Oxygen Demand (COD)
- Chloride
- Color
- Conductivity
- Cyanide
- Detergents
- Fecal Coliform Bacteria
- Fluoride
- Hardness
- Herbicides*
- Iron
- MBAS
- Magnesium
- Manganese
- Metals
  - Arsenic
  - Barium
  - Cadmium
  - Chromium
  - Copper
  - Lead
  - Mercury
  - Selenium
  - Silver
  - Zinc
- Nitrogens:
  - Organic
  - Ammonia
  - Nitrate
  - Nitrite
- Oil and Grease
- pH
- pHs
- Pesticides*
- Phosphorus:
  - Soluble
  - Total
- Potassium
- Radionuclides:*
  - Gross Beta-gamma
  - Gross Alpha
  - Radium-226
  - Strontium-90
  - Tritium
- Sodium
- Silica
- Solids, settleable
- Solids:
  - Total—fixed and volatile
  - Dissolved—fixed and volatile
  - Suspended—fixed and volatile
- Stability Index
- Sulfate
- Sulfide

* Initial contact should be made with:
State Hygienic Laboratory
Medical Laboratory Building
The University of Iowa
Iowa City, Iowa 52240

WASTEWATER TREATMENT PLANT EFFLUENT SURVEY (BOD)

INTRODUCTION

The mail order BOD (Biochemical Oxygen Demand) service is provided by the Des Moines Branch Laboratory to the State Department of Health's Environmental Engineering Service 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 State Department of Health.

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:

1. Collect the original effluent sample in a larger container (one quart minimum) and *mix* thoroughly before filling the pint plastic sample bottle.
2. 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.
3. Cap the bottle tightly so no leakage will occur during shipment.
4. 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 State Department of Health 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 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 Pollution Control 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.

Analysis of pesticide and mercury concentrations in fish, water and other parts of the aquatic environment are being performed on a statewide basis in cooperation with the Iowa Conservation Commission.

REPORTING OF LABORATORY RESULTS

Written reports of the studies with subsequent recommendations for appropriate action are submitted to the Iowa Water Pollution Control 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 ac-

Figure 12-5 BOD collection kit

cording to the U.S.P.H.S. 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 12-6).

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 $2.00 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 three 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.
4. Time of sampling should coincide with the peak load.
5. 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 52240

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 in the case of public-use facilities.

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 Inspection Division 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.*

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

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 Inspection Division include the following types of samples and examinations:

1. Processed milks
   a) Standard Plate Count
   b) Coliform Count

Figure 12-6 Swimming pool water collection kit

c) Butterfat Content
d) Phosphatase Test
e) Antibiotic Residual

2. Other dairy products (cheeses, ice creams, dips)
   a) Standard Plate Count
   b) Coliform Count
3. Adult and infant formulas from University Hospital
   a) Standard Plate Count
   b) Coliform Count
4. 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.

BREATH AND BLOOD ALCOHOL PROGRAM

INTRODUCTION

The State Hygienic Laboratory and the Department of Public Safety have developed a statewide breath and blood alcohol program to curb the increasing accident toll associated with the intoxicated driver. To implement the program, the state has been divided into three zones for testing purposes (Figure 14-1). Each zone has a branch of the State Hygienic Laboratory to test breath and blood alcohol specimens from that zone. Specimen kits, which are provided by the Iowa State Highway Patrol Office in Des Moines, and the State Hygienic Laboratory, include the necessary information and where to mail the specimens.

The following information is intended to provide a basic understanding of the Breath Alcohol Program and how it is used to detect intoxicated drivers.

FAIT—Field Alcohol Indicator Test

A small tube containing a calibrated amount of dichromate/sulfuric acid is used first as an indicator of the presence of alcohol (included in the kit shown in Figure 14-2). Any alcohol passing through the tube changes the yellow dichromate, by a chemical reaction, to a green color. When the dichromate is totally changed, it will indicate a breath sample that contains at least a 0.10 percent alcohol concentration or more. These tubes are factory sealed to prevent environmental contamination. After the tips are broken off for use, they must be used or discarded within eight hours.

Indium Tube Encapsulation

Indium is a soft, relatively inert metal. When crimped or pressed together under a nominal amount of pressure, it fuses together or forms what might be called a cold weld which is air tight. This is the principle of tubular indium collection for breath samples. The tube is approximately four to 4¾ inches long and has a ⅛-inch internal diameter. After the breath sample is passed into the tube, it is crimped into three equally sized portions. These portions are exactly 1.00 inch in length, with each containing ¼ cc of the breath specimen. At this point, the three specimen samples are equal in size and are ready for confirmatory analysis. For preservation of the sample, the inside of the tube has been coated with a bacteriostatic substance. This substance safeguards the sample and prevents bacteria from decomposing any alcohol in the sample. The preserved sample has been shown to display no change in alcohol content up to five days after collection. The kit prepared for this procedure is shown in Figure 14-3.

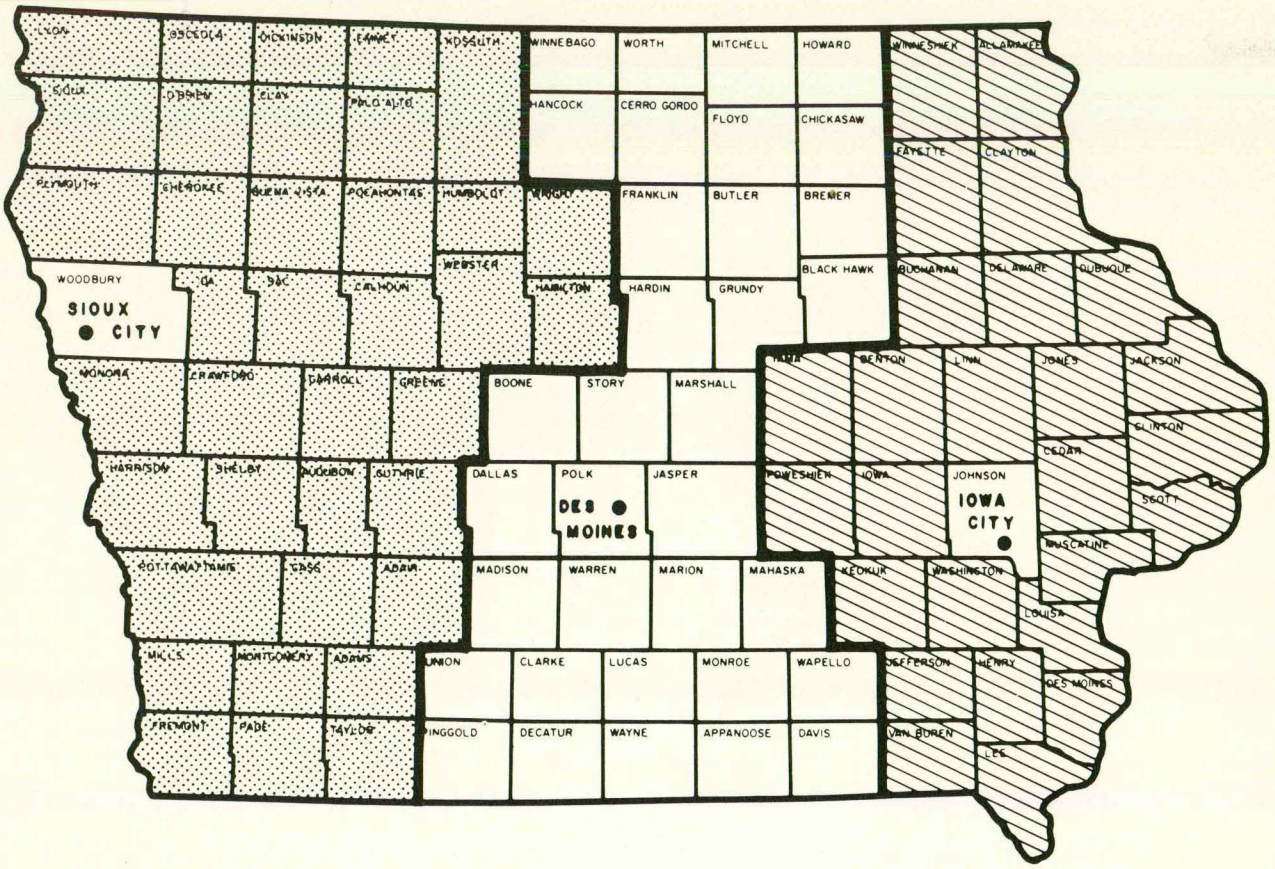
Laboratory Analysis of Breath

The instrument used for testing in all three laboratories is called a "gas-liquid chromatographic intoximeter" (GCI). Simply, this is a device which takes the sample in each indium capsule, identifies its alcohol content and quantitates the amount in each capsule. All three specimen samples are analyzed in this manner. A standard sample is then run exactly in the same manner as the three capsules. A standard is prepared from an "artificial" breath device called a breath simulator. This contains a known, precalculated concentration of gaseous ethyl alcohol. The standard is crimped or encapsulated in an indium capsule at the Laboratory and is then passed through the intoximeter as was the unknown. The measurement of the unknown specimen is calculated against the known standard. Briefly, the intoximeter is a device which can separate, detect and quantitate an organic substance. Separation is performed within the instrument in a long, narrow column which allows many substances to "filter" through at different speeds. These speeds are known and predictable to a competent operator. After separation, the alcohol is detected in a sensing device by a principle called electronic-flame ionization. An electronic signal is passed into an amplifier and then displayed onto a chart paper called a chromatogram. The chromatogram then becomes the authentic evidence of "what" and "how much" the sample capsule contained and can be used in court as evidence of suspected alcohol consumption.

Laboratory Analysis of Blood

The analysis of blood is essentially the same as that mentioned above for breath alcohol examination. The technic used for breath employs the indium tube method and the blood employs head space technic. Two milliliters (ml) of whole blood are added to a 60 ml serum bottle and tightly capped with a rubber gasket and seal. This then is placed in a 37°C waterbath for 15 minutes and allowed to equilibrate. From this, 2 ml of air is taken and injected into the column of the chromatograph. From this point on, including

CHEMICAL ANALYSIS TESTING LABORATORY LOCATION & ZONING MAP



ZONE 1
Sioux City Branch Lab.
Sioux City Health Dept.
City Hall
6th & Douglas
• Sioux City, Iowa 51102
Phone (712) 277-2121

ZONE 2
State Hygienic Branch Lab.
405 State Office & Lab. Bldg.
East 7th & Court
• Des Moines, Iowa 50309
Phone (515) 281-5371

ZONE 3
State Hygienic Lab.
The University of Iowa
Medical Laboratory Bldg.
• Iowa City, Iowa 52240
Phone (319) 353-5990

Figure 14-1 Breath alcohol zone map

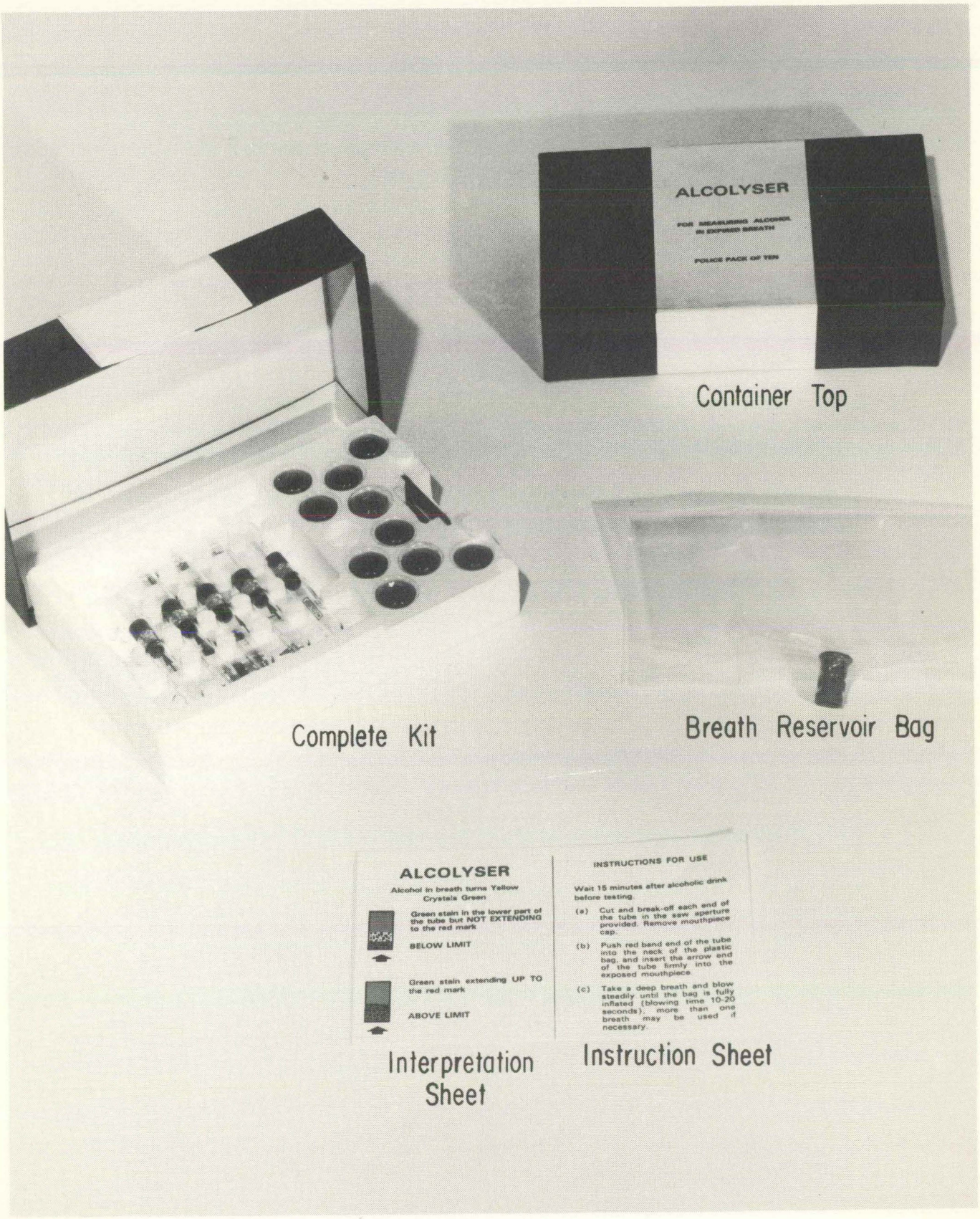


Figure 14-2 FAIT kit

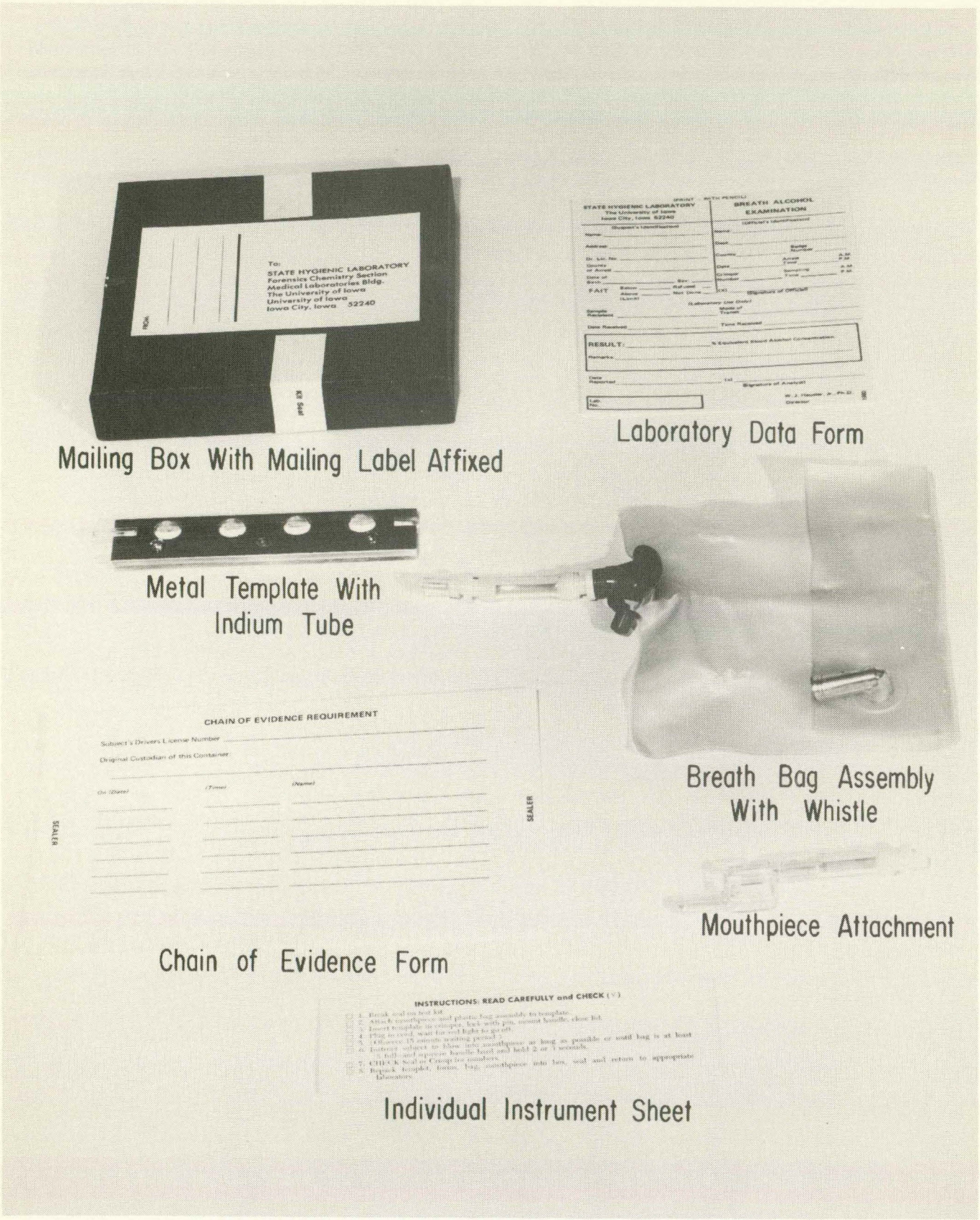


Figure 14-3 Indium tube encapsulation kit

calculations, the method is the same as for breath. This choice of technics maintains uniformity of technic and simplicity of technic in both breath and blood alcohol determinations.

COLLECTION AND SHIPMENT OF SPECIMENS

Breath Collection

FAIT. This test comes in a small box of 10 testing tubes, 10 mouthpieces and one 1,000 ml plastic bag (Figure 14-2).

1. Each tube is sealed and must have its ends broken off before use.
2. The mouthpiece and the plastic bag are then assembled tightly.
3. After a 15-minute waiting period, instruct the suspect to blow into the apparatus and fill the bag.
4. Upon filling the bag, observe the yellow color of the *dichromate* to detect a change to a green color. If the yellow color crystals have completely changed to green (that is to the line depicted on the tube), the suspect has demonstrated a breath alcohol content of approximately 0.10 percent concentration and suggests further consideration.

The tube can be kept for further evaluation by the officer if the ends of the tube are adequately sealed with tape. However, the tube *cannot* be used on a second suspect, because a partial reaction may have occurred and would, therefore, be additive when used for the second time.

Indium Tube Encapsulation Operation. The indium tube comes in a special container and is fixed in a frame or template ready for use (Figure 14-3). (Note: The encapsulation by the indium tube is the focal point of the system; therefore, one must pay special attention to its usage.)

1. Remove the template with the tube from the container. A mouthpiece and a one-way valve with bag are attached to the ends depending on how the officer wishes to collect the sample.
2. Place the template assembly in the crimper box (Figure 14-4), attach the handle grip in the proper manner and close the box.
3. Plug the external heater cord 12 vdc or 110 vac into its appropriate receptical. The red pilot light on the outside of the box will turn off to indicate when the box is up to temperature, i.e., 34°C (92°F), or breath temperature. The heating of the box is necessary to simulate normal breath conditions of the suspect and thus to prevent unreal conditions in sampling.
4. After the suspect has breathed a desired amount (1,000-2,000 cc or one full breath) through the sample tube, squeeze the hand-grip crimper, thereby collecting and sealing three specimen samples.
5. Remove the specimen tube and template from the crimper box and note the crimped area. There will be four numbers imprinted thereon, one at each crimp or sealed area. These numbers are individual box numbers and also indicate that a good seal has been made on the tube.
6. Place the template with its sample tube in the mailing box, with the completed breath alcohol examination form, close and seal for mailing.
7. Mail all breath specimens to the Laboratory as first class mail with a chain of evidence form on the outside of the box.

Blood Collection

1. It is preferred that the Vacutainer Collection Kit, blood alcohol determination #BD 4990 be used solely for this purpose. All forms, all labels, and Chain of Evidence form are included.
2. If #BD 4990 kit is not available, blood may be drawn and shipped, using specially prepared sodium fluoride tubes. These are available from Becton-Dickinson.
3. If you prepare your own tubes, they must contain 20 milligrams (mg) of sodium fluoride per milliliter of whole blood.
4. All blood alcohol and allied forms must comply with good evidence practices. This must include the chain of evidence form.

REPORTING OF LABORATORY RESULTS

In the Laboratory, the breath and blood samples are examined by gas chromatography and reported as blood alcohol equivalent concentrations. Section 321.-281 of the Code of Iowa states that more than 0.10 of one percentum by weight of alcohol in one's blood shall be admitted as presumptive evidence in a court of law that the defendent was under the influence of an alcoholic beverage at the time of sampling. The original copy of the results is kept in the Laboratory, in the event the case is taken to court. A copy is sent to the county attorney's office serving the area in which the arrest was made, and the arresting officer is informed of the results by letter or telephone.

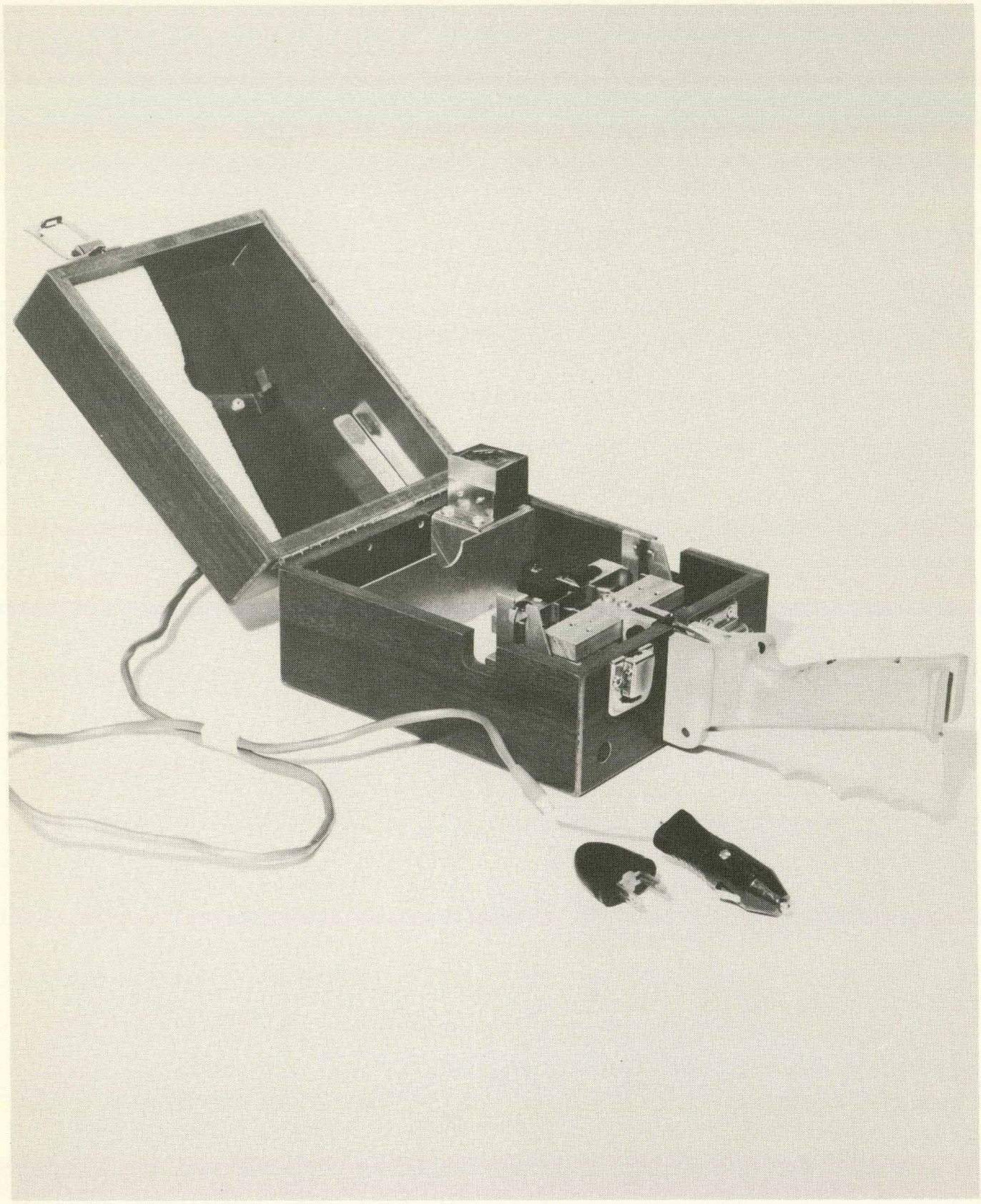


Figure 14-4 Crimper box

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.

1. 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.
3. 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 Services. The Guthrie screening 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 15-1). Most of the Guthrie screening tests conducted are performed on specimens from newborn infants delivered at the University of Iowa Hospitals and Clinics. The PKU test is performed in compliance with the Iowa law recommending testing of infants for phenylketonuria.

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 of this manual.

This PKU Screening and Confirmatory Testing Service is a significant part of a program designed to prevent mental retardation.

STATE HYGIENIC LABORATORY
THE UNIVERSITY OF IOWA
MEDICAL LABORATORIES BUILDING
IOWA CITY, IOWA 52240

Please print with BLACK MEDIUM SOFT lead pencil only

Miscellaneous Examination

Patient JOHNNY INFANT Age – Sex M

Address BIGTOWN, IOWA

Physician (Name and Address)

Dr. T. HOWARD
MEMORIAL HOSPITAL
BIGTOWN, Iowa
Zip Code 50000

PLEASE PRINT PLAINLY

Examination Desired: PKU

○ Primary Culture
○ Subculture

Source of Specimen: Blood

CLINICAL DIAGNOSIS

Laboratory Report

<2mg% phenylalanine

Date Reported

LAB NO.

W. J. Hausler, Jr., Ph.D.
Director

1311

Figure 15-2 PKU report form

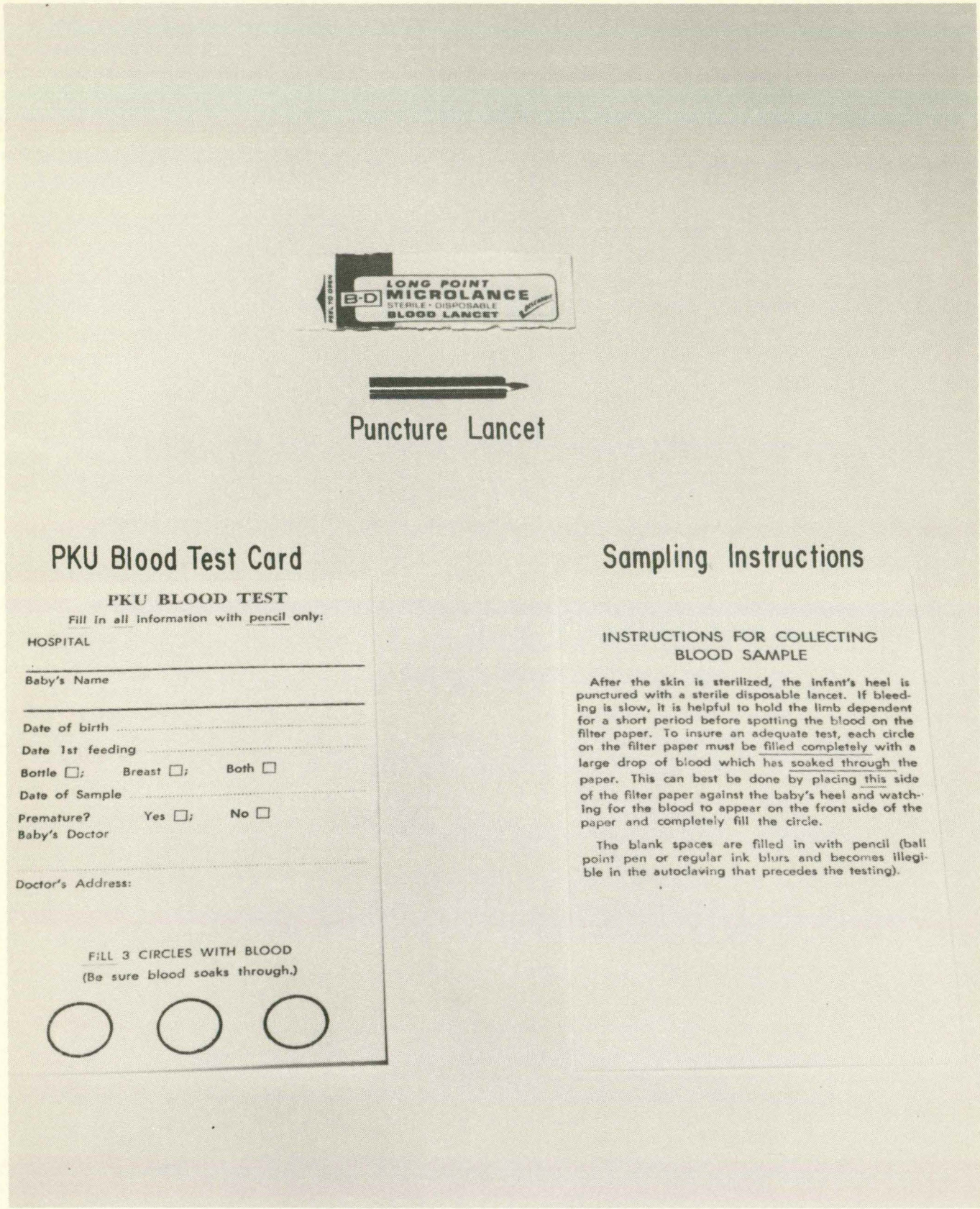


Figure 15-1 PKU blood test kit

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 15-2) 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 drug 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. It will also analyze samples of street drugs in solid or liquid dosage forms to determine their content, contamination and the possible potential for untoward reaction. 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.

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.

The information of common name, source, location, date of collection and any other valuable comments are required for each street drug sample sent to the laboratory for identification.

A fee will be charged for each specimen and should accompany the sample unless previous billing arrangements have been made with the laboratory for quarterly or monthly statements.

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.