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This publication is prepared and disseminated for the specific purpose of providing epidemiological information within the general scope of the USAF Aerospace Medicine Program (AFM 161-2, dated 10 October 1962).

The USAF Epidemiological Services are concerned with the relationships of various factors and conditions which contribute to injuries and to the frequency and distribution of all illness among Air Force personnel (AFR 161-12, dated 25 September 1962).

The information contained in this publication is a part of the consultation service being rendered to the USAF medical services and is considered to be expert professional opinion. The use of such information for purposes other than as specified above relieves the authors and the USAF of any responsibility or obligation.

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FOREWORD

This publication was prepared by the following personnel of the USAFE Epidemiological Flight (TUSLOG Detachment 36):

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ABSTRACT

This publication identifies and discusses the epidemiological services available to USAF medical facilities. The scope and mode of operation is presented. Further, the laboratory capabilities are discussed with detailed instruction on the collection and shipping of specimens.

This publication has been reviewed and approved.



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CONTENTS

| | <u>Page</u> |
|--|-------------|
| INTRODUCTION | 1 |
| Functional Organization - Figure 1 | 2 |
| REQUEST FOR SERVICES | 3 |
| FOOD ANALYSIS | 4 |
| Bacterial Examination of Food Items | 4 |
| Perishable Items, Canned Foods, Dairy Products | 4 |
| Request Forms | 5 |
| ZOONOTIC AND HUMAN DISEASES | 5 |
| Rabies | 5 |
| Request for Laboratory Diagnosis of Rabies - Figure 2 ---- | 6 |
| Parasitology | 5 |
| Animal | 5 |
| Human | 7 |
| Shipment of Fecal Specimens | 7 |
| Kala-Azar | 7 |
| Malaria | 7 |
| Adult Parasites | 7 |
| Anthrax | 8 |
| Brucellosis, Leptospirosis, Q Fever | 8 |
| Histoplasmosis, Toxoplasmosis, Trypanosomiasis | 8 |
| ANALYSES OF OTHER MATERIALS FROM HUMAN PATIENTS -- | 8 |
| Packaging of Specimens or Materials for Mailing | 9 |
| Bacteriology | 9 |
| Specimens To Be Examined for Acid-Fast Bacilli | 11 |
| Mycology | 11 |
| Specimens from Dermatophytic Lesions | 11 |
| Formula - Cycloheximide - Chloramphenicol Media | |
| for the Selective Isolation of Pathogenic Fungi | 12 |
| Specimens from Systemic Mycotic Infections | 13 |
| WATER ANALYSIS | 14 |
| MEDICAL ZOOLOGY | 15 |
| Collection Procedures | 15 |
| Mosquitoes - Larvae and Adults | 15 |
| Flies | 16 |
| Ectoparasites | 16 |
| Other Arthropods | 17 |
| Snails, Terrestrial and Aquatic | 17 |
| Reptiles | 17 |
| Shipment of Specimens | 18 |

THE USAFE EPIDEMIOLOGICAL SERVICE

1. INTRODUCTION

Under the provisions of AFR 161-12, dated 25 September 1962, the Epidemiological Services for USAFE are established. TUSLOG Detachment 36, located in Izmir, Turkey, is the medical unit designated to be staffed and equipped to carry out these responsibilities. The purpose of this writing is to discuss the present capabilities of the unit and to define the scope of service which can be expected within these capabilities.

Epidemiology is not a medical specialty in itself, but is the combining of several medical and allied specialties or sciences under the team concept. Each individual contributes to the group, thereby giving depth and range to the total capability. Epidemiology is, therefore, the scientific study of the behavior of disease and injury in groups of people by relating all living organisms in an environment at a particular time and place. This has also required the additional consideration of cultural, economic, and social factors in the particular area under study. During the seven years in which this unit has been in Izmir, considerable information has been accumulated concerning the Eastern Mediterranean Area. This information will be the basis for future publications of this unit.

The professional staff assembled to accomplish this mission consists of a preventive medicine officer, a bacteriologist, a medical entomologist, an industrial hygiene engineer, and a veterinarian. It can be visualized with a minimum of explanation how these individuals form the epidemiological team approach to the study of disease and injury in groups of people. This team, except under unusual circumstances, must work together as a whole to accomplish its mission. It is realized, of course, that certain diseases or circumstances may drop particular members from the team temporarily. To assist these individuals, administrative, supply, laboratory, etc. personnel are assigned to the unit.

Presently, the activities of the various sections cover epidemiological assistance visits to bases, environmental health surveys, in-house research, education and training, special survey projects, and development of better and newer techniques and

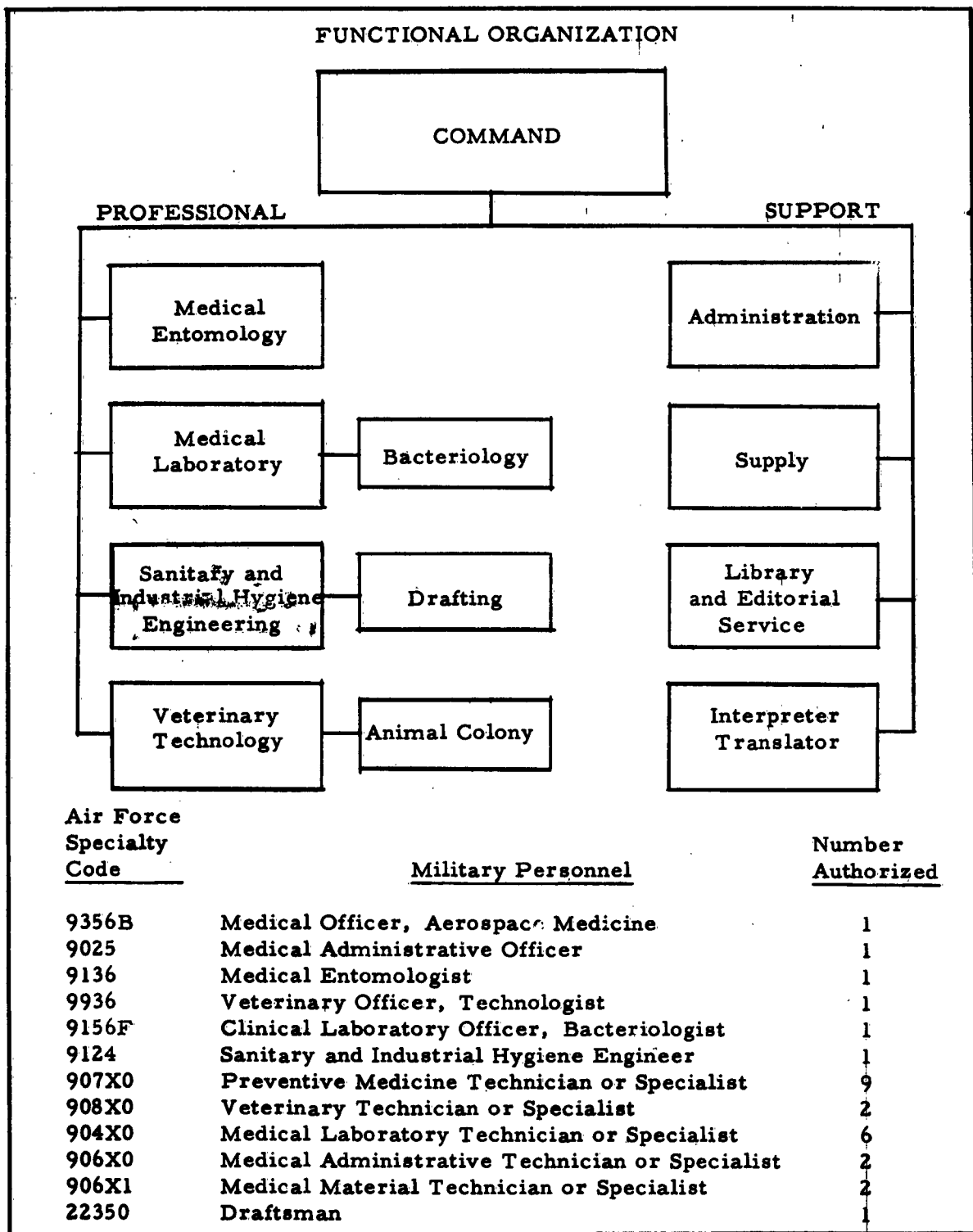


FIGURE 1

procedures relative to the accomplishment of the mission. Information procured but never made known is useless; therefore, it is planned to follow this introductory brochure with selected epidemiological papers of current Detachment 36 research activities, military public health information. etc.

The unit is established in a four-story building facing Culture Park in the modern residential section of Izmir, Turkey. The building was used by the U. S. Navy as a hospital until 1956. Detachment 36 secured it at that time, as the Navy moved its operations to Naples, Italy. The ground floor is occupied by the unit command, administration, supply, and library. The clinical laboratory and veterinary services share the second floor. The third floor is divided between the entomology and engineering services. The top floor is primarily animal colonies, with a division between healthy and isolation areas. A necropsy and animal inoculation room is also located on the fourth floor.

The nature of the type of service expected from an epidemiological unit requires an ability to function under field conditions. Field adapted laboratory equipment has been procured to maintain full capabilities while under tentage in isolated areas. As presently field equipped, the unit accomplished two independent epidemiological field surveys during the summer of 1962 in two widely separated provinces of Turkey. These field surveys demonstrated the unit's ability to perform its mission under actual field conditions. Any one of the specialty sections of the unit also possesses independent field capability. Further, a team formed of selected individuals from two or more sections for the purpose of studying a specific problem would possess this same capability. This last configuration is the type of team which is required in most instances.

2. REQUEST FOR SERVICES

Directors of base medical services in USAFE may send a request for epidemiological services direct to the Commander, Detachment 36, APO 224, US Forces, Europe, under the provisions of AFR 161-12, dated 25 September 1962. Information copies of all requests must be forwarded to Headquarters, USAFE. A copy to the intermediate headquarters would be considered appropriate, although not required by regulation.

It is suggested that the request contain specific information insofar as possible concerning the nature of the problem, time elements, number of persons involved, environment, and measures already taken. This information will aid in establishing the epidemiological team's personnel and equipment requirements prior to arrival.

Request may also be made for information only. Detachment 36 has established and maintains a constantly enlarging cross-reference file on epidemiological field surveys, research projects, base environmental health surveys, disease incidence statistics, and pertinent literature abstracts covering the activities of this unit since 1956 in the Near and Middle East. The information coverage is not complete nor always up to date; however, it is available and constantly being improved.

Many medical facilities have found it convenient to send specimens to Detachment 36 for analysis, identification, processing, etc. Although Detachment 36 is not established as an area laboratory for accomplishing routine clinical laboratory work, it is available as a back-up for any procedure which is beyond the capability of most medical facility laboratories.

3. FOOD ANALYSIS

a. Bacterial Examination of Food Items. A complete bacterial examination of food items is performed by this Detachment.

(1) Perishable Items. All perishable items to be sent to this laboratory must be iced down prior to shipment and sent so they will arrive the same day. Representative samples must be sent and, in addition, preferably a representative sample of one of the normal appearing items.

(2) Canned Foods. Canned foods submitted for examination for sterility keeping purposes or causes of spoilage are to be representative of the pack. Samples should include two or more abnormal and two normal appearing cans of the same batch or code whenever possible, and must be packed to avoid damage in shipment.

(3) Dairy Products (Milk, Cream, Other Fluid Dairy Products, Ice Cream, and Cottage Cheese). Collect samples of fluid

milk products, ice cream, or cottage cheese in original containers at time of delivery. Refrigerate with cracked ice. Place milk and cottage cheese containers in an upright position, and in such a manner that the refrigerant liquid will not come in contact with the lip or cap of the containers. Better still, use milk cartons or sealed cans filled with water and frozen, instead of loose cracked ice. Samples preferably should be delivered to the laboratory within four hours of the time of collection.

b. Request Forms. All requests for laboratory analysis of foods will be submitted on DD Form 389, original and two copies, with samples.

4. ZOO NOTIC AND HUMAN DISEASES

a. Rabies. Whole dead animal bodies will be shipped if the distance is not over 25 miles or time of delivery is not more than one hour. In case of greater distance, the dead animal's head will be removed, skin left intact, and placed in a container with a crimped lid (no screw tops) not air-tight. This container will be placed in a larger container and iced or refrigerated, as indicated in paragraph 3a(3), above, on dairy products. The outer container will be marked with a label, plainly visible, containing the word "DANGER" in letters not less than 3/4-inch high. An envelope containing a narrative summary will accompany the container. Figure 2 gives the format for a "Request for Laboratory Diagnosis of Rabies."

b. Parasitology.

(1) The following are routinely examined for in the laboratory:

(a) Animal.

Ancylostomiasis
Ascariasis
Diphyllobothriasis
Giardiasis
Strongloidiasis
Amebiasis
Taeniasis
Others

REQUEST FOR LABORATORY DIAGNOSIS OF RABIES

ANIMAL OWNER

| | |
|--------------------------------------|-------------|
| Name, Rank & Organization | Date |
| | |

| |
|--------------------------------|
| Address & Phone No. |
| |

HUMAN EXPOSURE

| | |
|-------------------------------------|---|
| Patient's Name & Address | Sponsor's Name, Rank, & Organization |
| | |

| | |
|--|-------------------------|
| Report of Incident (Description of bite wound or exposure & location) | Date of Exposure |
| | |

ANIMAL DATA

| | | | | | |
|----------------|--------------|------------|------------|---------------|--------------|
| Species | Breed | Sex | Age | Weight | Color |
| | | | | | |

| | | | |
|---|------------------------|-------------|---------------------|
| Animal Vaccinated against Rabies | Type of Vaccine | Date | Stray or Pet |
| | | | |

| |
|---|
| Possible exposure to Rabies (fighting, other animals involved, date) |
| |

| | | | |
|--|--|--|----------------------|
| Date Animal submitted to Quarantine | Died _____ Euthanasia _____ | Sign of Rabies _____ Healthy _____ Death & No Signs _____ | Date of Death |
| | | | |

| |
|---|
| Veterinarian's tentative clinical diagnosis if Veterinarian is available on base |
| |

| | |
|--|-----------------------|
| Submitting Veterinarian if Veterinarian is available on the base & Organization | Date Submitted |
| | |

(b) Human. Stool specimens from food handlers and patients will be examined for cystic stages of ova and parasites. Request slip 514G should accompany each specimen (see paragraph 5).

(2) Enclose any significant signs or symptoms that the animal or human shows at the time of collection of the fecal sample.

(3) Shipment of Fecal Specimens. Fecal specimens may be submitted in Merthiolate-Formalin (MF) solution without deterioration of ova. MF solution is prepared and used as follows:

| | | | | |
|-------------------------|-----|-----------|-----------|---------|
| Distilled Water | ... | ... | ... | 250 mls |
| Tincture of Merthiolate | ... | (1:1000), | Lilly #99 | 200 mls |
| Glycerol | ... | ... | ... | 5 mls |
| Formaldehyde (USP) | ... | ... | ... | 15 mls |

Thoroughly mix the above ingredients and dispense in 14 ml amounts to 22 ml, screw-capped vials. Using wooden applicators, add about 0.5-1.0 grams of fecal sample to the MF solution and prepare a complete immersion. After sealing, the vial is then packed for shipment. MF solution vials prepared for future use should be stored in the refrigerator to prevent evaporation of the contents.

(4) Kala-Azar. Smears may be submitted from peripheral blood (thin smears), spleen pulp, lymph node juice, liver pulp, sternal bone marrow, or nasal secretions. Ship as described in paragraph 4e, below. Hamster inoculation may also be performed if so desired. Submit chilled specimens for latter analysis as shown in paragraph 5a(1). Narrative summary must accompany specimens.

(5) Malaria. All smears for malaria should be prepared of blood collected at the peak of fever. After the blood film has dried, fix in absolute methyl alcohol for 10 minutes, then carefully pack for shipment as outlined in paragraph 4e, below. A minimum of five smears should be prepared and submitted in this manner, along with a narrative summary.

(6) Adult Parasites. Wash in physiological saline until clean, then fix in 5% formalin heated to 80° C. After cooling, the specimen may then be shipped in a leak-proof vessel with the formalin.

c. Anthrax. Whole blood or vital organs may be sent to the laboratory, chilled or frozen, for culture of anthrax bacteria. (Extreme caution should be employed when collecting samples.) Ship specimens as described in paragraph 5a(2).

d. Brucellosis and Leptospirosis (in addition, Q Fever on a limited basis due to the scarcity of antigen). Sterile blood serum must be submitted, paired serum if possible. The serum may be chilled or frozen.

e. Histoplasmosis, Toxoplasmosis, and Trypanosomiasis. A minimum of five blood smears on microscopic glass slides will be sent to the laboratory. After the blood film has dried, fix in absolute methyl alcohol for 10 minutes. Preferably, slides will be put in a slide box, which will be returned to the sender on arrival at this laboratory. In lieu of a slide box, slides may be placed together with the blood smears facing one another, and match sticks placed at the ends to hold the slides apart. Tape may then be used to hold the slides together.

5. ANALYSES OF OTHER MATERIALS FROM HUMAN PATIENTS

Request slips of the 514 series and/or appropriate narrative summaries must accompany specimens or materials forwarded for laboratory studies. The following information will be included:

1. Patient's full name, rank, serial number, ward number, and APO. If a dependent, include name, rank, and serial number of sponsor.
2. Type of specimen and manner obtained.
3. Type of examination requested.
4. Date specimen was obtained.
5. Name or numerical designation of the requesting agency.
6. Signature of the requesting officer.
7. Provisional diagnosis.

In the event a quick report is desired on a given specimen, the notation "EMERGENCY" should be written on the request.

a. Packaging of Specimens or Materials for Mailing.

(1) Any infectious liquids, pure cultures, or substances other than those listed in paragraph 5a(2), below, must be enclosed in a stout glass receptacle with a leak-proof stopper or lid. Prescription bottles, screw-capped tubes, or similar vessels of appropriate size may be employed. The stoppers or caps of specimen containers should be tightly applied, secured with adhesive tape, and dipped into melted paraffin to insure effective sealing. Each specimen is identified to correspond with the information on the request slip, by securely attaching a gummed label to the exterior wall of the container. Specimens prepared in this manner which do not require refrigeration are wrapped in adequate cotton insulation and placed in a metal or heavy cardboard container. The latter is then placed in a larger box or mailing container, with adequate packing material, and secured by binding or other suitable means. A round pathological shipping container or dental shipping box will usually suffice. Specimens requiring refrigeration, such as clinical materials for bacteriological analysis, must be submitted in the chilled or frozen state. In these instances, receptacles containing specimens must be placed in a leak-proof, nonbreakable container with adequate insulating material, and then placed in a larger vessel with a mixture of cracked ice and salt.

(2) Cultures and infectious material of plague, cholera, anthrax, undulant fever, and tularemia may be submitted if enclosed in a stout glass receptacle, hermetically sealed and packed in a larger container with cotton saturated with four percent formalin. After securely sealing the outer vessel, the specimen is prepared for shipment as described above. (NOTE: Clinical specimens must be shipped in the chilled or frozen state and clearly marked "FRAGILE, PATHOLOGICAL SPECIMENS.")

b. Bacteriology.

(1) General Considerations. Clinical materials which cannot be analyzed locally may be forwarded for bacteriological examination. In addition, strains of bacteria presenting a problem in identification may be forwarded in pure culture on appropriate carrier media. As a general rule, 18-24 hour growth is optimal

for most organisms, but the more fastidious pathogens such as Brucella, Hemophilus, and Pasteurella species usually require a longer period of incubation before shipment. For most pathogenic bacteria, trypticase soy blood agar slants or stabs will serve as an ideal shipping medium. Stab tubes of cystine trypticase agar (CTA - Baltimore Biologics Laboratory) are excellent for growth and maintenance of fastidious organisms, including gonococci, meningococci, pneumococci, brucellae, and corynebacteria. If Pasteurella pestis is suspected, slants of trypticase soy agar may be used as a shipping medium, while cystine glucose blood agar slants (cystine heart agar - BBL manual) are required for growth and transmittal of Pasteurella tularensis. In order to insure viability of referral cultures and expedite their identification, careful attention should be given to selection of media for transporting laboratory isolates. The following culture media are suitable for transport of the bacteria listed:

| | |
|--------------------------------|--|
| <u>Neisseria gonorrhoeae</u> | Chocolate agar slant (Incubated in candle jar) |
| <u>Neisseria</u> species | Chocolate agar slant or 5% blood agar slant (Incubated in candle jar) |
| <u>Hemophilus</u> species | Chocolate agar slant or 5% Sheep blood agar slant |
| <u>Enterobacteriaceae</u> | TSI, nutrient, or 5% blood agar slants |
| <u>Corynebacterium</u> species | Loeffler's slant or chocolate agar slant |
| <u>Brucella</u> species | Trypticase soy or tryptose agar slants containing 5% blood (Incubated in 10% carbon dioxide) |
| <u>Streptococci</u> | 5% blood agar slants (Incubated in candle jar) |
| <u>Listeria</u> species | Loeffler's slant or chocolate agar slant |

It is imperative that cultures submitted be pure. Therefore, considerable care must be exercised in picking colonies to avoid mixing of Proteus Pseudomonas, Bacillus and other contaminants with the organism under study. The use of Petri dishes for transporting cultures should be avoided.

(2) Procedures accomplished at this laboratory include serological identification of streptococci, Salmonella spp., Shigella spp., and pathogenic E. coli strains. Facilities are also available for laboratory diagnosis of acid-fast bacilli in clinical specimens and phage typing of coagulase positive staphylococci.

(3) Specimens To Be Examined for Acid-Fast Bacilli. Sputum, urine (first morning specimen), and gastric washings are mixed with an equal amount of 23% trisodium phosphate in a sterile, screw-capped vessel. The mixture is then incubated at 37° C for 24 hours with periodic shaking. After centrifuging for 30 minutes at 3,000 rpm, the supernate is decanted and the sediment neutralized with 8% hydrochloric acid solution using brom thymol blue indicator. The specimen is then prepared for shipment as described in paragraph 5a(1). After treated in this manner, acid-fast bacilli will remain viable for Guinea pig inoculation for about one week without having to be refrigerated. "Deep" specimens such as pleural spinal or pericardial fluids, etc. are collected and shipped aseptically, since they are usually free of extraneous contamination. Tissue specimens are handled in the same manner. Specimens of the latter two categories, however, must be forwarded in the chilled or frozen state (see paragraph 5a(1)).

c. Mycology.

(1) Specimens from Dermatophytic Lesions. With lesions involving the skin and nails, the area is washed with 70% alcohol to remove surface contaminants and any medication. Following evaporation of the alcohol, a flame-sterilized scalpel blade is used to obtain scrapings from the active border of lesions. Fragments of finger or toenails may be plucked with flame-sterilized tweezers, as may be infected hairs, in cases of tinea capitis. These specimens are placed in a sterile, screw-capped vial and prepared for shipment. Samples are best collected from weeping or purulent lesions with a sterile swab. The latter is inoculated directly to a Sabouraud's Dextrose agar slant which is allowed to incubate during

the shipment. Isolation of the pathogen is more certain when Sabouraud's agar is prepared according to the following formula:

CYCLOHEXIMIDE - CHLORAMPHENICOL MEDIA FOR
THE SELECTIVE ISOLATION OF PATHOGENIC FUNGI

Sabouraud - cycloheximide - chloramphenicol medium*

PURPOSE: For the routine isolation of most pathogenic fungi.

Composition:

| | |
|---|---------------------------|
| Sabouraud dextrose agar (2% agar content) | |
| Cycloheximide** | 0.5 mg/ml (0.5 gm/liter) |
| Chloramphenicol*** | 0.05 mg/ml (.05 gm/liter) |

*Two essentially similar media, in dehydrated form, are available commercially: Mycosel Agar (Baltimore Biological Co.), Mycobiotic Agar (Difco)

**Acti-dione (The Upjohn Co.)

***Chloromycetin (Parke Davis & Co.)

Preparation: (1 liter)

1. Suspend 65 gm dehydrated Sabouraud dextrose agar (to which has been added 5.0 gms agar to bring to 2% agar content) in 1000 ml distilled water. Heat to boiling.
2. Add chloramphenicol (50 mg suspended in 10 ml, 95% alcohol) to boiling medium. Remove quickly from heat and mix.
3. Add cycloheximide solution (500 mg in 10 ml acetone).
4. Mix well and distribute into tubes.
5. Autoclave at 118° C for 10 min (no longer). Slant and allow to harden.

Brain heart infusion - cycloheximide - chloramphenicol

PURPOSE: For the isolation of nutritionally fastidious fungi at 37° C. (Histoplasma capsulatum and Blastomyces dermatidis).

Composition:

Brain heart infusion agar (2% agar content)
Cycloheximide 0.5 mg/ml
Chloramphenicol 0.05 mg/ml

Preparation: (1 liter) Prepare as described above using 37 grams of Brain heart infusion medium and 20 grams of agar.

USE OF MEDIUM

The cycloheximide medium is of particular advantage in the isolation of pathogenic fungi from materials heavily contaminated with bacteria and saprophytic fungi (such clinical specimens as skin scrapings, toenail clippings, sputa, pus, and from such materials as soil, shoes, etc.). On this medium the pathogenic fungi develop with their typical morphology and pigments, and can usually be identified without further subculture. It is recommended for the isolation of most of the fungi pathogenic to man with the following exceptions:

| | | |
|--------------------------------|---|------------------------------|
| <u>Allescheria boydii</u> |) | |
| <u>Madurella grisea</u> |) | Sensitive to cycloheximide |
| <u>Aspergillus fumigatus</u> |) | |
| <u>Cryptococcus neoformans</u> |) | |
| <u>Trichosporon cutaneum</u> |) | |
| <u>Candida sp (some)</u> |) | Sensitive to chloramphenicol |
| <u>Actinomyces bovis</u> |) | |
| <u>Nocardia asteroidis</u> |) | |

GEORG, L.K., AJELLO, L. and PAPAGEORGE, C. 1954, Use of Cycloheximide in the Selective Isolation of Fungi Pathogenic to Man. Jour. Lab. Clin. Med. 44:422-428.

(2) Specimens from Systemic Mycotic Infections. Specimens from suspected systemic or "deep seated" mycoses (sputum, spinal

fluid, joint fluid, pleural and pericardial fluids, etc.) may either be shipped directly in sterile, leak-proof containers in the chilled state or inoculated to culture media and forwarded after incubation. Since many systemic fungi are diphasic, the latter should be carried out at 37° C and room temperature. Several slants of both Sabouraud's agar and Brain Heart Infusion agar with and without the inhibitors are recommended for specimens from suspected systemic mycoses.

(3) It is imperative that selective media such as described above be incorporated when examining any specimen from a contaminated source, otherwise troublesome saprophytic fungi and bacteria may overgrow any pathogenic fungi present.

6. WATER ANALYSIS

a. A limited chemical and physical analysis of water samples is performed by this unit. Tests for the following chemical impurities in water can be made: Carbon Dioxide, Total Hardness, Magnesium, Sulfate, Chlorides, Fluorides, Calcium, Iron, Manganese, Nitrate Nitrogen, Nitrite Nitrogen and Ph value.

b. The following physical characteristics can be evaluated: Color, Odor, Dissolved Oxygen, and Oxygen Consumed.

c. All samples should be submitted in at least one-liter volumes, two liters being preferred. Sample containers should be made of Pyrex, hard rubber, or other inert material. They should be carefully cleaned before each use. Glass bottles may be cleaned with chromic acid or with alkaline permanganate solution. Hard rubber and polyethylene bottles may be cleaned with detergent or concentrated hydrochloric acid. After cleaning, rinse the bottles thoroughly with distilled water. Bottle stoppers, caps, and plugs should be resistant to chemical change and contribute no contamination to the sample. Tin-wrapped cork stoppers are suitable for many samples. Metal, screw caps are poor because of ready corrosion. Glass stoppers are unsatisfactory for highly alkaline solutions. Rubber stoppers are excellent for alkaline solutions, but poor for organic solvents.

d. Prior to filling, the bottle should be thoroughly rinsed with the water that is to be sampled. Bottles should be packed to avoid breakage and shipped to reach the laboratory in the shortest possible time.

7. MEDICAL ZOOLOGY

a. General. Insects, other arthropods, and mammals of medical importance should be collected whenever possible in order that control measures can be adopted for the species concerned, and that representative collections may become available from all geographical areas where military personnel are stationed. The importance of accurate identification of arthropod and other animal species associated with disease transmission or nuisance abatement cannot be overemphasized.

b. Collection Procedures.

(1) Mosquitoes.

(a) Larvae. Concentrate all of the larvae from a single collection point (do not mix collections from different breeding areas) in one inch of water in a small test tube. Heat with a candle or burner until bubbles begin to reach the surface. Pour into a small container. Pick up the larvae on the point of a needle and drop them into a procaine tube (obtainable from dental facilities) filled with 70% ethyl alcohol. Push a small loosely compacted piece of cotton into the tube to a point just above the larvae and well below the surface of the alcohol. Write the collection data with a lead pencil on a bond paper label and push it into the tube above the cotton. Insert the top procaine tube stopper using a needle to release the compressed air. Make certain that no bubbles are trapped in the section of the tube containing the larvae. Re-examine the tubes one week following preservation. If bubbles have formed, remove the top stopper and release the trapped air with a long needle. Wrap the tubes carefully in cotton or other soft packing material for mailing.

(b) Adults. Mosquito adults are delicate and must be handled carefully to avoid loss of scales or appendages essential to their identification. Moisture will cause scales to lose their natural color. Condensation of moisture on the inside of a chloroform collecting tube occurs quickly if the tube is left in the sun or a heated place. Consequently, mosquitoes should be removed from killing tubes as soon as killed. Reared specimens should be kept alive for at least 12 hours to allow them to harden. Adult mosquitoes should be packed in pill boxes. Prepare the pill boxes

for receipt of specimens by cutting two slips of fine paper tissue (e. g. lens paper) the diameter of the lid. Place a thin layer of cotton in the bottom of the box. Insert one paper slip over the cotton. Pour in the mosquitoes from the collection and cover them with the other paper slip. Place a layer of cotton of sufficient thickness to barely engage the lid when closed over the top paper slip. Do not pack mosquito adults between layers of cotton, cellucotton, or similar fibrous and heavy materials because the threads become entangled with the legs and other body structures. Write the collection data on the top of the box.

(2) Flies. Delicate flies, such as sand flies, culicoides, eye gnats and black flies may be tubed in 70% ethyl alcohol as described for mosquito larvae. Domestic flies and related types should be preserved dry in pill boxes as detailed above for mosquito adults. Immature flies (maggots) may be placed in 70% alcohol.

(3) Ectoparasites. Particular effort should be made to collect ectoparasites from rodents suspected of being reservoirs of disease. Since fleas leave the host immediately after its death, it is best to capture the rodent alive and kill it with chloroform in a container from which the detached insects can be collected. At this time the fur should be brushed with cotton moistened with ether or chloroform and the anesthetized fleas combed onto white paper. Tube specimens in 70% alcohol as described for mosquito larvae. Parasitic mites may be collected by scraping the skin or, in the case of a dead animal, portions of the infested skin may be removed and preserved in alcohol. Ticks may be collected by examining all parts of the host animal. Care must be taken in removing ticks in order that the mouthparts do not break off in the skin of the host. Place specimens in alcohol. A second procedure found to be quite satisfactory and relatively simple involves placing the live host in a soap and water solution and submerging it until it is drowned. The specimen is washed in the solution for several minutes and then withdrawn from the container. The soap solution is poured into a funnel made of filter paper suspended in a ring stand or top of a can. The filter paper can be folded when dry, the collection data printed on the outside, and then packed in a box for mailing. Lice may be picked from clothing and bedding with forceps or combed from hair with a fine-toothed comb and placed in alcohol. Bed bugs may be similarly preserved.

(4) Other Arthropods. All insects, scorpions, centipedes, millipedes, and spiders to be forwarded should be preserved in 70% alcohol, either ethyl or isopropyl.

(5) Snails, Terrestrial and Aquatic. Live snails should be placed in a glass container, and the level of specimens should never exceed 2/3 of the capacity of the container. The container should be filled with water, allowing a meniscus to form at the top, and the cap affixed to exclude air. After 24 hours the water should be drained and the jar filled with boiling water. The container should be drained after 10 minutes and filled with 70% alcohol. A layer of cotton should be placed over the snails to prevent excessive shaking and damage to the specimens. Either paste a label on the outside of the container or place one inside it so that the collection can be identified.

(6) Reptiles.

(a) Lizards and snakes are usually killed during capture; however, those captured alive may be drowned in formalin. Formalin should be injected liberally with a syringe at 2-inch intervals along the belly of the animals to deter degeneration of stomach products and preclude rotting of the specimen. Snakes may be captured alive by means of a slip noose of cord or fine wire on the end of a stick. They are carried best in small bags of heavy muslin or lightweight canvas, one animal to a bag. **CARE MUST BE TAKEN IN HANDLING, TRANSPORTING AND TRANSFERRING THE SPECIMENS. IT MUST BE ASSUMED AT ALL TIMES THAT THE ANIMALS CAN BITE AND ARE POISONOUS!**

(b) The animals should be left in formalin for three to four days, then removed from the tank and rinsed thoroughly in running cold water. After two days of soaking in distilled water, they should be transferred to museum jars or other permanent containers, and preserved in 70% alcohol. The position of the animal can be arranged at this time. Some collectors prefer to cut 1/2-inch slits at 2-inch intervals on the ventral surface of the animals to insure preservation.

(c) Keep specimens from different localities in different jars, or wrap those from separate localities in thin cloth to avoid mixing them. Each specimen should be labeled with the

locality, date, collector, and also any observations on food, habitat or breeding. Be sure to make a label that will be legible when the specimen arrives at its destination. Do not use ordinary ink, as it will dissolve in the preserving fluid. If specimens are to be carried about before packing for final shipment, wrap them in cloth so that the label will not rub and become illegible.

(d) Jars of preserved specimens should be kept in a dark place, as light bleaches the original scale colors. The jars should be inspected daily in hot weather. If any soft spots develop - usually greenish sunken areas above the stomach or intestines - a deep puncture of the skin above the soft places should be made and preserving fluid added with a syringe.

c. Shipment of Specimens.

(1) Collections of individual specimens should be sent to the Commander, TUSLOG Detachment 36, for identification. Complete collection data for each lot of specimens including date, locality, elevation, host habitat, and name of collector should accompany the shipments. All questions for which answer are specifically required by the shipper should be contained in a letter to accompany each shipment.

(2) Invertebrate specimens should be placed in tightly-sealed containers. Alcohol should be added until no air space remains. Where corks are used to close vials, it is recommended that these be sealed with paraffin to prevent loss of fluid through evaporation. If a small amount of glycerin is added to each vial, the specimens will not become dry and shrunken should the alcohol be lost accidentally during transit. Large, hard-bodied insects are normally preserved dry in pill boxes, but they can also be placed in containers with alcohol. Shipping in a loose, dry state is hazardous. Specimens may be beaten against the sides of the container and appendages or other structures required for identification may be lost or broken, thereby destroying the value of the specimen. A label clearly printed with a soft lead pencil on white bond paper should be inserted into the container with the specimen. Label collections as to date collected and name of collector, as well as the place where the collection was made. The container should be placed in a mailing tube with sufficient packing to prevent breakage.

(3) Large reptiles should be wrapped loosely and tied in a piece of white cheesecloth or other lightweight cloth, with the label attached to each specimen or group of specimens coming from a single locality. The cloth should be saturated with preserving fluid and the specimens placed in a clean tin, the top of which may be soldered shut when it is full. The epidermis of the specimens must be protected from coming into contact with the tin, for the latter will rust and stain the specimen. The specimens should not be packed too tightly; packing material can be used to keep them from shaking about in the can. Only white or light-colored packing material should be used, as dark-colored material may discolor the specimens and labels. Small specimens may be shipped in vials or bottles filled with fluid and packed in boxes in straw or other packing material. Ink may be used on labels pasted on the outside of the containers, but experience has taught that soft pencil is better, as fluid from a broken bottle may render inked labels illegible.