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**METHODS OF PREPARING PATHOLOGIC SPECIMENS
FOR STORAGE AND SHIPMENT**

**DEPARTMENTS OF THE ARMY, THE NAVY, THE AIR FORCE AND THE
VETERANS ADMINISTRATION**

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CHAPTER 1

GENERAL

Section I. GENERAL

1. Purpose and Scope

In pathology the properly chosen and prepared tissue specimen furnishes diagnostic evidence, records and changes produced by a given disease, and preserves the relationship of the tissue elements as they existed in life. After choosing the best sections of tissues, it is equally important that proper fixation be completed. Embedding tissue in paraffin blocks, proper preparation of microslides, and furnishing complete information on the forms submitted by hospitals and laboratories are essential if a case history is to be of value to the patient and the medical profession. The methods described in this manual will assist personnel of contributing activities in the preparation, storage, or shipment of microslides, paraffin blocks and wet tissue to histopathology centers, references laboratories and/or the Armed Forces Institute of Pathology (AFIP). These methods should establish procedures to eliminate waste and prevent loss of valuable material through improper preparation, storage or shipment. This manual has been prepared for reference purposes and it includes supply lists to assist personnel in securing both standard and nonstandard items.

2. Histopathology Centers

a. Histopathologic material is authorized to be sent to certain hospitals and medical laboratories that have been designated as histopathology centers, consultation centers, or reference laboratories. (AR 40-31/BUMEDINST 6510.2D/AFR 160-55).

b. In addition to providing diagnostic and consultative services in closer geographic relationship to Armed Forces hospitals and other military installations which are not adequately equipped with facilities or personnel qualified to do this work, the histopathology centers will screen all material prior to shipment to the AFIP (see chapter 1, section III). Military hospitals with a board-certified pathologist as Chief of Pathology may ship directly to the AFIP, rather than through a Histopathology Center, subject to prior coordination with the Director, AFIP and the respective Surgeon General.

3. Veterans Administration

Veterans Administration pathologists who desire consultation or assistance from the AFIP are referred to VA Department of Medicine and Surgery Manual M-2, Part VI, Change 41, chapter 4.

Section II. UNIFORM SYSTEM OF NUMBERING PATHOLOGIC MATERIAL

4. Description of System

A uniform system of numbering pathologic materials and the accompanying forms has long been needed by the agencies contributing material to the AFIP. The system described below is for use by all installations, and may be readily adapted to fit the system already in use. At the close of each calendar year all chronological numbering systems should be terminated and a new series of accessioning numbers should be initiated. The following procedures govern all pathologic ma-

terials and accompanying forms, using the calendar year method:

a. *Hospital Laboratories.* Use the letter "S" to denote surgical and the letter "A" to denote autopsy, followed by the case number and the last two digits of the calendar year.

EXAMPLES: S-1-77, S-2-77, S-3-77

A-1-77, A-2-77, A-3-77

b. *Histopathology Centers.* When serving as a laboratory for the hospital in which the histopathology center is located, the same system de-

DOE, James A.					S-196-77 A-3-78
PATIENT'S NAME (LAST-FIRST-MIDDLE INITIAL)					
SERVICE NO. 123-45-6789			GRADE M/Sgt		
AGE 35	SEX M	RACE W	VA CLAIM NO. n/a		
SPECIMEN Lung					SPECIMEN NUMBER(S)
DIAGNOSIS Epidermoid carcinoma, lung					
SPONSOR'S NAME (Last-First-Middle initial)			SERVICE NO.	GRADE	
ADDRESS OF NONMILITARY PATIENT					
General Services Administration and Interagency Committee on Medical Records FPMR 101-11.806-8 October 1975					SPECIMEN RECORD 542-102 8-340-1

Figure 1. Properly prepared SF 542, Specimen Record.

scribed in "a" above is used. When serving as a histopathology laboratory for other medical installations, the prefix "HP" (histopathology) should be used to distinguish between these cases and those originating in the center.

EXAMPLES: HPS-6-77, HPS-7-77,
HPS-8-77
HPA-6-77, HPA-7-77,
HPA-8-77

5. Records and Files Maintained

a. Separate folders for SF 515 (Clinical Record-Tissue Examination) and SF 503 (Medical Record—Autopsy Protocol) should be established for each calendar year. These standard forms should be maintained in folders in specimen number sequence (S-1-77, S-2-77, S-3-77). A registry of surgical and autopsy specimen numbers may be maintained in ledger form or any other perma-

nent type of numbering series, listing all materials on hand for each case, being certain to leave sufficient space for additional information which may be considered necessary.

b. SF 542, Specimen Record, should be used to control tissue specimens. This record will be maintained in alphabetical sequence by patient's name and will include the assigned specimen number(s), Social Security Account Number, VA Claim Number, if applicable, grade, age, race, and sex. Diagnoses may also be included. For nonmilitary personnel, enter sponsor's name, grade, and social security account number, and the address of the nonmilitary patient. (See fig. 1.) As an alternative, a computerized filing system may be used for this purpose when the capability exists.

c. Microslides, blocks, and tissue should be labeled with the laboratory accession number and the unit activity, segregated by case, and filed in numerical sequence.

Section III. PATHOLOGIC SPECIMENS TO BE SENT TO AFIP

6. Surgical Specimens

a. Surgical specimens on all malignancies and other conditions which may have future scientific, administrative, or follow-up value should be sent to the AFIP. (AR 40-31/BUMEDINST 6510.2D/AFR 160-55). In addition, any other type case may be submitted when the contributing pathologist desires consultation. A listing of desired specimens is published here as appendix C.

b. Surgical specimens which are unsatisfactory for definite diagnosis should *not* be forwarded.

Examples of the most frequently contributed inadequate specimens are: Biopsies from areas peripheral to the actual lesion, fragments of endometrial tissue of insufficient quantity, biopsies of normal bone, cartilage and joint capsules, and poor blood and bone marrow smears. A specimen inadequate for the contributing pathologist to diagnose is also impossible for the AFIP staff to diagnose.

c. Surgical specimens which should *not* be forwarded are listed in appendix D.

7. Autopsy Specimens

All completed autopsies should be forwarded. It is emphasized that a completed autopsy includes a complete narrative summary, autopsy protocol, including a description of gross and microscopic findings, stained microslides, paraffin blocks, and wet tissue. Pertinent surgical pathology material which has not been previously forwarded to the AFIP should be included with the autopsy material; it should not be sent after submission of the autopsy unless specifically requested by the AFIP staff. "Completed" also implies the inclusion of pertinent x-rays, electrocardiograms, photographs, and investigative reports (Forensic Pathology cases). Gross organs should *not* be forwarded routinely, but should be sent only if

they are essential for research or teaching. The duplicate wet tissue should be representative and not over 0.5 cm in thickness. Halves of large organs should *not* be sent. A qualified pathologist should review the pathologic material before it is shipped. In addition to the instructions on the kind and amount of wet tissue to be sent, the pathologist should review and eliminate all microslides and blocks not pertinent to the case. Pathologists in the Veterans Administration will comply with the current Department of Medicine and Surgery Manual M-2, Part VI, chapter 4, and related directives. Review of a random sample of VA autopsy cases will be performed by the AFIP, according to VA Department of Medicine and Surgery Circular 10-75-109, 9 June 1975.

CHAPTER 2

PREPARATION OF SPECIMENS FOR SHIPMENT

Section 1. MICROSLIDES

8. Labeling

a. The importance of properly labeling microslides before they are packed for shipment should be called to the attention of all laboratory personnel. A paper label, 22 mm square, bearing the name of the contributing activity, surgical or autopsy number, and the year, should be placed on one side near the end of the slide. A blank spacer label should be placed on the opposite end of the slide to reduce breakage and to prevent slides from adhering to each other. Nothing should be written on the spacer labels.

b. Contributors forwarding microslides through a histopathology center or reference laboratory should be certain to provide sufficient space on the microslide label to allow addition of the number assigned by the histopathology center or reference laboratory. Likewise, the histopathology center or reference laboratory should be certain that the original contributor's data are legible before forwarding the microslides, as data from both units are necessary for proper processing and recording at the AFIP. (See fig. 2.)

9. Packing and Shipping

There are three methods of properly packing microslides for shipment. The standard items of issue are the "Box, Microscope Slide, Plastic, 5 Slide" (see fig. 3), and the "Box, Microscope slide, 25 Slides or 100 Slides" (see fig. 4). The use of the nonstandard item "Cardboard Slide Holder" (see fig. 5) or the improvised toilet tissue methods are to be substituted only when standard items are not available. Phone, TWX and RUSH cases should not be packed in bulk shipments, but instead sent individually packaged.

10. Shipping Small Quantities of Microslides— Methods A and C (see figs. 3, 5 and 7).

a. The standard item of issue "Box, Microscope Slide, Plastic, 5 Slide," should be used when only

a few microslides are being shipped. If this item is not available, the nonstandard item, "Cardboard Slide Holder," should be used and may be procured at a nominal cost by direct purchase (see appendix B).

b. The properly labeled microslide should be placed in the appropriate size plastic slide box or cardboard holder on strips of paper toweling. The microslide should be covered with additional strips of paper toweling before the container is closed and sealed with masking or brown paper tape. The contributor's activity, shipment number, and a listing of the microslides enclosed, showing surgical or autopsy number and year, together with the quantity, should be marked on the outside of the container. Standard Forms 503, 515 and 543 (Contributor's List of Pathologic Material) should be wrapped around the container. The package should be wrapped, properly addressed, and labeled "FRAGILE." Microslides or microslide holders should never be mailed in envelopes which are passed through post office cancelling machines.

11. Shipping Large Quantities of Slides— Method B (see fig. 6)

The standard item of issue, "Box, Microscope Slide, 25 Slides or 100 Slides," should be used when large number of microslides are being shipped. These boxes are the most desirable means of shipping microslides and should be used whenever available. Paper toweling should be fitted into the base of the box to act as a cushion before inserting the properly labeled microslides. Paper toweling should also be placed in the lid before the box is closed and sealed with masking or brown paper tape. The contributor's activity, shipment number, and a listing of the enclosed microslides, showing patient name, surgical or autopsy number and year, together with the quantity, should be marked on the outside of the box. All microslide

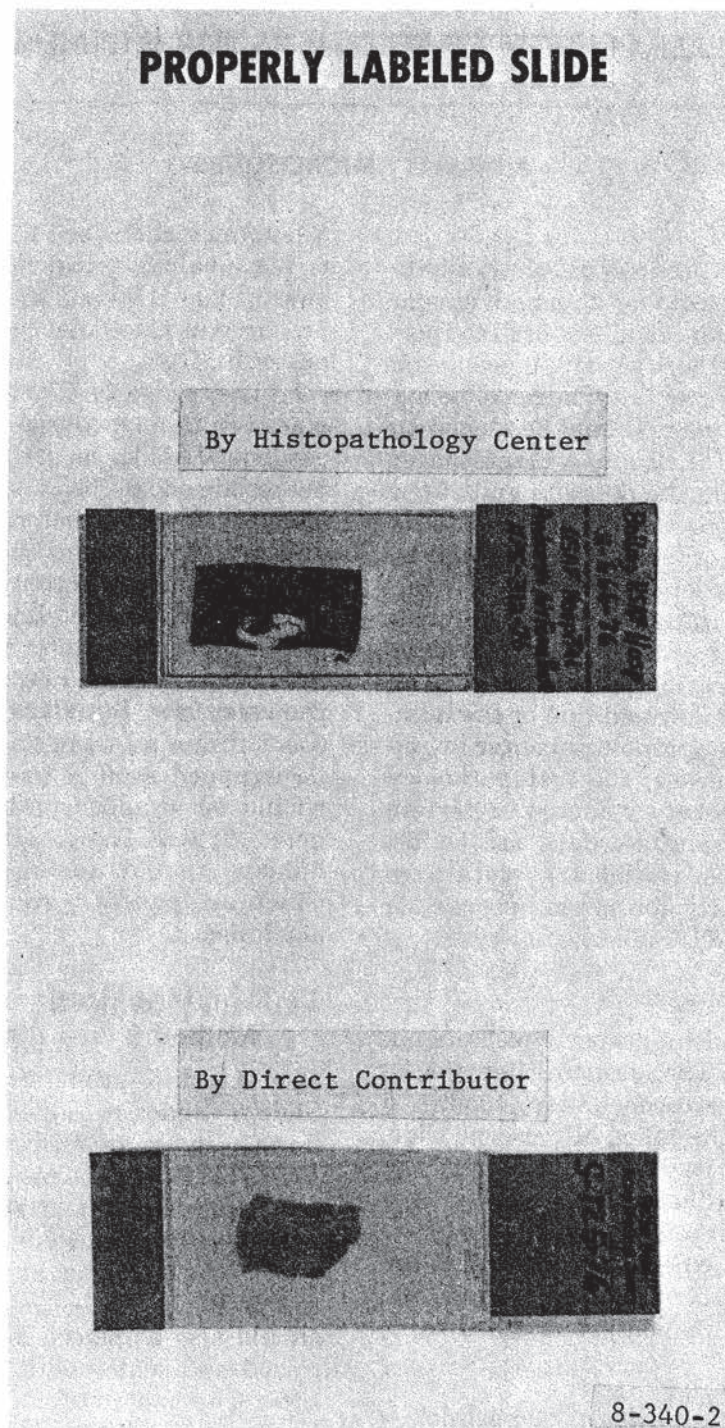


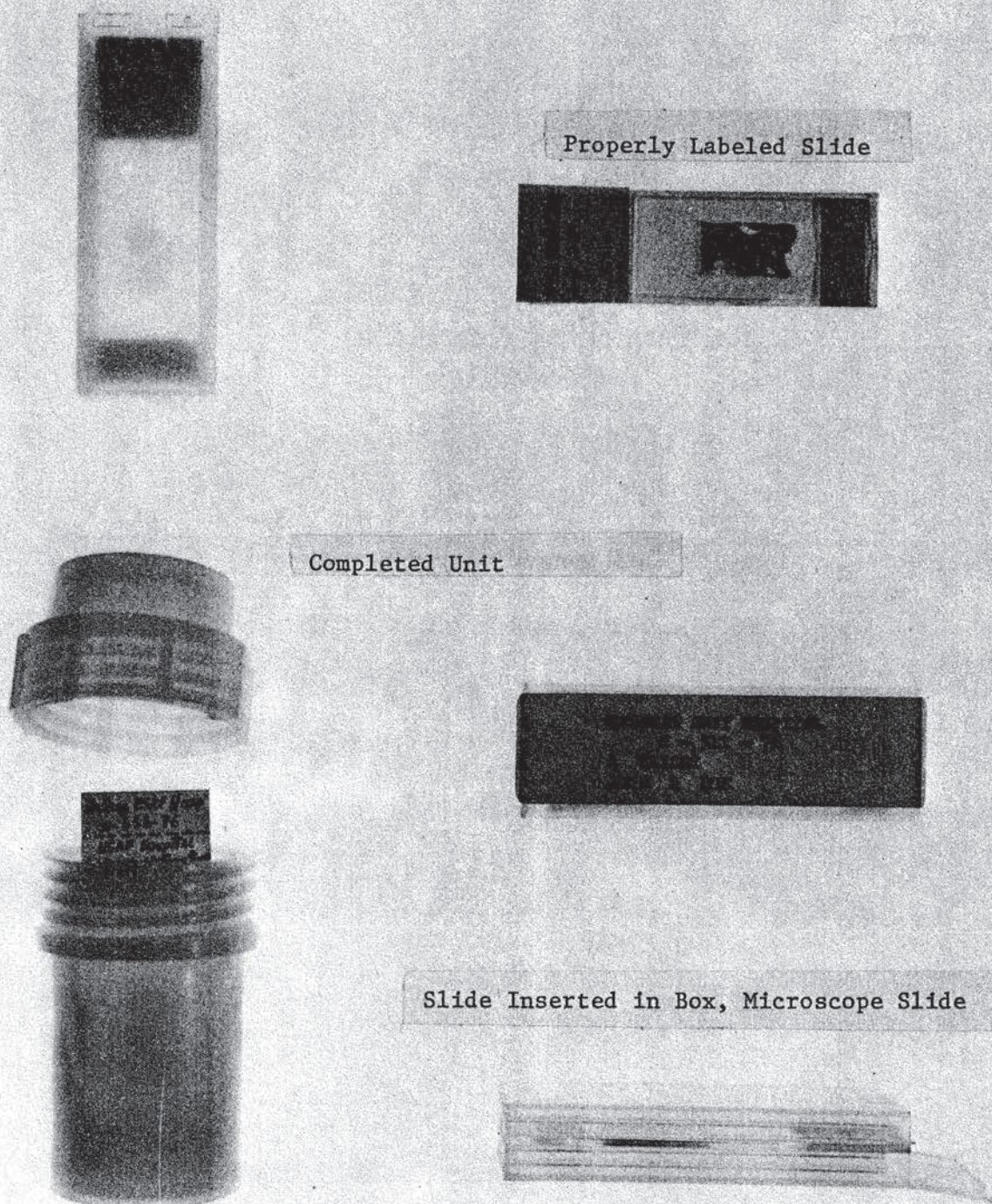
Figure 2. Properly labeled slides.

METHODS FOR PACKING MICROSCOPE SLIDES

Box, Microscope Slide, Plastic, 5 Slides

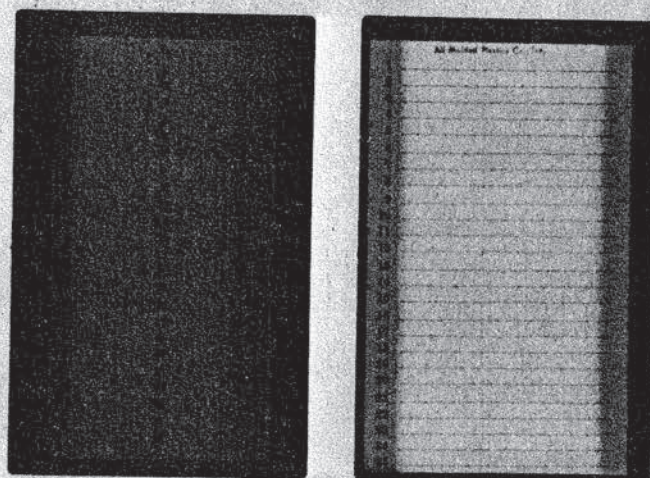
(6640-00-241-0138)

METHOD "A"

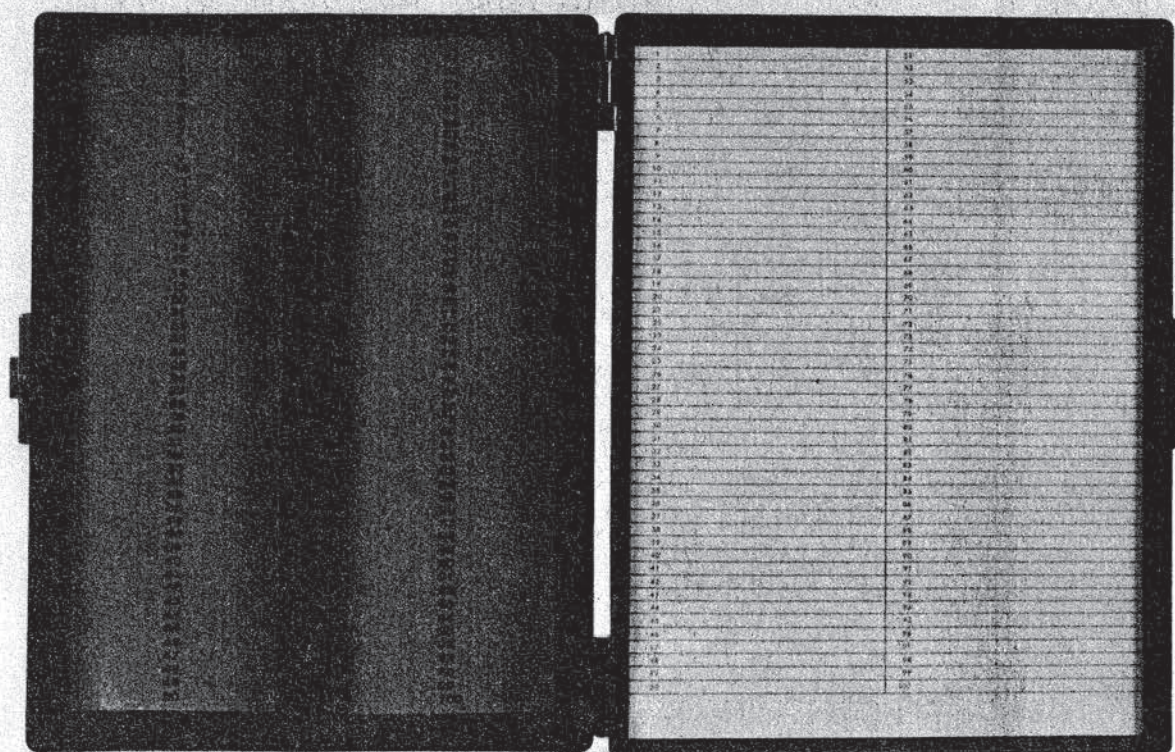


8-340-3

Figure 3. Method A for packing microslides in 5 slide plastic box.



**Box, Microscope Slide, Plastic, 25 Slides
(6640-00-684-1345)**



**Box, Microscope Slide, Plastic, 100 Slides
(6640-00-408-9915)**

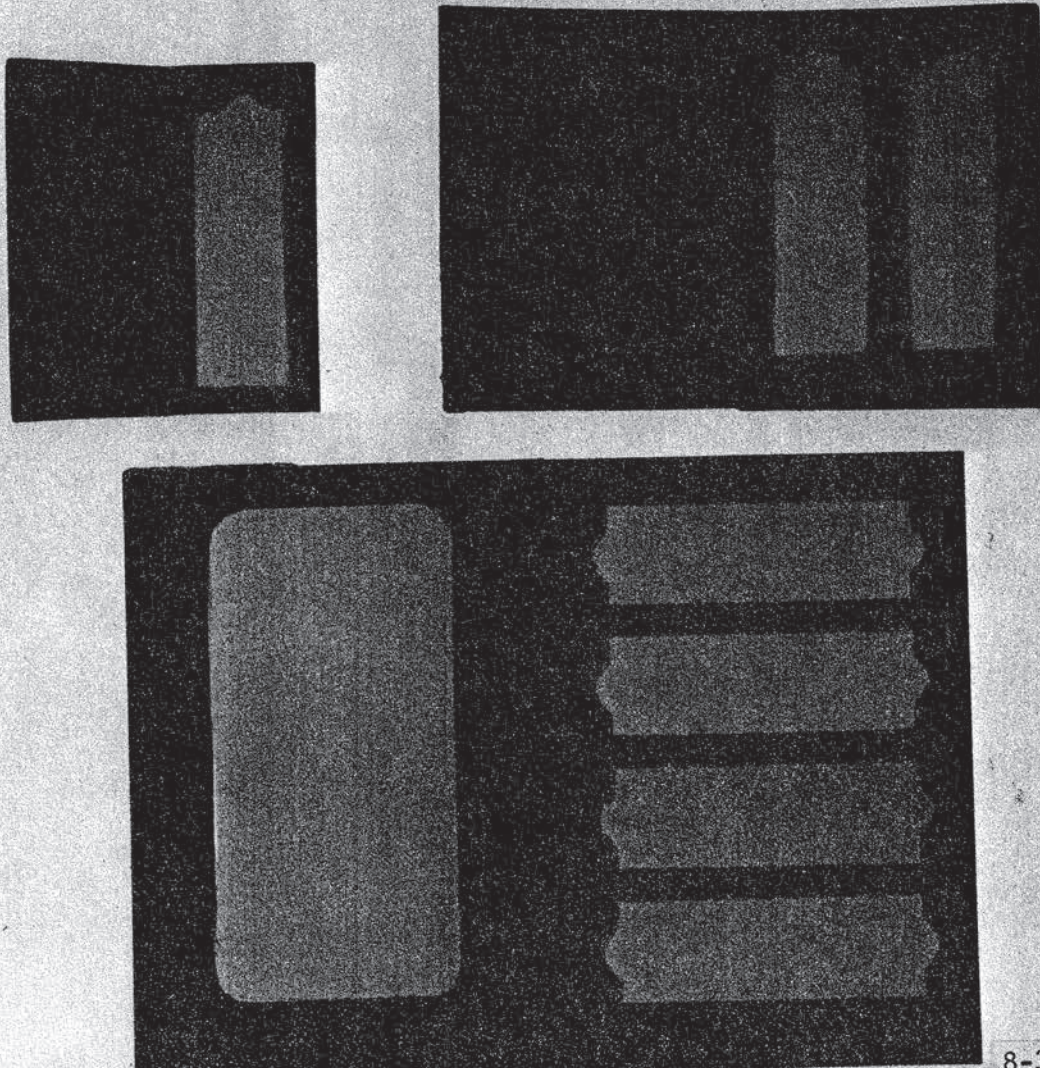
8-340-4

Figure 4. Box, microscope slide, 25 slides and box, microscope slide, 100 slides.

**BOX, MICROSCOPE SLIDE, PLASTIC, 5 SLIDE
(6640-00-241-0138)**



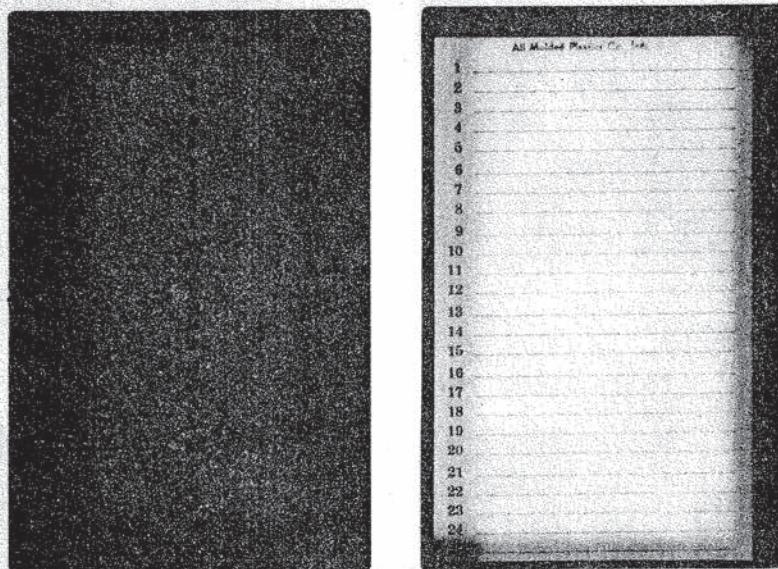
Non-Standard Cardboard Slide Folders



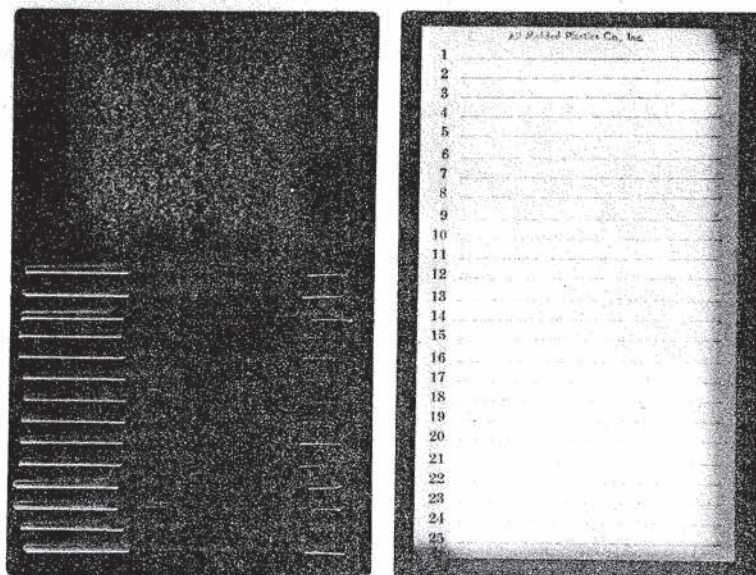
8-340-5

Figure 5. Plastic microscope slide box (5 slide) and nonstandard cardboard slide holders.

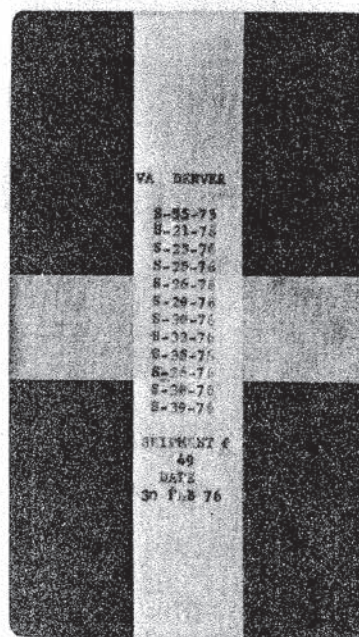
METHOD "B"



**Box, Microscope Slide, Plastic 25 Slides
(6640-00-684-1345)**



Inserted in Slide Box



Completed Slide Box

8-340-6

Figure 6. Method B for packing microslides, Box, Microscope Slide, 25 slides.

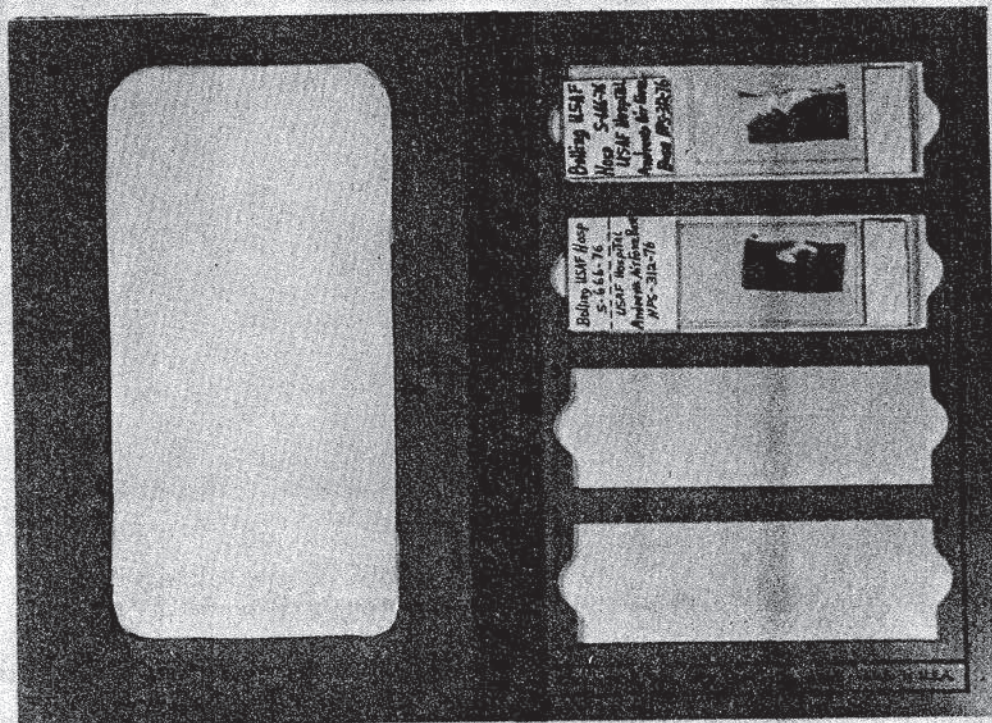
METHOD "C"

Standard Form 595
Revised August 1954
Bureau of the Budget
Circular A-22 (Rev.)

CLINICAL RECORD		AUTOPSY	
DATE AND HOUR USED August 9, 1974 10:00 AM		DATE AND HOUR AUTOPSY PERFORMED August 9, 1974 1:00 PM	
PROSECTOR Paul H. Strausbaugh, M. D.		ASSISTANT	
CLINICAL DIAGNOSES (Including operations) 1. Hypernephroma, metastatic to lung and liver.			
1. NO. 545 2. 1961 3. 1962 4. 1963 5. 1964			
DONOR'S LIST OF PATHOLOGIC MATERIAL		DATE 19 JAN 76	
RECIES INSTITUTE OF PATHOLOGY		FROM VA HOSP. DENVER	
NAME	LABORATORY NO.	SLIDES	PHASES FOR BLACK
Doe, John W.	5-232-76	X	X
Anderson, Mary L.	5-234-76	X	X

Sealed Side Holder

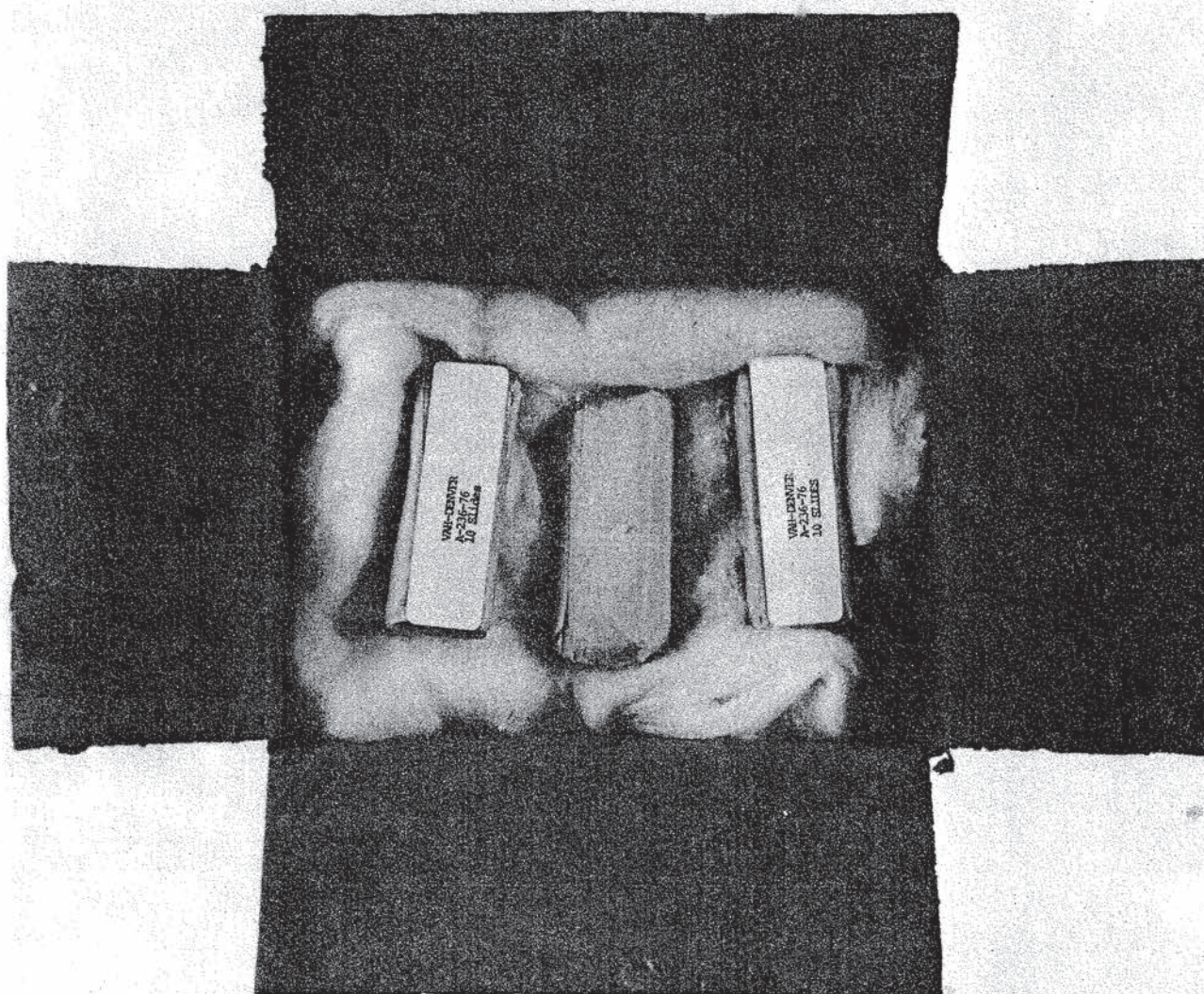
Non-Standard Cardboard Holder



8-340-7

Figure 7. Method C for packing microslides, nonstandard cardboard slide holder.

PROPERLY PACKED MICROSCOPE SLIDES SHIPPING CONTAINER



8-340-8

Figure 8. Properly packed microslide shipping containers.

boxes prepared in this manner should be placed in one large shipping container which should be sufficiently padded to protect the contents (see fig. 8). Standard Forms 503, 515 and 543 should be placed inside the shipping container, after which it should be sealed, properly addressed, and labeled "FRAGILE."

12. Improvised Method for Packing Slides— Method D (see fig. 9)

If neither slide boxes nor cardboard holders are available, an improvised method using toilet tissue may be substituted.

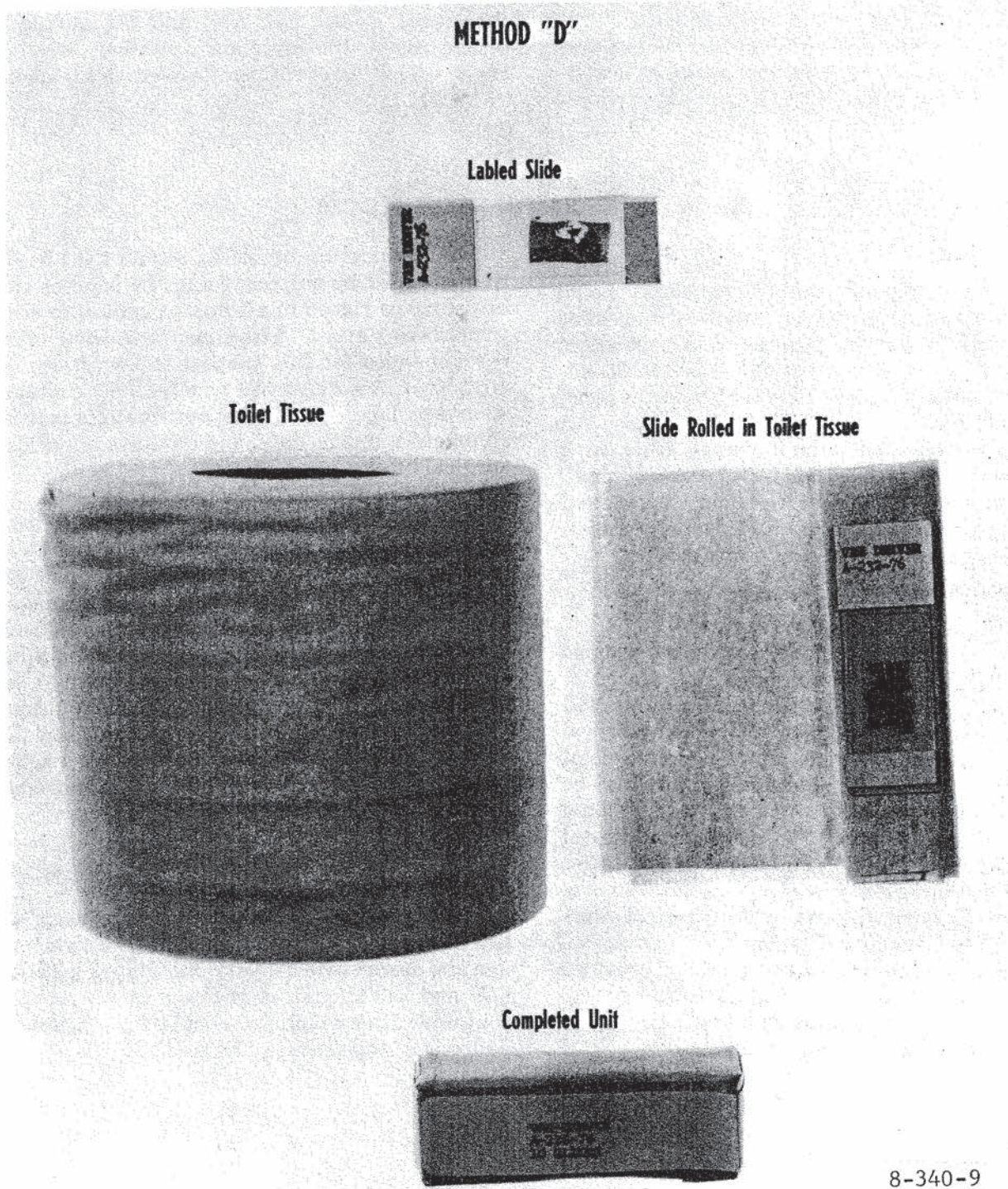


Figure 9. Method D for packing microslides.

a. Unroll a strip of toilet tissue about 4 feet in length; place a properly labeled microslide on one end and roll it over tightly several times. Add another microslide and repeat this operation until about twenty microslides have been encased in the tissue. Seal the group of microslides by placing

brown paper tape first around the center and then lengthwise around the pack. The contributor's activity, shipment number, and a listing of enclosed microslides showing patient name, surgical or autopsy number and year, together with the quantity, should be marked on the tape.

b. A shipment prepared in this manner will provide a group of well-packed, identified microslides. Packets of microslides should be placed in a well-padded shipping container to assure safe arrival.

Standard Forms 503, 515, and 543 should be placed inside the shipping container, which is then sealed, properly addressed, and labeled "FRAGILE."

Section II. PARAFFIN TISSUE BLOCKS

13. General

All properly prepared paraffin tissue blocks should have the surgical or autopsy numbers embedded in the paraffin for the purpose of identification. Should a block become separated from the Standard Form 503, 515, or 543, the number on the block is the only means of identifying the material. All excess paraffin should be trimmed from each block before it is packed for storage or shipment. All cut surfaces should be resealed with melted paraffin to protect the tissue against dehydration and to make subsequent sectioning easier.

14. Packing

a. *Surgical Tissue Blocks* (see fig. 10).

(1) Surgical tissue blocks should be grouped for shipment by surgical number and placed in boxes of appropriate size, one box containing all blocks of the same number. Blocks on surgical cases are normally small and few in number, therefore blocks for each surgical number may be packed either in the standard item, "Box, Folding Chipboard, size $1\frac{1}{2} \times 15\frac{1}{16} \times 15\frac{1}{16}$ inches," or in one of the nonstandard white chipboard boxes, sizes $15\frac{1}{16} \times 1\frac{1}{2} \times 2$ inches or $15\frac{1}{16} \times 2 \times 3$ inches. The contributor's activity, patient name, surgical number, and year should be stamped or marked on the outside of each box. Space should be provided for inclusion of the histopathology center or reference laboratory number, if the material is routed through one of these installations.

(2) Surgical tissue blocks should then be further grouped by shipment and the smaller chipboard boxes placed in a shipping container of appropriate size. The nonstandard item recommended for this purpose is a kraft covered box, $4\frac{3}{16} \times 3\frac{5}{16} \times 1$ inches in size. This container should be labeled with the contributor's activity, shipment number, date, and quantity of enclosed surgical blocks.

b. *Autopsy Tissue Blocks* (see fig. 11).

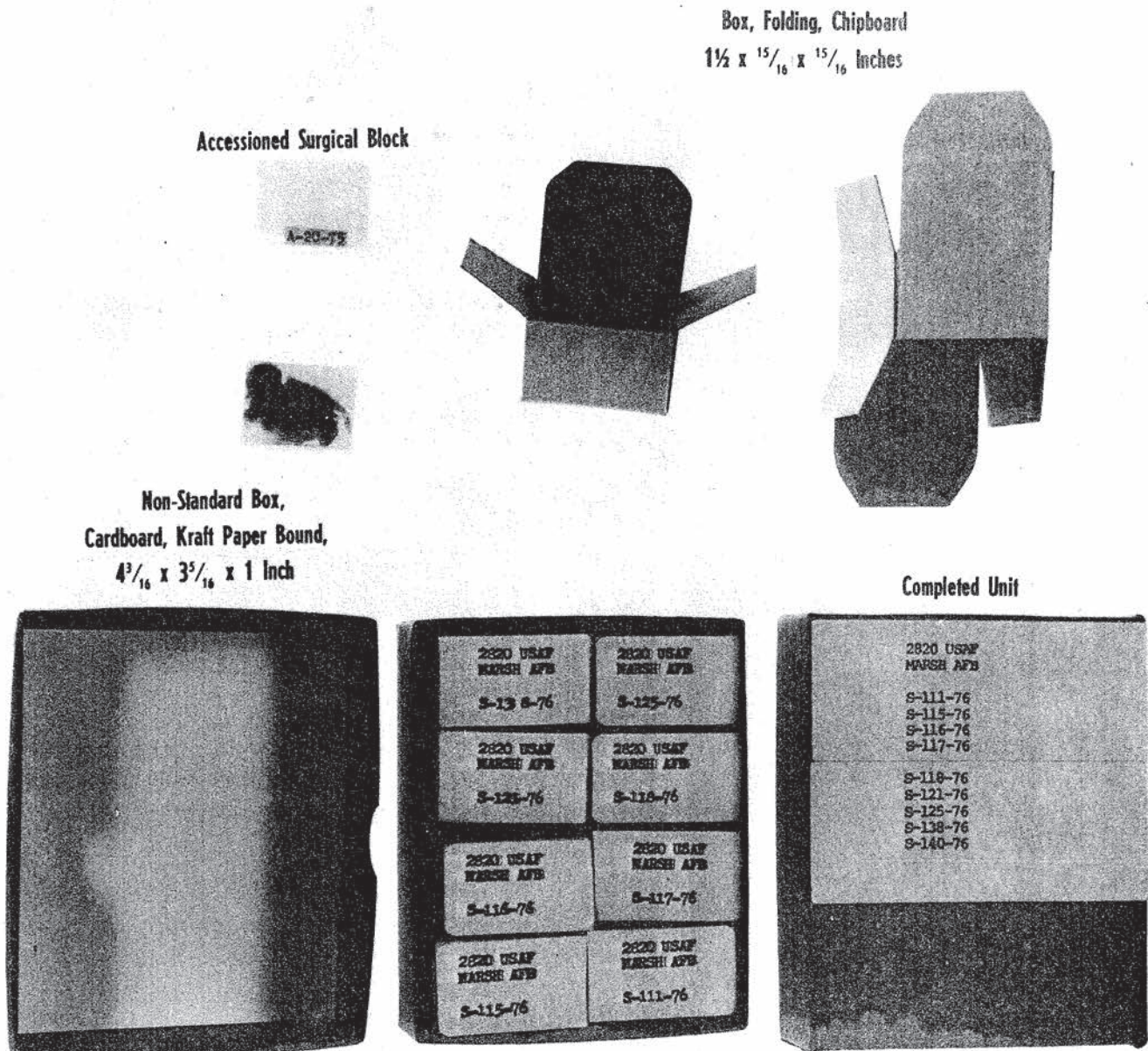
(1) Due to the usual large number of blocks prepared on each autopsy case, all of the blocks pertaining to a case should be grouped and shipped in one container (approximately $4\frac{3}{16} \times 3\frac{5}{16} \times 1$ inches). This container should be labeled with the contributor's activity, patient name, autopsy number and year, shipment number, and the number of blocks making up the shipment.

(2) Materials such as ground cork, sawdust, or spun glass should never be used for packing paraffin blocks, since it could become embedded in the blocks and damage the specimens.

15. Shipping

All blocks should be properly packed, using sufficient paper or waste cotton to make a firm package and assure safe delivery. Standard Forms 503, 515, and 543 should be enclosed in the shipping container after which it should be sealed, properly addressed, and marked "FRAGILE."

METHOD FOR PACKING SURGICAL BLOCKS



8-340-10

Figure 10. Method for packing surgical blocks.

METHOD FOR PACKING AUTOPSY BLOCKS

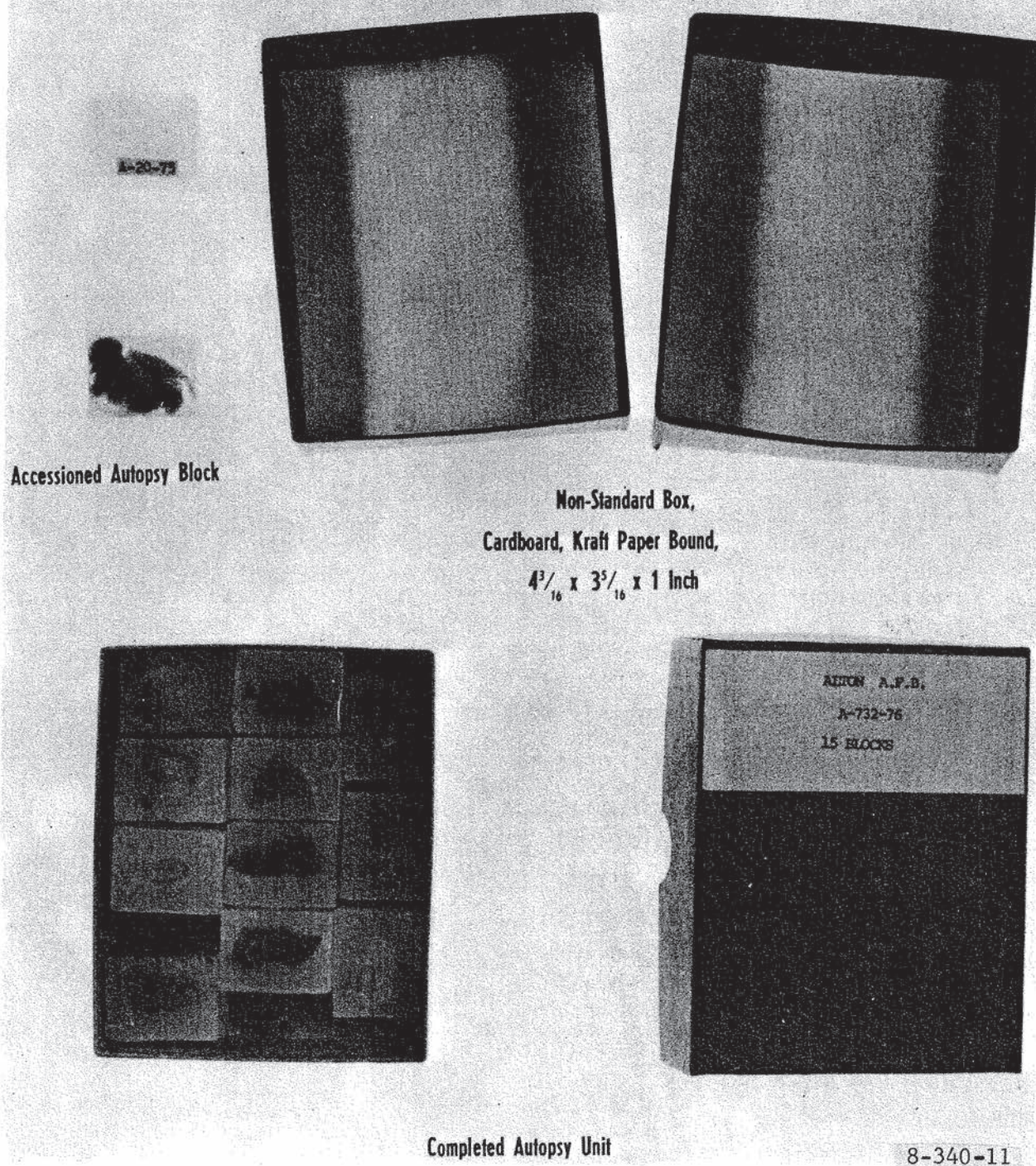


Figure 11. Method for packing autopsy blocks.

Section III. WET TISSUE

16. Solutions for Fixation of Wet Tissue

Fixation is the process of preserving the structure of tissue as closely as possible to its conditions during life. The first phase of preservation is the rapid penetration of the tissue; the second is the hardening of tissue. It is necessary that the tissue, immediately upon removal from the body, be placed in the fixative to enable the fixing agent to penetrate quickly and so preserve the relationship of the tissue elements as they normally existed in life. The hardening process should be of such a degree that the tissue components and architecture will be unaffected by any subsequent procedures. The fixing agent must not cause the tissue to become brittle, and it should be a product which is relatively stable and inexpensive. A few of the commonly used fixatives with their particular advantages and disadvantages are described below:

a. Formalin, 10 Percent Solution, Buffered.

(1) First Method.

Formaldehyde concentrated (37-40%)	100 ml
Distilled water (or tap water)	900 ml
Sodium acetate	20 g

(2) Second Method.

Formaldehyde, concentrated (37-40%)	100.0 ml
Distilled water (or tap water)	900.0 ml
Sodium phosphate, monobasic	4.0 g
Sodium phosphate, dibasic (anhydrous)	6.5 g

Formalin has certain advantages and is extensively used for routine purposes. It quickly fixes and hardens tissue, including red blood cells; it permits a variety of staining methods; it preserves fat and myelin; it is inexpensive, simply prepared, and available globally. It also has certain disadvantages, such as dissolving uric acid and sodium biurate crystals, and changing bile from a yellow to a green color. It also produces precipitated hemoglobin in tissue unless properly buffered.

Note. In order to ensure proper fixation, tissue blocks not more than 0.5 cm in thickness should be selected from representative parts of the specimens and fixed in approximately 20 times their volume of 10 percent formalin.

b. B-5 Solution.

Distilled water	900 ml
Mercuric chloride	60 gm
Sodium acetate (anhydrous)	12.5 gm
Formaldehyde, concentrated (37-40%)	100 ml

Fixation in this mixture is adequate in 12-24 hours for tissue measuring 4 mm or less in thickness. However, longer exposure does not seem to over harden tissue. Tissue is placed directly into 70% alcohol after fixation, and may remain in this solution for storage. The advantages and disadvantages of alcohol fixatives are given in para c below.

c. Alcohol Solutions, 80 Percent Alcohol Solution.

Ethyl alcohol, anhydrous	800 ml
Distilled water	200 ml

Alcohol, when used as a fixative, may be used in various strengths, with the 80 percent solution being the one most commonly used. The advantages of alcohol are that it preserves glycogen, hemosiderin, pigments, blood, blood smears, bacteria, fibrin and many cytoplasmic structures, and it is available globally. The disadvantages of alcohol are that solutions weaker than 80 percent hemolyze red blood cells and inadequately preserve white blood cells; it penetrates slowly, causes shrinkage, is volatile, flammable and expensive.

d. Zenker's Solution.

Potassium Dichromate	25 g
Mercuric Chloride	50 g
Distilled Water	1000 ml

Note. At time of use, add 50 ml Glacial Acetic Acid. If Mercuric Chloride is not available, 25 gm Zinc Chloride may be substituted. When used for fixation of bone marrow in which there are spicules of bone, add 100 ml of Glacial Acetic Acid to decalcify the specimen.

Note. The addition of acetic acid will remove iron from the section. Therefore, do not expect to demonstrate iron in bone marrow specimens when using this fixative.

Zenker's Solution is a popular fixative, and tissue preserved by this method stains well with many techniques, provided it has been thoroughly washed after fixation. It is generally used on tissue which is to be studied for protozoa or for inclusion bodies in viral diseases. It is also preferred on tissue for finer cytologic studies and for Masson's trichrome and the Giemsa stains. The hematoxylin stain may require a longer staining time, while the counterstain may have to be diluted and the slides left in for a shorter time. Tissue should not be left in this solution longer than 24 hours or overfixation will occur. When the specimen is removed from

the fixative, it should be rinsed in running water for 24 hours and then transferred to 80 percent alcohol for storage.

e. Schein Solution.

Sodium phosphate, monobasic	--	89.1 g
Sodium phosphate, dibasic	-----	112.5 g
Formaldehyde, concentrated		
(37-40%)	-----	950 ml
Distilled water to make a total		
of 19000 cc		

This method of fixation is preferred for use on tissue which is to be used for museum specimens. It is a special color-preserving fixative, which effectively fixes tissue for histopathologic study as well as gross presentation. To obtain adequate fixation, it should be noted that the time required is two to four times greater than when formalin is used.

f. Kaiserling Method: The Kaiserling method requires three steps for preservation of natural colors and preparation of museum mounts:

Step 1. After the specimen is rinsed and all excess blood removed, it is fixed for 3 to 7 days in the following solution, after which it is washed in running water for 12 to 24 hours:

Kaiserling Solution I

Potassium acetate	-----	170 g
Potassium nitrate	-----	90 g
Formaldehyde,		
concentrated (37-40%)		1600 ml
Water	-----	8000 ml

Step 2. The washed specimen from step 1 is then placed in Kaiserling Solution II for 6 to 8 hours or until full development of the natural red color occurs. The specimen is washed again in running water for two hours:

Kaiserling Solution II

Alcohol 80 percent

Step 3. The washed specimen from Step 2 is placed in Kaiserling Solution III, the final mounting solution:

Kaiserling Solution III

Potassium acetate	-----	172 g
Glycerol	-----	200 ml
Water	-----	1000 ml
Phenol	-----	22 ml

Note. Phenol may be replaced by thymol, menthol or sodium salicylate, 2 grams, dissolved in the glycerol.

g. Regaud Method.

Potassium dichromate, 3 percent	80 ml
---------------------------------	-------

Formaldehyde, concentrated	
(37-40%)	----- 20 ml
	(mix before use)

This method is used for the demonstration of micro-organisms in tissue. The specimen is placed in this solution for 24 to 48 hours, with the fluid being changed daily. The specimen is then transferred to running water for another 24 hours, after which it is placed in a solution of 80 percent alcohol.

h. McDowell and Trump Solution.

Fixative for Diagnostic Light and Electron Microscopy

Distilled water	-----	88.0 ml
Sodium phosphate, monobasic		
(NaH ₂ PO ₄ H ₂ O)	-----	1.16 g
Sodium hydroxide, pelletized	---	0.27 g
Formaldehyde, concentrated		
(37-40%)	-----	10. ml
Glutaraldehyde, 50%, biological		
grade	-----	*2.0 ml

This fixative is stable for 3 months and should be stored at 4° C.

Formaldehyde—Glutaraldehyde combinations are found to be easily prepared and provide satisfactory preservation for routine automated histologic processing as well as preserving tissues for electron microscopic studies following prolonged (12 months) storage at room temperature.

Optimal fixation is best obtained if the tissue is flooded with fixative solution as soon as it is obtained (surgical or biopsy source) and again during gross dissection. Blocks or strips of tissue not in excess of 3.0 mm in thickness should be prepared and placed in fresh fixative. Sections selected for electron microscopy must be taken from the outer portion of the grossed material. Fresh fixative solution should be exchanged after 24 hours if the tissue is to be stored before processing.

17. Identification Labels for Wet Tissue Specimens

a. Plastic Labels

(1) Labels for identification of wet tissue specimens should be prepared from pyralin cellulose nitrate, .010 gauge, white, 50 × 20 inch sheets. These sheets may be cut into 2 × 20 inch strips

*The concentration of glutaraldehyde is minimal and should not alter the PAS or other reactions encountered in histologic staining procedures.

for less difficult storage and easier preparation of labels. One sheet of this plastic will be sufficient for preparation of 250 labels (2 × 2 inch). This label is preferable over other types due to its simplicity of preparation and its permanency when immersed in fluids.

(2) The plastic strip should be labeled with a lead pencil, or a typewriter. Data should include Hospital Name, Patient's Name, Surgical or Autopsy Number and Type of Specimen; i.e., kidney, lung, etc. After this information is completed, the label should be dipped in acetone and rinsed in water. The acetone acts as a solvent and embeds the lettering in the plastic. Rinsing in water stops the solvent action of the acetone, and the finished product is a permanently legible label. All corners of the label should be rounded to prevent the piercing of the plastic storage bags.

(3) A word of caution is necessary concerning specimens of excessively fatty tissues or specimens that have been cleared in oil of wintergreen. These materials act as solvents and will damage this type of plastic label. Labels for such specimens should be sealed in separate plastic bags or placed into the outer or second plastic storage bag.

(4) This label should not be used with frozen specimens for toxicology studies. The camphor ingredient of the label will permeate throughout the sealed container and could lead to false determinations.

b. Cotton Tape, India Ink, Paraffin Dipped Label.

This type label, widely used for many years, is time-consuming and difficult to prepare. It is acceptable when plastics are not available.

Using India ink for legibility, the identifying data are written on cotton tape. The tape is dipped in melted paraffin and hung to dry. Any number of labels can be prepared on a continuous strip of tape, the individual labels being cut off after drying.

c. Cardboard Labels. Cardboard labels are undesirable and should never be used with wet tissue unless other materials are not available for labels. In such cases, all data should be typed or written with a lead pencil. Wax crayons or ink will smear much faster when wet than the pencil or typewritten data. The cardboard label should then be sealed in a separate plastic bag for protection against fluids before being placed into the specimen container. Such labels also are used with frozen specimens being sent for toxicology studies.

18. Wet Tissue Specimens

The correct fixation and proper labeling of wet tissue are essential before preparing the specimen for either storage or shipment.

a. Fixation is accomplished by placing the specimen in the desired preservative for a sufficient length of time to allow for adequate penetration of the fixing fluid. The size of the specimen governs the time necessary for complete fixation (approximately 3 to 5 days). Fixatives will not penetrate beyond a few centimeters; therefore, large specimens should be properly cut to permit orientation and to ensure complete fixation. The amount of fixative should be twenty times the volume of the specimen. After fixation is accomplished, the specimen should be removed from the fixative solution, rinsed in water for several hours, replaced in a fresh solution of the preservative, and then prepared for storage or shipment.

b. Proper labeling should provide the necessary identifying information and must remain with the specimen at all times. The specimen container should be labeled with the following information:

- (1) Name of contributing activity
- (2) Surgical or autopsy number and year
- (3) Patient's full name
- (4) Date
- (5) Type of specimen (if gross)

19. Methods for Packing

Various methods have been used for packing wet tissue specimens, the procedures depending on the availability of materials. If wet tissue specimens are to be placed in storage or packed for shipment to an area laboratory, histopathology center, or the AFIP, the following methods are offered to assist personnel concerned with packing specimens:

a. Plastic Bag Method (see figs. 12, 13, 14, and 15).

(1) Plastic bags are recommended for shipping wet tissue specimens. These bags are included in the Federal Supply Catalog. This method is preferred because of the lower cost of materials and the savings in shipping costs due to weight differences. Less preservative is required, and the plastic bag is lighter than other shipping containers. Shipments in plastic bags have also shown a lower rate of damage in transit.

(2) The preserved specimen should be wrapped in paper toweling, gauze, or placed in a white cotton bag. These materials will assure a moist area around the specimen at all times. Bone specimens with sharp edges should then be wrapped in cotton to prevent piercing of the plastic storage bags.

The wrapped specimen and label should then be placed in the proper size plastic bag. Sufficient formalin should be added to saturate the paper toweling, gauze or cotton bag. The excess formalin is poured off and as much air as possible evacuated before heat-sealing the plastic bag.

A good method for creating a vacuum within the plastic bag is to heat seal all but one half inch of the opening of the bag. Manipulate the specimen to expel all the air and part of the fixing fluid; then heat-seal the small opening. This method is less difficult to do, and leaves less margin for defects in preparation of the specimens. A properly sealed and packaged specimen will be one in which the plastic bag clings to the tissue. A defective or poorly sealed specimen is one in which the plastic bag leaks fluids or is expanded away from the tissues.

(3) In preparing a shipment, all specimens pertaining to one case should be grouped together and resealed in one larger plastic bag. This contains the case and prevents any part of it from being lost or mistaken during the preparation of the contributor's listing.

In cases where cardboard labels are being used, it is necessary to provide a separate pocket in the plastic bag to seal the label against damage from fixing fluids. All other procedures are the same as for other specimens sealed in plastic bags.

Standard Forms 503, 515 and 543 should accompany all tissue shipments. These forms should be grouped and sealed in a plastic bag to prevent them from being damaged in case an accident should occur while in transit. Tissue cases should be grouped and shipped according to weight. No package should weigh more than 35 pounds. All cases for shipment should be placed in one larger plastic bag which can be sealed with masking tape. The larger plastic bag should then be placed in a well-insulated box which is labeled with proper address and marked as "FRAGILE," "Laboratory Specimens."

(4) Many experiments have been conducted with various types of plastic materials to determine the type best suited for prolonged storage of preserved specimens, as well as the type most economical for shipping. The plastic which has proven the most durable, pliable, and suitable for this kind of work is polyethylene, .005 gauge, clear plastic. Plastic bags cost less than glass jars and they solve a storage problem. Several thousand plastic bags can be stored in less space than is required for one case of glass jars.

(5) Each installation must establish procedures for its own program and needs for processing or storing of tissue specimens. Conditions,

such as quantity and length of time to be stored, would govern whether the lighter gauge standard tissue plastic bag can be used, or if it is necessary to procure the heavier .005 gauge polyethylene bag. In some instances, it is considered advisable to procure polyethylene, .005 gauge, lay-flat tubing from which individual bags can be cut and sealed. This is recommended where larger quantities of wet tissue are processed frequently, as it is more economical to cut the plastic and make the bags to fit the specimens.

(6) Fresh tissue specimens used for toxicology studies should be packaged in .005 gauge polyethylene plastic bags. This type and gauge of plastic acts as a deterrent against CO₂ (dry ice) penetrating the plastic and permeating the tissue. In preparing the case for shipment, the tissue should be sealed in one plastic bag and the identifying data in a second plastic bag. Specimens should be prepared in this manner and packed in sufficient dry ice to arrive in a satisfactory condition at their destination.

(7) Bone specimens create another problem for storage and shipment in plastic bags, due to the necessity for keeping all parts of the specimen exposed to the fixative. It is suggested that such specimens be wrapped in absorbent cotton which is saturated with the desired fixative. Caution should be taken to ensure that all sharp or ragged edges of the bone are heavily padded in the cotton to prevent cutting of the plastic bag. When as much air as possible has been evacuated from the bag and it has been heat-sealed, these specimens can be stored indefinitely or shipped without being damaged. (See paras 20 through 23 on bone specimens.)

(8) The use of .005 gauge polyethylene plastic bags for permanent storage of celloidin blocks is also recommended, since this method eliminates the constant maintenance of alcohol levels required by other types of storage containers. This method also effects a savings in both the cost of alcohol and the manhours necessary for the care of such blocks. A firm piece of cardboard should be tied to the specimen to prevent warping, and the specimen should be inserted in a plastic bag of appropriate size with sufficient alcohol added to keep the celloidin in a firm state. As much air as possible should be evacuated from the bag, after which it should be heat-sealed, and checked for possible leakage. The properly sealed specimen and an appropriate identification tag are then sealed in a second plastic bag.

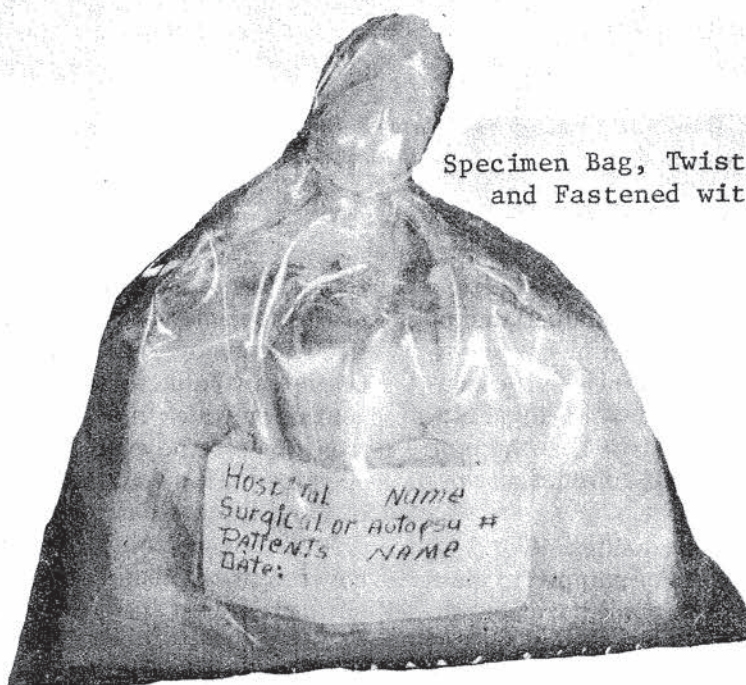
(9) There are other advantages to storing pathologic materials in plastic bags, such as protection from the danger of ants or other insects

METHODS FOR PACKING WET TISSUE

Plastic Bags Heat Sealed



Specimen Bag, Twisted, Doubled Over, and Fastened with Rubber Band



8-340-12

Figure 12. Specimens packed in plastic bags.

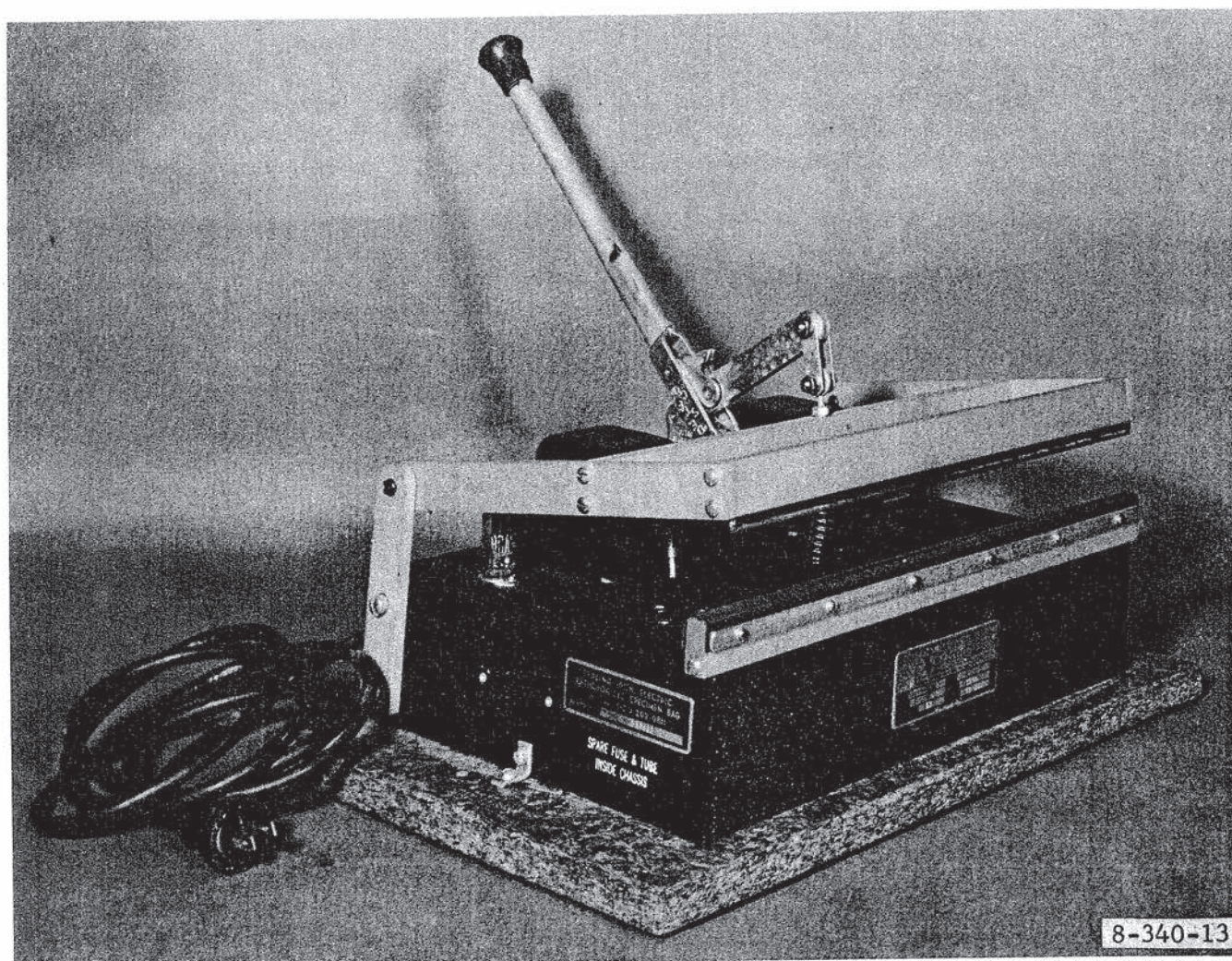


Figure 13. Sealing iron, portable—standard item of supply.

which destroy such materials, and the protection of paraffin blocks or microslides from dust, dampness or dryness. In storing paraffin blocks or microslides, it is necessary only to enclose this material in plastic bags, add the identifying data, and heat seal. Materials stored in this manner will remain in good condition for an indefinite period of time.

b. Glass Container Methods (see figs. 16 and 17)

(1) Wet tissue being stored or shipped in glass containers requires the same preparation for proper fixation and identification as tissue packed in plastic bags. It is emphasized that personnel responsible for packing the tissue be impressed with the importance of using only straight, wide-mouth containers. These will permit easy access

to the tissue and prevent damage or mutilation of the specimen. It is also important to stress the need for using a container of proper size, since tissue can be distorted if forced into too small a container which has insufficient space to permit free flow of the preservative around the entire specimen. This could also cause the tissue to dry out and lose its pathologic value.

(2) The bottom of each container should be covered with absorbent cotton which has been saturated with the fixing fluid, allowing all parts of the tissue to remain exposed to the preservative. The specimen, with the identifying tag attached, should be inserted in the glass container with sufficient fluid to cover the specimen. More cotton should be placed in the top of the container

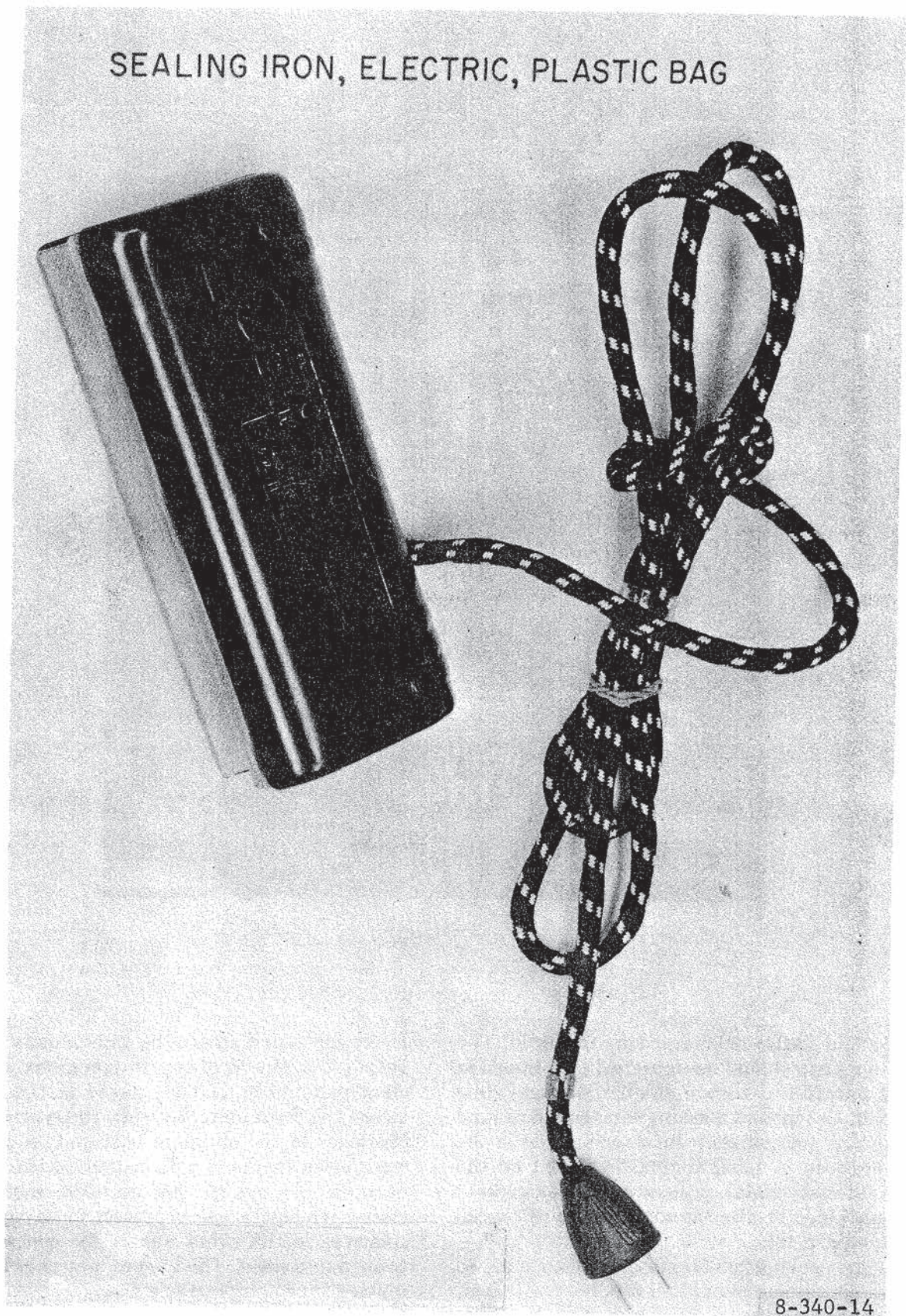


Figure 14. Sealing iron, hand operated—nonstandard item of supply.

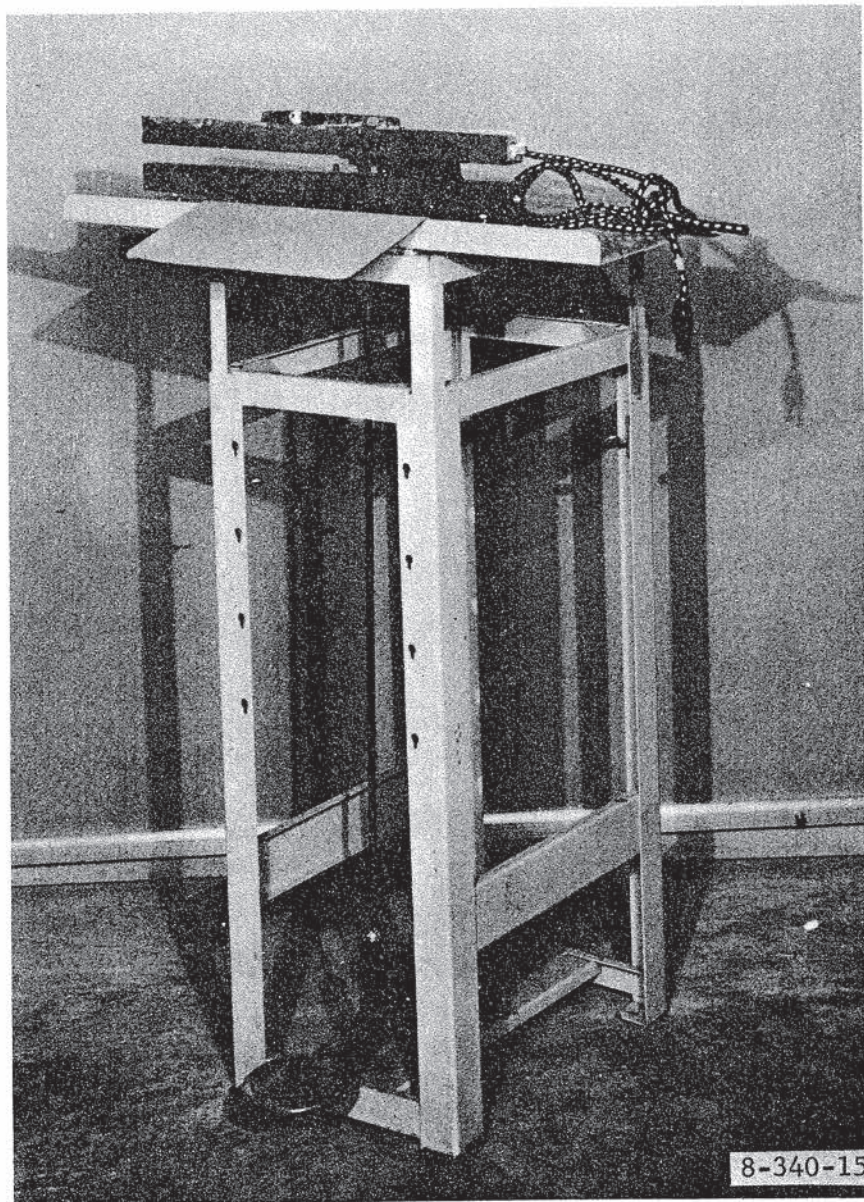


Figure 15. Sealing iron, foot operated—nonstandard item of supply.

to keep the tissue submerged in the fluid. The container's cap should be tightened and dipped in melted paraffin to ensure against leakage while in transit. Cellophane masking tape bound around the cap and part of the container will serve the same purpose. A label should be placed on the outside of each container, bearing the same data as is contained on the tag attached to the specimen (see para 18b).

(3) The packing box for material being shipped in glass containers should be prepared with particular attention to the corners of the box, this is where most of the damage occurs while in transit.

Each container should be individually wrapped before it is placed in the shipping box, and sufficient padding should be placed at the base, between the containers, between the containers and the sides of the shipping box, and on top of the containers to make a firm and secure package. Standard Forms 515, 503 and 543, should be inclosed in a plastic bag to protect these papers from fixatives in the event any of the containers are broken in transit. The papers may then be placed inside of the shipping box; the box is sealed, properly addressed, and labeled "GLASS-FRAGILE."

(4) It is desirable that Standard Forms 515,

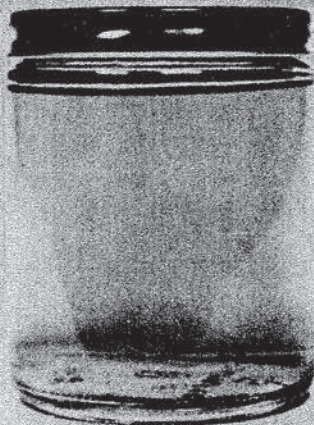
GLASS CONTAINERS



Jar, Screw Cap,
Ointment, 4 oz, Glass, 12's
(6530-00-430-5410)



Jar, Screw Cap,
Ointment, 1 lb, Glass
(6530-00-430-5436)



Jar, Screw Cap,
Ointment, 8 oz, Glass
(6530-00-430-5420)



Jar, Spring Fastened Cap,
Specimen, 1 Pint
(Non-Standard Item)

8-340-16

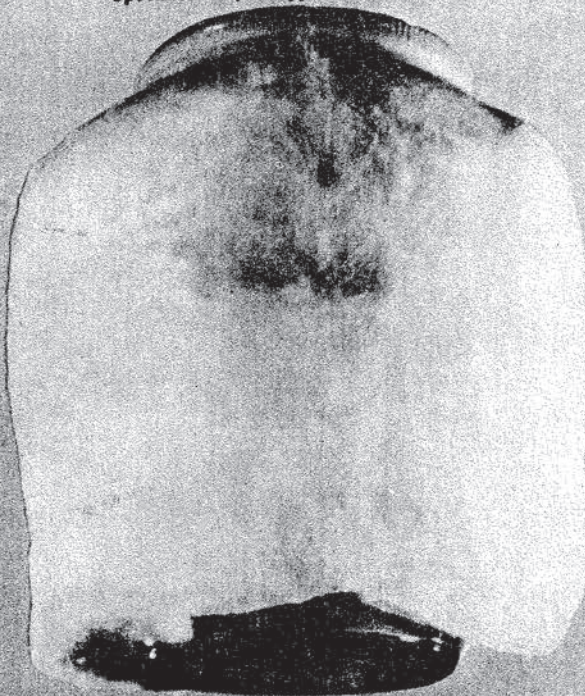
Figure 16. Glass containers.

METHODS FOR PACKING WET TISSUE

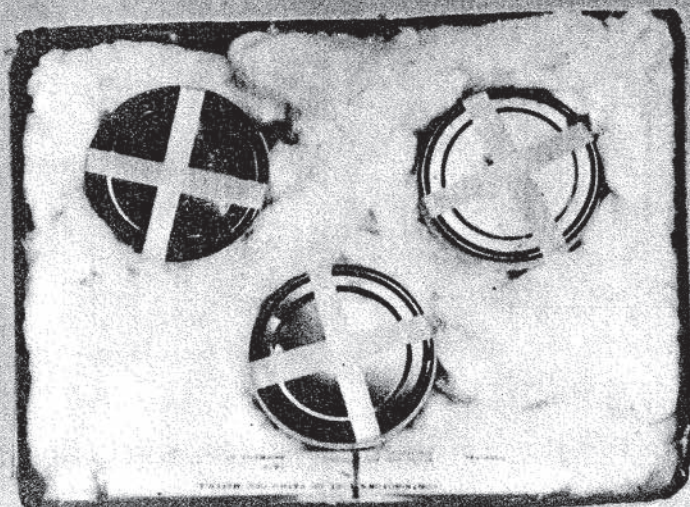
Properly Labeled Specimen Jar



Specimen Jar, Wrapped in Cotton



Packing Box With Glass Jar Specimens and
Protocols Sealed in Plastic Bag



8-340-17

Figure 17. Properly labeled glass containers and method for packing.

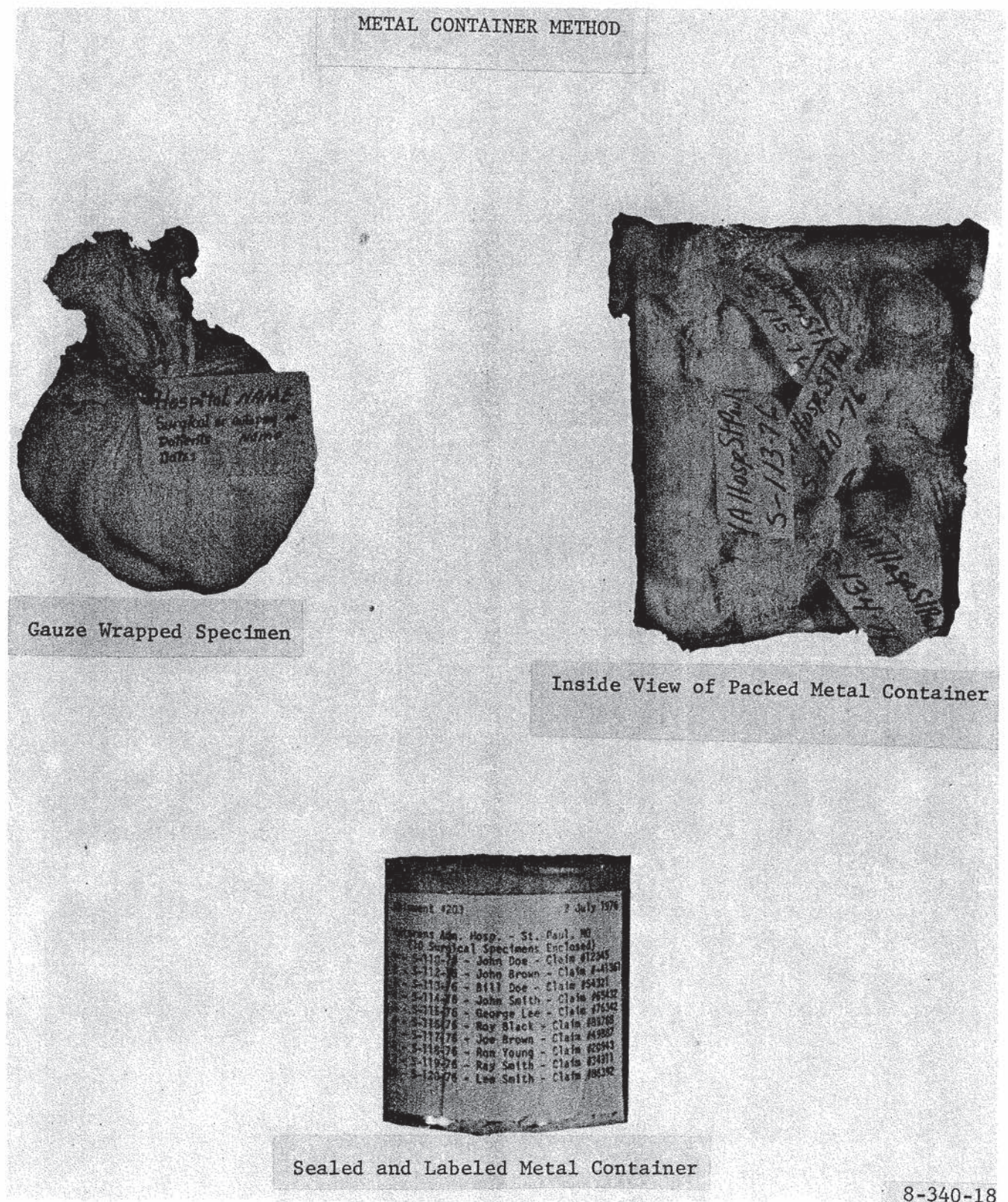


Figure 18. Properly labeled metal container and proper method for packing.



Figure 19. Small specimen shipping container.

503 and 543 accompany all shipments, but if plastic bags are not available to permit their inclosure inside of the shipping box, they may be placed in an addressed manila envelope and securely attached to the outside of the shipping box. If the Standard Forms 515, 503, 543 are too bulky, a separate package should be forwarded by mail. If this is done, sufficient information should be included with the shipment to provide identification on its arrival.

(5) The size of the shipping box used should never exceed 2 square feet, and it should not weigh more than 35 pounds when complete. Boxes of larger dimensions or heavier weight create problems in shipping, handling, personnel safety, and possible damage to the enclosed specimens, since they may be dropped or roughly handled during transit.

c. Metal Can Method (see fig. 18).

(1) Metal cans may be used in preference to glass jars, when plastic bags are not available. Several types of metal cans may be requisitioned from standard items of supply. A more economical method however, is the use of cans in which other items have been previously packed. Such cans may be available from mess halls, X-ray clinics, and dental or photographic laboratories.

(2) Wet tissues being stored or shipped in metal cans require the same preparation for proper fixation and should carry the same proper identification as tissue packed in plastic bags or glass containers (see para 18b).

(3) The base of the metal can should be covered with a layer of absorbent cotton which has been saturated with the fixing fluid. The properly prepared and tagged specimen should be placed in the can and covered with another layer of cotton, after which sufficient preservation should be added to saturate the top layer of cotton. The lid should be placed on the can, tightly closed, and sealed. A label should be placed on the outside of the can bearing the same data as is contained on the tag attached to the specimen. It should be noted that most preservatives react against base metals and cause rust to form, therefore storage in metal containers should be for a short duration only.

(4) Properly prepared cans should be placed in a well-padded shipping box, with sufficient padding on the bottom, between, and on top of the cans to ensure safe arrival.

(5) Standard Forms 515, 503, and 543, if in-

closed in a plastic bag, should be packed inside the shipping container; otherwise, these papers should be placed in an addressed manila envelope and the envelope securely attached to the outside of the shipping container. The shipping box should be sealed, properly addressed, and labeled "CAUTION—HANDLE WITH CARE."

d. Method for Shipping Small Wet Tissue Specimens (see fig. 19).

(1) In cases where small, individual wet tissue specimens, such as eyes, bone particles, or minute sections of tissue, are being prepared for shipment, the standard supply item, "Container Assembly, Sample and Specimen Shipping," should be used. This unit comes in two sections, one to inclose the specimen, and the other to mail the specimen. The specimen section should be cushioned with cotton before inserting the properly tagged and sealed specimen. Cotton should be packed around the specimen before securing the lid. Standard Forms 515 or 503, and 543 should be folded and wrapped around the specimen container section by means of a rubber band. The container section is then inserted in the outer section for mailing. The outer lid should be secured with cellophane tape, properly addressed, and labeled "FRAGILE."

(2) Contributors are requested to place the eye specimen, immediately after removal, in a 10 percent formalin solution of 20 times its volume. Normally one eye requires 300 ml of 10 percent formalin. Enucleated eyes should not be opened. After 48 hours, the eye specimen should be wrapped in 10 percent formalin saturated cotton, placed in a small glass container with a pyralin identifying tag inserted, and the container then sealed. A label, containing the same data as that shown on the identifying tag (see para 18b), should be placed on the outside of the container. The small glass container should be placed inside the specimen section of a mailing assembly and properly packed before securing. The balance of the shipping assembly should be completed as described in (1) above.

CAUTION. Plastic bags should never be used to store or ship eye specimens, since any undue pressure against the specimen could cause it to rupture or become distorted. An eye specimen should never be suspended in formalin since damage can occur while in transit due to its shifting against the sides of the container.

Section IV. BONE SPECIMENS

20. General

Amputation specimens of all types, except those caused by gangrene due to arteriosclerosis, diabetes, and Buerger's disease, are needed for further research and study. Many autopsy protocols are received on cases where there is a disease involving the skeleton, but little or none of the skeletal material which was removed has been received with the case history. There is need for such material, especially in all childhood and metabolic diseases, affecting the skeleton.

21. Preparation of Specimen

Immediately following amputation, the specimen should be skinned and all excessive soft tissue removed, except that tissue involved with the disease. The specimen should then be placed in a 15 percent formalin solution for a period of 10 to 14 days. A 15 percent formalin solution is prepared by adding 1.5 parts of formaldehyde (40%) to 13.5 parts of water. Fixation and preservation are much better if the specimen can be sawed in half longitudinally—a band saw may be used for this purpose. The AFIP is interested not only in the diseased portion of the specimen but in the entire amputation specimen, since the normal portion is useful for study of developmental changes.

22. X-Rays

X-ray films or photographic copies of the films should accompany all bone or joint specimen cases. If photographs are made, the date of the particular X-ray which is photographed should be indicated on the copy to prevent misunderstanding

ing or perhaps incorrect diagnosing. The original X-rays are preferred, and a complete series of the films guarantee the best diagnosis, because changes from one date to another are a part of the diagnostic criteria. X-ray films sent with a case to the AFIP will be copied and the originals will be returned to the contributor.

23. Method for Packing (see fig. 20)

When a bone specimen is ready for shipment with the identifying tag attached (see para 18b), it should be wrapped in cotton or gauze which has been saturated with formalin. Special attention should be paid to the sharp edges of bone specimens; they should be well padded or wrapped, in order to prevent the cutting of plastic bags. The specimen should be inserted in a plastic bag, sufficient 15 percent formalin solution added for protection, and the bag heat-sealed or closed by means of a rubber band or cellophane tape. The bag should be checked to be certain there is no leakage of formalin. When the specimen is placed in the shipping container, sufficient padding should be used to give complete protection to the specimen while in transit. Standard Forms 515, 503, and 543 should be inclosed in a separate plastic bag and included in the shipping container. The shipping container should then be sealed, properly addressed, and labeled "FRAGILE—LABORATORY SPECIMENS." X-ray films should be mailed in a separate container, with sufficient information furnished to identify them with the proper specimen. Slides should *not* be sent in the X-ray container.

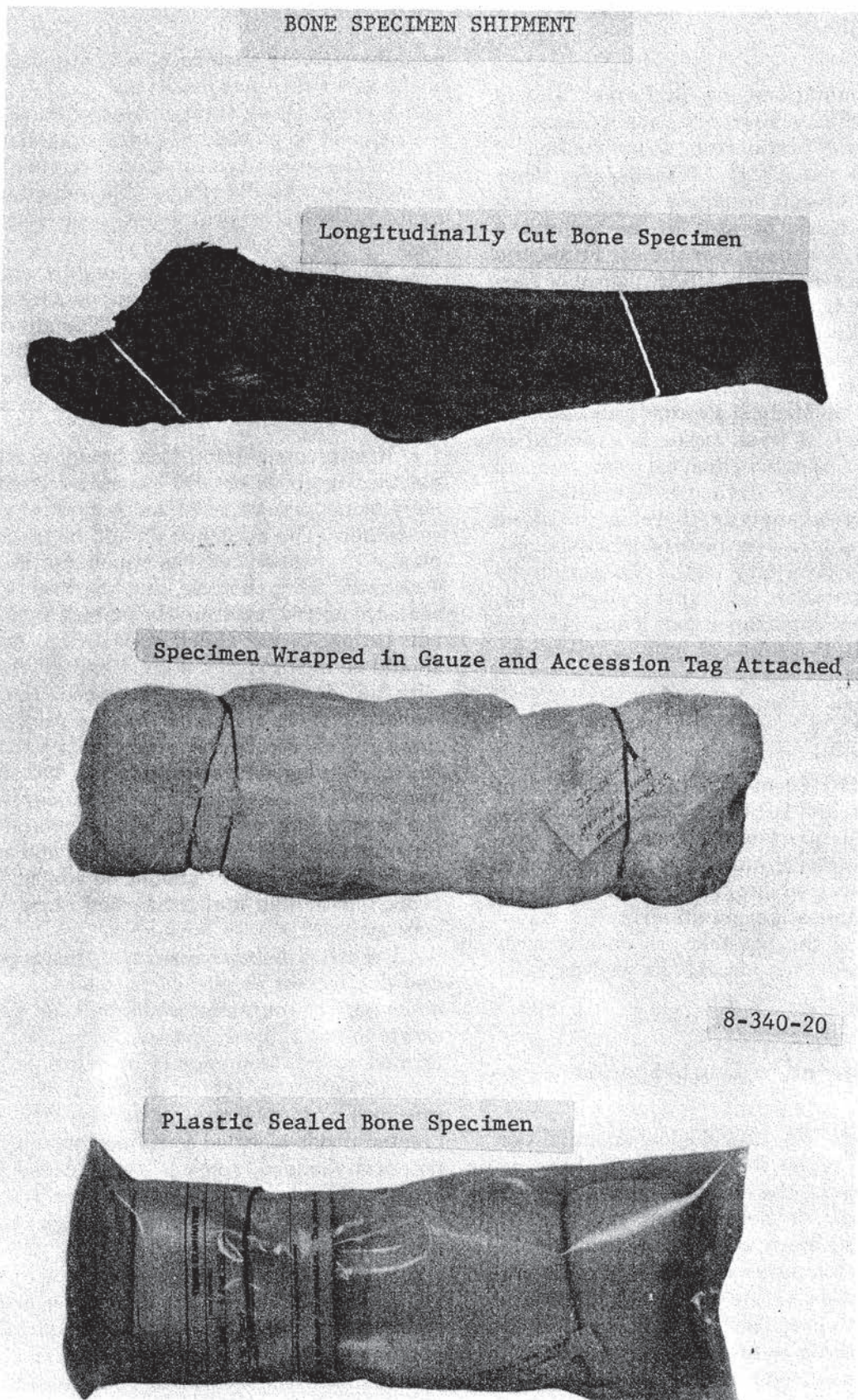


Figure 20. Bone specimen shipment.

Section V. FROZEN SPECIMENS FROM AIRCRAFT ACCIDENTS

24. General

Toxicology examinations are performed at the AFIP on all military aircraft accident cases. In addition, all Army postmortem tissue toxicology is to be sent to the AFIP. All toxicology, other than that from aircraft accidents and Army postmortem cases should be sent to the respective Service toxicology centers. For the Air Force, the specimens should be sent to USAF School of Aerospace Medicine (EP), Brooks Air Force Base, TX 78235; for the Navy, to the Chief, Toxicology Laboratory, Department of Laboratory Medicine, National Naval Medical Center, Bethesda, MD; for the Army, to the Medical Center Laboratories. Prompt collection of fresh tissue is essential in order to protect it against chemical or mechanical change. Chemical preservatives invalidate results of toxicological analysis; therefore, no fixing fluid (formalin) should ever be used for tissue protection. Refrigeration (dry ice) is the prescribed method of preservation, and rapid transportation is of the utmost importance (see para 113, TM 8-300/NAVMED P-5065/AFM 160-19, "Autopsy Manual").

25. Toxic Agents

In completing DD Form 1322 (Aircraft Accident Autopsy Report) and DD Form 1323 (Toxicological Examination—Request and Report), when toxicological studies are requested, it is important to indicate any suspected intoxicants or drugs. Every medical officer investigating an aircraft accident must be alert to the possible presence of toxic agents associated with aircraft as well as those not so associated.

26. Preparation and Packing of Specimens (see fig. 21)

Tissue specimens for toxicological examination will be collected under the supervision of the pathologist performing the autopsy and will consist, whenever possible, of the following: Liver, brain, kidney, lung, bone marrow, blood, urine and stomach contents. Precautions should be taken to prevent contamination of the specimen during the course of the autopsy. Thorough toxicological examination requires approximately 250 to 500 grams each of brain, liver, kidney, and lung, 20 ml of blood, and all urine available. The amount of tissue available will govern the amounts submitted. Red bone marrow and lung tissues are es-

pecially useful in such cases where decomposition of the soft tissue has occurred.

a. Individual tissue specimens, such as brain or liver should be placed in separate plastic bags. In view of the quantity of material required, it may be necessary to distribute the individual specimens between several latex rubber or plastic bags.

b. Blood and body fluids may be shipped in either screw-top polypropylene centrifuge tubes (50 ml) or in latex rubber bags. The air should be carefully evacuated prior to closing the bag by knotting or other means. As an added precaution, this type of bag should be inclosed in a second bag.

c. It is recommended that heavy polyethylene plastic bags (.005 or .006 gauge) or latex rubber bags (condoms) be used as individual specimen containers. The specimen should be placed in the plastic or rubber bag, as much air as possible evacuated from the bag, and the bag then heat-sealed, knotted, or securely fastened with a rubber band. As an added precaution, the tissue should be inclosed in a second bag in which a tag with all identifying data is placed. *It is recommended that a paper label only be used in identifying frozen specimens, since plastic labels may cause camphor odors to permeate the specimens and give false determinations.* Heat seal or fasten the second bag, as indicated above, and prepare for shipment. DD Forms 1322, 1323, and any other available information should be sealed in a separate plastic bag and forwarded along with the specimen.

WARNING: *It is imperative that frozen specimens and dry ice not be packed in sealed cans or any other type of container which will not permit the escaping gas to pass through its walls.* Dry ice is formed under tremendous pressure; it requires approximately 230,000 ml of carbon dioxide under pressure to form pound of dry ice. The pressure created inside a sealed container presents a great potential hazard, since it could cause the container to burst. Do not inclose dry ice in a thermos bottle unless holes are drilled through the stopper of the thermos.

d. When packing for shipment, the specimen and DD Forms 1322 and 1323 should be placed in a stout cardboard box filled with pieces of dry ice and enough filler (sawdust, styrofoam, etc.) to fill and insulate the box. The box should be large enough to hold sufficient dry ice for a shipping time of 24 to 36 hours. It should be sealed with tape and wrapped in several layers of heavy pa-

FROZEN TISSUE SHIPMENT

DEPARTMENT OF THE NAVY
NAVAL AIRSPACE & REGIONAL MED. CTR.
MILITARY 2269
PENSACOLA FLORIDA 32512

TO: THE DIRECTOR
ARMED FORCES INSTITUTE OF PATHOLOGY
ALTER BUILDING MEDICAL CENTER
WASHINGTON, D.C. 20306

NOTE: SPECIMENS FOR TOXICOLOGICAL STUDIES
PACKED IN DRY ICE-1540 HOURS-10-30-76
DO NOT DELAY

Completed Shipping Container



Specimen Sealed in
Polyethylene Plastic Bag

TOXICOLOGICAL EXAMINATION, REQUEST AND REPORT

TO: Aerospace Division, Toxicology Branch, Armed Forces Institute of Pathology, Washington, DC 20306

FROM: 284th USAF Hospital, Bedford, Pennsylvania 22033

SECTION A - MEDICAL REPORT

NAME OF PATIENT (Last, First, Middle Initial): DOE, John D.

DATE OF EXAMINATION: 66-12-1009

EXAMINATION: Bedford, Pennsylvania

DATE OF REPORT: 15 Oct 76

TIME: 1500

DATE: 1-76

AIRCRAFT Accident Case

FROM: 284th USAF Hospital
Bedford Penn.

Patient: Doe, John D.
A-123-76

All Specimens Placed in Second Bag with
Identification Sealed in Top

8-340-21

Figure 21. Frozen tissue shipment.

per. A plastic-insulated box is available in the Federal Supply Catalog with the nomenclature "Box, Plastic, Insulated; Meat, Dairy Products, and Laboratory Samples."

e. The packing box containing specimens for toxicological examination should be labeled "FRAGILE—RUSH—SPECIMENS FOR TOXICOLOGICAL EXAMINATION (AIRCRAFT ACCIDENT)," and forwarded by military or commercial air freight to The Director, AFIP, WASH DC 20306. This special labeling must be recorded on DD Form 1387-2, Special Handling Data/Certification, if the package is to be shipped by military air freight. Correct designation should be clearly written to ensure prompt delivery.

Section VI. MEDICAL MUSEUM SPECIMENS

28. General

Specimens forwarded to the AFIP for museum use are grouped in two categories:

a. Specimens intended only for gross mounting and museum use with no consultation or report desired of the AFIP pathologists.

b. Specimens intended for the dual purpose of consultation and report by the AFIP pathologists and for permanent preservation in the Museum.

c. See chapter 7 TM 8-300/NAVMED P-5065/AFM 160-19 (Autopsy Manual).

29. Identification

The category of each specimen should be clearly indicated by notation on the Standard Forms 503, 515 and also on the label on the specimen container. The label on the specimen container should also indicate data as outlined in paragraph 18b. To avoid possible confusion and loss of identifying information, in case labels may loosen and become detached, it is advisable to have an identifying tag attached to the specimen or placed inside the container.

30. Preparation and Packing of Specimen

a. Specimens intended primarily for museum use should be placed in a special color preserving solution, such as Schein's:

Schein's Solution

Sodium Phosphate, Monobasic	----	44.5 g
Sodium Phosphate, Dibasic	-----	56.2 g
Formaldehyde (40%)	-----	475 ml
Distilled water	-----qs ad	9500 ml

27. Dry Ice Machine

The following item has been added to the Federal Supply Catalog: 6640-00-585-1801 Ice Making Machine, Carbon Dioxide, Disk, Laboratory— for use with compressed carbon dioxide gas, with blow check, safety, and gas control valves; tubing and fittings for connection to gas cylinder; and bolt flange for mounting on table. This is a small compact, cylindrical-shaped, automatic machine for the production of solid carbon dioxide with a temperature of -114° F. It is approximately 10 inches high and 10 inches in diameter, and produces a disk of approximately 12 ounces. It is equipped with a handle to raise and lower the lid and lock it securely when solid carbon dioxide disks are being made.

This solution effectively fixes tissue for both histopathologic study and gross presentation; however, adequate fixation may take from two to four times as long as that required by the use of 10 percent formalin. The initial solution described above does not retain colors, so during the mounting procedure which will be performed at the AFIP, specimens will be treated with a second solution which will restore colors.

b. Drying will quickly spoil a specimen, therefore it should be carefully washed with normal saline and placed in the initial solution as soon as possible. It may remain therein during transit to the AFIP if the solution remains clear. If it becomes cloudy, one or two changes of the solution should be made. Cotton should be placed in the bottom of the container so that the solution will come in contact with every part of the specimen.

c. The usual precautions applicable to gross tissue fixation should be followed, such as filling of hollow organs with cotton and avoidance of unnecessary specimen mutilation. Cotton should not be packed too firmly in a cavity, because after fixation, the lining of the cavity will appear merely as a mold of the cotton. If a hollow organ must be held open, distend it with fixing fluid for a day or two before cutting it. If this is not possible, it may be propped open with cotton inserted very loosely.

d. Glass containers are suitable for shipping specimens, if they are large enough to contain the specimen and if they are properly sealed. Forcing specimens into inadequate bottles will lead to distortion of the specimen. For display purposes, a glass jar with parallel surfaces

should be used; the American Medical Museum jar is the one in general use. The size of the jar should be large enough to contain the specimen, to prevent it from coming into contact with the sides of the jar, and to allow for overlying fluid of from one to several centimeters. Before covering the jar, it should be filled with the solution to within 5 mm of the top. Polyethylene plastic

bags are permissible, if the specimen is kept moist and free from distortion.

e. When an item is to be mounted and returned to the sender for a specific purpose, the installation desiring this service should clearly indicate this fact when the material is submitted to the AFIP. Directions for mounting should be included.

Section VII. SPECIMEN STORAGE AND FIXATION OF MATERIALS FOR IMMUNOFLOUORESCENT STUDIES

31. Tissue Storage and Shipment for Immunofluorescent Studies

a. Tissue fragment, portion of biopsy material or an entire biopsy specimen must be frozen as soon as possible after obtaining the specimen. Rapid freezing on a cryostat or on a fragment of dry ice is optimal. Freezing is best performed by placing the specimen on a pre-frozen drop of water and then covering it with water, allowing the specimen to freeze entirely. Once the specimen has been frozen, it should be kept in the frozen state until the time of cutting of the frozen section.

b. Frozen specimens should be shipped by the fastest method available, packed in dry ice. For shipment over any distance, a minimum of 30 to 50 pounds of dry ice is required.

32. Alternate Method of Examining Tissue for Immunofluorescence

a. Prepare an ammonium sulfate fixative in the following manner:

(1) Buffer—

1M Potassium citrate buffer
(pH = 7.0) ----- 2.5 ml

0.1M $MgSO_4$ ----- 5.0 ml
0.1M N-ethylmaleimide ----- 5.0 ml
Distilled water ----- 87.5 ml
Adjust pH to 7.0 with 1M KOH

(2) Fixative—Dissolve 55 grams of $(NH_4)_2SO_4$ in 100 ml of buffer.

b. Specimens for immunofluorescence may be placed in this fixative and sent to the examining facility at room temperature. This fixative is known to be adequate for evaluation of immunoglobulins in tissue for up to 10 days. (Reference: Beno, Michael, et al., *Preservation of Tissue-Fixed Immunoglobulins in Skin Biopsies of Patients with Lupus Erythematosus and Bullas Disease—A Preliminary Report*. J. of Inv. Derm. 59:449-452, 1973.)

c. Of the above two means of transporting tissue, the latter (para 32) is of greatest benefit, however, speed and immediacy of shipment of the specimen is necessary to minimize the time period involved. The apparent principle involved in the latter procedure is that immunoglobulins will be precipitated in the tissue at the site of their localization.

Section VIII. HANDLING AND SHIPMENT OF TISSUES FOR ELECTRON MICROSCOPY

33. All Tissue Submitted for Electron Microscopy Must Be:

a. Fixed as rapidly as possible to prevent sub-cellular degeneration

b. Thin sectioned to allow for immediate fixation. As rapidly as possible, a less than 1 mm section of the tissue to be examined for electron microscopy should be taken with a fresh, sharp razor blade and placed in the EM fixative. For larger specimens, 1 mm or less thin slices should be made of the tissue followed by immediate fixation. The critical factor is the thinness of the section, allowing for penetration in two plains.

34. Electron Microscopy Fixatives

a. *Paraformaldehyde picric acid (PAF) fixative for light and electron microscopy.* This fixative, which represents a combination of paraformaldehyde and picric acid in a buffered solution, has the advantages of allowing a high speed of penetration, stabilizing cellular proteins, is not easily destroyed by tissue fluids, may be used without postosmication, is very stable and not light sensitive, and has a shelf life of at least 1 year at room temperature.

b. *Formula for PAF fixative.*

Paraformaldehyde ----- 20.0 g

Saturated and filtered aqueous solution of Picric acid 150 ml
Heat above mixture to 60° C. to dissolve paraformaldehyde and convert it to formaldehyde.

Sodium hydroxide solution, 2.5% Add dropwise to the above solution so as to make it alkaline (solution should be clear).

Filter and allow to cool.

Phosphate buffer, add sufficient volume to make 1000 ml.

Phosphate buffer

NaH ₂ PO ₄ ·H ₂ O	3.31 g
Na ₂ HPO ₄ ·7H ₂ O	33.77 g
(or Na ₂ HPO ₄)	(17.88 g)
Distilled H ₂ O	1000 ml

The PAF fixative should have a final pH of 7.3 and an osmolality of 900 mOsm/kg.

(Reference—Stefanini, M., DeMartino, C. and Zamboni, L., *Nature* 216:173, October 14, 1967).

c. *Glutaraldehyde Fixative* This fixative is a slightly harsher fixative, however, equally as good as the PAF fixative noted above. The following special handling should be maintained:

Fixative solution must be stored at all times in a refrigerator and away from bright light.

Formula: Glutaraldehyde Fixative
Glutaraldehyde, Biological

Grade, 50% 6 ml

Sorensen's Phosphate Buffer,

0.1 M 94 ml

The final pH of this fixative should be 7.2.

Sorensen's Phosphate Buffer

Prepare the 0.2 M stock solution as follows:

Sodium monobasic phosphate	27.6 g
Distilled water	1000 ml
Sodium Dibasic Phosphate ..	28.4 g
Distilled water	1000 ml

Note: Each solution should be thoroughly mixed and then stored in a refrigerator.

Working Strength Sorensen's Buffer

Sodium Monobasic Phosphate

Solutions, 0.2 M 23 ml

Sodium Dibasic Phosphate

Solution, 0.2 M 77 ml

Distilled water 100 ml

The pH of this buffer solution is 7.2.

CHAPTER 3

SHIPMENT OF PATHOLOGIC SPECIMENS

Section I. GENERAL

35. General

a. Material should be prepared for shipment in accordance with its durability. Blocks and microslides should never be included in a shipping container with wet tissue specimens, even though plastic bags are used for shipping the wet tissue specimens. Segregation of materials will eliminate the possibility of damage from solutions in case leakage or breakage should occur while the shipment is in transit. The necessary forms accompanying wet tissue shipments should always be inclosed in a sealed plastic bag for protection.

b. Proper packing is necessary to ensure successful shipment. Accidents occurring to mixed shipments have resulted in materials becoming saturated with fixing fluids: thus, accessioning data on block boxes becomes illegible, paper gummed labels are loosened from microslides, and accompanying forms absorb fluid and become illegible or disintegrate. This results in the loss of valuable medical material, data and information, making it impossible for the AFIP to process the material or forward the requested medical information.

36. Federal Regulations (see fig. 22)

a. The following sections of the United States Code of Federal Regulations provide specific guidance and limitations on the shipment of biological and etiological agents: Title 18, USC, Sections 831-835 and 1716; Title 32 (Public Health), USC,

Part 72 and Title 49 (Transportation) USC, Part 173. Also, the US Postal Service Manual, sub-chapters 121 and 124, provides additional guidance on the mailing of biological and etiological agents.

b. Shippers of specimens to the AFIP who use good scientific and medical judgement in consonance with the above cited portions of the United States Code and United States Postal Service Regulations should have no problem in meeting all of the requirements for safe shipment of infectious specimens to the AFIP for examination.

37. Size of Shipment

"Routine" cases should be shipped to the AFIP every 90 days, or in groups of at least 25 cases. Processing of cases at the AFIP is more readily accomplished when a shipment consists of 25 cases or more. Large installations will have no difficulty in meeting this requirement. Smaller installations, where it would require some time to accumulate this number may send cases accumulated over a 90-day period.

a. "TWX" or "RUSH" cases *should not* be included with regular shipments, but should be forwarded separately to ensure priority in processing (see para 39).

b. Microslides and paraffin blocks in each instance, and wet tissue when available, should be submitted for each surgical or autopsy protocol.



Figure 22. Damaged shipment of mixed pathologic material.

Section II. FORMS ACCOMPANYING SHIPMENT OF PATHOLOGIC MATERIAL

38. General

a. It is imperative that all forms accompanying a shipment of pathologic material be legible and complete in order that the staff of the AFIP may render sufficient service to the contributor. Incomplete information could cause a delay in reporting the diagnosis or double accessioning of a case when new material is submitted. It could present a problem of identification of a case or patient selected for special review or research study and/or followup. It could cause errors in recording the status of a patient; i.e., a Navy patient being accessioned as a civilian dependent when service number is not furnished, an Air Force patient being accessioned as Army when treated in an Army Hospital. No shipments of material should go forward without inclusion of SF 543 and the accompanying SF's 503 and/or 515. A complete listing of materials shipped legibly prepared, with full details on the identifying data is essential for the proper processing of each case.

b. Forms and corresponding material (microslides, blocks, tissue, photographs and X-rays) should be marked with the identifying specimen number (see para 4), and forms should contain the complete information as outlined in paragraph 40. Many hospitals employ the Addressograph plate imprint system which ensures a record of the necessary identifying information on all forms completed for a patient in a hospital. When a case is forwarded to a histopathology center or reference laboratory, the number assigned to it as well as the name of the contributing laboratory should be added to all forms and material for each case. The histopathology center or reference laboratory may reclassify the priority when submitting a case to the AFIP for diagnosis. The priority should be clearly stamped on the case history and all other designations lined out (see para 39).

c. If more than one surgical procedure has been performed on a patient, an SF 515 should be prepared and a new accession number assigned to each separate specimen.

39. Priority

The large volume of material received at AFIP precludes the immediate sorting and evaluation of all specimens. Cases requiring a "TELEPHONE," "TWX," or "RUSH" confirmation should be forwarded separately from routine (regular) shipments. Such cases should include Standard Forms 503 or 515, SF 543, slides, blocks and tissue.

To serve the contributor better, one of the following terms, designating the priority of the required reply, should be stamped or marked in red at the top and bottom of each Standard Form 503, 515, and 543.

a. "TWX" or "TELEPHONE." Protocols so stamped will receive prompt attention and take priority over all other cases; reports will be sent by cable, radio, telegraph, or telephone.

b. "RUSH." Protocols so stamped will be handled as speedily as possible and are second in priority; reports will be sent via airmail.

c. "COMMENT." Protocols so stamped will be handled in the usual routine manner; reports will be sent by ordinary mail.

d. "ROUTINE." Receipt of such cases will be acknowledged, but comments will not be sent unless requested by the contributor, or unless the reviewing pathologist at the AFIP deems comments are pertinent. This designation should be used only when a contributor does not desire comments from the AFIP.

Note. "TWX," "Telephone" or "Rush" cases should be sent by the most expedient method, airmail if possible. In addition to marking each protocol form "TWX," "TELEPHONE" or "RUSH," it is imperative to stamp or mark "TELEPHONE," "TWX" or "RUSH" case on the outer wrapping of the package. If this is done, immediate attention will be given such a case, thus affording the least possible delay in securing a diagnosis or confirmation from the AFIP. The contributor should include his/her telephone number.

40. Standard Forms 503, 515 (see figs. 23 and 24)

The following information is essential and must be included on these forms:

a. Patient's status—Military, dependent of military, or civilian. In cases of military dependents, identifying information should include the name, grade, and social security account number of military sponsor or person on whom dependent (to be placed in the space provided for patient's name and to appear directly below the patient's name).

b. For VA patients, enter the service, social security and veteran's claim numbers along with the patient's name.

c. Priority of reply required (Telegram, Telephone, Rush, Comment or Routine).

Standard Form 503
Revised August 1954
Bureau of the Budget
Circular A-32 (Rev.)

ROUTINE

CLINICAL RECORD		AUTOPSY PROTOCOL			
DATE AND HOUR DIED	0815	A. M.	DATE AND HOUR AUTOPSY PERFORMED	1000	A. M.
29 March 1976		P. M.	29 March 1976		P. M.
PROSECUTOR		ASSISTANT		CHECK ONE	
J. B. Cook, Maj, MC				FULL AUTOPSY	HEAD ONLY
				X	TRUNK ONLY
CLINICAL DIAGNOSES (Including operations)					

1. Myocarditis of unknown cause.
2. Hypochloremia and hyponatremia with hypovolemia.
3. Cardiac insufficiency, secondary to Dg. 1 and Dg. 2.

S
A
M
P
L
E

PATHOLOGICAL DIAGNOSES

CARDIOVASCULAR SYSTEM: 1. MYOCARDIAL HYPERTROPHY, IDIOPATHIC.
2. Interstitial fibrosis.
3. Myocarditis, focal, chronic, slight.
4. Atherosclerosis, aorta, minimal.

RESPIRATORY SYSTEM: 1. Chronic passive congestion.
2. Pulmonary edema.
3. Atelectasis, partial, left lung.
4. Interstitial fibrosis, left lung.

SPLEEN AND HEMATOPOIETIC SYSTEM: Chronic passive congestion.

LIVER: 1. Contolobular anoxic necrosis.
2. Chronic passive congestion.

GALLBLADDER AND BILE DUCTS: None

PANCREAS: Chronic passive congestion.

GASTROINTESTINAL SYSTEM: Acute duodenal ulceration, due to Candida albicans.

GENITOURINARY SYSTEM: None

ENDOCRINE GLANDULAR SYSTEM: None

BONES AND JOINTS: None

CENTRAL NERVOUS SYSTEM: None

MISCELLANEOUS: Ascites.

APPROVED—SIGNATURE

MILITARY ORGANIZATION (When required)	AGE	SEX	RACE	IDENTIFICATION NO.	AUTOPSY NO.
USAH, SHILO	23	M	Cau	415 09 7048	A-25-77
PATIENT'S IDENTIFICATION (For typed or written entries give: Name—last, first, middle; grade; date; hospital or medical facility)				REGISTER NO.	WARD NO.
				346921	17

ROSE, Julian B. SP4
29 March 1977 USAH, SHILO

ROUTINE

8-340-23

AUTOPSY PROTOCOL
Standard Form 503
503-104

Figure 23. Properly prepared SF 503, Clinical Record—Autopsy Protocol.

Standard Form 515
Rev. Jan. 1957
Promulgated
By Bureau of the Budget
Circular A-32

RUSH

CLINICAL RECORD	TISSUE EXAMINATION
SPECIMEN SUBMITTED BY Dr. Ehrenmarer	DATE OBTAINED 12 May 1977
SPECIMEN	

Growth from left nares

S A M P L E

BRIEF CLINICAL HISTORY (Include duration of lesion and rapidity of growth, if a neoplasm)

Small growth obstructing left nasal passage, present for several years, Chronic sinusitis left maxillary antrum.

PREOPERATIVE DIAGNOSIS

Fibroma

OPERATIVE FINDINGS

POSTOPERATIVE DIAGNOSIS

SIGNATURE AND TITLE

PATHOLOGICAL REPORT

NAME OF LABORATORY Histopathology Laboratory
4720 USAF HOSP., ZIPPY AFB, TX.

ACCESSION NO(S).
HPS-77-372

(Gross description, histologic examination and diagnoses)

Gross: The specimen consists of four pieces of spongy tan tissue, the largest of which measures 2 x 1 x 0.6 cm. The tissue appears to be covered with a papillary pale tan mucous membrane and is covered with much mucus.

Microscopic: Sections show a polypoid growth which is composed of numerous dilated vascular structures which are filled with abundant mucus. These acini are lined by small, dark, regular cells which somewhat resemble the epithelium of mucous secreting glands, in the nasal pharynx. A rare mitotic figure is seen. These acini are supported by a loose, fibro-vascular stroma. There is no evidence of malignancy in the sections.

Diagnosis: Adenoma of the nasal mucosa

COMMENT: These tumors are regarded a premalignant by some but this view is not generally accepted.
This case is referred to the AFIP RUSH.

(Continue on reverse side)

SIGNATURE OF PATHOLOGIST				DATE 15 May 1977	
WYLLE, Reed W. M/Sgt USAF	AGE 29	SEX M	RACE Cau	IDENTIFICATION NO. 321 77 7045	
PATIENT'S IDENTIFICATION (For typed or written entries give: Name—last, first, middle; grade; date; hospital or medical facility)			REGISTER NO. 497342	WARD NO. 4	

RUSH

8-340-24

TISSUE EXAMINATION
Standard Form 515
515-105-09

Figure 24. Properly prepared SF 515, Clinical Record—Tissue Examination.

STANDARD FORM NO. 543
FEBRUARY 1962
BUREAU OF THE BUDGET
CIRCULAR A-32 (REV.)

543-102

PAGE 1 OF 3

CONTRIBUTOR'S LIST OF PATHOLOGIC MATERIAL				DATE 15 October 1977		SHIPMENT NO. 25	
TO The Director, Armed Forces Institute of Pathology, Washington, D.C. 20306			FROM Walter Reed Army Medical Center Washington, D.C. 20012				
AFIP ACCESSION NO.	NAME	LABORATORY NO.	MICRO- SLIDES	PAPAT- FIN BLOCKS	WET TISSUE	GROSS ORGANS	MISCEL- LANEOUS
	KELLY, Foster R.	S-59-77	X	X	X		
	COVERTSON, Wayne	S-60-77	X		X		
	GRANT, Haywood R.	S-69-77	X	X		Kidney	Photos
	HULCHE, Louis F.	S-71-77	X	X	X		
	CRAWFORD, Alice G.	S-73-77	X	X	X	Ovary	
	HANDLEY, Eugene H.	S-1-77	X		X		
	GOODWIN, Albert A.	S-2-77	X	X			X-Rays
	MOORE, Elvin E.	S-3-77	X	X			
	ELDRIDGE, Egbert T.	S-6-77	X	X	X		
	BEAVER, Louis M.	S-7-77	X	X			
	JONES, William Q.	S-8-77	X	X	X		Photos
	SMITH, Frank T.	A-198-77	X	X	X		
	GARRIS, Nannette M.	A-199-77	X	X		Brain	
(	JENNINGS, Mary L.	A-3-77	X	X	X		
(	" " "	S-4-77	X	X			
(	" " "	S-99-77	X	X			
	BOLLINS, Louise	A-4-77	X	X	X		Photos
704568	CARRY, William M.	A-6-77	X	X		Heart	X-Rays
	THOMPSON, Susie S.	A-10-77	X	X	X		
905432	COCKRIDGE, Ethel T.	A-19-77	X	X	X	Lung Brain Spinal Cord	
	ROBINSON, John J.	A-20-77	X	X			
832745	HANBY, Robert	A-21-77	X	X	X		
	HOTCHKISS, Everett L.	A-22-77	X	X			
915632	DAVIS, Grace C.	A-23-77	X	X	X		Photos
	WILLIAMS, Martha A.	A-24-77	X	X	X		

8-340-25

CONTRIBUTOR'S LIST OF PATHOLOGIC MATERIAL
STANDARD FORM 543

Figure 25. Properly prepared SF 543, Contributor's List of Pathologic Material.

41. Standard Form 543 (see fig. 25)

Standard Form 543 will be used in forwarding shipments of pathologic materials to the AFIP. The original and four copies will be included with the shipment and the sixth copy will be retained by the contributor. Letters of transmittal are not required. The items on the form are self-explanatory except for the following:

a. AFIP Accession Number. Furnish AFIP Accession numbers for those cases previously accessioned by the AFIP.

b. Laboratory Number. List cases in numerical sequence by laboratory number and year, with surgical cases listed first and autopsy cases listed last. When a surgical case(s) is sent together with the autopsy case for the same patient, list autopsy number first, followed by the surgical number.

c. Microslides and Paraffin Blocks. Record number under appropriate column.

d. Wet Tissue. Record number of section(s) of tissue.

e. Gross Organs. List gross organs submitted; i.e., heart, brain, lung, breast. Organs which have been cut, such as brain sections, should be listed as tissue and not as gross organs.

f. Miscellaneous. This column will be used to denote that photographs, X-rays (complete series) or kodachromes are being submitted. All available material of this type is pertinent for proper diagnosis.

42. DD Forms 1322 and 1323

See paragraphs 91 and 113, TM 8-300/NAVMED P-5065/AFM 160-19, (Autopsy Manual).

APPENDIX A

STANDARD ITEMS OF SUPPLY

<i>National Stock Number</i>	<i>Nomenclature</i>	<i>Unit of Issue</i>
*3540-00-299-9811	Sealing Iron, Electric, Path. Spec. Bag, Heating surface 1½" × 4" -----	Each
*3540-00-457-2706	Sealing Iron, Heat, for sealing plastic bags, 24" width, motor driven -----	Each
6505-00-100-2470	Acetic Acid, Glacial, USP, 1 lb -----	Bottle
6505-00-104-8000	Alcohol, USP, 1 qt. -----	Each
6505-00-104-9000	Alcohol, USP, 5 gal -----	Each
6505-00-105-0000	Alcohol, Dehydrated, Analyzed, Reagent, 1 pt., (Absolute) -----	Bottle
6505-00-128-5200	Mercury Bichloride, NF, ¼ lb. (Powder) -----	Bottle
6505-00-133-4483	Paraffin, NF, 5 lb. -----	Each
6505-00-133-9920	Phenol, USP, 1 lb. -----	Bottle
6505-00-153-8220	Glycerin, USP, 1 lb. -----	Bottle
6505-00-153-8445	Wax, white, USP, 1 lb. -----	Package
6505-00-290-4433	Paraffin, USP, 1 lb. -----	Package
6510-00-201-4000	Cotton, Purified, Rolled, 1 lb. -----	Package
6510-00-202-2000	Gauze, Absorbent, 36 in × 25 yds. -----	Package
6510-00-204-2000	Wadding, Cotton, Non-Absorbent, 1 lb. -----	Package
6510-00-550-8501	Adhesive Plaster, Surgical, 2 in. × 10 yds. -----	Spool
6530-00-430-5410	Jar, Screw Cap, Ointment, 4 oz., Glass, 12's -----	Box
6530-00-430-5420	Jar, Screw Cap, Ointment, 8 oz., Glass -----	Each
6530-00-430-5436	Jar, Screw Cap, Ointment, 1 lb., Glass -----	Each
6640-00-074-4191	Slide, Microscope, Frosted end, 25×75 mm, 72's -----	Box
6640-00-241-0138	Box, Microscope Slide, Plastic, 5 slide, 144's -----	Package
6640-00-408-9915	Box, Microscope Slide Plastic, 100 slide -----	Each
6640-00-418-9000	Cover Glass, Microscope Slide, 22 mm Sq., ½ oz. -----	Box
6640-00-494-3893	Slide, Microscope, Plain, 25×75 mm -----	Box
6640-00-585-1801	Ice Making Machine, Carbon Dioxide, Disk, Laboratory -----	Each
6640-00-618-0066	Cover Glass Microscope Slide, 22 mm -----	Box
6640-00-618-0067	Cover Glass, Microscope Slide, 22×40 mm -----	Box
6640-00-685-5168	Mailing Case Sec., Microscope Slides, 12's -----	Package
6640-00-889-1594	Box, Shipping, Histopathological Specimens ----- (w/12 4 oz bottles)	Each
6640-00-684-1345	Box, Microscope Slide, Plastic, 25 slide -----	Each
6810-00-136-3000	Potassium Dichromate, ACS, ¼ lb. -----	Bottle
6810-00-141-3595	Sodium Acetate, Trihydrate, ACS, ¼ lb. -----	Bottle
6810-00-299-8153	Sodium Phosphate, Dibasic, Anhydrous, ACS, USP, Reagent, ¼ lb. -----	Bottle
6810-00-817-0353	Formaldehyde Solution, Analyzed, Reagent, 1 lb. -----	Bottle
*7510-00-224-6744	Ink, India, Drawing, Black, ¼ oz. -----	Bottle
*7510-00-297-6655	Tape, Paper, Water Resistant, 3 in. × 120 yds., Roll -----	Package
*8105-00-200-0195	Bag, Plastic, Polyethylene, 24×24 inches. 12's -----	Package
*8105-00-229-8532	Bag, Plastic, Polyethylene, 20×40 inches. 12's -----	Package
*8105-00-299-9800	Bag, Cellophane, Pathological Specimens, Polyethylene Lined, 8×10 inches. 25's -----	Package
*8510-00-299-9801	Bag, Cellophane, Pathological Specimens, Polyethylene Lined, 6×8 inches. -----	Each
*8105-00-299-9802	Bag, Cellophane, Pathological Specimens, Polyethylene Lined, 4×6 inches. 25's -----	Package
*8105-00-299-9817	Bag, Cellophane, Pathological Specimens, Polyethylene Lined, 12×20 inches. 25's -----	Package
*8110-00-178-8280	Can, Screw Cap, Metal, 1 Pint -----	Each
*8110-00-412-4090	Container Assembly, Sample and Specimen, Shipping -----	Each
*8110-00-879-7182	Can, Screw Cap, Metal, 1 Gal. -----	Each
*8115-00-408-9750	Box, Folding, Chipboard, 1½×15½ inches. 144's -----	Package
*8115-00-682-6525	Box, Plastic, Insulated, Meat and Dairy Products, Laboratory Samples -----	Each
*8135-00-271-1446	Tape, Gummed Paper, Kraft, Opaque, Brown 2½ in. -----	Roll
*8135-00-782-7459	Plastic Tubing, Polyethylene, .004" thick, 4 inch Width -----	Roll
*8135-00-782-7460	Plastic Tubing, Polyethylene, .004" thick, 7½ inch Width -----	Roll
*8135-00-782-7461	Plastic Tubing, Polyethylene, .004" thick, 12 inch Width -----	Roll
*8315-00-958-0744	Tape, Textile, 1 inch Width -----	Roll
*9330-00-684-1823	Plastic Sheet, Cellulose Nitrate, Opaque, White, 0.010 in. Thick, 20×50 in. -----	Each

*Identifies those stock listed items which are found in non-medical Federal Stock catalogs.

APPENDIX B

NONSTANDARD ITEMS OF SUPPLY

1. General

The nonstandard items mentioned in this manual are commercial products which are used by the AFIP after tests have proven them to be most satisfactory for the purpose intended. These items may be procured through most laboratory equipment, laboratory supply, or plastic engineering companies. The names of such companies can be ascertained by consulting the yellow section of any telephone directory.

2. Microslides and Labels

a. The cardboard microslide holders are available in sizes suitable for one, two, six, and ten slides. It is recommended that no larger than the six-slide type be used for mailing or shipping microslides. These holders may be procured through most laboratory supply companies.

b. A self-adhering microslide label is used at the AFIP. This label is made of 10-point white card stock with a self-adhesive (rubber base) backing. These labels may be secured in microslide label size ($1\frac{5}{16} \times 1\frac{5}{16}$ inches) or spacer label size ($\frac{3}{8} \times 1\frac{5}{16}$ inches), and may be imprinted with the name of the contributor at a small additional cost.

3. Paraffin Tissue Blocks

a. The nonstandard white chipboard boxes recommended for storing or packing surgical tissue blocks, which are larger than the standard item of supply, are of two sizes; one, $1\frac{5}{16} \times 1\frac{1}{2} \times 2$ inches, and the other $1\frac{5}{16} \times 2 \times 3$ inches.

b. The box, Kraft covered, size $4\frac{3}{16} \times 3\frac{15}{16} \times 1$ inches, has proven to be the one best suited for maintaining and packing paraffin tissue blocks. These boxes may be procured through most local wholesale box and paper companies.

4. Wet Tissue Specimens

a. Most chemicals necessary for the preparation of fixing and preserving solutions are available through the federal supply system. If the type

available is not of sufficient refinement, the desired type can be procured through any laboratory supply company or drug firm.

b. The bag used in packing or maintaining specimens is "Bag, Cotton, White, with 1 Drawstring," sizes 2×2 inches, 2×4 inches, 4×6 inches, 6×8 inches, and 10×12 inches. These are available through most cotton textile companies.

c. Where plastic bags are not available from the Federal supply system, these items may be purchased locally. The nomenclature is "Plastic Bag, Polyethylene, Specimen, .005 Gauge." Various sizes are available from most laboratory supply companies.

d. "Plastic Polyethylene, Lay-Flat Tubing, .005 or .006 Gauge," in widths of 2, 4, 6, 8, or 10 inches, can also be obtained through most plastic engineering companies or laboratory supply companies if stocks are not available from the Federal supply system.

e. A hand-operated electric sealing unit—Sealer, Electric, Hand-Operated, Clamco Hand Electric Sealer (see fig. 14) is satisfactory for routine procedures, such as packing specimens for shipment from one unit to another. Its use is not recommended for permanent storage procedures, due to difficulties encountered in evacuating sufficient air from plastic bags. It is favorably considered because of its simplicity of operation; it is lightweight, transportable, and sufficiently adequate for the requirements of most units.

f. A larger foot-operated electric sealing unit—Sealer, Electric Foot-Operated, Crimpmaster, Model B-T, with 10-inch Jaws and Adjustable Stand (see fig. 15) is essentially the type required to seal specimens in plastic for permanent storage. This model frees the operator's hands for manipulation of the plastic bag so as to properly evacuate the air, which is necessary for proper storage of specimens. The machine requires no special maintenance, is lightweight, easily transferable from one area to another, requires 110-volt current, and requires no special training to operate.

APPENDIX C

CURRENT MATERIALS DESIRED

C1. Aerospace Pathology

a. Autopsy of surgical material from: (a) Aircraft accident victims, (b) individuals who are or have been military or civilian pilots, whether killed in aircraft accidents or not.

b. Autopsy material from deaths caused by aeroembolism.

c. Autopsy material from accidents in hyperbaric chambers.

d. All temporal bones from any individual with hearing or equilibrium difficulties or known middle and inner ear diseases.

e. Autopsy material from personnel on flight status, particularly those who die of non-traumatic causes.

C2. Cardiovascular

a. Case material from all heart tumors; active rheumatic heart disease; rheumatoid arthritis involving heart; vasculitis, acute and chronic; periarteritis nodosa; granulomas of the heart; glycogen disease of the heart; all congenital heart lesions; myocarditis; parasitic heart disease, and idiopathic cardiac hypertrophy; thrombotic thrombocytopenic purpura; all collagen diseases; Whipple's Disease involving the heart; all storage disease involving the heart; all arteritis.

C3. Dental Oral

a. Fibro-osseous lesions of the jaws to include Cherubism.

b. Odontogenic cysts and odontogenic tumors.

c. Primary Oral Fibrosarcomas and Rhabdomyosarcomas.

d. Salivary gland disease and neoplasms.

e. All kinds of salivary gland lesions.

f. Any and all materials relating to Forensic Dentistry, such as: (1) Base histories involving dental identifications in mass disasters, (2) texts of trials involving testimony of an expert witness in Forensic Dentistry, (3) significant clinical photographs of bite marks and stone models, (4) pre- and post-mortem related X-rays, and (5) saliva washing analysis of significance to the case, etc.

Special Requirements—Request block resections of mandible and maxilla with retained teeth and mucosal attachments in animals or humans.

C4. Ear, Nose, Throat

a. Intact larynx from all ages, diseases and non-diseased individuals.

b. Salivary gland diseases and neoplasms.

c. All kinds of salivary gland lesions.

C5. Forensic

a. Autopsy cases in deaths resulting from acute intoxication due to drug abuse.

b. Autopsy cases from sudden, unexpected, or undetermined causes of death in basic trainees.

c. Autopsy material from accidents during deep-sea diving operations.

d. Autopsy material from unusual household accidents.

e. Autopsy material from accidents involving venomous or dangerous marine animals.

f. Unusual missile wounds; bullet emboli, ricochet, blank ammunition, military practice ammunition, cherry bombs, stud guns. (Material to include formalin fixed tissue from wound track, circumstances of injury, technical data on the causative projectile.)

g. Autopsy cases in which death resulted from untoward events in swimmers using self-contained underwater breathing apparatus (SCUBA).

h. Autopsy cases in which death resulted while in confinement or custody.

i. Autopsy cases in which death resulted from environmental haphazards or exposures.

j. Autopsy cases in which death resulted in therapeutic accidents.

Special Requirements—All submissions must include pathologic material consisting of either slides or blocks or formalin-fixed tissue and related records to include investigative information related to history of drug abuse (if applicable) and circumstances of death, report of autopsy and results of toxicological studies. X-rays, photographs of the scene, and any other materials required for

evaluation of a particular case, when available. This includes cases in Forensic Pathology, aircraft accident, vehicular accident, aquatic accident, wound ballistics, toxicology, and medico-legal cases.

C6. Gastro-Intestinal

Special Requirements—All material should be accompanied by appropriate X-rays on a loan basis.

C7. Genitourinary

- a. Hamartomas of the GU tract.
- b. Renal cases of Wegener's granulomatosis, scleroderma, periarteritis nodosa, etc.
- c. Renal transplants.
- d. Testicular tumors in children.

Special Requirements—(1) Kidney—Paraffin blocks for thin sections are required. Material will be accepted for electron microscopy and fluorescent microscopy. Instructions will be sent on request. (2) For the co-operative Study of Testicular Tumors, the remaining wet tissue should accompany slides and paraffin blocks.

C8. Geographic Pathology and Infectious Diseases

- a. All material regarding mycotic diseases and particularly those which develop in immunosuppressed patients.
- b. All materials relating to tropical and parasitic diseases, particularly those presenting diagnostic problems clinically or histopathologically.
- c. Any specimens representing any of the immunodeficiency diseases.
- d. Any case materials on the following: (1) Bancroftian filariasis, (2) glanders, (3) leptospirosis, (4) melioidosis, (5) onchocerciasis, (6) plague (Pneumonic or Bubonic), (7) relapsing fever, (8) babesiosis-piroplasmiasis, (9) bartonellosis, and (10) septicemic diseases.

Special Instructions Early autopsy; for small blocks of tissue in 20x volume of fresh buffered neutral refrigerated formalin and change solution whenever it becomes discolored by blood. Fix some small blocks in glutaraldehyde or preferably buffered paraformaldehyde* for electron microscopy.

*Reference: Recent Improvements of Histologic Techniques for the Combined Light and Electron Microscopic Examination of Surgical Specimens. Lynn, J.A. Martin, J.H. and Race, G.J., Amer. J. Clin. Path., Vol. 45:704-713, 1966.

Geographic Zoonoses

Glanders (Equine)

Hematologic & Lymphatic

a. Hematologic material for use in proposed circulating study set on hematology. (Special instructions: 30 slides of peripheral blood and/or bone marrow smears stained with Wright's stain and brief clinical history including pertinent laboratory data).

b. Lipid storage and histiocytic disease (Gaucher, Niemann-Pick, Metachromatic Leukodystrophy, Fabry's disease and Tay-Sachs). Request formalin fixed tissue as well as touch preparation from surgical and autopsy material. (AFIP can send containers to contributor to get fresh frozen material.)

c. Touch-imprint preparations of lymph nodes and spleen on hematologic cases. Cases should also include pertinent hematologic data, films of the peripheral blood and bone marrow, unstained film of bone marrow for iron stains, and sections of the bone marrow particles.

Special Requirements—(Reticulo-endothelial System)—Include hematologic data, serum proteins and fractions, peripheral blood smears, unstained bone marrow smears, particle sections and blocks, if applicable and available.

Hepatic

a. Liver biopsy or autopsy material (slides, blocks and/or wet tissue) from documented or suspected cases of Wilson's disease (hepatolenticular degeneration) in all age groups.

b. Primary neoplasms of the liver, benign or malignant, in children.

Special Requirements—Case material for a above is to be accompanied by family history, clinical and laboratory data. Facilities for quantitation of hepatic copper stores in material obtained by percutaneous, surgical or autopsy material are available at AFIP and interested pathologists should contact us for special-washed containers and instructions for handling and shipping of this material. Case material for b above must include slides, blocks and/or wet tissue, clinical and laboratory data and operative findings. Finally, serum for hepatitis-associated antigen testing and antimitochondrial, anti-smooth muscle and antinuclear antibodies from patients from whom hepatic biopsy material has been accessioned, should be frozen (dry ice) and shipped Air Express.

Obstetrics, Gynecology & Breast

- a. Interstitial cell tumors in children.
- b. Functioning ovarian lesions of all types.

Ophthalmic

Whole eyes obtained post-mortem from patients dying of systemic diseases in which ocular or neuro ophthalmologic manifestations were known to have been present during life.

Special Requirement—Enucleated eyes obtained surgically or at autopsy should be unopened and intact.

Orthopedic

All lesions of bones and joints, cysts, and odontogenic tumors.

Special Requirement—All cases must be accompanied by appropriate X-rays which will be copied and returned.

Pediatric

- a. Surgical or autopsy slides, blocks and/or wet tissue from documented cases of genetic diseases, both those due to chromosomal aberrations and those due to point mutations.
- b. Gonadal dysgenesis of all types.
- c. Testicular biopsies for infertility and chromosome abnormalities.

Pulmonary & Mediastinal

- a. Uncommon lung tumors, such as carcinosarcomas, blastomas, lymphomas, and bronchial adenomas.
- b. Mesotheliomas of all types with an occupational history relating to asbestos exposure.
- c. Primary mediastinal tumors of all types.
- d. Uncommon interstitial lung diseases (eosinophilic granuloma, lymphoid interstitial pneumonia, Wegener's granulomatosis, etc.).
- e. Pneumoconioses of all types (with a detailed occupational history) and other inhalational diseases (byssinosis, bagassosis, etc.).

Special Requirements—Wet tissue, or if not available paraffin blocks, should be submitted with all cases, since the evaluation of several special stains is usually required for diagnosis. All case materials must be accompanied by appropriate X-rays on a loan basis.

Radiation Pathology

- a. Slides, blocks and tissue on irradiation changes in brain and spinal cord.
- b. Slides, blocks and tissue on irradiation changes in stomach and intestine.

Special Requirement—The complete dosimeter data and accurate dose information, size of field, and circumstances of radiation therapy or exposure must be included.

Skin & Gastrointestinal Pathology

- a. All types of dermatitis and dermatoses.
- b. Eosinophilic gastritis.
- c. Malignant melanoma in children.

Special Requirement—Material must include a complete clinical history, a detailed clinical description, surgical pathology report, a complete set of slides and paraffin blocks, and previous relevant material. Material poorly documented will not be accepted. Clinical pictures, wet tissue and epon embedded material are desired.

Soft Tissue Pathology

Soft Tissue tumors, benign and malignant: Epon embedded material for electron-microscopy.

Special Mycobacterial Diseases

- a. Biopsy material on leprosy or suspected leprosy.
- b. Biopsy or autopsy material on proved atypical mycobacterial lesions.
- c. Biopsy or autopsy material on mycobacterial disease—non-tuberculous, non-pulmonary.

Tissue Reaction To Drugs

- a. Breast lesions from women taking oral anti-ovulants.
- b. Cases of thrombo-embolism in women receiving oral contraceptives.
- c. Cases (surgical and autopsy) in which drug-induced disease is suspected or proved, and deaths due to drug overdose. Material must include slides, blocks and/or wet tissue, a detailed drug-history (dates, dosage, rate of administration and duration of therapy) and clinical and laboratory data.
- d. Renal and vesical lesions induced by drugs.
- e. Cases of drug reactions in the pediatric age group, including congenital anomalies that might be due to maternal medication during pregnancy.
- f. Adverse drug reactions in the cardiovascular system.
- g. Burn patients with unexplained lung or CNS lesions.

Veterinary Pathology

- a. Animal diseases of comparative significance.
- b. Ophthalmic neoplasms.

APPENDIX D

SURGICAL SPECIMENS NOT DESIRED BY BY THE ARMED FORCES INSTITUTE OF PATHOLOGY

Normal Tissues

Skin and Dermal Appendages

- Simple scars
- Sebaceous cysts (except those in oral cavity)
- Simple lipomas of skin and subcutaneous tissue (except those in oral cavity)
- Pilonidal cysts and sinuses
- Fibrous polyps and tags of the skin
- Ordinary nevi in adults (except those in oral cavity)

Ear, Nose and Throat

- Tonsils and adenoids
- Nasal allergic mucous polyps
- Chronic allergic rhinitis and sinusitis

Gynecology, Obstetrics and Breasts

- Cervical and endometrial biopsies showing no important pathologic change
- Ordinary leiomyomata of the uterus
- Fibroadenomas of the breast
- Papanicolaou smears without biopsy tissue
- Material from uncomplicated abortions
- Mastectomy without biopsy or residual tumor

Hematologic and Lymphatic System

- Smears of normal peripheral blood and bone marrow
- Negative LE preparations
- Bone marrow smears with minor abnormalities; e.g., eosinophilia

Pulmonary and Pleura

- Lungs resected for proven tuberculosis
- Lungs resected for bronchiectasis
- Negative biopsies

Cardiovascular System

- Varicose veins

Gastrointestinal System

- Appendices
- Hemorrhoids and anal tabs
- Segments of intestines showing no significant change
- Segments of stomach removed because of a duodenal ulcer
- Hernial sacs

Central and Peripheral Nervous System

- Normal nerves and sympathetic ganglia

Orthopedic Specimens

- Sequestra
- Chondromalacia of patella
- Single small skeletal osteochondromas (except those in oral cavity)
- Digits, arms and legs amputated for arterio-sclerotic gangrene
- Intervertebral disks and bone fragments
- Torn menisci from knee joints
- Small bone fragments from fractures
- Nonspecific inflammation of muscle, traumatic

APPENDIX E

REFERENCES

ARMY

- AR 40-31 MEDICAL SERVICE, The Armed Forces Institute of Pathology and
Armed Forces Histopathology Centers
- AR 40-441 MEDICAL SERVICE, Joint Utilization of Certain Armed Forces Med-
ical Laboratory Facilities
- TM 8-300 Autopsy Manual

NAVY

- ~~BUMEDINST 6200.1D~~ ^{*cancel*} MEDICAL SERVICE, Joint Utilization of Certain Armed Forces Med- ^{*served its purpose.*}
ical Laboratory Facilities - *CANC. by BM NOTE 5215 of 4 JAN 90*
- BUMEDINST 6510.2 ~~NF~~ MEDICAL SERVICE, The Armed Forces Institute of Pathology and
Armed Forces Histopathology Centers
- NAVMED P-5065 Autopsy Manual

AIR FORCE

- AFM 160-19 Autopsy Manual
- AFR 160-55 MEDICAL SERVICE, The Armed Forces Institute of Pathology and
Armed Forces Histopathology Centers
- AFR 160-109 MEDICAL SERVICE, Medical Investigation of Aircraft Accident Fa-
talities
- AFR 161-40 MEDICAL SERVICE, Joint Utilization of Certain Armed Forces Med-
ical Laboratory Facilities

VETERANS ADMINISTRATION

- M2 PART VI DEPARTMENT OF MEDICINE AND SURGERY MANUAL, Pa-
thology and Allied Sciences

The proponent agency for this regulation is the Office of the Surgeon General. Users are invited to send comments and suggested improvements on DA Form 2028 (Recommended Changes to Publications and Blank Forms) direct to HQDA (DASG-PSG-L) WASH, DC 20310.

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