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□ TECHNICAL METHODS

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Applications of clinical laboratory tests to the autopsy

A practical guide for specimen collection*

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ABSTRACT Clinical laboratory tests performed on autopsy specimens have become of increasing legal and clinical importance. Methods of specimen collection and handling, as well as selection of tests, are discussed.

The hospital autopsy is usually conceptualized by pathologists and clinicians alike as a thorough gross and microscopic examination of a body that eventually produces a neatly typed list of gross and microscopic diagnoses and an anatomic cause of death. With the exception of blood and lung cultures, few hospital pathologists collect specimens at the autopsy that can be used in clinical laboratory tests. This state of affairs is sad indeed when one considers the sophisticated determinations made on living patients and the possibility of further follow-up data. In addition, it is not unthinkable in today's litiginous atmosphere that a hospital pathologist might be asked a serum drug level or the distribution of a drug determined at autopsy. Specimens for chemical analysis can be collected at the autopsy with a minimum of additional time and effort expended, and when the finality of the autopsy is considered, a few extra minutes expended may answer many future inquiries from clinicians and attorneys alike.

Many common clinical laboratory tests can be performed and interpreted on samples obtained at necropsy. Vitreous humor and large quantities of cerebrospinal fluid, bile, and whole organs not obtainable during life are also readily procured at the time of a postmortem examination. As with specimens obtained from living patients, all examples should be placed in properly labeled containers; a chain-of-custody must be maintained in all medi-

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FIGURE 1
The apex of the heart is reflected cephalad, and blood is drawn through a 12- or 14-gauge needle from the inferior vena cava. The area of puncture is sterilized with a heated spatula.

colegal cases, and specimens should be stored frozen and, when necessary, shipped to reference laboratories in that frozen state.

Before beginning a postmortem examination, it should be remembered that specimens from the deceased may still be in the clinical laboratory. These specimens may be of great value and should therefore be retained by the pathologist or laboratory supervisor.

The following is intended as a practical outline, and the bibliography should be consulted for details.

BLOOD

There are many methods advocated for obtaining blood postmortem. In our experience, the easiest method for obtaining large quantities of blood in a sterile fashion is by opening the pericardium, reflecting the apex of the heart cephalad, and sterilizing the inferior vena cava with a red-hot spatula or soldering iron. Blood is then drawn through a sterile 12- or 14-gauge needle into a 50-cc syringe (Fig. 1). (Smaller-gauge needles tend to clog; we have never clogged a large bore needle.) The needle is then changed to a sterile 18- or 21-gauge needle, and blood cultures are inoculated. The remainder of the sample is injected into evacuated blood collection tubes, and more blood is drawn from the inferior vena cava if needed.

TABLE 1.

Useless Determinations on Postmortem Blood

1. CBC
2. Electrolytes
3. Enzymes
4. Glucose
5. Coagulation studies

Since blood coagulates and then reliquifies at an unpredictable rate following death, coagulation studies, hematocrit, and cell count determinations are meaningless. Hemolysis begins shortly after death, rendering electrolyte and enzyme determinations meaningless. Hepatic glycogenolysis continues for some time postmortem, and glucose determinations do not reflect blood levels at the time of death (Table 1).

Postmortem blood is useful for cultures and yields meaningful values comparable to those obtained antemortem for serum protein electrophoresis,[1] BUN, and creatinine.[2] Cholesterol and triglyceride

FIGURE 2
Vitreous humor is obtained from the lateral canthus of the eye. The tip of the needle is visible through the pupil.

values are comparable to those obtained in living, nonfasting subjects, but in our experience patterns of lipoprotein electrophoresis are indistinct on postmortem sera. Blood is of paramount importance in all toxicologic studies, and postmortem sera can be used for serologic studies (Table 2).

VITREOUS HUMOR

The posterior chamber of each adult eye contains approximately 2 ml of clear, colorless vitreous humor, which is a low protein-content ultrafiltrate of plasma. The vitreous is in equilibrium with the plasma with regard to low molecular weight solutes. Since the eyes are protected by their anatomic location, the vitreous humor is frequently preserved when blood or urine is not.

Vitreous is obtained by inserting an 18- or 21-gauge needle, attached to a 10-cc syringe at the lateral scleral canthus, and by slowly and gently aspirating the fluid (Fig. 2). The vitreous may be replaced with an equal volume of water or saline for cosmetic reasons if desired by the morticians. In bodies where the eyes have desiccated and collapsed, we have been able to recover drugs by injecting 2–3 ml of saline into each eye and aspirating after 30–60 minutes. Vitreous, even when appearing crystal clear, should be centrifuged prior to analysis to prevent clogging of sampling tubes in instruments.

Vitreous is useless for protein electrophoresis, lipoprotein determinations, and bilirubin levels (Table 3). Vitreous potassium levels rise gradually

TABLE 2.

Uses of Postmortem Blood

1. Cultures
2. Serum protein electrophoresis
3. BUN, creatinine
4. Cholesterol and triglycerides
5. Toxicology and therapeutic drug monitoring
6. Bilirubin
7. Serology
8. Hemoglobin electrophoresis
9. Certain hormones

TABLE 3.

Useless Determinations on Vitreous Humor

1. Enzymes
2. Protein electrophoresis
3. Cholesterol, triglycerides
4. Bilirubin

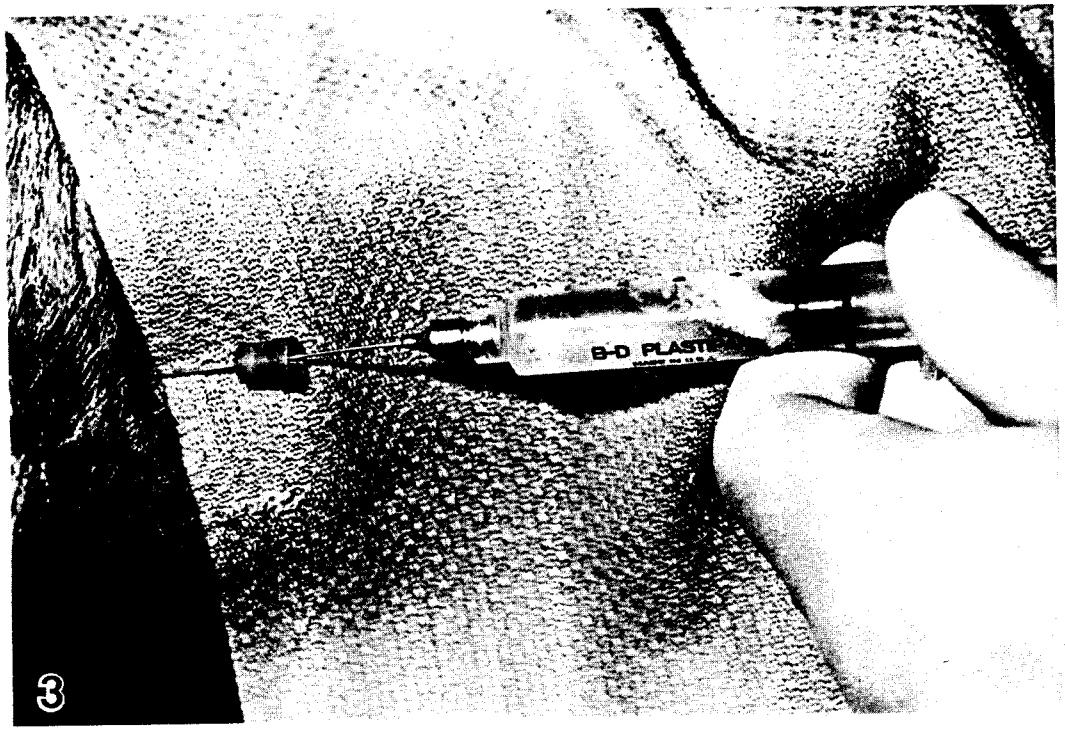

FIGURE 3
Cerebrospinal fluid is easily obtained from the cisterna magna.

FIGURE 4
Larger quantities of cerebrospinal fluid are available if an anterior tap of the lumbar cistern is made.

in the first 12 hours following death, and the postmortem interval may be roughly estimated by using the following formula:

$$T = \frac{V_K - 3.4}{1.7}$$

Where T = postmortem interval in hours, and V_K = vitreous potassium in meq/L. A more accurate estimation of postmortem interval is possible if specimens from each eye are drawn several hours apart, and a more complicated calculation employed.[3] Sodium, chloride, BUN, and creatinine levels in the vitreous change little following death and reflect antemortem values.[2] Vitreous glucose decreases in an unpredictable fashion following death, but high vitreous glucose levels are indicative of antemortem hyperglycemia (Table 4). Ethanol and most drugs can be detected in the vitreous, and the vitreous chemistry is little affected by embalming.[4]

URINE

Urine is of particular importance in toxicologic studies, and inherited metabolic disorders have been diagnosed by using postmortem urine.[5] The easiest route for obtaining urine is via the placement of a Foley catheter. Otherwise we advocate puncture of the bladder with a large-bore needle and using direct visualization, since blind bladder taps may yield fluids other than urine. The bladder in embalmed bodies may contain urine which should be collected.

CEREBROSPINAL FLUID (CSF)

Cerebrospinal fluid is easily obtained through an external tap of the cisterna magna via a spinal needle (Fig. 3). If larger quantities are required, a needle may be inserted anteriorily into the lumbar cistern through the L_2–L_3 interspace before the cranial vault is opened (Fig. 4). Cerebrospinal fluid is a second choice for chemical analysis if vitreous is not available. A "bloody tap" is presumptive evidence of intracranial hemorrhage. If meningitis is suspected, cultures of CSF, obtained in a sterile fashion, may be of great value.

TABLE 4.

Uses of Vitreous Humor

1. Estimation of time of death
2. Sodium and chloride
3. Glucose (if high)
4. Ethanol and drugs (even in embalmed bodies)
5. BUN, creatinine

BILE

Since many drugs and particularly narcotics are excreted in bile, it is of critical importance in toxicologic studies. Bile is easily obtained through aspirating the gallbladder (Fig. 5). If the gallbladder is absent, it is possible to obtain small quantities of bile by aspirating the common bile duct.

GASTRIC CONTENTS

Stomach contents should be preserved *in toto* in all cases of sudden death or suspected poisoning. The distal esophagus and pylorus are clamped with large clamps before removing the stomach, which is then opened inside a large container. Alternatively, the entire stomach can be submitted unopened for toxicologic analysis.

SAMPLES OF ORGANS

It is seldom the case that more than a few grams of any organ are needed for histopathologic examination, but this is not so for toxicology. At least 50 g of lung, the equivalent of one kidney, 200 g of liver, and 200 g of brain should be submitted unfixed and in separate containers for toxicologic analysis. As discussed above, blood, vitreous, urine, and bile should also be submitted (Table 5).

MICROBIOLOGIC STUDIES

We do blood cultures routinely on all autopsies, since the expenditure of time and money is quite small. Lungs and abscesses are also cultured. Postmortem sera can additionally be employed in serologic studies and have been increasingly valuable in the investigation of Legionnaire's disease and other infectious diseases. An excellent and thorough review of postmortem microbiology is found in Koneman's article.[6]

LABELING AND CHAIN-OF-CUSTODY

It is a constant source of amazement that specimens from autopsies are frequently submitted without labels when the laboratory rejects unlabeled speci-

FIGURE 5
Bile is aspirated from the gallbladder before the liver or bowel is removed.

TOXICOLOGICAL EXAMINATION - REQUEST AND REPORT

To: Chief,Laboratory Medicine Service
ATTN: Mr.J.Jones, Toxicology Unit
Clinical Chemistry Section
NNMC,Bethesda, MD 20011

From: Chief, Laboratory Medicine Service
USS Ironsides
FPO New York 09220

SECTION A - MEDICAL REPORT

1. NAME OF PATIENT	2. SERVICE NUMBER	3. AGE	4. SEX	5. RACE
Example, Arthur G.	123-45-6789	24	Male	CAU

EXAMINATION

6. HOUR	7. PLACE	8. DATE	9. TIME & DATE OF DEATH
0940	USS Ironsides	9 Jan 88	0130 hrs,8 Jan 88

RECENT MEDICATION

10. PRESCRIBED OR ADMINISTERED
None

11. IN POSSESSION OF PATIENT
5 ml syringe

12. CONTAINERS FOUND IN PROXIMITY OF PATIENT
None

SPECIMEN COLLECTION

13. HOUR AND DATE

SPECIMEN	AMOUNT	PRESERVATIVE (Preceding preferred)
14a. Liver	200 grams	Frozen
14b. Kidney	100 grams	Frozen
14c. Lung	100 grams	Frozen
14d. Urine	38 ml	Frozen
14e. Blood	15 ml	Frozen
14f.		
14g.		
14h.		

15. SUMMARY OF EXAMINATION AND/OR AUTOPSY (Include all brief history, any routine or special laboratory tests performed, and other pertinent information which may suggest drug or poison ingestion)

A 24 year old male was found comatose in the ship's quarters. A syringe was found near him. Patient expired 15 min. later. The patient allegedly abused drugs.

16. DATE	17a. NAME AND TITLE OF REQUESTOR	17b. SIGNATURE
10 Jan 88	Doctor, Another CDR,USNR,MC	A. DOCTOR

SECTION B - CHAIN OF CUSTODY

	SIGNATURE	ORGANIZATION	HOUR	DATE	CONDITION OF SPECIMEN
18a.	A. Doctor	USS Ironsides	0940	9 Jan 88	Good
18b.					
18c.					
18d.					
18e.					

SECTION C - TOXICOLOGY REPORT

19. LABORATORY: Toxicology
Dept of Laboratory Medicine Service
NNMC,Bethesda, MD

20. DATE: 29 Jan 88

21. CASE NUMBER: 79-10018

LABORATORY ANALYSES

22. GASES: Hydrogen Cyanide; Carbon Monoxide	Blood: less than 1% COHb
23. VOLATILES: Cyanide; Ether; Ethanol; Acetaldehyde; Methanol; Formaldehyde; Chloral Hydrate; Chloroform; Phenols and Cresols; Methyl Salicylate; Common Aromatic Hydrocarbons, (e.g. Benzene, Acetone, etc.)	Ethanol: Blood - NEGATIVE
24. ACIDIC COMPOUNDS: Barbiturates; Salicylates; Diuretics; Acetaminophen; Antipyrene; Tab and Dilantin; Phenols; Theophylline; Caffeine	Liver: No Acid Drugs Detected No Neutral Drugs Detected
25. BASIC COMPOUNDS: Alkaloids, Amphetamine; Alkaloids, Codeine, Morphine and Morphine Derivatives; Antihistamines; Tranquilizers	Liver: Codeine: 30 mcg/ml
26. METALS AND METALLOIDS: Antimony; Lead; Mercury; Silver; Bismuth; Arsenic	
27. CORROSIVES: Sulfuric Hydrochloric and Nitric Acids; Sodium and Potassium Hydroxides and Carbonates	
28. INORGANIC NONMETALLIC COMPOUNDS: Bromides, Fluorides, Borates	
29. SPECIAL ANALYSES: Any analyses not included above which are specifically requested or warranted by case history	Urine: Codeine: 26 mcg/ml Morphine: 0.8 mcg/ml

30. REMARKS
Approximately 5 per cent of "ingested" codeine may be metabolized to morphine in urine.

31. DATE COMPLETED	32. TOXICOLOGIST	33. SUPERVISOR-IN-CHARGE
29 Jan 8	JAMES W. JONES	Dr. S.

S/N 0102-LF-013-7000

FIGURE 6
Completed chain-of-custody form, with toxicology report as an integral part of form. The original is filled out by hand, and a final report is then typed from the original.

TABLE 5.

Samples to Submit for Toxicology

Specimen	Amount
Fluids	
Bile	10 cc or more
Blood	20 cc or more
Cerebrospinal[a]	20 cc or more
Gastric	Entire stomach contents
Urine	Entire bladder contents
Vitreous	Entire contents of both eyes
Organs	
Brain	100 g
Kidney	100 g
Liver	200 g
Lung	50 g

[a] Use if vitreous fluid is not available.

mens from living patients. Specimens from autopsies should be labeled completely and clearly. Perhaps the most confusing area of specimen collection for the nonforensic pathologist is the chain-of-custody. A chain-of-custody *must* be maintained with *all* specimens intended for use as evidence. It is simply a record showing who had a specimen in his or her possession, proves to a court the integrity of a specimen, and that it came from the stated source. We use a chain-of-custody as a specimen catalog and toxicology report. (An example is seen in Fig. 6.)

CONCLUSIONS

Since the autopsy represents the ultimate diagnostic procedure, we feel that the collection of specimens for chemical and microbiologic studies is an integral part of the postmortem examination in every hospital or forensic case. Clinical laboratory studies are not intended to replace careful anatomic studies but are intended to provide complimentary and confirmatory data. The investment of time and money is small, and the information obtained is invaluable. □

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