

SPECIMEN PROCUREMENT AND HANDLING

ORDERING TESTS

A tissue requisition form must accompany all specimens. The following information must be provided:

- \$ Patient=s full name
- \$ Patient=s medical record number
- \$ Patient=s age or date of birth
- \$ Patient=s sex
- \$ Requesting physician
- \$ Indicate clinic (e.g., MOP, ER, OPS)
- \$ Requisition date/time
- \$ Specimen source
- \$ Clinic history (e.g.,: Abdominal pain, Rectal bleed)
- \$ Post-Op Diagnosis (e.g.,: Gastritis, Appendicitis)

A stamped plate is adequate for patient identification

SPECIMEN IDENTIFICATION AND HANDLING

All specimens must be labeled properly with the following required information:

- \$ Patient=s full name
- \$ Patient=s medical record number
- \$ Location (e.g.,: floor, clinic)
- \$ Date/time of collection
- \$ Tissue identification
- \$ Initials of person collecting specimens

After collecting specimen, label the specimen container. The laboratory will reject any specimen that is not properly labeled (See Specimen Rejection Policy). If a specimen cannot be recollected, the person who collected the specimen must properly label the specimen and sign a Specimen Misidentification Form.

SPECIMEN REJECTION POLICY

Proper specimen identification and handling are essential to obtain valid, timely laboratory results. All requisitions and specimens must meet the defined criteria for processing. If any specimen does not meet the criteria, the physician, resident, or nursing staff will be notified immediately so corrective action can be taken.

Criteria for Rejection

Inadequately labeled specimens

1. Unlabeled

Any specimen is considered unlabeled if the specimen container does not have the patient's first and last name, and patient's medical record number directly affixed to the specimen.

2. Mislabeled

A specimen is mislabeled when the patient's name or medical record number differ from the name or medical record number on the tissue requisition form.

3. Improperly/Incompletely Labeled

4. Corrective Action

All specimens that are unlabeled, mislabeled, or improperly/incompletely labeled without exception, should be recollected.

If a specimen cannot be recollected (e.g., surgical tissue, CSF, fluid aspirates) the nurse or other health care worker that can verify the identity of the specimen must come to the laboratory to properly label the specimen and sign a Misidentification form accepting responsibility for the laboratory's performing a test on a unlabeled or mislabeled specimen.

This process of clarification will cause a delay in the processing of the tissue and the issuance of the Final Report, and **MAY COMPROMISE PATIENT CARE.**

SPECIMEN DELIVERY POLICY

University Medical Center Operating Room

1. Routine Specimen

All routine specimens should be placed in formalin containers and properly labeled. The specimen and completed tissue requisition must be brought to the Pathology door and left on the stainless steel table. A specimen label must be placed in the book on the stainless steel table. This may be done 24 hours a day, everyday. Specimens picked up by 4:30PM will be processed that day.

2. Fresh or Frozen Specimens

A. Working Hours

Enter the Pathology door and ring the bell on counter. Wait for the technician or physician to accept the specimen and requisition. **DO NOT** leave the tissue without notifying the Pathology Personnel.

B. After Hours

If an after hours specimen is anticipated during working hours, notify Anatomic Pathology (3-2155). Should this occur late night or weekends/holidays, page the Anatomic Pathologist on call through the UMC operator.

3. Fresh Amputations and Large Fresh Specimens

A. Pathology door is locked after hours. The key is on the OR Supervisor=s key ring.

B. Walk specimen through pathology to the gross room and place the labeled and wrapped specimen in the lower left drawer of the stainless steel refrigerator.

C. Leave the specimen requisition on the stainless steel table in the OR and write "Specimen in Refrigerator" on requisition.

SPECIMEN DELIVERY POLICY (continued)

All other departments and TTUHSC Clinics

1. Routine Specimens

A. All specimens should be placed in the sealable compartment of a Double Lumen bag. Place completed tissue requisition in second outside compartment, not in some compartment as specimen, which may be contaminated or may leak formalin.

B. Working Hours

1. Deliver during working hours to Anatomic Pathology, Room 1A115, prior to 4:30PM each working day.
2. Laboratory pickup is available through the Clinical Laboratory (Page 104-573). The tissue must be delivered prior to 4:30PM for processing that day.

C. After Hours

Deliver to Clinical Laboratory [open 24 hour]. The specimen will be processed the following business day.

2. Specimens requiring special handling

Notify: Anatomic Pathology [3-2155] should be notified during working hours if an after hours specimen requiring special processing is anticipated. **The Anatomic Pathologist “on call” should be paged.**

HANDLING OF MATERIAL LEAVING DEPARTMENT FOR REVIEW

The Attending physician should make requests to Anatomic Pathology

Give one-day notice; if possible, when patient is to hand carry material or physician is to pick up in the Department; proper identification, and a request letter from physician will be required.

Please supply the following information:

§ Patient name and medical record number

- § Date of procedure or Surgical Pathology Report number
- § Attending Physician name
- § Destination of material: Address and Name of receiving physician
- § Method of delivery (by mail or pick-up)

If a request for outside consultation is made after the admission during which the procedure was done, a Release of Information form signed by the patient is required.

We will request the return of all borrowed material from other institutions. Prompt return of slides reviewed outside the Anatomic Pathology Department is requested. If not promptly returned, the requesting Attending physician will be contacted.

TISSUE/MATERIAL TO BE RETURNED TO PATIENT

Usually requested: Gallstones, orthopedic implants, pins, etc.; amputated legs for burial.

We will return any tissue, stone, etc. to a patient on request after it has been grossly examined and appropriate sections taken for diagnosis as necessary. However, specimens are routinely disposed of two week after the final report is issued.

NOTE: Immediate dictation of Agross-only@ specimens at the time of the procedure such as hardware and gallstones will be done on request so that the specimen can be returned immediately to the patient. OR personnel should come to Anatomic Pathology, request the dictation, and wait for the return of the specimen; this only takes 2-3 minutes.

Request that the specimen be saved for the patient on the tissue requisition and it will be set-aside after processing. The attending physician will be notified that he or a designee can pick up the specimen.

ROUTINE SPECIMENS

All routine specimens should be submitted in 10% neutral buffer formalin to the Anatomic Pathology Laboratory.

SPECIMENS FOR SPECIAL HANDLING

The following pages contain specimens that require special handling.

BREAST BIOPSIES; MASTECTOMIES FOR MALIGNANT TUMOR

Breast biopsies must be evaluated fresh for evidence of tumor by gross, touch prep and/or frozen section examination.

Tissue for estrogen and progesterone receptors and DNA analysis is collected at this time.

Mastectomy specimens are evaluated in the same manner, even if tissue for receptors has previously been harvested from biopsy material. Initial receptors may fail due to extensive tissue necrosis, and additional tissue may need to be harvested.

Mastectomies may also be done occasionally without a tissue biopsy in cases of overt tumor or a fine needle aspiration diagnosis of cancer. In each of these cases, tissue for receptors and/or DNA analysis will need to be collected.

CARDIAC BIOPSIES

Cardiac biopsies are helpful in looking for cardiomyopathy and infectious disease

Minimum volume 3 segments measuring 0.2 cm in diameter each

Specimen Requirements

1. Cardiac biopsies need to be scheduled in advance if possible. **You may schedule a cardiac biopsy by calling Anatomic Pathology [3-2155] and speaking to the pathologist on duty.**
2. Send the fresh, unfixed biopsies to Anatomic Pathology as soon as possible. The surgical pathologist will then divide the specimen for the following tests:
 - 11.1 Light Microscopy, submitted in formalin
 - 11.2 Electron Microscopy, submitted in 3% gluteraldehyde
 - 11.3 Cultures, submitted fresh

Consultation

If an outside consultation is needed, the slides will be sent to Armed Forces Institute of Pathology (AFIP).

CERVICAL CONE BIOPSIES

Specimen Requirements

Cervical cone biopsies require:

1. Quadrant orientation
- 1.2 Pre-fixation pinned flat rather than in the original cone shape

Deliver the fresh cone with requisition to pathology:

1. If intact, a suture should mark 12:00 or elsewhere, with the site of the suture noted on the requisition form. The cone will then be opened and pinned flat for fixation. The margins of cold cones will be inked.
1. If in pieces, the tissue should be oriented. Radial orientation on a telfa pad is preferred.

Note: A multipiece cone cannot be oriented as to the quadrant, nor can it routinely be oriented so as to sample, both endocervical and ectocervical lines of excision.

CYTOGENETICS

Submit fresh, unfixed specimen to Anatomic Pathology as soon as possible.

The pathologist will contact the Cytogenetics Department upon arrival of specimen.

ESTROGEN AND PROGESTERONE RECEPTORS, HER2^{neu} SPECIMENS

Use Some tumors are responsive to hormonal influences, and clinical prognosis/treatment may be affected by the identification of certain hormone receptors in tumor tissue. Tissue for receptors is most often collected/evaluated pathologically for confirmed breast carcinomas, but tissue from endometrial carcinomas and potential breast metastases may also be evaluated.

Minimum Volume 0.5cm in diameter, fresh unfixed tumor

Specimen Requirements

- 1.1 The entire unfixed, fresh tissue specimen (e.g., breast biopsy, mastectomy) should be transported immediately to Anatomic Pathology Laboratory and given directly to laboratory personnel.

- 1.2 The tissue requisition should be completed as usual; however, AFor estrogen and/or progesterone receptors@ should also be written on the form.

Processing

1. The tumor/suspected tumor tissue will be snap frozen at the time of submission.
2. After confirmation of the diagnosis of breast carcinoma, the tissue will be submitted to Quest Diagnostic Laboratory for receptor analysis.

Reporting

A separate Estrogen/Progesterone Receptor report will be issued, and should be in the patient=s chart within 5-7 days. A copy of the report will be available in the Anatomic Pathology Laboratory.

Additional Information

There are times when there is an insufficient volume of malignant fresh tissue for routine estrogen/progesterone receptors. Quantitative in-situ receptor analysis, done by an immunoperoxidase method on formalin fixed tissue sections, is presently available from an outside laboratory and has a 90% correlation with standard procedures. This test will automatically be done if the standard procedure fails for any reason. A separate report will be issued.

FETUSES

A fetus, of any gestational age, who has breathed and therefore been considered a live birth, is treated as an autopsy. Gestational age can be estimated only to within about 2 weeks of actual gestational age, even by ultrasound techniques. We therefore use an additional criteria based on weight and foot length to determine the legal status of borderline cases.

Specimen Requirements

- 2.1 In borderline cases:

A fetus above 550-600 gms birth weight and/or with a foot length of more than 4.0 cm will be considered to fall under autopsy rules.
- 2.2 If the fetus falls under autopsy rules:
 - 2.2.1 A completed autopsy permit is required and the evaluation treated as an autopsy. An Autopsy report will be issued. No charge will be made for the autopsy on a patient born at University Medical Center Hospital.
 - 2.2.2 Normal funeral arrangements must be made by the parents. TTUHSC does not have facilities for cremation or disposal of bodies in autopsy cases.

- 2.2.3 The parents may transport the body themselves to a funeral home and avoid transportation costs. A Burial Permit is available in Nursing Administration. It must be carried by the parents or the designee, and must be presented to Morgue personnel when picking up the body.
- 2.3 If the fetus falls under routine surgical pathology rules:
 - 2.3.1 A tissue requisition form must accompany the fetus to Anatomic Pathology. Evaluation closely following the normal autopsy procedure will be followed. The staff will, however, respect any restrictions placed on examination/dissection by the parents.
 - 2.3.2 The parents may wish to bury the fetus. Please notify Pathology if this is the case. A burial permit must be presented when picking up the body in Pathology.
 - 2.3.3 In the absence of other instructions, the fetus will be disposed of in the usual fashion 2 weeks after the issuance of the final Surgical Pathology Report.
 - 2.3.4 If the parent(s) sign a Consent to Dispose of Dead Fetus, Anatomic Pathology will dispose of the fetus in accordance with customary medical practices. All claims to the body are relinquished by signing the Consent to Dispose of Dead Fetus.

IMMUNOPHENOTYPIC ANALYSIS BY FLOW CYTOMETRY

Synonyms Flow

Special Instructions Specimen **must** be received fresh and unfixed

Minimum Volume 0.5 cm in diameter of tumor tissue; more will be needed if tumor necrosis appears extensive

Collection Tissue will be harvested (using sterile technique) from unfixed, fresh tissue specimens received in the Anatomic Pathology Department on appropriate specimens.

The fresh tissue will be saved in RPMI [special medium] and held at room temperature in the Anatomic Pathology Department.

After examination of histologic sections and clinical consultation, the tissue will be submitted for Flow Cytometric analysis as necessary.

Reporting A separate interpretative report will be issued by the Flow Cytometry Laboratory. A copy will be added to the file copy of the Surgical Pathology Report

DNA Analysis (Send-Out) This procedure includes a listing of other neoplasms with documentation of clinical utility for flow cytometric DNA analysis. The prognosis refers to that associated with aneuploidy and/or high S-phase activity.

Since the clinical utility of DNA analysis may vary among tumors and patients, DNA analysis will be done after consultation between the pathologist, operating surgeon and/or oncologist involved in the case.

See the following page for list of common neoplasms. This list is not meant to be inclusive, and information is available in the literature on the other neoplasms. Other uses include adjunctive screening of fluid specimens for cytology [e.g., urine], and use in the discrimination between metastases and second neoplasms.

Two areas in which ploidy analysis are of little clinical use are in tumors of the thyroid and parathyroid. In these organs, there is a high incidence of aneuploidy in both benign adenomas and malignancies. For lymphomas, DNA analysis is of much less importance than is immunophenotypic.

PRESENT INFORMATION ON CLINICAL UTILITY OF FLOW CYTOMETRY

ADULT	Aneuploidy and/or increased S-phase
Breast	Decreased survival [indicator for adjuvant chemotherapy in Stage I, node negative]
Colonic	Decreased survival
Ovarian	Worsened prognosis [epithelial neoplasms]
Endometrial	Worsened prognosis
Bladder	Worsened prognosis, increased risk for disease progression invasion
Lung	Decreased survival in non-small cell carcinoma
Prostate	Worsened prognosis, increased risk for local or systemic progression
CHILDHOOD	Aneuploidy

ALL	Good prognosis [responsive to chemotherapy]
Neuroblastoma	Improved survival
Medulloblastoma	Improved survival

FRESH, UNFIXED SPECIMENS

FROZEN SECTION

These specimens require **immediate** handling and/or processing and should **never** be placed in fixative or left in Anatomic Pathology unattended.

3.If possible, phone Anatomic Pathology [3-2155] during working hours prior to the Frozen section. Please provide the patient=s name and approximate time of procedure.

After hours the Anatomic Pathologist and Resident on call must be paged. The schedule is in the OR main office.

4.Bring the fresh specimen, a completed tissue requisition and the patient chart to the Pathology door in the OR hall [or to Anatomic Pathology, 1A115 if from clinics] and ring the bell in the Frozen Section room. Do not leave until a technologist or pathologist is in attendance. Please provide the OR number on the requisition.

5.The pathologist or resident with the Frozen section report will return the chart to the OR.

6.Medical personnel are always welcome in the Frozen Section Room during the evaluation of the tissue by the Pathologist.

7.If on site viewing of the lesion to be biopsied would be helpful, please request that a Surgical Pathologist be present in the Operating Room.

RESECTIONS OF MALIGNANT OR SUSPICIOUS NEOPLASM

Resections of malignant neoplasms or masses suspicious for malignancy should **ALWAYS** be delivered fresh to Anatomic Pathology.

- 7.1 Fresh tissue is required for DNA analysis by Flow Cytometry, a diagnostic tool being used routinely in many neoplasms and being evaluated in others. [See Flow Cytometry for list of neoplasms].
- 7.2 Special studies requiring fresh tissue is routine for specific neoplasms
- 7.3 Fresh resection specimens can be opened, initially evaluated and then photographed for documentation and teaching purposes.
- 7.4 We request that tumor resection specimens **Not** be opened in the Operating Room because margins may be destroyed. We will show the opened specimen to the operating surgeons either in Anatomic Pathology or in the Operating Room.
- 7.5 If specimen resection will occur **after hours**, please call Anatomic Pathology to alert the Surgical Pathologist or Page the Anatomic Pathologist on call, depending on the hour.

GROSS DIAGNOSIS ONLY

To clarify the list of objects, organic material and sometimes tissue that will routinely be evaluated only grossly without microscopic sections.

Virtually all foreign bodies or tissue removed from the body for legal documentation, in most cases, will be evaluated for pathological processes.

The majority of specimens submitted to pathology undergo gross evaluation and microscopic sectioning to confirm normality, identify tissue removed and/or define pathology. The exceptions are:

7.5.1 Inorganic material and rare organic material unamenable to sectioning.

7.5.2 Tissue where microscopic examination has been shown not to contribute to the gross evaluation based on long national experience.

Gross Only Always

1. Calculi [gallstones, urinary tract]
2. Toenails, fingernails
3. Teeth without significant gum tissue
4. Orthopedic hardware, external or internal

5. Intravenous and other catheters [ventricular shunts, etc.]
6. Abdominal trochars
7. Dorsal column stimulators, batteries
8. Pacemaker batteries [tissue membranes submitted with battery may be sectioned]
9. Foreign bodies

Gross Only Except On Physician Request

- 9.1.1 Foreskin [routine circumcisions in infants]
- 9.1.2 Varicose veins without gross thrombosis
- 9.1.3 Lens [eye]
- 9.1.4 Repeated eschar from burn debridements **ON SURGEON REQUEST** without gross evidence of purulence. A random section may be taken for documentation purposes only.
- 9.1.5 Traumatic amputations
- 9.1.6 Bone from bunions
- 9.1.7 Loose bodies
- 9.1.8 Bone from tibial osteotomies
- 9.1.9 Ribs removed incidentally [thoracotomies, etc.]
- 9.1.10 Nasal cartilage [deviated septum]
- 9.1.11 Acute traumatic fracture fragments [NOT FEMORAL HEAD]

Special Cases:

1. Burn Eschar

Eschar submitted by the surgeon for **Gross Diagnosis Only** will be billed as such in most cases. A representative section will be taken and examined to >catch= the rare case of invasive fungus that may occur without obvious gross change. If more extensive sectioning is required based on initial examination, routine microscopic charges will be billed.

We will gladly examine eschar microscopically on request.

2. Normal placentas

Placentas submitted without clinical indication [See Placentas] will be examined grossly, sectioned and submitted in permanent paraffin blocks

saved without sectioning for use in the future as needed. A gross diagnosis will be issued.

LIVER TRANSPLANT PERCUTANEOUS BIOPSIES

Use Rapid same day processing, review and sometimes outside consultation of repeated liver transplant biopsies after operation is sometimes necessary to identify and evaluate the progress of rejection and to identify infective and obstructive processes.

Minimum Volume 2 cm core

Personnel Involved

- 2.1 Requesting Physician: Gastroenterologist
- 2.2 Performance of liver biopsy: Gastrointestinal Division
- 2.3 Pathologist: Dr. Ruc Manh Tran
- 2.4 Outside consultation

Dr. Jack Demetrius
Presbyterian University Hospital
Department of Pathology
Transplant Division, Room A519
Desoto at O=Hara St.
Pittsburgh, PA 15213
[412] 624-6645 [Ask for Mary Ann]

Surgical Requirements

Pathology must be notified in advance of the biopsy being done so that the processing sequence may be set up on the tissue processor. [Rapid processing allows processing and interpretation on the same day]

3. The percutaneous liver biopsy should be received by 9:30 AM. 12:00PM [noon] is the latest we can start the rapid processing except in cases of emergency. This allows processing, in house interpretation, along with the Gastroenterologist, and packaging for Federal Express for overnight delivery for outside consultation.
4. The biopsy should be delivered to Anatomic Pathology fresh unless CMV cultures are specifically not requested or required.
5. A completed tissue requisition must accompany the specimen.
6. A member of the Gastrointestinal Division must provide a copy of the current Laboratory Value and medication flow sheet.
7. Any amount of biopsy tissue will be processed but the following should be considered:
 - 7.1.1 CMV shell culture [rapid early and late antigen detection; reported at 24 and 48 hours] and full CMV culture: 1 cm core biopsy
 - 7.1.2 Histological examination: 2 cm core biopsy

****Note:** If less than a total of 2 cm core biopsy is received, a decision will be made on optimum division. A regular CMV culture only needs a small amount of tissue, but rapid shell cultures may need to be sacrificed for enough tissue for histological examination.

LYMPH NODES

Use Evaluation of Lymph nodes for decision on proper processing/special procedures needed for diagnosis.

Specimen Requirements

The labeled **sterile fresh** lymphoid tissue with completed requisition should be delivered **immediately** to the Frozen Section Room.

If microbiology tests [bacterial, myobacterial, fungal, viral cultures] are anticipated, a completed microbiology requisition should accompany the tissue. **It is best if a separate specimen is collected and sent directly to microbiology.**

The patient chart must accompany the tissue. A frozen section and/or touch preps may be done, and initial diagnoses/differentials will be entered in the chart.

Special studies will be done based on initial evaluation, and the appropriate additional forms completed by the Surgical Pathologist.

- 7.2 Immunophenotypic analysis [to Flow Cytometry Lab] in suspected lymphoproliferative disorders
- 7.3 Cultures in suspected infectious disorders
- 7.4 Special fixation of tissue for histologic processing
- 7.5 Immunoperoxidase stains for leukocyte markers on the processed tissue
- 7.6 Electron microscopy [occasional cases; to Electron Microscopy Lab]

A specific diagnosis of lymphoma should not be expected based on frozen section tissue; evaluation of permanent sections is usually necessary for the diagnosis of lymphoproliferative disorders.

MUSCLE AND PERIPHERAL NERVE BIOPSIES

Muscle and peripheral nerve biopsies, which require special studies for the diagnosis of neuromuscular disease, are presently being forwarded for processing and diagnosis to

Quest Diagnostic. This is a highly specialized procedure, which requires the coordinated efforts of the operating surgeon, pathologist, and requesting physician [usually a neurologist, neurosurgeon or orthopedist].

Muscle and nerve biopsies are scheduled procedures; **at least 24 hour notification** so staff will be prepared for snap frozen.

Rapid immediate transport to Anatomic Pathology is required

Specimen Requirements

Requisition and Clinical Information:

7.6.1A completed tissue requisition form is required with each specimen

7.6.2Muscle and peripheral nerve biopsies are done by a surgeon at the request of another clinician [Neurologist, Orthopedic Surgeon, other]. This clinician, not the operating surgeon, should provide a detailed clinical summary and clinical or differential diagnosis. A legible handwritten summary or copy of a complete clinical evaluation is acceptable.

7.6.3Clinical summary is required. The biopsy will not be sent without it. Direct conversation with Quest Diagnostic is encouraged in complex cases; the Quest number is available from Anatomic Pathology.

Billing Information

8.Billing for the muscle and/or peripheral nerve biopsy will be done directly by the Quest Clinic. Sufficient information for billing [Insurance, Cerebral Palsy authorization, etc.] must be provided with the biopsy.

9.A complete examination may require light, histochemistry, electron microscopy, immunofluorescence and immunohistochemistry. Other biopsies may not require all these techniques. Charges are available from Anatomic Pathology

Additional Information

Preferred biopsy sites:

Biceps, deltoid, quadriceps, or as specified by the neurologist. Generally from the proximal portion of the muscle.

Note: Avoid muscle that has had EMG manipulation, or severely wasted muscle. Avoid lidocaine infiltration of the muscle to be biopsied [epinephrine depletes muscle glycogen and phosphorylase activity].

Premedication

Sedation such as IV Demerol and Valium

Specimen Collection

9.1.1Local infiltration with lidocaine. **Do not infiltrate the muscle.**

9.1.2Use self-retaining retractor if possible.

MUSCLE AND PERIPHERAL NERVE BIOPSIES (continued)

9.1.3 Take the biopsy from the muscle belly. Sharply dissect a block of muscle parallel to the muscle fibers. **Avoid blunt dissection.**

1. If the muscle biopsy is taken >delicately=, clamps are not necessary. Once removed, the biopsy should be laid on a damp sponge and allowed to relax.

2. Clamp technique; Introduce the lower jaws of the isometric clamp under the dissected muscle. **First close the clamp and then cut off the specimen.** Specimens have been rendered useless by attempting to place the muscle specimen in the isometric clamp after resection from the muscle.

3. Applicator technique; Lay a sterile wood applicator stick parallel to the muscle fibers. Use 2-0 chromic to tie muscle to tick, undermise and put on a second tie. Excise the biopsy.

10. For complete studies, **a biopsy of approximately 1.5x1.0x1.0 cm is adequate.** Smaller biopsies may be taken in the case of small children or atrophic muscle.

Peripheral Nerve Biopsies

10.1.1 The sural nerve, at the ankle, is usually biopsied and should be handled delicately.

10.1.2 **Minimum of 1 cm is adequate.**

PLACENTAS

Use The histologic examination of placentas in selected cases may produce findings that can be helpful in evaluating maternal gestational problems or problems that arise in a newborn.

Examination of the placenta is also used to document the pattern of membranes in multiple gestations.

Indication for Submission

10.2 Maternal

- 10.2.1 Diabetes Mellitus
- 10.2.2 Pregnancy induced hypertension/chronic hypertension
- 10.2.3 Premature rupture of membranes
- 10.2.4 Preterm delivery [less than or equal to 32 weeks]
- 10.2.5 Post-term delivery [greater than or equal to 42 weeks]
- 10.2.6 Unexplained fever
- 10.2.7 Poor previous obstetrics history
- 10.2.8 History of drug abuse, including cocaine

10.3 Fetus/newborn

- 10.3.1 Stillborn
- 10.3.2 Neonatal death
- 10.3.3 Multiple gestations
- 10.3.4 Prematurity [<32 weeks]
- 10.3.5 Intrauterine growth retardation
- 10.3.6 Congenital anomaly
- 10.3.7 Erythroblastosis fetalis
- 10.3.8 Transferred to Neonatal Intensive Care Unit
- 10.3.9 Ominous fetal heart tracing
- 10.3.10 Meconium staining
- 10.3.11 Apgar score [less than or equal to 5 at 1 minute; less than or equal to 7 at 5 minutes]

10.4 Placenta/Umbilical Cord

- 10.4.1 Extensive infarctions [greater than or equal to 25%]
- 10.4.2 Abruptio placenta
- 10.4.3 Placenta previa
- 10.4.4 Abnormal appearance of placenta or umbilical cord [abnormal insertion, knot, etc.]

Submission

Placentas should be placed **fresh** into a red plastic bag and labeled. They should be transported in a second covered plastic container together with paperwork to Anatomic Pathology per usual procedure.

PLACENTAS (continued)

In addition to the completed tissue requisition, a **Placenta Information form must be complete and accompany the specimen.** The form provides additional clinical information and is a check list of the above noted indications.

RENAL BIOPSIES

Renal biopsies are handled by Dr. Ruc Manh Tran. For optimal results, **Dr Tran should be notified 24 hours in advance of the procedure** to allow him or his designee to be present at the site of the biopsy for immediate selection and processing of the biopsy.

Please call Dr Tran, 743-2155, to schedule a renal biopsy, providing the time and site of biopsy and the name of the patient. A renal biopsy Clinical Information form will be completed by Dr Tran and the Clinician at the time of the biopsy.

The Surgical Pathologist on duty will be present at the biopsy in Dr Tran's absence.

The routine slides and special stains will usually be available for review the following workday. The final report will be issued by Dr Tran in 3-5 days after the evaluation of immunofluorescence and electron microscopy as necessary.

URINARY TRACT STONES

Proper handling of urinary tract [renal, ureteral, bladder and urethral] stones to assure accurate chemical analysis.

All urinary tract stones must be submitted unfixed

Urinary tract stones are routinely submitted for Chemical Analysis after gross description, and fixation interferes with chemical analysis.

A Surgical Pathology report with a gross diagnosis will be issued by TTUHSC Anatomic Pathology.

A separate Chemical Analysis Report will be issued by the UMC Clinical Laboratory

DEPARTMENT OF PATHOLOGY
LUBBOCK, TEXAS

TEXAS TECH UNIVERSITY-HSC
SPECIMEN COLLECTION MANUAL

DEPARTMENT OF CYTOLOGY
LUBBOCK, TEXAS

TEXAS TECH UNIVERSITY-HSC
SPECIMEN COLLECTION MANUAL

SPECIMEN PROCUREMENT AND HANDLING:

ORDERING TESTS

A requisition form must accompany all specimens. The following information must be provided:

\$Patient=s full name

\$Patient=s medical record number

\$Patient=s age or date of birth

\$Patient=s sex

\$Requesting Physician

\$Indicate clinic (e.g.,MOP, ER, OPS)

\$Requisition date/time

\$Source of specimen (e.g., sputum, left nipple discharge, cervical smear)

\$Clinical diagnosis

SPECIMEN LABELING REQUIREMENTS:

Each specimen received by the cytology department **must have** a proper label with the following information:

\$Patient=s first and last name

\$Patient=s medical record number

\$Date/time of collection

\$Source of specimen (e.g., sputum, left nipple discharge, cervical smear)

All prepared slides should have Patient=s name written in pencil on frosted end of slide.

All samples should be labeled in the presence of the patient.

Note:See Specimen Rejection Criteria which follows.

SPECIMEN REJECTION CRITERIA:

Proper specimen identification and handling are essential to obtain valid, timely laboratory results. All requisitions and specimens must meet the defined criteria for processing. If any specimen does not meet the criteria, the physician, resident, or nursing staff will be notified immediately so corrective action can be taken.

Criteria for Rejection

Inadequately labeled specimens

1. Unlabeled

Any specimen is considered unlabeled if the specimen container does not have the patient=s first and last name, and patient=s medical record number directly affixed to the specimen.

2. Mislabeled

A specimen is mislabeled when the patient=s name or medical record number differ from the name or medical record number on the requisition form.

3. Improperly/Incompletely Labeled

4. Corrective Action

All specimens that are unlabeled, mislabeled, or improperly/incompletely labeled without exception, should be recollected.

If a specimen cannot be recollected (e.g., CSF, fluid aspirates) the nurse or other health care worker that can verify the identity of the specimen must come to the cytology department to properly label the specimen.

Rejection of specimens after initial processing

1. Grossly bloody
2. Too clotted for adequate preparation
3. Insufficient volume
4. Grossly contaminated with food or medication
5. Not fresh

SPECIMEN DELIVERY POLICY

All cytologic specimens taken between the hours of 8:00AM to 4:30PM Monday-Friday should be delivered to the Cytology Department, located on the first floor of the TTU-HSC in Anatomic Pathology, Room 1A-115.

After-hours or weekends specimens should be delivered to the Clinical Pathology Department.

All fresh, unfixed cytology specimens, such as CSF, urine, sputum, bronchial washing, and pleural fluid should be **delivered as soon as possible** to the Cytology Department, as degeneration of the cells in these samples tends to occur rapidly.

If delivery of a specimen is delayed, fluids must be refrigerated.

NOTE:

All cytology specimens submitted as smears, such as cervical smears or bronchial brushing, are spray-fixed or fixed by immersion in 95% ethanol at the time of the procedure. Once fixed, these samples are stable and may be delivered to the cytology department when convenient.

SUPPLIES AVAILABLE FROM CYTOLOGY DEPARTMENT

- §Cervical Brush
- §Cytolyt7 Solution for non-gyn fixation
- §Slides and slide folders
- §Thin Prep7 Pap supplies
- §Spray fixative
- §Requisitions

BETHESDA (2001) REPORTING

Interpretation/Results

A. Negative for intraepithelial lesion or malignancy

1.1.1 Organisms

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with *Candida* spp
- Shift in flora suggestive of bacterial infection
- Bacteria morphologically consistent with *Actinomyces* spp

- Cellular changes consistent with Herpes Simplex virus (HSV)
- 1.1.1Other non-neoplastic findings
 - Reactive cellular changes associated with
 - inflammation (includes typical repair)
 - radiation
 - intrauterine contraceptive device (IUD)
- A.Epithelial cell abnormalities
 - 2.1.1Squamous cell
 - i.Atypical squamous cells
 - of undetermined significance (ASC-US)
 - cannot exclude HSIL (ASC-H)
 - ii.Low grade squamous intraepithelial lesion (LSIL)
 - encompassing: HPV/mild dysplasia/CIN 1
 - iii.High grade squamous intraepithelial lesion (HSIL)
 - encompassing: moderate and severe dysplasia, CIS/CIN II and CIN III
 - with features suspicious for invasion (if invasion is suspected)
 - iv.Squamous cell carcinoma
 - 2.1.1Glandular cell
 - i.Atypical
 - endocervical cells (NOS or specify in comment)
 - endometrial cells (NOS or specify in comment)
 - glandular cells (NOS or specify in comment)
 - ii. Endocervical adenocarcinoma *in situ*
 - iii. Adenocarcinoma
 - endocervical
 - endometrial
 - extrauterine
 - not otherwise specified (NOS)
- B.Other malignant neoplasms (specify)
- C.Other

UTERINE CERVIX, CANCER SCREENING

Synonyms PAP

Materials Needed Cervix Brush, Clean frosted end glass slides, lead pencil, gloves, speculum (without lubricant), spray fixative, slide folder, Perserv Cyt7

Use Screen women for malignant and premalignant cervical disease

Limitations This technique is most accurate when the squamocolumnar junction is thoroughly sampled, since this is where most cervical neoplasms originate

Specimen Requirements

The bottle of fixative should be open and readily accessible before the specimen is obtained. Cells dry rapidly once they are spread on the glass slide, so the slide must be fixed immediately.

Talcum powder or starch should be wiped from the gloved fingers before the smear is made to prevent obscuring of the cells by this material .

The speculum must be introduced without lubricant, although it can be dipped in saline or water for moistening. Lubricant jelly will mask the cells on the smear.

The presence of bleeding or inflammatory exudate is not a contraindication to the taking of cervical smears. However, these conditions may sometimes result in a unsatisfactory sample, and the patient should be advised that it may be necessary to repeat the study.

All slides must have the patient=s name written in pencil on the frosted end of the slide. Unidentified slides will be rejected by the laboratory.

Collection

ONE SLIDE METHOD

- §Have fixative and glass slide labeled with patient name on it at hand
- §Expose the uterine cervix to view and identify the cervical os
- §Using gentle pressure, insert the long central bristles into the cervical os until the lateral bristles flatten against the ectocervix
- §Maintain gentle pressure and rotate the Cervex-Brush by rotating the handle between the thumb and forefinger 3-5 times to the left and right
- §Transfer the sample to the glass slide with a single paint stroke action;
 - a Apply first one side of the bristles
 - 11.1 Turn brush over
 - 11.2 Then paint the slide again in exactly the same area
- §Apply fixative to the slide immediately, then send slide and completed requisition to the Cytology laboratory

THINPREP7 METHOD

- 1.Expose the uterine cervix to view and identify the cervical os
- 2.Using gentle pressure, insert the long central bristles into the cervical os until the lateral bristles flatten against the ectocervix

3. Maintain gentle pressure and rotate the Cervex-Brush by rotating the handle between the thumb and forefinger 3-5 times to the left and right
4. Rinse the Cervex-Brush into the PreservCyt7 Solution vial by pushing the brush into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the brush vigorously to further release material. Discard the collection device.
5. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
6. Record the patient's name and medical record number on the vial, and the patient information and history on the cytology requisition form.
7. Place the vial and requisition in a specimen bag for transportation to the Cytology laboratory.

Interpretation

1. At TTU-HSC the smears are interpreted using the Bethesda System as of Spring 1993. A Translation Outline is available. A pathologist will be happy to help you translate; please call Anatomic Pathology [3-2155]
2. If endocervical cells metaplastic squamous cells are absent, this is noted on the report at a sub-optimal specimen. Endocervical cells should be present if the entire squamocolumnar junction has been sampled. Their absence signifies a sub-optimal smear.
3. Sub-optimal quality may also be reported if there is significant blood or inflammation present, or the number of the cells present is limited in quantity.
4. A completely unsatisfactory smear is noted
5. Interpretation is enhanced by the screener's knowledge of certain aspects of the clinical history, and accurate completion of the Cytology Requisition form is necessary. If the clinical history of question is complex, please call Anatomic Pathology and ask for the Pathologist on Cytology.

BRONCHIAL BRUSHING/BRONCHIAL WASHING

Material Needed Balanced salt solution [e.g. Normosol], Cytolyt7 solution

Use Bronchial brushing and washing specimens are used as an aid in diagnosing bronchial tree lesions. Usually obtained in conjunction with bronchial biopsies, the combined diagnostic yield of biopsy, brushing, and washing specimens is greater than any of these samples alone. Bronchial brushing and washing specimens are also used in the diagnosis of *Pneumocystis carinii* pneumonia.

Specimen Requirements

Bronchial brushing specimens must be fixed in Cytolyt7 solution immediately. Bronchial washing specimens should be immediately brought to Cytology laboratory or refrigerated.

Collection

Bronchial Brushing

6. Bronchial brushing is performed most frequently by the use of the flexible fiberoptic bronchoscope, allowing the operation to view the primary and lobar bronchi as well as many of the segmented bronchi.
7. Prior to the procedure, the Cytolyt7 vial should be labeled with the patient name, site of procedure (e.g., Left lower lobe) and medical record number.
8. Under bronchoscopic visualization, the area to be sampled is selected and the specimen is taken with a bronchial brush.
9. After the brush is withdrawn, the brush is immediately cut off and submersed into Cytolyt7 for fixation. The vial containing the brush should be delivered to the Cytology laboratory immediately.
10. If brushing are taken from more than one site, properly labeled and use a separate Cytolyt7 vial for each site.

Note: If Cytolyt7 is not available:

The material should be spread on at least two slides by rolling the brush across the slides, then immediately fixed by spraying with fixative. Air drying must be avoided.

Bronchial Washing

- 10.1 This is done by instilling 5-8 ml of balanced salt solution through the bronchoscope and retrieving it with suction into a trap.
- 10.2 If there are two separate bronchial washing, they should be placed in separate traps, and labeled as to side (e.g., right, left).
- 10.3 Bronchial wash specimens should be delivered as soon as possible to the Cytology laboratory. If immediate delivery is impossible, the cells can be temporarily preserved by placing the sample in a refrigerator.

CEREBROSPINAL FLUID

Minimum Volume 1-3 ml

Use Cytologic examination of the cerebrospinal fluid is an important part of a complete neurologic evaluation, particularly of cancer patients with clinical evidence of central nervous system involvement. Malignant cells may be found in the CSF when there is seeding of the leptomeninges by leukemia, lymphoma, metastatic carcinoma or medulloblastoma.

Specimen Requirements

Physicians should try to schedule collection of CSF specimens during the hours of 8AM-4PM, Monday-Friday. CSF specimens collected after hours or on weekends are not recommended. If unavoidable bring specimen to the Clinical laboratory and refrigerate immediately.

A separate vial of CSF specimen should be sent for cytologic evaluation to obtain optimal results.

Processing

The specimen is centrifuged, then slides are made from the sediment. Most often cell blocks cannot be made from CSF specimens because of the small quantity and low cellularity of these specimens.

EFFUSIONS, PARACENTESIS FLUID (PLEURAL , PERICARDIAL, PERITONEAL)

Volume ≥10ml

Minimum Volume 1 ml

Use Cytologic examination of effusion fluids is useful in discriminating between benign vs. malignant effusions.

Specimen Requirements

4. **Do not add formalin or other fixative.** All fluid samples should be sent to the laboratory as soon as possible. However, if a delay is anticipated, most specimens will still produce satisfactory results if kept refrigerated.
5. A separate sample container should be sent for each requested test. In general, the cytology laboratory should receive any remaining fluid after samples for culture, chemistry, and hematology have been taken.
6. It is recommended, but not required that effusion fluids for cytology be collected in a heparinized container (1 ml of 1:1000 per 100 ml of specimen) in order to prevent clotting. However, heparin should not be used in the collection of a sample that will be later shared with microbiology, since heparin may adversely affect the recovery of microorganisms.

Processing

The specimen is centrifuged and cytopins are made from the sediment for a Wright-Giemsa Stain. The remainder of the sediment is placed in PreservCyt7; additional slides are then made and stained with the Papanicolaou Stain (PAP Stain).

If there is sufficient quantity and/or cellularity, a cell block is also made from the sediment.

Interpretation

1. In chronic effusions, cells may undergo considerable degeneration *in vivo*. A second or third tap may be requested if degenerative changes interfere with interpretation.
2. Certain conditions may produce cellular changes that cause interpretive problems. Therefore a history of any of these should be noted on the requisition slip:
 - a. An infarction of any organ adjacent to the cavity containing the effusion
 - b. A liver disease, such as cirrhosis
 - 1.1.1 Radiation or chemotherapy
 - 1.1.2 Connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus)
 - 1.1.3 Traumatic irritation (e.g., chest tube)
 - 1.1.4 Chronic inflammation of very long duration

ESOPHAGEAL OR GASTRIC BRUSHING

Materials Needed Cytolyt7, Two frosted-end slides, spray fixative, balanced salt solution [e.g. Normosol], gloves

Use Brush cytology of suspicious upper gastrointestinal lesions is an adjunct to direct biopsy. In the detection of malignancy, these procedures combined have a higher diagnostic yield than either procedure alone.

Specimen Requirements

- 1.2 Prior to the procedure, the Cytolyt7 vial should be labeled with the patient name, site of procedure (e.g., esophageal, gastric) and medical record number.
- 1.3 After brush is withdrawn, the brush is immediately cut off and submersed into the Cytolyt7 for fixation.

Note: If Cytolyt7 is not available:

The material should be spread on at least two slides by rolling the brush across the slides, then immediately fixed by spraying with fixative. Air drying must be avoided.

- 1.4 The specimen should be transported to the Cytology laboratory immediately.

NIPPLE DISCHARGE, CYTOLOGY

Materials Needed Clean frosted-end glass slides, lead pencil, spray fixative, gloves

Use Nipple discharge is a common complaint of women with both benign and malignant breast disease. A nipple discharge is considered pathologic if it is spontaneous and persistent in a non-lactating woman. Cytology of the nipple discharge is utilized to help rule out ductal carcinoma as the cause of the discharge.

Specimen Requirements

Properly fixed smear, on frosted-end glass slide(s) labeled with patient=s name and left or right.

Procedure

1. Using gentle pressure, express the discharge from the nipple and subareolar collecting ducts. If no secretion appears at the nipple with this gentle compression, do not manipulate further.
2. Allow a Apea size@ droplet of fluid to collect on the nipple
3. Immobilize the breast and using the nipple, smear the material across the glass slide
4. Immediately spray fix the slide. For best fixation, the smearing of the material across the slide and the spraying of the fixative should be accomplished with one motion.
5. Make as many smears as the amount of secretions will allow.
6. **Label slides according to right or left breast**

SPUTUM CYTOLOGY

Materials Needed

Use Sputum cytology is utilized in the screening for cancer cells in patients with abnormal chest X-ray, hemoptysis or chronic cough. In immunosuppressed patients with suspected *Pneumocystis carinii* pneumonia, sputum induced by hypertonic saline inhalation may demonstrate the organism in some cases, avoiding the necessity for fiberoptic bronchoscopy with alveolar lavage and/or transbronchial biopsy.

Specimen Requirements

- 6.1 The specimen must be a deep-cough specimen, and not saliva containing only squamous epithelium from the oral cavity.
- 6.2 The specimen must be submitted promptly to the Cytology laboratory. Delays will result in cellular deterioration and bacterial overgrowth which render the specimen unsatisfactory.

Collection

It is recommended to collect three separate early morning specimens on three consecutive days to maximize the detection of cancer cells.

7. The sputum specimen should be obtained in the morning, soon after the patient awakens
8. The patient should be instructed to first rinse mouth and gargle with water in order to reduce the number of contaminating oral epithelial cells and food particles
9. The patient should then inhale repeatedly to full capacity, and exhale the air with a deep expulsive cough into a collection container; 30 ml of Cytolyt7 should immediately be added.
10. The outside of the container should be disinfected with alcohol after sputum collection

Induced Sputum: This is used in the collection of sputum for *Pneumocystis screening*. Prior to sputum collection, the patient inhales a mist of 3-5% saline generated by an ultrasonic nebulizer for 10 minutes.

Interpretation

- 10.1 Dust cells (carbon-bearing histiocytes) and columnar cells must be present in a sputum specimen in order for the sample to be considered satisfactory. These cells indicate that the sample is truly sputum, rather than saliva.
- 10.2 Sputum improperly fixed, or heavily contaminated with food particles or bacteria are considered unsatisfactory.

URINE CYTOLOGY

Minimum Volume 50 ml

Use Urine may contain cells exfoliated from urethelial malignancies involving the renal pelvis, ureter, urinary bladder or urethra. Urine cytology may also detect cytomegalovirus inclusions in infected newborns.

Specimen Requirements

Cells degenerate rapidly in urine; therefore, urine samples should be transported as soon as possible to the Cytology laboratory for immediate processing. If a delay in transport is unavoidable, addition of equal amount of Cytolyt7 is recommended fixation if specimen cannot be delivered immediately. In the event Cytolyt7 is not available refrigerate specimen until it can be brought to the Cytology laboratory.

Collection

1. Urine cytologies specimens are usually of spontaneously voided clean-catch urine or following instrumentation (catheterization). Voided urine should be collected on **three consecutive days**. Do not catch the first void of the morning, the second void should be collected for optimal results.
2. **If the specimen is achieved by catheterization, it is very important to note this on the requisition slip**

Interpretation

Instrumentation of the bladder (catheterization) will cause sheets of transitional cells to be sheared off of the bladder lining. In the urinary specimen these cell fragments can resemble papillary fragments of transitional cell carcinoma. **Therefore, it is important to note if the specimen is a catheterized specimen** in order to avoid possible misinterpretation of these tissue fragments.

****Interpretive difficulties** may also be caused by artifactual changes induced by urinary tract infection, in dwelling catheters, urinary calculi, local radiation therapy or instillation

of chemotherapeutic agents into the bladder. Therefore, a history of any of these conditions should also be noted.**

FINE NEEDLE ASPIRATION

Materials Needed 22 gauge needle, sterile disposable 10-20 cc syringe, clean frosted-end glass slides, lead pencil, spray fixative, alcohol wipes, 95% alcohol fixative, Thin Prep vials (Cytolyt), sterile container for collection of culture material, cell culture media for flow cytometry

Use Fine needle aspiration biopsies of masses are used in the diagnosis of primary or metastatic malignant disease, as well as the evaluation of benign masses. The aspiration of superficial masses [e.g., breast, thyroid] are routinely performed by a Pathologist or the Clinician; a Cytotechnologist will be present at the bedside to assist in the procedure. In the case of CT directed aspiration of a deep mass, the procedure is routinely done by a radiologist.

Specimen Requirements

The specimen must be properly prepared and properly fixed. This generally requires the assistance of a Cytotechnologist during the procedure.

For scheduling, call 743-2167 [Cytology laboratory]. A Pathologist will usually be available to perform superficial aspirations, but the Clinician should call before-hand to check on availability.

Collection

Deep masses are aspirated by a radiologist with CT or ultrasound guidance. Superficial palpable masses are aspirated by a physician trained in this procedure. The slides are immediately prepared at the bedside by a Cytotechnologist or other trained personnel.

- 2.1 The skin is cleaned with alcohol or iodine
- 2.2 A local anesthetic may be used, but this is generally not necessary for the aspiration of superficial masses
- 2.3 Using sterile technique, the needle is attached to the syringe
- 2.4 Superficial masses are fixed with one hand, between the index finger and thumb
- 2.5 The needle is introduced into the subcutaneous tissue and then into the target. A distinct change in consistency of the tissue is felt when a subcutaneous lesion is entered.
- 2.6 With the needle within the lesion, the tissue is aspirated by drawing the plunger of the syringe backwards. At the same time, the needle tip should be moved back and forth (with short movements) within the target in order to loosen cells from the target tissue.

- 2.7 Without releasing the negative pressure on the syringe, the needle is redirected within the target by withdrawing it a few millimeters (but not beyond the boundaries of the target lesion), then reinserting it at a slightly different angle. At least three needle passes should be performed within the target.
- 2.8 The plunger is allowed to return to the resting position before the needle is withdrawn from the lesion (Otherwise, air will be aspirated through the specimen as the needle is withdrawn, ruining the sample)
- 2.9 A drop or two of the sample is gently squirted onto one pre-labeled glass slide and the material is spread with a second slide. One slide is allowed to air dry while the other is immediately fixed in 95% alcohol or spray fixed. The needle should be rinsed into Cytolyt7 solution.
- 2.10 Presence of a Pathologist or Cytotechnologist allows for immediate evaluation for diagnostic material to establish if the procedure should be repeated.

Interpretation

- 2.1 The accurate interpretation of this type of cytologic specimen is dependent on the close cooperation of the clinician, radiologist, Cytotechnologist, and pathologist. An accurate and detailed clinical history of the patient and radiologic or clinical appearance of the lesion should be available so that an appropriate differential consideration can be made.
- 2.2 An interpretation cannot be made unless the sample is adequate and representative:
 - 2.2.1 Samples taken from the center of necrotic, hemorrhagic or cystic lesions usually do not yield diagnostic material.
 - 2.2.2 Fibrotic lesions may yield only a few cells, which often are non-diagnostic.
 - 2.2.3 The chances of achieving an adequate sample are optimized by sampling a lesion in several locations.
3. The sample must be properly prepared and fixed. This requires immediate processing of the sample at the patient's bedside by trained personnel. The Cytotechnologist should be advised of the suspected diagnosis so that he/she can use the best processing procedure. For example, preparation of at least one air-dried smear is useful in the cytologic evaluation of possible lymphoma.

**DEPARTMENT OF PATHOLOGY
LUBBOCK, TEXAS**

**TEXAS TECH UNIVERSITY-HSC
SPECIMEN COLLECTION MANUAL**

**DIAGNOSTIC LABORATORY SERVICES
LUBBOCK, TEXAS**

**MONTFORD FACILITY
SPECIMEN COLLECTION MANUAL**

ORDERING LABORATORY TESTS:

Laboratory services are available to licensed physicians and other authorized individuals upon written request. All requests for laboratory services, both routine and stat, must be written in the patient's medical record. Test requests are initiated on the patient care unit. A written request/requisition **must** follow all telephone orders within 30 days (CLIA >88).

SPECIMEN PROCUREMENT AND HANDLING:

The blood specimens tested in the laboratory are collected by the nursing staff. They are responsible for accurate patient identification, accurate and complete blood specimen collection, and labeling and special arm banding as required.

COLLECTION OF SPECIMENS OTHER THAN BLOOD:

All non-blood specimens (i.e., urine, sputum, swabs, stool, body fluids, tissue, etc.) are collected by the nursing staff or physicians. To avoid specimen degradation, all specimens should be delivered to the laboratory as quickly as possible.

CRITERIA FOR NOT PERFORMING A VENIPUNCTURE:

The nursing staff are instructed not to draw blood samples from the following:

- §Patients not properly identified
- §Patients indicate refusal to have blood drawn (see TDCJ Policy/Procedure)
- §The arm in which a patient is receiving an I.V. unless specifically directed by the physician; if the arm must be used, the specimen must be drawn well below the I.V. site; Blood Drawn On I.V. Arm must be noted on the specimen and also must be indicated on the accessioning information of that specimen
- §The arm of a patient with a canula, fistula, or vascular graft
- §The arm on the same side as mastectomy surgery
- §The bruised area or site of hematoma unless distal to this area
- §Certain arterial puncture sites or femoral punctures

SPECIMEN LABELING REQUIREMENTS:

Each specimen received by the laboratory **must have** a permanently attached label with at least the following information:

- \$Patient=s first and last name
- \$Date of collection
- \$Time of collection
- \$Initials of the person obtaining the sample
- \$TDJC Identification (ID) number

All samples should be labeled at the patient=s bedside.

Note: See Specimen Rejection Criteria which follows.

SPECIMEN REJECTION CRITERIA:

The following criteria will be used in determining specimen rejection for testing in the STAT Laboratory Services or for testing at an approved reference laboratory. These guidelines will cause immediate rejection and/or delayed analysis upon receipt in the laboratory.

Other rejection criteria may also exist in each section as technologists begin their in depth analysis. If any of these criteria apply to the specimen, the specimen will be set aside and classified either as a AReplaceable@ specimen or ADifficult to Replace@ specimen. The testing personnel will notify the appropriate patient care unit and/or ordering physician.

- \$Unlabeled specimen
- \$Mislabeled specimen
- \$Improperly/Incompletely labeled specimen
- \$Specimen improperly collected and/or preserved
- \$Specimen sample volume not sufficient (QNS) for require test protocol or proper blood to anticoagulant ratio
- \$Specimen inappropriately handled with respect to temperature, timing, or storage requirements
- \$Specimen submitted in syringes with needles attached
- \$Cracked or leaking containers with external contamination (infection hazard)
- \$Specimen submitted on tissue paper, foil, plastic wrap, etc.
- \$Hemolysis

§Lipemia

§Patient not properly prepared for test sample requirement

DEFINITIONS:

AReplaceable Specimen@

The following specimens are considered AReplaceable Specimens@:

§Random blood specimen

§Random urine specimen

§Specimens submitted to the Transfusion Service Department

The laboratory will immediately notify the patient care unit and will explain the nature of the problem and inform them that the test(s) has been delayed due to a rejection problem and that the specimen should be recollected. If after discussion with the patient care unit it is determined that the specimen cannot be replaced or is difficult to replace, then the specimen will automatically be processed according to the ADifficult to Replace@ specimen protocol. If the specimen is to be recollected, see the ARejected Specimen Recollect Procedure@ which follows.

ADifficult to Replace@ Specimen

The following specimens are considered ADifficult to Replace@:

§Timed specimens

§Specimens obtained by

- tap
- catheterization
- surgical procedure
- swabs
- arterial punctures

§Specimens submitted for Microbiology Testing

The laboratory will process the specimen to maintain its integrity and stability (e.g., iced, refrigerated, covered, room temperature, plated as appropriate@ until the problem has been resolved.

The laboratory will immediately notify the patient care unit of the nature of the problem and inform them that the test should be recollected if possible.

If the unit indicates that recollection is not possible, the Director of Technical Operations will determine how to permit processing of the specimen without compromising patient care. A qualifying statement will be annotated to the result report.

REJECTED SPECIMEN RECOLLECT PROCEDURE:

Nurse Collected Specimens

§When a ANurse Collected Specimen@ has been deemed unsatisfactory for testing the technologist will notify the patient care unit. Test request for that specimen should be reviewed by all laboratory sections.

§If the nursing staff or physician determines that a recollect is not possible and the test is not to be performed, then the appropriate specimen rejection protocol, which is to cancel and credit, should be done with documentation of the reason for cancellation and credit.

TURNAROUND TIME AND TEST ORDERING PRIORITIES:

Turnaround time (TAT) is the time elapsed from receipt of specimen within the laboratory until the result is available within the laboratory. This denotes the time during which the laboratory has control of the sample, performs the analysis, and produces the test result. ARequest report time@ refers to the time interval between ordering and receipt of laboratory reports.

ORDERING PRIORITIES:

All testing performed by Diagnostic Laboratory Service/Montford Facility is considered STAT and will be performed according to the following guidelines.

STAT: Designates an ancillary process, study, and/or order to be performed immediately or as soon as physically or technologically possible (less than 1 hour) due to the patient being in a real or potentially life-threatening situation (i.e., studies or services absolutely essential to the immediate medical management of the patient). Receipt of the requested process, study and/or order will result in action within a reasonably short time which may alter either the course of the disease or the patient=s outcome. A table of approved laboratory stat procedures is maintained in the laboratory and is available on each patient care unit. Additional copies may be requested by contacting the laboratory.

CRITICAL/ALERT VALUES:

Critical/Alert values are those test results that indicate a current or imminent emergency. After confirmation of the accuracy of the value, immediate action should be taken to inform the responsible medical personnel of the critical/alert value. The protocol established by the Laboratory Medical Directory requires the laboratory staff to report critical/alert value test results to a person responsible for the care of the patient.

TEST	REFERENCE RANGE		CRITICAL	
		UNITS	LOW	HIGH
CBC				
WBC	3.5-11.0	k/ Φ L	2	40
RBC	4.0-5.2	m/ Φ L		
HGB	12-16	gm/dL	5.0	20.0
HCT	35-47	%	19.8	60.0
MCV	83-92	fL		
MCH	27-34	pg		
MCHC	31-36	gm/dL		
Platelets	150-450	k/ Φ L	30	
Neutrophils	46-77	%		
Lymphocytes	16-43	%		
Monocytes	0.0-10.0	%		
Eosinophils	0.0-6.0	%		
Basophils	0.0-2.0	%		
CHEMISTRY				

**DEPARTMENT OF PATHOLOGY
LUBBOCK, TEXAS**

**TEXAS TECH UNIVERSITY-HSC
SPECIMEN COLLECTION MANUAL**

Calcium	8.5-11.0	mg/dL	6	14
Chloride	94-108	mEq/L	70	120
Creatinine	0.5-1.7	mg/dL		7.5
Glucose	60-110	mg/dL	40	700
Potassium	3.5-5.5	mEq/L	2.7	6.5
Sodium	135-145	mEq/L	120	160
Urea Nitrogen, Blood (BUN)	6-23	mg/dL		40
Magnesium	1.7-3.0	mg/dL	1.5	5.0

TEST	REFERENCE RANGE		CRITICAL	
		UNITS	LOW	HIGH
THERAPEUTIC DRUGS				
Carbamazepine	4-10	Φg/mL		15
Phenobarbital	15-40	Φg/mL		50
Phenytoin (Dilantin ⁷)	10-20	Φg/mL		40
COAGULATION				
PT	11.0-13.0	Seconds		25
INR	<2.0			6
APTT	25.0-36.0	Seconds		70
URINALYSIS				
Color	Yellow			
Appearance	Clear			
pH	5-8			

Specific Gravity	1.005-1.030			
Ketones	Negative			3+
Protein	Negative			
Glucose	Negative			3+
Blood	Negative			
Bilirubin	Negative			
Urobilinogen	0.2-1.0			
Nitrite	Negative			
Leukocyte Esterase	Negative			
URINE MICROSCOPIC				
WBC	<3.0			
RBC	<3.0			
EPI=s	<3.0			
Bacteria	None			

TESTING TRIAGE:

Certain tests are not performed in the laboratory due to lack of space, time, technical difficulty, or volume considerations. Low volume highly technical tests are sent out because the laboratory cannot maintain proper proficiency when performing a test only rarely. Certified laboratories are carefully chosen to perform referral tests.

All requests and specimens for tests or services that are not performed in the clinical laboratory must still go through the stat laboratory for processing and accessioning. This is necessary so that the laboratory has a record of all tests sent out and can verify that the test was done, and can approve payment.

REFERENCE LABORATORIES (UNLISTED TESTS AND PROCEDURES):

Reference laboratories are utilized by TTUHSC-STAT laboratories to provide services and capabilities not currently available in-house.

If a particular test or service is not found in the specimen collection manual, special arrangements can be made with the reference laboratory for test processing. A clinical pathologist may consult with the requesting physician to select the best test or procedure for the particular situation.

If tests are sent to a laboratory in another state, the referral laboratory must have a CLIA license applicable to the specialty or sub-specialty of services requested.

TEST/PANEL	VIAL CODE	MINIMUM SAMPLE SIZE	SPECIAL COLLECTION REQUIREMENTS
BUN	1 red top	3 mL blood	
Calcium	1 red top	3 mL blood	
Carbamazepine (Tegretol ⁷)	1 red top	3 mL blood	
Chloride	1 red top	3 mL blood	
Complete blood count (CBC)	1 purple top 5 mL 3 mL Microtainer ⁷	2 mL blood 1 mL blood 250 Φ L	Mix gently to prevent clotting
Creatinine, serum	1 red top	3 mL blood	
Dilantin ⁷	1 red top	3 mL blood	
Glucose	1 grey top or 1 red top	3 mL blood	Grey top for tolerances and postprandial if analysis is to be delayed over 1 hour
Magnesium	1 red top	3 mL blood	
Phenobarbital	1 red top	3 mL blood	
Potassium	1 red top	3 mL blood	
Prothrombin Time (PT/INR)	1 blue top	4.5 mL blood	Gently mix to prevent clotting. If requesting PT & PTT, minimum specimen requirement is 4.5 mL. If delayed longer 2 hrs, separate plasma and put plasma on ice
PTT	1 blue top	4.5 mL blood	Gently mix to prevent clotting. If requesting PT & PTT, minimum specimen requirement is 4.5 mL. If delayed longer than 2 hrs, separate plasma and put plasma on ice

Sodium	1 red top	3 mL blood	
Urinalysis		2 mL urine	Keep refrigerated

BLOOD GASES, ARTERIAL

Synonyms ABG; Arterial Blood Gases

Applies to Blood Chemistry

Test Includes Measured parameter include pH, pCO₂; calculated parameters include bicarbonate (HCO₃), base excess or deficit (BE), standard bicarbonate, standard base excess, alveolar to arterial oxygen gradient (p_[A-a]O₂)

Laboratory Nursing Service

Availability Daily, 24 hours

Turnaround Time 10 minutes

Special Instructions Transmittal must indicate patient percentage of O₂.

Specimen Adequately heparinized (sodium or lithium heparin) arterial or Aarterialized@ blood sample.

Minimum Volume 3 mL for syringe samples.

Container Plastic syringes (1-10 mL) may be used. Glass is preferred if sample measurement is delayed more than 1 hour.

Collection Specimens are collected by nursing staff. Specimen is placed on ice and tested immediately. Syringe sampling: assemble the following equipment: syringe, on needle (20- and 25- gauge); anticoagulant (e.g., sodium heparin - 1000 units/mL); sterile gauze sponges or cotton; skin antiseptic (e.g., 70% alcohol and Betadine⁷ swab); rubber stopper or needle cap; adhesive bandage. Don disposable gloves as an infection control measure.

Preheparinized glass or plastic syringes are available in blood gas kits from several manufacturers. Syringes preheparinized with liquid heparin should be held vertically, needle up, and the liquid heparin should be pushed into the dead space of the syringe and needle, expelling all air out the dead space. If preheparinized syringes are not used, the following procedure should be used.

Fit a needle into the syringe and draw the anticoagulant into it. Draw 0.5-1 mL. Remove the original needle and replace with a sterile needle. Pull and push syringe plunger several times to coat syringe surface. Holding the syringe with the needle up, expel all liquid heparin into needle cap, taking care not to leave any air bubbles in the dead space of the needle.

Perform the Allen test for collateral circulation to determine the best site for puncture (see patient preparation below). Sites listed in order of preference are the radial, brachial, and femoral arteries. Palpate and visualize the artery. Apply antiseptic to the sampling site. Palpate the site once again, trying to stabilize the artery. Slight hyperextension of the wrist or elbow can be achieved by placing a rolled up towel under the joint; this can aid palpation and stabilization of the artery. Hold the syringe so the bevel of the needle faces upward, keeping the needle at a 25° to 30° angle to the artery.

Storage Instructions Specimen not analyzed within 10 minutes should be stored in an ice-slush mixture (approximately 2°C). Syringe barrel plunger assembly should be sufficiently tight to prevent specimen dilution with the ice-slush mixture.

Patient Preparation Patient preparation includes assessment of peripheral circulation on both arms, (the Allen test). Both the radial and ulnar arteries should be compressed at a level approximately 1 cm proximal to the wrist joint while the patient makes a tight fist for approximately 5 seconds. The patient is then instructed to open the fist in a relaxed fashion. The palmar surface of the hand should be blanched. Release compression on the ulnar artery. The palmar surface should flush within 5 seconds. Prolonged delay before flushing indicated decreased ulnar artery flow. Radial arteries lacking collateral ulnar circulation should be avoided as puncture sites if possible. If the radial artery is unsuitable as a puncture site, the brachial artery is the second choice, followed by the femoral artery. If the need for repeated measurements over several days exists, placement of an arterial catheter is indicated. The skin over the puncture site should be swabbed, first with a Betadine7 solution, followed by an alcohol swab.

Aftercare The puncture site should be compressed for a minimum of 5 minutes, longer if the patient is taking anticoagulant therapy, aspirin, or has a prolonged prothrombin or partial thromboplastin time. After 5 minutes, the puncture site should be inspected for several seconds to ensure that clotting has taken place. During this inspection, palpate the pulse proximal and distal to the puncture site to assess the presence of arterial spasm. Notify physician if spasm continues after removing needle from artery. A sterile bandage should be placed over the puncture site to keep the puncture site clean while healing. **WARNING:** A bandage is **not** a substitute for compression of the puncture site.

Causes for Rejection Large air bubbles will cause all values to be erroneous, the magnitude of the error will be determined by the size of the air bubble, sample and sample air bubble interface, length of time bubble was in contact with sample before analysis and the gradient between sample gas tensions and room air gas tensions. Small bubbles, if immediately expelled will generally not cause any significant error. Samples with large (more than 0.2 mL) bubbles should be discarded and a new, anaerobic sample obtained.

Reference Range Normal values (arterial blood), pH: 7.35-7.45; pCO₂: 36-45 mm Hg. The normal partial pressure of oxygen in arterial blood at sea level is generally considered to be >80 mm Hg. Several factors compound interpretation of pO₂ by this simple means, notably altitude of residency, age, hypoventilation, and hyperventilation. The most common causes of hypoxemia are:

- § Ventilation-perfusion (V/Q) abnormalities in the lungs
- § Physiologic shunting
- § Alveolar-capillary diffusion defects
- § Alveolar hypoventilation
- § Decreased inspired oxygen concentration

Interpretation of blood gases should start with the assessment of the ventilatory status by classification of the pCO₂. A low pCO₂ (<30 mm Hg) indicates alveolar hyperventilation. A

high pCO₂ (>50 mm Hg) indicates ventilatory failure. A pCO₂ in the range of 30-50 mm Hg represents an acceptable level of alveolar ventilation. Because the lungs and the kidneys work together to achieve acid-base homeostasis, inspection of the arterial pH in conjunction with the pCO₂ will allow determination of the origin of the acid-base disturbance. Acid-base disturbances can be a primary ventilatory problem (respiratory acidosis, respiratory alkalosis) or a primary metabolic problem (metabolic acidosis; metabolic alkalosis). Respiratory acid-base disturbances present for more than 24 hours will result in renal compensation by increasing or decreasing the plasma bicarbonate to normalize pH. Metabolic acid-base disturbances will result in partial or complete compensation by the respiratory system which increases or decreases alveolar ventilation (and thus the pCO₂).

Use Assess oxygenation of arterial blood and the blood=s acid-base balance.

Limitations Erroneous values can result from improper (aerobic) sample handling; excessive storage at room temperature (more than 10 minutes) before measurement, excessive storage at 2°C before measurement, improper calibration of the blood gas analyzer, and measurement errors. Potentially hazardous clinical judgments based solely on blood gas values without clinical correlation are discouraged.

Methodology Electrodes and reagents

Contraindications Relative contraindications include peripheral artery spasm.

Additional Information Heparinized blood is required. Sodium heparin is generally used as an anticoagulant, it may be used as a powder or liquid. Errors have been reported from the excessive dilution of the sample with liquid heparin. Syringes using powdered heparin obviate the need for concern regarding heparin dilution errors. Disposable kits are available that supply needles with inner cannula that minimize syringe-needle combined dead space, making a minimum sample size of 0.5 mL sufficient.

CALCIUM, SERUM:

Synonyms Ca, Serum

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Blood

Volume 7 mL

Minimum Volume 1 mL serum or plasma

Container Red top tube/SST tube

Storage Instructions Refrigerate

Causes for Rejection Gross hemolysis, incorrect collection tube (i.e., purple, blue, or gray top tube)

Reference Range 8.5 - 11.0 mg/dL

Critical Values #6.0 mg/dL, \geq 14.0 mg/dL

Use Work up hyperparathyroidism and calcium metabolism

Limitations Sodium citrate, EDTA, and sodium fluoride/potassium oxalate interfere. Gross hemolysis falsely elevates results.

Methodology Cresolphthalein complexone

Additional Information Primary hyperparathyroidism is one of the most commonly causes of increased serum calcium. Hyperparathyroidism may coexist with other endocrine tumors (multiple endocrine adenomatosis syndromes). In the differential diagnosis of hypercalcemia, serum calcium should be measured on at least three occasions. In primary hyperparathyroidism, parathyroid hormone, serum chloride, and urine calcium are increased. Calcium rises, then phosphorus falls, then alkaline phosphatase rises. Carcinoma with or without bone metastases, is another common cause of increased calcium. Tumor induced hypercalcemia is seen especially in primary squamous cell carcinoma of lung, head, and neck, but other important tumors include primaries in the kidney, liver, bladder, and ovary. It is probably caused by parathyroid hormone-like peptides. The most common solid tumors causing bone metastases are primaries in the breast and lung. Laboratory results which would favor malignancy include anemia, increased LD and alkaline phosphatase, decreased serum albumin, and chloride and chloride/phosphorus ratio < 29 . The third common cause of increased calcium is drug-induced hypercalcemia. Thiazide diuretic administration may cause increased serum calcium. Low albumin and low total protein relate to common, usually slight decreases of calcium. Since the metabolically active form of calcium is the ionized state, the patient's serum protein level should be considered when interpreting a calcium result. Causes of hypocalcemia include renal failure, hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, rickets, malabsorption, malnutrition with interference with vitamin D and/or calcium absorption, acute pancreatitis, and hypomagnesemia.

CARBAMAZEPINE, TOTAL

Synonyms Tegretol[®]

Laboratory Chemistry

Availability Monday - Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Special Instructions Include date and time specimen drawn.

Specimen Blood

Volume 10 mL

Minimum Volume 0.5 mL serum

Container Red top tube (specimen cannot be used if collected in EDTA tube), **Do not use a gel barrier tube**

Collection Oral: peak: 3-5 hours after dose; trough: immediately prior to next dose. Due to variability of absorption, the trough level may not represent the lowest drug level during the dosing interval, so multiple determinations may be necessary in some patients.

Storage Instructions Separate serum and refrigerate

Reference Range Therapeutic: 4-10 μ g/mL

Critical Values Toxic: >15 μ g/mL

Use Monitor therapeutic drug level; evaluate toxicity

Limitations Fluorescent dyes invalidate this assay

Methodology Fluorescence polarization immunoassay (FPIA)

Additional Information After beginning therapy with carbamazepine, more than 2 weeks are required to reach steady-state levels. Blood half-life is 10-25 hour, but clearance may increase during initial therapy (autoinduction). Co-administration of phenytoin, barbiturates, benzodiazepines, succinimides, and valproic acid may induce lower serum levels, but seizure control is possible because the active metabolite, carbamazepine epoxide has anticonvulant activity. Clinical effects are difficult to predict. Likewise carbamazepine administration may decrease plasma levels of phenytoin and warfarin.

Toxic effects may occur with apparently therapeutic levels if given with phenytoin or barbiturates. Carbamazepine can rarely produce serious side effects which may not be dose related. The most widely feared side effect is bone marrow depression. Although this problem is rare, a CBC and platelet count are desirable before starting and at regular intervals during treatment (if possible, weekly for the first 3 months, then monthly for at least 2-3 years). The drug should be withdrawn at the first sign of leukopenia or thrombocytopenia. Also, liver damage accompanied by abnormalities in liver function tests and jaundice may occur. Cardiovascular disease may be precipitated or worsened by this drug.

CHLORIDE, BLOOD

Synonyms Cl, Blood

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Blood

Volume 10 mL

Minimum Volume 3 mL serum or plasma

Container Red top tube/ Gel barrier tube

Collection Routine venipuncture

Storage Instructions Separate serum or plasma and refrigerate

Causes for Rejection Gross hemolysis, lipemic specimen

Reference Range 94-108 mEq/L

Critical \neq 70 mEq/L, \neq 120 mEq/L

Use Differential diagnosis of acidemias and alkalemia; evaluate electrolytes, acid-base balance, water balance.

Limitations Interference from bromide

Methodology Ion-selective electrode (ISE)

Additional Information Chloride increases and decreases with plasma and serum sodium in most cases. Chloride is **increased** in dehydration, with ammonium chloride administration, with renal tubular acidosis (hyperchloremic metabolic acidosis) and with excessive infusion of normal saline. Chloride is higher in hyperparathyroidism than in some of the other causes of hyperclacemia, but a great deal of overlap exists. Chloride is **decreased** with over hydration, congestive failure, syndrome of inappropriate secretion of ADH, vomiting, gastric suction, chronic respiratory acidosis, Addison=s disease, salt-losing nephritis, burns, metabolic alkalosis, and in some instances of diuretic therapy.

CREATININE, BLOOD

Synonyms Creat, blood

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Blood

Volume 7 mL

Minimum Volume 3 mL serum or plasma

Container Red top tube/Gel barrier tube

Collection Routine venipuncture

Storage Instructions Refrigerate serum or plasma

Causes for Rejection Hemolysis

Reference Range 0.5-1.7 mg/dL

Critical \neq 7.5 mg/dL

Use Renal function test, providing a rough approximation of glomerular filtration

Limitations Concentration of creatinine only becomes abnormal when approximately one-half the nephrons have stopped functioning. Causes of increased serum creatinine result from noncreatine substances, including ketonemia (acetonemia), hydantoin, ascorbic acid, cephalosporin antibiotics. Creatinine reaction is inhibited by bilirubin, histidine, glucose, and guanidino compounds.

Methodology **Kinetic** alkaline picrate (Jaffe= reaction)

Additional Information The serum creatinine level is proportional to lean body muscle mass. It is unaffected by most diet or activity, and is freely filtered by the glomerulus. Both BUN and creatinine are often ordered to follow renal problems. Creatinine overall is the more reliable index, but each has pitfalls. As creatinine increases in chronic renal failure, the hematocrit decreases, total carbon dioxide and bicarbonate fall, and serum phosphate and BUN increase.

Causes of high creatinine include renal disease and insufficiency with decreased glomerular filtration (uremia or azotemia if severe); urinary tract obstruction; reduced renal blood flow, including congestive heart failure; shock; and dehydration. Rhabdomyolysis causes high serum creatinine, which may be elevated out of proportion to BUN, or to the reduction in renal function.

Causes of low creatinine include small stature, debilitation, decreased muscle mass, some complex cases of severe hepatic disease. In advanced liver disease, low creatinine may result from decreased hepatic production of creatinine and inadequate dietary protein as well as reduced muscle mass.

COMPLETE BLOOD COUNT (CBC)

Synonyms Blood Cell Profile; Blood Count; CBC; CBC With Differential

Test Includes White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and differential (diff).

Laboratory Hematology

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Blood

Minimum Volume 1.5 mL in a 5 mL purple top (dry EDTA-K₂) tube or 0.25 mL in a 0.6 mL purple top (dry EDTA-Na₂) capillary collection system

Container Purple top (dry EDTA-K₂) tube for venipunctures

Collection Routine venipuncture or fingerstick. Invert tube several times immediately after collection to ensure mixing and prevent clotting. Deliver to laboratory within 4 hours of collection.

Storage Instructions Up to 4 hours at room temperature; up to 24 hours at 4° C.

Causes for Rejection Specimen hemolyzed, clotted, or diluted with I.V. fluid; tube not filled with minimum volume

Reference Range

\$WBC 3.5-11.0 k/ Φ L
\$RBC 4.0-5.2 m/ Φ L
\$HGB 12-16 gm/Dl

COMPLETE BLOOD COUNT (CBC) (continued)

\$HCT 35-47%
\$MCV 83-92 fl
\$MCH 27-34 pg
\$MCHC 31-36 gm/dL
\$PLT 150-450 k/ Φ L
\$NEUTROPHILS 46-77%
\$LYMPHOCYTES 16-43%
\$MONOCYTES 0.0-10.0%
\$EOSINOPHILS 0.0-6.0%
\$BASOPHILS 0.0-2.0%

Critical Values

\$WBC #2.0 k/ Φ L, \exists 40.0 k/ Φ L
\$HGB #5.0 gm/dL, \exists 20.0 gm/dL
\$HCT #19.8%, \exists 60.0%
\$PLT #30 k/ Φ L, \exists 1000 k/ Φ L

Use Evaluate anemia, leukemia, reaction to inflammation and infections, state of hydration and dehydration, polhcythemia, and ABO incompatibilities; determine qualitative and quantitative variations in white cell numbers and morphology, morphology of red cells and platelet evaluation; evaluate anemia, leukemia, infections, inflammatory states, myeloproliferative disorders, and inherited disorders of red cells, white cells, and platelets.

Methodology Automated blood cell counter. **Note:** A CBC includes an automated differential. A manual differential is performed by the technologist if the results fail defined instrument criteria. Manual differential: 100 cell differential of the white blood cells, RBC morphology, platelet evaluation.

GLUCOSE, RANDOM

Synonyms Blood Sugar, Random; Random Blood Glucose

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Blood

Volume 7mL

Minimum Volume 3 mL serum

Container Red top tube or gray/SST tube

Collection Routine venipuncture

Storage Instructions Glucose will decrease 5-10 mg/dL hour in unseparated, room temperature blood in a red top tube. Remove serum promptly.

Patient Preparation Patient should have been fasting for 8 hours

Causes for Rejection Blood stored overnight on clot, gross hemolysis

Reference Range 60-110 mg/dL

Critical #40 mg/dL, \exists 700 mg/dL

Use Evaluate carbohydrate metabolism; evaluate acidosis and ketoacidosis, evaluation of dehydration; work up alcoholism or apparent alcoholism; work up coma, neuroglycopenia. Hypoglycemia, if present, should be investigated with insulin levels as well. Determination of blood glucose on admission in patients who have had an out-of-hospital cardiac arrest can serve as a predictor of neurologic recovery. Higher levels are indicative of more severe brain ischemia and difficult resuscitation.

Limitations Glucose will decrease in samples left on the clot, and in tubes other than fluoride prior to analysis.

Methodology Hexokinase

Additional Information For the diagnosis of diabetes mellitus in nonpregnant adult subjects, random glucose of >200 mg/dL is required. In pregnant women a value >105 mg/dL usually prompt further investigation. If glucose is >400 mg/dL, an acetone examination should be done. A fasting and a 2 hour postprandial specimen is preferable to a random specimen for evaluation of possible diabetes mellitus.

PARTIAL THROMBOPLASTIN TIME, ACTIVATED

Synonyms Activated Partial Thromboplastin Time; APTT; PTT; aPTT

Test Includes Patient time in seconds

Laboratory Hematology

Availability Monday-Friday, 8hours

Turnaround Time Stat: 1 hour

Specimen Blood

Minimum Volume 4.5 mL

Container Blue top (sodium citrate) tube

Collection If multiple tests are being drawn, draw coagulation studies second or third. If only coagulation studies are being drawn, draw 1-2 mL, discard, and collect coagulation studies. This procedure avoids contamination fo specimen with tissue thromboplastin.

Blood may be used from an arterial line or a line previously flushed with heparin providing the first 15 mL blood is drawn off before coagulation studies are drawn. Please indicate such specimens. All coagulation tests are, to some degree, sensitive to heparin contamination.

Storage Instructions If test cannot be done immediately, spin 10 minutes, separate plasma, and freeze. Thaw quickly and perform test within 1 hour after thawing.

Causes for Rejection Specimen clotted, hemolyzed, drawn above and I.V., contaminated with heparin (i.e., drawn with blood gases), received more than 2 hours after collection; tubes not full

Reference Range 25.0-36.0 seconds

Critical \geq 70 seconds

Use Evaluate intrinsic coagulation system, monitor heparin therapy; screen for presence of classical hemophilia A and B; congenital deficiency of factors II, V, VIII, IX, X, XI, and XII; dysfibrinogenemia; vitamin K deficiency; congenital deficiency of Fitzgerald factor; congenital deficiency of prekallidrein (Fletcher factor).

Additional Information If the activated partial thromboplastin time (APTT) is performed in conjunction with a prothrombin time (PT), its diagnostic utility is increased. A normal PTT in the presence of an abnormal PT suggests a possible factor VII deficiency. Conversely, an abnormal PTT in the presence of a normal PT suggests a possible deficiency of factor(s) VIII, IX, XI, and/or XII. The pattern of an abnormal PT and PTT suggests a possible deficiency of factor(s) I, II, V, and/or X. A prolonged PTT can be caused by inherited factor deficiency (I, II, V, VIII-XIII), Fletcher or Fitzgerald, Coumadin type therapy, liver disease, circulating anticoagulant (heparin, lupus anticoagulant, fibrin breakdown products), specific factor inhibitor (rheumatoid arthritis, penicillin reaction, occasional hemophiliacs), or intravascular coagulation.

PHENOBARBITAL, SERUM

Synonyms Luminal⁷, Phenobarb; Phenobarbital, Level, Blood

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Special Instructions Include date and time specimen drawn

Specimen Blood

Volume 10 mL

Minimum Volume 3 mL serum

Container Red top tube/ **Do not use gel barrier tube**

Collection Peak: 4-12 hours after dose; trough, immediately prior to next dose; after change in dose: 1 week

Storage Instructions Separate serum and refrigerate

Reference Range Therapeutic 15-40 $\mu\text{g/mL}$

Critical $\geq 50 \mu\text{g/mL}$

Use Monitor therapeutic drug level; evaluate toxicity; use with primidone levels to monitor therapeutic drug level of primidone

Limitations Mephobarbital (Mebaral[®]) interferes significantly; it is metabolized to phenobarbital. Phenobarbital level may increase when phenytoin is given. Fluorescent dyes invalidate this assay.

Methodology Fluorescence polarization immunoassay (FPIA)

Additional Information Excretion is faster in infants and children, who may need higher doses. Phenobarbital is a long-acting barbiturate with a half-life of 53-140 hours. If dose is doubled, it will take 5.5 half-lives to reach a new steady-state (up to 3 weeks). Phenobarbital is a metabolite of primidone and mephobarbital. Phenobarbital affects the metabolism of phenytoin and succinimides. It is recommended that these levels be monitored when the phenobarbital dose is changes. **Phenobarbital may increase the effect of other drugs bound to protein, such as warfarin.** Used with valproic acid excretion diminishes because of urine acidification by valproic acid. In addition to legitimate therapeutic applications phenobarbital, like other barbiturates, is a drug of abuse. Ethanol and a barbiturate are a potentially lethal combination.

PHENYTOIN

Synonyms Dilantin[®]; Diphenylhydantoin

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Special Instructions Include date and time specimen drawn

Specimen Blood

Volume 10 mL

Minimum Volume 3 mL serum

Container Red top tube/ **Do not use gel barrier tube**

Collection I.V. 2-4 hours after loading dose; oral: peak 3-9 hours after dose; trough: immediately prior to next dose. Optimal resampling after change in dosage: 48 hours

Storage Instructions Separate serum and refrigerate

Causes for Rejection Hemolysis

Reference Range Therapeutic: 10-20 $\mu\text{g/mL}$

Critical $\geq 40 \mu\text{g/mL}$

Use Monitor therapeutic level; evaluate toxicity

Limitations Fluorescence dyes invalidate this assay

Methodology Fluorescence polarization immunoassay (FPIA)

Addition Information Phenytoin is indicated for the control of tonic-clonic and simple partial and partial complex seizures and prevention and treatment of seizures occurring during or following neurosurgery. The half-life is 20-40 hours in adults and around 10 hours in children (who need more frequent phenytoin administration). Phenytoin interacts with several medication, including dicumarol and phenobarbital. Phenobarbital may increase or decrease phenytoin levels. It is recommended that these levels be monitored when the phenobarbital dose is changed. In uremia, and with concomitant salicylate, phenylbutazone or thiazide treatment protein binding is decreased, and serum levels of the drug are increased. INH may inhibit degradation of phenytoin to parahydroxyphenylhydantoin (HPPH). Chloramphenicol and dicumarol may also increase serum levels. Carbamazepine or alcohol may decrease serum levels. Phenytoin also has effects on the serum levels of several other drugs, increasing phenobarbital and decreasing digitoxin, dicumarol, cortisol, and folate. Drug levels or clinical effects of these agents should be checked following institution or change in phenytoin therapy. There are reports of increased clearance of phenytoin (with resultant decreased blood level and seizures) during infectious mononucleosis and in one case as the presenting feature of this disease.

POTASSIUM, BLOOD

Synonyms K⁺, K, Blood; serum Potassium

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Specimen Blood

Volume 7 mL

Minimum Volume 3 mL serum

Container Red top tube/ Gel barrier tube Avoid very small needles if possible. Avoid stasis, use of tourniquet, hand-clenching, if possible, and potassium-containing tubes such as potassium oxalate.

Storage Instructions Separate serum and refrigerate within 4 hours

Causes for Rejection Hemolysis, specimen collected in potassium oxalate tube

Reference Range 3.5-5.5 mEq/L

Critical #2.7 mEq/L, ∃6.5 mEq/L

Use Evaluate electrolyte balance; potassium level should be followed especially in elderly patients, those on intravenous hyperalimentation, in patients on diuretic therapy, and in cases of renal disease, particularly renal failure, patients on hemodialysis, and those with interstitial nephritis or nephropathy; evaluate hypertension; potassium should be monitored during treatment of acidosis, including ketoacidosis in diabetes mellitus; evaluate muscular weakness and irritability, mental confusion, weakness, manage leukemia, diseases of gastrointestinal tract; evaluate and prevent

cardiac arrhythmia; evaluate alcoholism with delirium tremens; detect, diagnose, and manage mineral-corticoid excess (primary aldosteronism, Cushing=s syndrome, tumor with ectopic ACTH production, some cases of congenital adrenal hyperplasia).

Limitations Since platelets release potassium during coagulation, samples from patients who have thrombocytosis (e.g., some cases of polycythemia vera and other myeloproliferative disease) will yield spuriously elevated potassium concentrations. Such Pseudohyperkalemia@ may also occur in cases of leukemia with high WBC count. For such patients it is best to assay potassium on a heparinized sample (green top tube).

Methodology Ion-selective electrode (ISE)

Additional Information Low potassium occurs with endogenous or exogenous increase in other corticosteroids, including that in Cushing=s syndrome as well as with dietary or parenteral deprivation of potassium (e.g., parenteral therapy without adequate potassium replacement). Hypokalemia occurs with vomiting, diarrhea, fistulas, laxatives, diuretics, burns, excessive perspiration, Bartter=s syndrome, some cases of alcoholism and of folic acid deficiency, in alkalosis, and in renal tubular acidosis, as well as in other entities.

Hyperkalemia (high potassium) reflects generally inadequate renal excretion, mobilization of potassium from the tissues or excessive intake or administration. Hyperkalemia occurs with hemolysis, trauma, with administration of potassium salts of some drugs, Addison=s disease, acidosis, insulin lack, with increased osmolality (e.g., glucose, mannitol), and in other entities as well as with renal diseases. Increased potassium can occur with potassium sparing diuretic, nonsteroidal, anti-inflammatory drugs, especially in the presence of renal disease.

PROTHROMBIN TIME

Synonyms Prottime; PT

Test Includes Patient time, INR

Laboratory Hematology

Availability Stat: 1 hour

Specimen Blood

Minimum Volume 4.5 mL

Container Blue top (sodium citrate) tube

Collection If multiple tests are being drawn, draw coagulation studies second or third. If only coagulation studies are being drawn, draw 1-2 mL, discard, and collect coagulation studies. This procedure avoids contamination of specimen with tissue thromboplastin. Blood may be used from an arterial line or a line previously flushed with heparin providing the first 15 mL blood is drawn off before coagulation studies are drawn. Please indicate such specimens. All coagulation tests are, to some degree, sensitive to heparin contamination. Transport specimen to laboratory immediately.

Storage Instructions If test cannot be done immediately, spin 10 minutes, separate plasma, and freeze. Thaw quickly and perform test within 1 hour after thawing.

Causes for Rejection Specimen clotted, diluted with I.V. fluid, hemolyzed, or contaminated with heparin; tubes underfilled; excessive delay in transport.

Reference Range Normal range is established with each new lot number of reagents.

Current range 11.0-13.0 seconds

Critical ≥ 25 seconds

Use Evaluate extrinsic coagulation system; screen for congenital deficiencies of factors II, V, VII, X; deficiency of prothrombin; dysfibrinogenemia; afibrinogenemia (complete); heparin effect; coumarin or warfarin effect; liver failure; disseminated intravascular coagulation (DIC); screen for vitamins

Limitations Prothrombin times drawn less than 2 hours after heparin administration may be prolonged. Drugs which enhance the hypoprothrombiemic action of the coumarins include salicylates phenylbutazone, clofibrate, barbiturates, chloral hydrate, chloramphenicol, ethchlorvynol, glutethimids, phenylamidol, quinidine allopurinol, anabolic steroids, MAO inhibitors, antipyrine, phenytoin, propylthiouracil, antacids, sulfonamides, carbamazepine estrogenic contraceptives, and griseofulvin. Drugs lowering the PT include ethchlorvynol, glutethimide, anabolic steroids, antacids, estrogenic contraceptives, and griseofulvin.

Additional Information The prothrombin time determination is affected by the plasma to anticoagulant ratio. That is, if enough blood is not added to the liquid citrate-containing tube then a falsely elevated prothrombin time may result.

An increased (prolonged) prothrombin time may be seen in association with septicemia, Whipple's disease, herpes, simplex, viral hepatitis, malaria, leishmaniasis, leptospirosis, malignant neoplasm of liver and pancreas, secondary malignant neoplasm for liver, myelocytic leukemia (acute and chronic), monocytic leukemia, myelofibrosis, Zollinger-Ellison syndrome, vitamin K deficiency, celiac sprue disease, malabsorption, lactosuria, abetalipoproteinemia, cystic fibrosis, hepatocellular degeneration, congenital dysfibrinogenemia, congenital afibrinogenemia, disseminated intravascular coagulopathy, factor II deficiency, factor V deficiency, factor X deficiency, factor VII deficiency, congestive heart failure, mesenteric artery embolism, ulcerative colitis, acute and subacute necrosis of liver, cirrhosis of liver, liver abscess (pyogenic), chronic active hepatitis, toxic hepatitis, hepatic encephalopathy, Reye's syndrome, hepatic failure, extrahepatic biliary obstruction, eclampsia, systemic lupus erythematosus, hemolytic disease of newborn, jaundice due to hepatocellular damage, toxic effects of venom, effects of x-ray, heat stroke, exercise, diet (vitamin K deficiency), drug therapy (including acetaminophen and carbenicillin), dextran, aspirin, laxatives, penicillin, and sulfisaxazole.

A decreased PT may be seen in association with ovarian hyperfunction and regional enteritis or ileitis.

A normal PT may be seen in association with polycythemia vera, Tangier disease, hemophilia, Christmas disease, factor XI deficiency, factor XII deficiency, von Willebrand disease, factor XIII deficiency, ITP, thrombathenia, and thrombotic thrombocytopenic purpura.

Heparin has a negligible effect on PT.

SODIUM, BLOOD

Synonyms Na⁺, Blood

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Specimen Blood

Volume 7 mL

Minimum Volume 3 mL serum and plasma

Container Red top tube/ SST tube

Storage Instructions Refrigerate serum or plasma

Causes for Rejection Gross hemolysis

Reference Range 135-145 mEq/L

Critical #120 mEq/L, ∃160 mEq/L

Use Evaluate electrolytes, acid-base balance, water balance, water intoxication, diagnose dehydration

Limitations Increased values may be seen with interference from anabolic steroids, boric acid, calcium, corticosteroids, fluorides, or iron. Decreased values may be seen with interference from hemolysis, heparin, laxatives, sulfate, and diuretics.

Methodology Ion-selective electrode (ISE)

Additional Information Hyponatremia occurs in dehydration. Hyponatremia without obvious cause may relate to Cushing=s syndrome, central or nephrogenic diabetes insipidus with insufficient fluids, primary aldosteronism, and other diseases. Severe hypermatremia may be associated with volume contraction, lactic acidosis, xzotemia, weight loss, and increased hematocrit as evidence of dehydration.

Hyponatremia occurs with nephrotic syndrome, cachexia, hypoproteinemia, intravenous glucose infusion, congestive heart failure, and other clinical entities. Serum sodium is a predictor of cardiovascular mortality in patients in sever congestive heart failure.

Hyponatremia also may occur with hypothyroidism, the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), renal failure, or renal sodium loss.

The differential diagnosis of hyponatremia includes Addison=s disease, hypopituitarism, liver disease, including cirrhosis; hypertriglyceridemia; and psychogenic polydipsia. Diuretic and other drugs may also cause hyponatremia.

UREA NITROGEN, BLOOD (BUN)

Synonyms Blood Urea Nitrogen; BUN

Laboratory Chemistry

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour upon arrival

Specimen Blood

Volume 7 mL

Minimum Volume 3 mL serum or plasma

Container Red top tube/ SST tube

Storage Instructions Separate specimen and refrigerate if it cannot be processed within 24 hours. Freeze after 3 days.

Reference Range 6-23 mg/dL

Critical \geq 40 mg/dL

Use Renal function test. Urea nitrogen reflects the ratio between urea production and clearance. Increased BUN may be due to increased production or decreased excretion.

Limitations Uremia is best evaluated with creatinine as well as by urea nitrogen. In both prerenal and postrenal azotemia, for instance, BUN is apt to be increased somewhat more than creatinine. In chronic progressive renal disease, approximately 75% renal parenchyma must be damaged or destroyed before azotemia develops.

Methodology Enzymatic using urease

Additional Information High BUN occurs in chronic glomerulonephritis, pyelonephritis, and other causes of chronic renal disease, acute renal failure, and decreased renal perfusion (prerenal azotemia) as in shock. With urinary tract obstruction BUN increases (postrenal azotemia). Example of obstructions include neoplastic infiltration of the ureters, hyperplasia, or carcinoma of the prostate. BUN is useful to follow hemodialysis and other therapy. Other causes of increased BUN include severe congestive heart failure, catabolism, tetracyclines with diuretic use, hyperalimentation, ketoacidosis, and dehydration as in diabetes mellitus, but even moderate dehydration can cause BUN to increase. Corticosteroids tend to increase BUN by causing protein catabolism. Bleeding from the gastrointestinal tract is an important cause of high urea nitrogen, commonly accompanied by elevation of the BUN/creatinine ratio. Nephrotoxic drugs must also be considered. Borderline high values may occur after recent ingestion of high protein meal or with muscle wasting.

Low BUN occurs in normal pregnancy, decreased protein intake, administration of intravenous fluids and some antibiotics, and in some but not all instances of liver disease.

URINALYSIS

Synonyms Macroscopic and Microscopic Examination, Urine; Routine Urinalysis; Routine Urine; UA; Routine

Test Includes Opacity, color, appearance, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, urobilinogen, leukocyte esterase, nitrite. Microscopic examination of **urine sediment**

Laboratory Urinalysis

Availability Monday-Friday, 8 hours

Turnaround Time Stat: 1 hour

Specimen Random urine

Volume 20 mL

Minimum Volume 6 mL

Container Sterile plastic urine container

Collection A voided specimen is usually suitable. If the specimen is likely to be contaminated by vaginal discharge or hemorrhage, a clean catch specimen is desirable.

Storage Instructions Transport specimen to the laboratory as soon as possible after collection.

Refrigeration preserves formed elements in the urine, but may precipitate crystals not originally present.

Causes for Rejection Specimen more than 4 hours old, fecal contamination, decomposition or bacterial overgrowth, insufficient quantity of urine, improper labeling of specimen

Reference Range

§Specific gravity: 1.005-1.030

§pH: 5.0-8.0

§Protein: negative

§Bilirubin: negative

§Urobilinogen: 0.2-1.0

§Glucose: negative

§Ketones: negative

§Occult blood: negative

§RBC=s: 0-3/hpf

§WBC=s 0-3/hpf

§Bacteria: none

§Leukocyte esterase: negative

§Casts: 0-4 hyaline casts/hpf

§Squamous epithelial cells: 0-3/hpf

§Crystals: interpreted by physician and/or referral laboratory

URINALYSIS (continued)

Critical

§Glucose: 3+

§Ketones: 3+

Use Screen for abnormalities in urine; diagnose and manage renal diseases, urinary tract infection, urinary tract neoplasms, systemic diseases, and inflammatory or neoplastic diseases adjacent to the urinary tract.

Limitations Insufficient volume, less than 2 mL may limit the extent of procedures performed. Metabolite and Pyridium⁷ may interfere with the dipstick reactions by producing color interference. High vitamin C intake may cause an underestimate of glucosuria, or a false-negative nitrate test. Survival of WBC^s is decreased by low osmolality, alkalinity and lack of refrigeration. Formed elements in the urine, including casts, disintegrate rapidly; therefore, the specimen should be analyzed as soon as possible after collection. Specific gravity is affected by glucosuria, mannitol infusion or prior administration of iodinated contrast material for radiologic studies (IVP dye). False positive tests for protein can also be due to contamination of the urine by an ammonium containing cleansing solution. Bence Jones proteins may not be detected by dipstick method.

Methodology Multistix⁷. Microscopic is done.

Additional Information

COLOR/CLARITY

Colorless urine may be normal or secondary to diuretic use, high fluid intake, diabetes insipidus, or diabetes mellitus. **Cloudy or hazy** urine may reflect the presence of phosphates, pyuria, or bacteriuria. On oxidation, development of a **black** color is evidence for alkaptonuria. Increased indican may cause the urine to blacken on standing. **Dark** urine is the second most common sign of acute intermittent porphyria. Very rarely, dark urine may indicate the presence of malignant melanoma. **Green** urine may be produced by indigo carmine, methylene blue, phenol, and in some cases of iodochlorohydroxyquin (clioquinol)-induced subacute myelo-optic neuropathy. Other causes of green urine are reported as *Pseudomonas* bacteremia, urinary bile pigments amitriptyline hydrochloride or methocarbamol ingestion, and breath freshener abuse. **Red** plasma and red urine indicate hemoglobin; clear plasma with red urine may indicate myoglobin, but may occur as well in congenital erythropoietic porphyria and cutanea tarda porphyria. Purple urine, after standing, may also be due to porphyrins. **Yellow to orange** urine may contain bile. Other causes of darker yellow to orange urine include increased concentration of urine or the presence of riboflavin, quinaerine (Atabrine⁷), rifampin (Rifadin⁷, Rimactane⁷), phenazopyridine (Pyridium⁷), or salicylazosulfapyridine (Azulfidine⁷). The plastic urine bag may discolor **purple** in the presence of the indican produced by *Providencia* or *Klebsiella* species.

DIPSTICK

Specific Gravity

Indicates the relative proportions of dissolved solid components to the total volume of the specimen. It reflects the relative degree of concentration or dilution of the specimen.

URINALYSIS (continued)

Blood in the urine is used to detect myoglobin, hemoglobin, or RBC=s in the urine. Hematuria and hemoglobinuria may represent a variety of conditions.

Glucose in the urine usually indicated significant hyperglycemia. A positive screening test for urine glucose is a significant sign and indicates a substantial likelihood of diabetes mellitus.

Leukocyte esterase determination gives the ability to identify the presence of leukocyte esterase in dilute urine specimens which have been subject to standing with lysis or the white cells.

Urine pH is a crude measure of the acid-base balance of the body. It may be helpful in determining subtle presence of distal renal tubular disease or pyelonephritis. Urine pH is useful for identifying crystals in urine and determining predisposition to form a given type of stone. Urine pH >7 is associated with calcium carbonate, calcium phosphate, and especially magnesium ammonium phosphate stones. Urine pH 7.5 most commonly indicated systemic alkali intake or urine infected by bacteria which split urea to ammonia. Acid urine is observed with high meat intake. Consistently acid urine, pH <5.5, is associated with xanthine, cystine, and uric acid stones. Calcium oxalate and apatite stones are not associated with any particular disturbance of urine pH.

Protein in the urine is a screen for nephritic syndromes, including complications of diabetes mellitus, glomerulonephritis, amyloidosis, and other diseases. Proteinuria is probably the single most important indicator of renal disease.

Ketonuria can occur in infants and children with febrile illnesses or toxic states with marked vomiting or diarrhea. Genetic disorders resulting in ketonuria include propionylcarb-oxylase deficiency, glycogen storage disease, branched chain ketonuria, and methylmalonic aciduria. In adult healthy men, a fast of 18 hours or longer produces in normal pregnancy, starvation, high protein diet, eclampsia thyrotoxicosis, and isopropanol ingestion.

Nitrite positive urine is strongly suggestive of urinary tract infection (i.e., $\geq 10^5$ organisms/mL). Therefore, when positive, a urine culture is recommended, but a urine culture is indicated in any case if the patient is symptomatic

MICROSCOPY

Crystalluria is uncommon despite maximal concentrations in warm, fresh urine because of the normal presence of crystal inhibitors, the lack of available fluid, and the time factor. When properly observed in fresh urine, crystals are diagnostically useful for a physician evaluating microhematuria, nephrolithiasis, or toxin ingestion.

In abundance, **calcium oxalate** and/or **hippurate crystals** may suggest ethylene glycol ingestion (if accompanied by neurological abnormalities, appearance of drunkenness, hypertension, and a high anion gap acidosis).

Calcium magnesium ammonium phosphate may be present in massive quantities in alkaline urine. They usually are associated with urine infected by urea splitting bacteria which cause urinary tract infection or struvite phosphate stones.

Cystine crystals can be associated with cystinuria (failure of renal tubular reabsorption) and cystinosis (an inherited metabolic defect). In either disorder, calculi can be formed.

Tyrosine and leucine crystals are found in acid urine, indicating abnormal metabolism. These crystals occur together in acute yellow atrophy and in other destructive diseases of the liver. Crystals may also provide a clue to the compositions of renal stones not yet passed.

Leukocyturia may indicate inflammatory disease in the genitourinary tract, including bacterial infection, glomerulonephritis, chemical injury, autoimmune diseases adjacent to the urinary tract such as appendicitis or diverticulitis.

White cell casts indicate the renal origin of leukocytes and are most frequently found in acute pyelonephritis. White cell casts are also found in glomerulonephritis, such as lupus nephritis, and in acute interstitial nephritis.

Red cell casts indicate renal origin of hematuria and suggest glomerulonephritis, including lupus nephritis. Red cell casts may also be found in subacute bacterial endocarditis, renal infarct, vasculitis, Goodpasture's syndrome, sickling, and in malignant hypertension.

Dysmorphic red cells are observed in glomerulonephritis. Dysmorphic red cells refer to heterogeneous sizes, hypochromia, distorted irregular outlines, and frequently small blebs extruding from the cell membrane. Nonglomerular urinary red blood cells resemble peripheral circulating red blood cells.

Crenated RBCs provide no implication regarding RBC source.

Dark brown or smoky urine suggests a renal source of hematuria.

A **pink or red urine** suggests an extrarenal source.

Hyaline casts occur in physiologic states (i.g., after exercise) and many types of renal disease.

Renal tubular (epithelial) casts are most suggestive of tubular injury, as in acute tubular necrosis. They are also found in other disorders, including eclampsia, heavy metal poisoning, ethylene glycol intoxication, and acute allograft rejection.

Granular casts are very finely granulated casts which may be found after exercise; coarse granular casts are abnormal and are present in a wide variety of renal diseases.

ADirty brown@ granular casts are typical of acute tubular necrosis.

SPECIMEN COLLECTION MANUAL

The blood and other specimens collected for analysis by the Histocompatibility Laboratory are collected aseptically, utilizing standard phlebotomy techniques, by certified laboratory or other personnel. They are responsible for accurate patient identification, accurate and complete specimen collection, and labeling.

SPECIMEN PROCUREMENT AND HANDLING:

Each specimen must be accompanied by a requisition form with the name of the requesting physician. Each specimen is reviewed upon receipt by the laboratory supervisor or medical technologists in order to assure adequacy of collection and identification. The laboratory director is notified of any discrepancies, and is responsible for the resolution of problems in specimen collection and test requisitions.

SPECIMEN IDENTIFICATION AND HANDLING

Each specimen received by the HLA laboratory must have a proper label with the following information:

- §Patient=s name
- §Patient=s medical record number
- §Date/time of collection
- §Initials of phlebotomist

SPECIMEN REJECTION CRITERIA

§Specimen containers do not have a label with the following information:

- 11.1Patient=s name
- 11.2Patient=s medical record number
- 11.3Date/time of specimen collection
- 11.4Signature or initials of person collecting specimen

§Specimens are not accompanied by a request form with the following information:

- 11.1 Requesting physician
 - 11.2 Hospital or clinic location
 - 11.3 Patient's medical record number
 - 11.4 Date of collection
 - 11.5 Clinical diagnosis
 - 11.6 Nature of the test to be performed or purpose of the specimen.
- § Blood is delivered to the laboratory more than 24 hours after collection. Cell preparations will be accepted for analysis if properly prepared and viability is adequate (>80%).
- § The sample produces a lymphocyte preparation with less than 80% viability as determined by the trypan blue exclusion test (See Cell Count and Viability Procedure).
- § Lymph nodes are delivered to the laboratory more than 24 hours after collection. Sterile cell preparations from a lymph node will be accepted for analysis if properly prepared and viability is adequate (>80%).
- § Splenic tissue is delivered to the laboratory more than 24 hours after collection. Sterile cell preparations derived from spleen specimens will be accepted for analysis if cell viability is adequate (>80%).
- § Blood is collected into media containing phenol or other substances harmful to cells.
- § Volume requirements- Minimal test volumes depend on test type, age of patient, patient illness or medication use which may influence WBC and lymphocyte counts. Insufficient sample size may be a cause of specimen rejection.
- § Sample Condition Requirements- Poor conditions requiring sample rejection may include clotting of whole blood samples, hemolysis of serum samples, broken tubes, and certain blood tubes received refrigerated or frozen.

TRANSPORTATION OF SPECIMEN TO THE HLA LABORATORY

Patient specimens must be packaged in closed tubes or containers. Sealed plastic bags are acceptable for transport so long as they are capable of containing a broken tube of blood without leaking its contents. Packing material must be capable of preventing breakage, and to this end, absorbent material should be used to absorb fluid in the event of leakage. Styrofoam mailers are a good choice for blood tubes. It is the sender's responsibility to protect the shipper from biohazardous exposure.

PERIPHERAL BLOOD

Specimen requirements

Blood collected in vacutainer tubes containing ACD-A is the preferred specimen to be obtained by venipuncture. The blood should be collected aseptically in ACD-solution A (yellow top) tubes. Heparinized tubes containing phenol are unacceptable.

A clotted blood specimen (red top tube) is preferred for the collection of serum. A 10 ml red top tube without separating gel should be utilized for adults.

Specimens collected during dialysis procedures are unacceptable. Specimen collections within one hour after a meal are best avoided.

The specimen requirement is procedure-dependent. The minimum sample size for most procedures is 10 ml for adults. A larger specimen (>30 ml) is required for patients with a decreased white blood count or leukopenia.

Peripheral blood is NOT suitable for HLA typing and cross-matching if a cadaveric donor has received steroids or blood transfusions as part of post-admission therapy or has a differential white count that shows lymphocytes less than 15% (normal range is 22-44%) or segs or granulocytes greater than 75% (normal range is 45-70%). In these cases, lymph node or spleen samples are required.

The specimen should be delivered to the Histocompatibility laboratory as soon as possible after collection (within) twenty-four hours. If possible, the specimen should be maintained at a temperature of 18-25°C during shipment.

Processing

Upon arrival in the HLA laboratory the sample is given an identification number unique to that sample. Whole blood samples will be maintained at room temperature. On arrival in the HLA laboratory specimens will be processed as soon as possible (within two hours).

LYMPH NODES

Volume 3-6 lymph nodes

Use Lymph nodes are the most desirable specimen from a cadaveric donor.

Specimen Requirements

Place lymph nodes into a solution of RPMI (from HLA). There must be enough fluid to completely cover the nodes immediately upon collection.

Place the specimen in a reduced temperature container and submit to the HLA laboratory as soon as possible. **The specimen must be received by the HLA laboratory within 24 hours of collection.**

Processing

Specimens will be refrigerated upon arrival in the HLA laboratory and processed within 2 hours of receipt.

SPLEEN

Specimen Requirements

Splenic tissue must be placed in a solution of RPMI (from HLA). There must be enough fluid to completely cover the splenic tissue immediately upon collection.

Place the specimen in a reduced temperature container and submit to the HLA laboratory as soon as possible. **The specimen must be received by the HLA laboratory within 24 hours of collection.**

Processing

Specimens will be refrigerated upon arrival in the HLA laboratory and processed within 2 hours of receipt.

