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The examination of body fluids provides essential information for the care and treatment of patients. This evaluation encompasses many disciplines, involves multiple steps, and requires the integration of numerous data elements into the analysis. The evaluation of body fluids typically includes gross examination, microscopic examination, cell and differential counts, chemical analysis and microbiology studies. In addition, numerous ancillary studies including flow cytometry, molecular analysis and cytogenetics have now become more readily available and are used more frequently in the evaluation of many body fluids.

Although many new testing modalities are currently available for the evaluation of body fluids, morphologic evaluation still remains the cornerstone in the workup and diagnoses of these specimen types. It is imperative that laboratory professionals develop and maintain morphologic expertise as it is the morphologic examination of body fluids that often directs and guides additional testing and serves as the foundation for accurate interpretation and diagnosis. Still today, morphologic evaluation in conjunction with chemical analysis and microbiology studies are the most important components of body fluid evaluation.

Important indications for the evaluation of body fluids are:
1. to determine the etiology of the fluid accumulation
2. to distinguish between benign and malignant processes

Appropriate laboratory testing and specimen utilization is therefore essential for differentiating between the numerous potential disease processes and etiologies. The following chapters provide both an overview of common testing for each body fluid type but also highlight the unique features that must be considered. The individual body fluid chapters provide review of the morphologic features, chemical analysis and microbiologic evaluation for each fluid type. Additionally, more complex testing techniques are also presented as is appropriate to the specific fluid type. Many of the body fluid types discussed are difficult to replace and would put the patient at increased risk of complications with recollection; therefore, appropriate care must be taken to properly handle and test all body fluid specimens. As often numerous tests are requested for an individual body fluid sample, it is important not to waste any of the submitted specimen to be sure that ample specimen is available to complete all of the requested testing. There will be occasions, however, in which the submitted sample will not be of sufficient volume to allow for all of the testing that has been requested. In such instances, there must be communication between the testing laboratory and the submitting clinician to prioritize testing. The treating clinician will be the most familiar with the patient and the diagnostic considerations and will be best able to make decisions regarding test prioritization.

Chapter 2 is dedicated to laboratory methodology. It provides an overview of testing considerations and reviews the basic principles in the processing and evaluation of body fluids. In contrast to the previous edition, this text presents the principles and description of body fluid laboratory testing methodologies together within a single chapter. It includes a brief review of synovial fluid crystal analysis. The remaining chapters cover specific body fluid types and their unique features. New chapters have been added, including a chapter on urine and specialized body fluids. The chemical analysis and microbiology sections of the chapters have been expanded to provide the reader with current testing options and strategies. The urine chapter
emphasizes morphologic evaluation and reviews many of the common findings that will be encountered during urinalysis. The chapter on specialized body fluids introduces and discusses basic concepts for the evaluation of saliva, vitreous and sweat, including a brief discussion on the workup of cystic fibrosis. The seminal fluid chapter has been expanded and includes testing recommendations and current testing strategies.

During the last several years, numerous tests for the evaluation of body fluids, which were previously manual and labor intensive, have now become automated. Part of this automation has come about due to the increased need of laboratories to become more efficient with cost containment. Such utilization measures are among the most current topics discussed by laboratory directors and administrators and will continue to be an important aspect of laboratory medicine and health care delivery. Similar to the testing of other specimen types, the use of automated analyzers in the testing of body fluids must strictly follow manufacturers’ guidelines and be employed only for those body fluid types cleared and specified in the “intended use” statement for the device. If the laboratory modifies the approved testing device or a testing kit, performance specifications, including accuracy, precision, sensitivity, specificity, reference intervals and reportable range of test results, must be established before specimen testing and reporting of patient results. Quality control measurements must also be in place to assure the proper functioning of the testing instrumentation.

The testing of body fluid specimens is subject to the same quality measures and patient safety risks as testing done in the clinical laboratory on all other specimen types. Numerous preanalytic, analytic and postanalytic variables influence sample testing and results. Examples of preanalytic variables include test ordering, patient and specimen identification, and specimen collection and transport. Analytic variables include specimen viability, instrument function and Q/C. Result reporting, result communication, error correction and specimen storage are postanalytic variables. Other factors that can affect laboratory testing and result reporting include administrative oversight and leadership, properly trained personnel, laboratory information systems, instrumentation, physical facilities and process control.

Furthermore, the evaluation and testing of body fluids requires the laboratory to follow the same laboratory standards and regulations, including test validation, quality assurance/quality control and proficiency testing (PT). Laboratory testing activities are under federal regulation through CLIA ’88. All laboratories must comply with all of the provisions of CLIA. Laboratories must be licensed and accredited according to both federal and state requirements. In addition, laboratories must adhere to the standards of the Health Insurance Portability and Accountability Act (HIPAA), which was enacted in 1996 and protects the confidentiality of health information while allowing its exchange in appropriate circumstances. Additionally, all clinical laboratories within the United States must have a designated laboratory director who is professionally responsible and legally accountable for the testing results. The laboratory director oversees all of the activities of the laboratory.

Numerous proficiency testing options are now available for body fluids from accreditation agencies such as the College of American Pathologists (CAP). Some of these require laboratory testing of specific analytes and others are pictures or images that require morphologic identification of the cellular elements. Similar to other proficiency testing, the submitted results are scored and a summary report is sent back to the testing laboratory. When proficiency testing is not available for specific analytes, interlaboratory exchange programs can be utilized for result comparison. In addition, as a given specimen may be evaluated in multiple areas of the laboratory, such as morphologic examination in both the hematology and cytology areas, it is important to have a system in place to regularly compare results within the same laboratory.

Throughout the last several years numerous important ancillary techniques have been developed and are more commonly utilized to evaluate body fluids. Many of these ancillary tests are now more universally available. These include flow cytometry, FISH analysis, karyotyping, and molecular testing. Furthermore, microbiology testing has also changed significantly and not infrequently utilizes molecular testing modalities. However, in spite of the numerous advancements in testing techniques and strategies now available, many time honored procedures, eg, the Gram stain and cytologic/morphologic examination, remain immensely important and should not be overlooked.

A thorough understanding of body fluid evaluation and testing by laboratory personnel is essential. Such knowledge provides the members of the laboratory team the ability to produce high quality testing results and assist their clinical colleagues with test selection, testing sequence and prioritization. It is through high quality laboratory testing that we contribute to the care of patients and serve as vital members of the health care delivery team. Morphologic examination along with appropriate laboratory testing serves as the cornerstone of patient care and directs the diagnosis and downstream therapies of patients.
Anatomy & pathophysiology

The pleura (Greek: side, rib) consists of a thin, serous membrane that envelops the lung as well as the chest wall, diaphragm, and mediastinum. The 2 layers are contiguous, and the space between them forms the pleural cavity, which is lined by a single layer of mesothelial cells (the mesothelium). The outer layer, the parietal pleura, is adherent to the chest wall, and the inner layer, the visceral pleura, invests the lung except at the hili where vessels, bronchi, and nerves enter the lung. Normally, the 2 layers of pleura are in apposition, separated only by a small amount of fluid that facilitates movement of the 2 membranes against each other. The pleural cavity, therefore, is not a true cavity but only becomes so in the presence of disease that causes the accumulation of fluid therein. [Light 2007]

Similar to the pleural cavity, the pericardial (Greek: around the heart) cavity is only a potential cavity formed by 2 serous membranes that are closely apposed to each other (one lining the heart [visceral pericardium] and the other lining the inside of the pericardium [parietal pericardium]) and separated by small amounts of serous fluid. This fluid allows the heart to move easily during contraction and relaxation. Following injury or the onset of disease, more fluid may accumulate within the cavity, causing a separation between the visceral and parietal pericardia.

The accumulation of fluid within the pleural or pericardial cavities is called an effusion, and fluids that accumulate in pleural or pericardial cavities are referred to as serous effusions. The aspiration of pleural fluid is called thoracentesis. A thoracentesis is indicated for any undiagnosed pleural effusion. In addition to its diagnostic uses, thoracentesis may have therapeutic benefit to relieve dyspnea (shortness of breath) caused by large effusions.

Pleural fluid is normally produced by the parietal pleura and absorbed by the visceral pleura. The fluid is formed by filtration of plasma through the capillary endothelium, and its presence is dependent on the hydrostatic pressure in capillaries, plasma osmotic pressure, lymphatic resorption, and permeability of capillaries. Fluid is reabsorbed by lymphatic vessels and venules in the visceral pleura. Changes in the rate of production or removal of fluid can explain the accumulation of fluid.

A pleural effusion develops from an imbalance or disruption in the homeostatic forces that control movement of fluid across pleural membranes t5.1. A pleural effusion develops when the amount of fluid that enters the pleural cavity exceeds the amount that can be removed (via the lymphatics). Thus, pleural effusions can result from increased pleural fluid formation, decreased lymphatic resorption from the pleural cavity, or a combination of these factors. Pleural effusions are typically diagnosed by physical exam and chest radiography.

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>90% of pleural effusions are caused by congestive heart failure (CHF), cirrhosis of the liver, pleural and/or pulmonary infection, malignancy, or pulmonary emboli t5.2. Sometimes, several factors play a role in the etiology of pleural effusions. CHF or chronic kidney disease (CKD) may change the hydrostatic and osmotic forces across the pleura and cause an effusion that is an ultrafiltrate of plasma. Such an effusion is called a transudate. A localized disease, such as bronchopneumonia, is associated with increased permeability of the capillaries in the pleura and causes an effusion rich in leukocytes and serum proteins, and sometimes microorganisms. Such an effusion is called an exudate.
5.2 Causes of pleural effusions

**Transudates**

- Atelectasis
- Cirrhosis
- Congestive heart failure
- Nephrotic syndrome
- Peritoneal dialysis
- Postsurgery
- Superior vena cava obstruction

**Exudates**

- Infections
  - Bacterial pneumonia, lung abscess, empyema
  - Fungal
  - Parasitic
  - Viral
- Tuberculosis
- Neoplastic disease
  - Metastatic malignancy: carcinoma, sarcoma
  - Primary intrathoracic malignancy: carcinoma of the lung, mesothelioma
- Lymphoma and leukemia
- Pulmonary embolization and/or infarction
- Rheumatologic diseases
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
- Gastrointestinal disease
  - Abscess: subphrenic, hepatic
  - Esophageal rupture
  - Pancreatitis
- Myocardial infarction
- Trauma
- Hemorrhage
- Chylothorax

**Chylous effusion**

- Trauma
- Malignancy: carcinoma, lymphoma
- Tuberculosis

Accumulation of fluid in the pericardial cavity (pericardial effusion) is most frequently caused by damage to the lining of the cavity associated with changes in the permeability of the membranes due to infection (pericarditis), malignancy, or metabolic injury (e.g., CKD leading to uremia) **5.3.** The procedure of aspiration of pericardial fluid is called pericardiocentesis. In acute pericarditis, a fibrinous exudate interferes with pericardial venous and lymphatic drainage and predisposes the patient to effusion.

The major infectious agents responsible for pericarditis and pericardial effusion are viruses, especially the Coxsackie and echoviruses. Purulent bacterial and tuberculous effusions are less common. CKD is commonly associated with pericardial effusion. Significant effusion is often associated with the postinfarction syndrome (Dressler syndrome). Cardiac rupture or acute aortic dissection rapidly produces a bloody effusion and cardiac tamponade. Metastatic tumors and, rarely, primary tumors of the pericardium (mesothelioma) are often associated with large effusions. Lung and breast carcinomas, together with lymphoma and leukemia, are the most common causes of secondary malignant pericardial effusions. Traumatic pericardial effusions caused by stab wounds or crush injuries frequently result in cardiac tamponade, which is often fatal.

**Specimen collection**

A thoracentesis is indicated for any undiagnosed pleural effusion or for therapeutic reasons when fluid causes significant dyspnea. Position papers of the American College of Physicians & the American Thoracic Society have summarized the general application, safety, efficacy, and cost of diagnostic thoracentesis [PMID 4051357, PMID 2751171].

The procedure of thoracentesis consists of inserting a needle, under local anesthetic, into the pleural cavity and aspirating fluid. The specimen should be collected in heparinized tubes to avoid clotting, particularly if bloody. For a cell count an EDTA tube should be used. Aliquots for aerobic and anaerobic bacterial cultures are best inoculated into blood culture media at the bedside. When malignancy, mycobacterial infection or fungal infection is suspected, at least 100 mL should be submitted.
If cytologic examination cannot be performed immediately, the specimen should be refrigerated. Reasonable morphologic features will be maintained for 48-72 hours at refrigerator temperature. For determination of pleural fluid pH, the sample should be collected anaerobically, in a heparinized syringe, and sent to the laboratory. Finally, venous blood should be obtained for total protein, LD, cholesterol, and possibly bilirubin for comparison with pleural fluid.

A diagnostic thoracentesis is generally a safe and low risk procedure, and serious complications are uncommon. Complications of thoracentesis include pneumothorax, hemotherax, reexpansion pulmonary edema, and rarely air embolism. Leakage of air into the pleural space through the needle is the most common cause of pneumothorax. Hemothorax may be caused by trauma to the intercostal blood vessels. It is estimated that ~2% of all pleural infections are due to contamination of the pleural space at the time of thoracentesis. Removal of >1,000 mL of pleural fluid at any one time may result in pulmonary edema or severe hypotension. A thoracentesis may be contraindicated in a severe coagulopathy or with severe thrombocytopenia.

Pleural biopsy

A biopsy of the pleura is indicated in patients with an exudative pleural effusion of undetermined origin. A pleural biopsy may especially be useful in the diagnosis of malignancy, eg, mesothelioma, or tuberculosis. If tuberculous pleuritis is considered, a portion of the pleural biopsy specimen should be cultured for Mycobacteria. The identification of granulomas in the biopsy usually suggests tuberculosis, but fungal disease, sarcoidosis, and rheumatoid pleuritis should also be considered.

Transudates & exudates

Effusions of pleural and pericardial (as well as peritoneal) cavities have classically been divided into transudates and exudates t5.4 [PMID 4642731]. In general, transudates indicate fluid that has accumulated because of a systemic disease. The effusion is usually bilateral. A common disorder associated with transudates is CHF. Exudates are usually associated with localized disorders involving the pleural surfaces such as inflammatory conditions, malignancy, or infection. CHF and cirrhosis of the liver cause most transudative pleural effusions, while cancer, pneumonia, and pulmonary emboli cause most exudative pleural effusions.

The main usefulness in defining a pleural effusion as a transudate or an exudate is to determine which effusions need further laboratory (or other) evaluation. If the effusion is a transudate, usually no further diagnostic procedures are needed. If the effusion is an exudate, more extensive diagnostic procedures are indicated to determine the cause of the effusion. Although transudates and exudates frequently differ in such characteristics as color, appearance (clear vs cloudy or bloody), and cell count, these assessments are unreliable in establishing which type of effusion is present.

The most reliable tests for differentiating between the transudates and exudates are the simultaneous analysis of pleural fluid and serum for total protein and lactate dehydrogenase (LD) concentration (Light criteria). In transudates, the ratio of pleural fluid total protein to serum total protein is <0.5, while the corresponding LD ratio is <0.6. Exudates have corresponding ratios higher than 0.5 and 0.6, respectively. It has also been suggested that transudates have LD activity that is >2/3 of the upper reference limit for serum LD, while exudates have LD activity <2/3 of the upper reference limit for serum LD.

Using the results of simultaneous testing of serum and pleural fluid protein and LD activity, together with those of a good clinical history and physical examination, an accurate determination of exudates vs transudates should be possible in >90% of patients with effusions.

Cholesterol levels in pleural effusions have been recommended in differentiating exudates from transudates. A pleural fluid cholesterol level <45 mg/dL is usually associated with transudates, while >45 mg/dL is usually associated with exudates. In addition, a pleural cholesterol:serum cholesterol ratio (PS CHOL) of <0.3 is usually associated with transudates, and a ratio of >0.3 is usually associated with exudates. Pleural fluid cholesterol levels and serum cholesterol levels correlate in patients with exudative lesions but not in patients with transudates. It is thought that elevated levels of pleural fluid cholesterol are related to increased permeability of the pleural capillaries. These cholesterol tests may have greater sensitivity and specificity than the previously recommended tests of protein ratio and LD ratio.

### Pleural fluid laboratory differentiation of transudates & exudates

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<th>Transudate</th>
<th>Exudate</th>
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<tbody>
<tr>
<td>Appearance</td>
<td>Clear, pale yellow</td>
<td>Cloudy, turbid, purulent or bloody</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>&lt;1.015</td>
<td>&gt;1.015</td>
</tr>
<tr>
<td>Pleural fluid protein:serum protein ratio</td>
<td>&lt;0.5</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Pleural fluid LD</td>
<td>&lt;2/3 of the upper reference limit for serum LD</td>
<td>&gt;2/3 of the upper reference limit for serum LD</td>
</tr>
<tr>
<td>Pleural fluid:serum LD ratio</td>
<td>&lt;0.6</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>Pleural fluid:serum bilirubin ratio</td>
<td>&lt;0.6</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>Pleural fluid cholesterol</td>
<td>&lt;45 mg/dL</td>
<td>&gt;45 mg/dL</td>
</tr>
<tr>
<td>Pleural fluid:serum cholesterol ratio</td>
<td>&lt;0.3</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Cells</td>
<td>Few</td>
<td>Many</td>
</tr>
</tbody>
</table>
None of these tests are 100% effective in separating transudates from exudates. The Light criteria misclassify ~25% of transudates as exudates, and most of these patients are on diuretics [PMID 11400391]. Inaccuracies in separating transudates from exudates are common when the measured biochemical values are close to the cutoff values. Cholesterol and/or bilirubin concentration values may be useful when the protein and LD activity in the pleural fluid are equivocal. The reader is referred to the sections on chemical analysis for a more detailed discussion of these test parameters.

When the specimen is classified as an exudate, other studies are necessary to define the disease process further. Gram staining, staining for acid-fast organisms, cultures, and counterimmunoelectrophoresis may be indicated if an infection is suspected. Cytologic tests and biopsy may be diagnostic in cases of a suspected malignant condition.

The 4 leading causes of pleural effusions in order of incidence are congestive heart failure, malignancy, pneumonia and pulmonary embolism. Congestive heart failure and cirrhosis cause most transudative effusions, and malignancy, bacterial and viral pneumonia and pulmonary embolism cause most exudative effusions.

Recommended tests

To minimize unnecessary laboratory studies it is important to have a clear differential diagnosis in mind [PMID 18195577]. The results of laboratory studies should always be correlated with the clinical findings and results of other studies to arrive at an accurate diagnosis.

The purposes of laboratory testing of serous effusions are
- to differentiate transudates from exudates
- to differentiate malignant from nonmalignant effusions in patients with exudative effusions
- to diagnose specific causes of a serous effusions, eg, infection

A similar mindset is applicable to pericardial effusions although the focus is often more focused on the etiology (2 and 3, above) rather than determining the nature of the serous effusion, ie, transudative vs exudative [PMID 9449572].

Table 5.5 lists useful in differentiating transudates from exudates and Table 5.5 lists the most useful laboratory tests in most patients and tests useful in selected patients.

Gross examination

The initial step in the investigation of pleural & pericardial effusions is the gross examination, which can play an important role in determining the pathogenesis of the effusion [14/6]. Transudates are usually clear and pale yellow, and do not clot. Exudates are cloudy to purulent, and often clot while standing because of the presence of the serum protein fibrinogen. Exudative fluid usually contains large numbers of leukocytes and elevated protein content. Occasionally, exudates may be straw colored, similar to transudates. A cloudy, purulent fluid is usually associated with an infectious process. The presence of clearly visible pus is diagnostic of empyema. Anaerobic bacterial infection of the pleura may produce a feculent odor. A green white, turbid fluid may be seen with rheumatoid pleuritis.

Hemorrhagic fluid may be present because of a traumatic tap, malignancy, pulmonary infarction, rupture of an aortic aneurysm, chest trauma, pancreatitis, or tuberculosis. Metastatic adenocarcinoma is sometimes associated with mucoid, bloodstained fluid. Blood tinged fluid may also be seen in transudates, and only 1-2 mL of peripheral blood in 1,000 mL of pleural fluid will produce a blood tinged appearance. A traumatic tap must be distinguished from other sources of blood in the pleural fluid. In a traumatic tap, the blood is usually not distributed consistently and gradually clear as aspiration proceeds. A hematocrit should be obtained on bloody fluids. A pleural fluid hematocrit >1% is usually associated with malignancy, pulmonary embolus, or chest trauma. A hematocrit level >50% of that of the peripheral blood is associated with a true hemotherax, as may be seen in chest injuries.
When the pleural fluid is turbid, milky, and/or bloody, the specimen should be centrifuged and the supernatant examined. If the turbidity clears with centrifugation, it was most likely due to an increased number of cells or debris. However, if the turbidity persists after centrifugation, the patient most likely has a chylothorax or a pseudochylothorax. Chylous and so called pseudochylous effusions are typified by milky white pleural fluid. However, the classic milky white appearance may be present in <50% of cases of chylous effusions. It may be yellow or green and turbid, or even bloody. Following trauma, the chylous is frequently bloody, which may mask the milky appearance of chylothorax. Following centrifugation, fluid from a chylothorax remains opaque, whereas the milky fluid of empyema becomes clear.

A true chylothorax is rare and results from leakage of the thoracic duct, which is most commonly caused by a malignancy such as lymphoma or carcinoma. In rare cases, it may be the presenting sign of a malignant lymphoma. The 2nd leading cause of chylothorax is trauma, especially associated with cardiovascular surgery. The 3rd most common cause of chylothorax is idiopathic, including most cases of congenital chylothorax. Chylothorax is the most common form of pleural effusion encountered in the newborn. The origin of congenital chylothorax is unknown.

A pseudochylous effusion does not result from disruption of the thoracic duct but is associated with chronic effusions (usually several years) as seen in rheumatoid pleuritis and tuberculosis. Pseudochylothorax is much less common than chylothorax. When turbid or milky pleural fluid is present in a patient with longstanding pleural effusion, one needs to consider empyema and chylothorax in addition to pseudochylothorax. As previously discussed, in empyema centrifugation results in a clear supernatant.

Normally, only a small amount of clear, pale yellow fluid is present in the pericardial cavity. A pericardial effusion due to infection or malignancy usually has a turbid appearance. Blood streaked, cloudy fluid is frequently caused by tumors and tuberculosis. A bloody effusion is seen in cardiac rupture or puncture. Renal failure with uremia is usually associated with clear, straw colored fluid. A milky fluid may be seen when damage to the lymphatic system has occurred.

### Cell count

Leukocyte counts have limited value in separating transudates from exudates. It is primarily useful in cases of infection, ie, an elevated white blood cell count with increased numbers of PMNs in bacterial disease. Neutrophilia may also be seen in pulmonary infarction, pancreatitis, early tuberculosis, and subphrenic abscess. A predominance of lymphocytes is often seen in tuberculosis, viral infection, malignancy, true chylothorax, rheumatoid pleuritis and uremic effusions.

### Microscopic examination

An examination of the cells present in pleural or pericardial fluids and a differential cell count should be performed on a stained smear made by cytocentrifugation or other methods. Morphologically, the cytocentrifuge preparation is usually superior to a push smear. For routine clinical laboratory evaluation, a Romanowsky (Wright stain) stain and a Pap stain should be utilized (Romanowsky stain is especially useful in hematologic malignancies). The Pap stain provides superior nuclear detail and is better for the identification of squamous cells as in squamous cell carcinoma. Cell blocks may be a useful addition when a malignancy is suspected, especially for immunohistochemical studies, eg, keratin expression. A higher rate of recovery of tumor cells has been demonstrated in specimens examined with a combination of smear and cell block techniques. However, since the majority of effusions are benign, the routine use of cell blocks may not be a cost effective method of detecting malignancy. Cell blocks should only be used if initial cytologic preparations are suspicious or if clinical history indicates a likelihood of malignancy.

The cell types encountered in pleural, pericardial, as well as peritoneal fluids are granulocytes (polymorphonuclear leukocytes [PMNs], eosinophils, and basophils), lymphocytes, plasma cells, mononuclear phagocytes (monocytes, histiocytes and macrophages), mesothelial cells, and malignant cells.
Mesothelial cells form the lining of pleural, pericardial, and peritoneal cavities. Because of their variable appearance, they frequently cause difficulty in the microscopic examination and may be mistaken for malignant cells. It is essential to become familiar with the wide range of appearances seen in benign mesothelial cells in order to separate out reactive and proliferative mesothelial changes from malignancy, both primary and metastatic.

During inflammatory processes, mesothelial cells undergo proliferation and often desquamate into the serous fluid. These cells are notoriously pleomorphic. They may occur singly, in flat sheets, or rarely in 3 dimensional clusters that may have a spherical or papillary appearance. The cells are large and measure from 15-25 µm in air dried specimens. With alcohol fixation, the mesothelial cells are smaller, measuring from 10-14 µm in diameter. They may, however, be smaller or considerably larger than the measurements given. The cytoplasm is usually abundant but may be scanty, is light gray to deep blue, and may have a perinuclear zonal pallor. Cytoplasmic vacuoles of variable size may be seen. The nucleus is round to oval and occupies...
5.8 Loose aggregate of mesothelial cells

5.10 Mesothelial cells with cytoplasmic vacuoles

5.9 Cluster of mesothelial cells having an orderly mosaic appearance

5.11 Mesothelial cells with cytoplasmic blebs

5.12 A binucleate mesothelial cell

5.13 2 multinucleated mesothelial cells connected to each other
Kjeldsberg’s Body Fluid Analysis

~1/3-1/2 the cell’s diameter. The nuclear contour is usually smooth and regular, but may be irregular. The chromatin is stippled, dark purple, and uniformly distributed. 1-3 spherical nucleoli may be seen. Some mesothelial cells may resemble large plasma cells.

Various attempts have been made to classify the mesothelial cells according to their appearance and terms such as reactive, activated, hyperplastic, proliferative, and atypical are used. Unfortunately, there is no general agreement on the definition of these terms and since they are usually not meaningful clinically, their use should be avoided. The term proliferative and hyperplastic mesothelial cells usually refers to clusters of mesothelial cells, typically with moderate to scant cytoplasm and hyperchromatic nuclei. The term reactive mesothelial cells usually refers to cells with slightly irregular nuclei and prominent nucleoli. The term typical mesothelial cells usually refer to cells that resemble malignant cells and that the cytopathologist cannot always exclude from malignancy. So called atypical mesothelial cells may be particularly associated with liver cirrhosis, irradiation, connective tissue disorders, foreign bodies, neoplasms, infarction, and chronic inflammation of long duration. Mesothelial cells may be multinucleated, occasionally containing 20 or more nuclei. At times they may form glandlike structures.

Mesothelial cells, when they appear in clumps, are usually in loose aggregates and often are connected to each other in a characteristic manner. The cell borders are often “squared off” with clefts or clear spaces (“windows”) between the cells. Sometimes the cytoplasm of one cell appears to be clasping (embracing) another cell. One may also see large sheets of mesothelial cells having an orderly mosaic appearance. When cells have multilayered formation or 3 dimensional spheroidal arrangements, they are more likely to be malignant. These types of arrangements are usually better appreciated with a Papanicolaou stain.

Clumps of mesothelial cells may be distinguished from malignant cells by comparing their appearance within the clump and with other more readily identified mesothelial cells in the same smear thereby recognizing the “family
The uniform regular arrangement of the mesothelial cell usually indicates a benign origin. Benign mesothelial cell nuclei usually have a smooth nuclear outline and evenly distributed fine chromatin. The nucleoli may be large and prominent, but they are usually uniform in size and shape. In contrast, malignant cells frequently show nuclear outline irregularities together with irregular chromatin disbursement and nucleoli of varying size and shape.

Sometimes it may be impossible to differentiate mesothelial cells from malignant cells. In such cases, immunohistochemistry and flow cytometry may be helpful. Mitoses may be present in benign mesothelial cells and in malignant cells, and are of little diagnostic significance except when obviously abnormal. Difficulties in the distinction of mesothelial cells from malignant cells may particularly occur in smears that are technically not well prepared. When the cell concentration, for example, is high or bloody, there is an increased tendency for clumping of the cells and such cells are frequently overstained. In such cases, the specimen must be diluted to obtain optimal morphology. When only a thick overstained preparation is available, the examination should be made by reviewing cells at the periphery of the smear where the staining reaction is not as dark.

Degenerative mesothelial cells may show pyknosis and karyorrhexis, and they may contain peculiar cytoplasmic inclusions of varying colors. Degenerative changes may also be seen in the form of ground glass appearance to the cytoplasm and a coarse, motley, salamlike nuclear chromatin pattern. In longstanding effusions, the cytoplasm of the mesothelial cells may be filled with vacuoles that sometimes fuse and push the nucleus against the cell membrane producing a signet ringlike appearance. It is important not to mistake these degenerating cells for metastatic adenocarcinoma cells. Mesothelial cells may show evidence of phagocytosis. Mesothelial cells also frequently show a morphologic transformation characterized by loss of basophilia and development of foamy vacuolation, which produces a morphologic appearance indistinguishable from macrophages. Mesothelial cells may transform into macrophages and it is common to see various morphologic intermediate forms between mesothelial cells and macrophages. It is generally of no clinical significance to differentiate mesothelial cells from macrophages.

Mesothelial cells are seen in variable numbers in most effusions and are increased with pneumonia, pulmonary infarction, and malignant disorders. In tuberculous pleurisy, or when heavy concentrations of pyogenic organisms are present within the serous cavities, mesothelial cells are characteristically scarce. This is probably due to a fibrinous exudate covering the mesothelial lining of the cavity. Unusually small numbers of mesothelial cells may also be seen in patients in whom a sclerosing agent has been used as treatment for recurring malignant effusions and in patients with rheumatoid pleuritis.

Mononuclear phagocytes (monocytes, histiocytes, and macrophages) are usually seen in variable numbers in pleural, pericardial, and peritoneal effusions. Since both histiocytes and mesothelial cells may be transformed into macrophages, the distinction between them is not always obvious. The terms macrophage and histiocyte are used synonymously in this book although some authors distinguish between them by defining the macrophages as those that show evidence of phagocytosis. Macrophages vary in size and have a diameter of 15-25 µm. The cytoplasm is pale gray, cloudy, and frequently vacuolated. The nuclei may be bean shaped, lobular, ovoid, or round. Sometimes large (up to 50 µm) macrophages may be seen. So called signet ring cells are formed when the small vacuoles fuse forming one or 2 large vacuoles that flatten the nucleus against the side of the cell membrane. Signet ring cell is a descriptive term and may be seen often in benign and malignant cells.

A variety of names have been given to the macrophages depending on the material they have phagocytosed. If the term lipophage is used, then phagocytosed free lipid material is present in tiny phagocytic vacuoles. When digestion
of the phagocytosed neutrophil occurs, the remnants may be seen in the form of purple granules or pigment. An erythrophage is a macrophage that contains phagocytosed red blood cells. As red blood cells break down, hemosiderin pigment is seen within the macrophage and the term siderophage is used. The hemosiderin appears as darkly stained blue black granules in air dried smears. Crystalline hematin pigment may also be present. It is important not to mistake ingested and digested cellular products within the macrophage for intracellular organisms. Bacteria and yeast that may be present in macrophages and neutrophils characteristically have a regular shape. Yeast may have a capsule with a small clear zone around each individual organism. Macrophages may also contain bile pigment that may appear black or yellow and carbon that is black.

Lymphocytes are seen in variable numbers in most serous effusions. They may be small, medium, or large in size, and they may exhibit reactive (transformed) changes. It is important to be aware of the various
morphologic features that lymphocytes have as they morphologically transform in response to many stimuli. Such lymphocytes may have an immature appearance, suggesting lymphoblastic leukemia or lymphoma if they represent the predominant cell type. Lymphocytic nucleoli are often more prominent in effusions than in peripheral blood and the nuclei may be cleaved. The irregularity of the nuclear contours that is sometimes seen is often caused by centrifugation during the concentration process, and therefore caution should be used in morphologic evaluation. A variable number of transformed or reactive lymphocytes may be present. These are large lymphocytes with abundant deep blue cytoplasm and often with several prominent nucleoli. Mitoses are fairly common in the transformed lymphoid cells and they should not be mistaken for evidence of malignancy. In difficult cases, flow cytometric analysis may be very helpful.

Tuberculosis and malignant effusions frequently show a predominance of small lymphocytes. In tuberculosis, the fluid characteristically shows lymphocytosis and only a few
mesothelial cells. However, the absence of lymphocytosis does not rule out the diagnosis of tuberculosis or malignant effusions. In non-Hodgkin B cell lymphomas, the malignant lymphocytes are generally uniform or monotonous in size, shape, and staining characteristics. This is in contrast to benign, inflammatory lymphocytosis in which there is usually a mixture of different types of lymphocytes, which may appear polymorphous. However, in many T cell lymphomas, like anaplastic large cell lymphoma, the cells may be very pleomorphic. Lymphocytic pleural effusions may also be associated with leakage of the thoracic duct (chylothorax). In such patients, the lymphocytes are usually small and uniform 5.28. A lesser degree of lymphocytosis may be seen in CHF and cirrhosis.

Immunophenotypic studies have shown that the majority of lymphoid cells in reactive effusions are T cells with the CD4:CD8 ratio being somewhat higher than that reported for the peripheral blood. Phenotypic analysis of pleural fluid lymphocytes is probably not helpful in distinguishing tuberculosis from other nonmalignant effusions. It has been observed that B cells could represent up to ~35% of cells in benign cases and as few as ~5% in a case of malignant B cell effusions. This suggests that the relative numbers of T cells and B cells alone may not be adequate to differentiate benign from malignant effusions. The presence of a monoclonal B cell population, however, is usually associated with a malignant lymphoma.

Plasma cells may be seen in fluid specimens of patients with rheumatoid arthritis, malignant disorders, tuberculosis, and other conditions associated with lymphocytosis. Neutrophils may differ in appearance in serous fluids 5.29. They may appear more or less identical to those seen in the blood or may be difficult to recognize as PMNs. In longstanding effusions, the granules may be decreased in number or lost. The nuclei may appear as densely stained spherical fragments and may be mistaken for nucleated RBCs. Occasionally, the cytoplasm may have a blue color so that the PMNs resemble lymphocytes. In effusions, due to infection, the PMNs may show evidence of degeneration in the form of vacuolation, loss of granules, and blurring of the nuclei 5.30-5.31.

Gram stains are useful in detection and identification of bacteria 5.32-5.33. *Echinococcus* from a ruptured hydatid cyst is clear without staining 5.34.

It is debatable how valuable the differential leukocyte count is in the differential diagnosis. The number of PMNs present in different effusions may vary but a predominance of PMNs suggests bacterial pneumonia, pulmonary infarction, or pancreatitis. Serous fluid neutrophilia is usually the initial cell reaction to these conditions. Later, a predominance of mononuclear cells and mesothelial cells may be noted.

Eosinophilic pleural effusion has been defined as an effusion in which there are >10% eosinophils 5.35. Pleural fluid eosinophilia may occur in a wide array of disorders including infections, neoplasms, pulmonary infarction, connective tissue disorders, and hypersensitivity states, in association with parasites and in pneumothorax. When peripheral blood eosinophilia is also present, one should consider the possibility of hydatid disease, Löffler syndrome, periarteritis nodosa, trauma, or Hodgkin lymphoma. Pleural fluid eosinophilia is thus not a helpful
diagnostic finding. It may, however, be a marker for a more favorable immune or inflammatory response to pleural disease [PMID 8915232]. Charcot-Leyden crystals have been described in eosinophilic pleural effusions.

Mast cells and basophils often accompany eosinophils, and 5%-10% may be seen in pleural fluid eosinophilia.

Lupus erythematosus (LE) cell formation has been reported in pleural, pericardial, and peritoneal fluids 15.36. Serous fluids allowed to stand at room temperature may have more LE cells than the same fluid examined shortly after it was aspirated which indicates that LE cells are formed in vitro and in vivo. So called “tart cells,” which are cells morphologically similar to LE cells, are more frequently seen in serous fluids. They represent small macrophages that have phagocytosed a nonhomogenized nucleus of another cell. The phagocytosed nuclear material in an LE cell is smooth and homogeneous.
Megakaryocytes and immature myeloid cells may be seen in pleural fluid associated with myeloproliferative disorders. It is important not to mistake these cells for metastatic carcinoma, malignant mesothelioma, or lymphomas.

Malignant cells from a variety of neoplasms may be encountered in pleural and pericardial cavities, and microscopic examination for malignant cells may be the most important part of the examination of an undiagnosed exudative fluid. A more detailed description of various malignant conditions is found under the section “Clinical considerations.”

As mentioned earlier, when performing the microscopic examination it is important to be aware that a variety of artifacts and cellular changes may be present in serous fluid specimens. The cytocentrifuge instrument is generally excellent for concentrating the cells on the slide. There are, however, frequently various cellular distortions caused by the spinning process.
5: Pleural & Pericardial Fluids


5.43 Metastatic lung carcinoma cells (arrow) surrounded by mesothelial cells & macrophages

5.44 Typical arrangement of small cell carcinoma of lung showing molding of nuclei (arrow)

5.45 Tumor cell phagocytosing another tumor cell (cannibalism°). Small cell carcinoma of lung.

5.46 Squamous cell carcinoma of lung. Papanicolaou stain.

5.47 Metastatic carcinoma of unknown primary (arrow)
Giant tumor cells in metastatic adenocarcinoma

Metastatic carcinoma of unknown primary with a "signet ring" tumor cell (arrow)

Malignant mesothelial cells. Cell block (H&E stain).

A large malignant mesothelial cell (arrow)

Metastatic adenocarcinoma. Cell block (H&E stain).

Malignant mesothelial cell (arrow). Papanicolaou stain.
Sheets of malignant mesothelioma cells resembling benign mesothelial cells

Metastatic melanoma cells containing multiple vacuoles resembling a mesothelioma

A papillary cluster of ovarian carcinoma cells

A melanoma cell identified with HMB45 immunohistochemical stain

Metastatic esophageal carcinoma

A cluster of metastatic neuroendocrine carcinoma (arrow)
Anatomy & pathophysiology

The urinary tract plays vital roles, largely through the kidneys, in normal human physiology including homeostasis of volume, electrolyte levels, and maintenance of acid-base balance. It includes the kidneys, ureters, urinary bladder, and urethra. The microscopic anatomy of the kidney is centered on the nephron, of which there are ~1,000,000 per kidney, and its microanatomy is well described in standard histology and anatomy textbooks. As such, the kidney shows remarkable reserve and loss of 50% of renal function is generally well tolerated. The renal pelves all the way to the urethra, with a large reservoir in the urinary bladder, principally serve as the conduit of urine to be excreted. This urinary system is lined by a unique epithelium, urothelium, which is distinct from squamous and glandular epithelium. It should be noted, however, that the distal urethra is lined by squamous cells. The urothelium is composed of 2 major cell types—superficial (sometimes referred to as cap, dome, or umbrella) cells and basal cells. By electron microscopy, the unique nature of this epithelium is readily identified where the intercellular junctions prevent “backflow” of urine.

Like other body fluids, urine may be viewed as an ultrafiltrate of blood (ie, plasma). The kidney is the principal site for this event receiving ~25% of the cardiac output. Normally, the kidneys produce ~1L of urine per day. The functional unit in the kidney is the nephron, which includes the glomerulus and the tubules. Under normal physiologic conditions, the glomerulus filters plasma by retaining proteins and other components. Under abnormal conditions (eg, damage from autoimmune disease) the glomerulus may not be able to properly serve as a filter and leakage occurs, as is the case with the loss of protein in the urine (proteinuria).

The history of urinalysis dates back to at least the Middle Ages. At that time, it was referred to as uroscopy and was based on the gross examination of urine for the identification of disease (eg, diabetes). However, it was also referred to as “pisse prophecy.” Modern urinalysis coincides with the development of the microscope, almost 200 years ago, and chemical analyses, ~100 years ago; the identification of microorganisms overlaps with these 2 approaches. Today, much of the analysis is automated but laboratorian involvement, especially microscopy, remains key.

A large number of diseases can affect the kidney and result in changes in the urine. Consequently, the examination of urine (urinalysis) can be of vital importance. For example, the identification of proteinuria leads to the development of a distinct differential diagnosis that requires further testing to identify the specific etiology. Similarly, hematuria, azotemia, pyuria, and abnormal urothelial cells requires additional investigation. In short, any of the major disease processes (eg, environmental, genetic, hemodynamic, immunological, inflammatory/infectious, neoplastic, nutritional, and thromboembolic) may affect and involve the urinary system and be detectable in some fashion by urinalysis.
Clinical indications & considerations & recommended laboratory studies

Examination of urinary sediment is important in certain clinical settings. Normal sediments either rule out certain diseases or make them very unlikely. Abnormal sediments, principally the identification of cells (eg, red blood cells [RBCs], white blood cells [WBCs]), casts (eg, hemoglobin, granular), or crystals (eg, uric acid, tyrosine) indicates significant disease, many of which involve the kidney. An approach focusing on the most common laboratory studies is presented in f9.2. Generally, a “shotgun” approach is not cost effective and the clinical history and physical findings should be incorporated into the decision making. Obviously, a reagent strip (or “dipstick”), despite its many limitations, is a relatively inexpensive, rapid, and useful way to screen specimens in the physician’s office. Based on this, samples may then be sent to the laboratory for more detailed and accurate analyses.

The specific clinical scenario will determine what testing will occur. For example the workup of hematuria, which has a long differential diagnosis, will likely include urinalysis [PMID 9135826]. Some of the diseases that should be considered include coagulopathy, stones, urothelial carcinoma, and glomerulonephritis. If cancer is a likely consideration, then cytology and further workup often by a urologist, is the course. Glomerulonephritis, of which there are many types, is typically evaluated by a nephrologist. However, it is worth noting that extensive workup for asymptomatic microscopic hematuria may not be cost effective.

One of the more common causes of hematuria is infection of the urinary bladder, which is associated with clinical symptoms such as dysuria, increased frequency, and pyuria (neutrophils in the urine). Bladder infections are often caused by Gram– bacilli (eg, E. coli).

Careful examination of the sediment for casts and crystals is important to identify a host of diseases that may be renal in origin or systemically based with secondary renal involvement.

There are several common indications for the examination of urine. One would be azotemia, an elevation in blood urea nitrogen (BUN) and an indication of a decrease in the glomerular filtration rate (GFR). There may be abnormalities in the specimen including proteinuria, hematuria, pyuria, casts, or crystals. A change in urinary volume (increased or decreased) warrants further examination. Other clinical indicators suggesting urinary tract disease, notably the kidney, include hypertension (with or without edema), electrolyte abnormalities, acid-base disturbances, as well as fever and pain. In short, there are a large array of diseases and disease processes that can involve the urinary tract, whether as the primary site or as a signal of systemic illness.

Specimen collection, requirements & stability

A variety of urine specimens may be collected and submitted to the laboratory. As one might predict, this is predicated on the clinical setting. Examples include a clean catch urine (often a random collection done in a physician’s office), first morning specimen (akin to an 8 hour collection), 24 hour urine specimen, a fasting specimen, a catheterized specimen, a specimen from a suprapubic aspiration (sometimes performed in young children), and a bladder wash (for cytoclogic evaluation of malignancy) t9.1. Patients must be given detailed instructions for the appropriate collection, which must be done in a sterile manner.

<table>
<thead>
<tr>
<th>Specimen collection, requirements &amp; stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A variety of urine specimens may be collected and submitted to the laboratory. As one might predict, this is predicated on the clinical setting. Examples include a clean catch urine (often a random collection done in a physician’s office), first morning specimen (akin to an 8 hour collection), 24 hour urine specimen, a fasting specimen, a catheterized specimen, a specimen from a suprapubic aspiration (sometimes performed in young children), and a bladder wash (for cytoclogic evaluation of malignancy) t9.1. Patients must be given detailed instructions for the appropriate collection, which must be done in a sterile manner.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common urinary specimen types used for morphologic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voided</strong></td>
</tr>
<tr>
<td>Inexpensive</td>
</tr>
<tr>
<td>Scant cellularity</td>
</tr>
<tr>
<td>Poor preservation</td>
</tr>
<tr>
<td>Samples entire urinary system</td>
</tr>
<tr>
<td>Readily obtained</td>
</tr>
<tr>
<td>Noninvasive</td>
</tr>
<tr>
<td>Contamination</td>
</tr>
<tr>
<td>Mostly single cells</td>
</tr>
<tr>
<td>Squamous cells, umbrella cells, inflammatory cells</td>
</tr>
</tbody>
</table>

*If indwelling, always contaminated
Ideally, specimens should be submitted to the laboratory within 2 hours of collection per Clinical and Laboratory Standards Institute (CLSI) guidelines. For specimens that may be delayed (eg, 24 hour urine or transport issues) refrigeration or preservatives may be used. A common preservative is boric acid and for environmental reasons, nonmercuric preservatives should be utilized; other preservatives that have been used include formalin, toluene, and chlorhexidine.

One recurring problem is ensuring proper labeling of the specimen and the provision of a fully executed laboratory online requisition that includes relevant historical and current clinical information.

Gross examination

Appearance
The gross examination of urine begins with its appearance, and color is the most notable.

Color
The yellow color of urine is due to urochrome, an end product of hemoglobin catabolism \(t9.2\). Red suggests blood and only a small amount is needed to be identified as gross hematuria. Other colors may be observed including brown (suggesting bile pigments), orange (urobilinogen), and dark brown (suggesting hemoglobin). Certain color changes may also be due to drugs or foods, such as beets.

<table>
<thead>
<tr>
<th>Color</th>
<th>Possible pathologic etiologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>Diabetes insipid</td>
</tr>
<tr>
<td>Hazy</td>
<td>Casts, crystals, microorganisms, cells</td>
</tr>
<tr>
<td>White</td>
<td>Pyuria, lipids, chyle</td>
</tr>
<tr>
<td>Yellow</td>
<td>Bilirubin and its metabolic derivatives</td>
</tr>
<tr>
<td>Pink-red</td>
<td>Hemoglobin, RBCs, myoglobin, porphyrins, beeturia</td>
</tr>
<tr>
<td>Brown</td>
<td>Methemoglobin, myoglobin, porphyrins</td>
</tr>
</tbody>
</table>

Clarity
Normally, urine is clear and free of particulate material. Various states of turbidity may be encountered and the etiologies include crystals, abundant cells (eg, WBCs or epithelial cells), mucin, and lipids.

Odor
There is an odor associated with normal urine but certain conditions can alter this. For example, bacterial overgrowth (fetid odor) and certain metabolic disorders (eg, maple syrup urine disease and phenylketonuria [mousy]) will produce characteristic odors.

Volume
Recording the volume of urine received in the laboratory is a critical part of the gross examination. This is in part an issue of specimen type; a random sample may be 30-60 mL, which will be quite different from a 24 hour urine collection. As noted, the normal daily urine output is ~1 L and there may be either less or more volume depending on the underlying disease process. Polyuria may be seen with diabetes mellitus and diabetes insipidus, the latter due to a deficiency of antidiuretic hormone (ADH). Decreased urine volume may be seen with dehydration or intrinsic renal diseases.

Specific gravity & osmolality
Specific gravity and osmolality both describe the density of a urine sample, which is related to the concentrating ability of the kidney and the patient’s state of hydration. Specific gravity is dependent on the number and weight of the total solutes, or dissolved solids, found within the urine. More precisely, it is defined as the ratio of the mass of a solution (eg, urine) to the mass of an equal volume of water. The solutes—urea, sodium chloride, phosphate and sulfate—contribute significantly to the specific gravity of urine in a healthy individual. As it is a ratio, specific gravity is dimensionless and normal values range from 1.003-1.035, with lower values representing dilute urine and higher values associated with very concentrated urine.

Urine with low specific gravity may occur in patients with impaired renal concentrating ability such as glomerulonephritis, diabetes insipidus, or pyelonephritis. Elevated specific gravity values may be associated with diabetes mellitus, dehydration, congestive heart failure, adrenal insufficiency, or liver disease. Increased concentrations of glucose and protein contribute to the density of urine and will therefore elevate the specific gravity. Administration of radiographic dyes or dextran solutions can result in extremely high specific gravity values that may confound interpretation. When the kidney has lost the ability to either concentrate or dilute the urine, the specific gravity will not vary considerably from 1.010 (the specific gravity of plasma) upon repeated analysis, which is known as isosthenuria.

Several methods exist for the measurement of specific gravity. Dipstick pads containing electrolytes along with color dyes that detect hydrogen ion release (pH) as the pKa of the electrolytes change in relation to the ionic concentration of the urine. Since this indirect measurement technique detects only ionic solutes, excess glucose, protein, or dyes reportedly do not interfere. A refractometer may be used to indirectly determine the refractive index of a urine sample as compared to water. The refractive index, or ability of light to bend as it passes through a substance, is directly proportional to the density of particles within the sample and is thus related to specific gravity. This nonspecific technique therefore includes large interfering solutes in the final value. Measurements are determined by viewing the sample through the refractometer as it is directed toward a light source. The refractometer must be precisely calibrated in order to provide accurate results. Urinometer and falling drop techniques also exist for the measurement of specific gravity of urine but are more cumbersome and not...
in voided urine (and hence abnormal) is not necessarily the same as in a first morning urine or a bladder wash.

### 9.3 Morphologic elements encountered in urinary specimens

<table>
<thead>
<tr>
<th>Cellular</th>
<th>Noncellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>Crystals</td>
</tr>
<tr>
<td>Urothelial cells—umbrella, basal</td>
<td>Casts</td>
</tr>
<tr>
<td>Squamous cells</td>
<td>Corpsa amylaceae</td>
</tr>
<tr>
<td>Glandular cells, including renal tubular epithelial cells</td>
<td>Artifacts</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>Talc</td>
</tr>
<tr>
<td>PMNs</td>
<td>Fibers</td>
</tr>
<tr>
<td>Macrophages, including giant cells</td>
<td>Pollen</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>PMN = polymorphonuclear leucocyte (neutrophil)</td>
</tr>
<tr>
<td>Microorganisms</td>
<td>Parasites—Trichomonas vaginalis, Schistosoma ova</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>Mycobacteria—bacillus Calmette-Guérin (BCG)</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
</tr>
<tr>
<td>Fungi (yeast)</td>
<td></td>
</tr>
</tbody>
</table>

### Epithelial cells

There are 3 major cell types that may be observed: squamous, urothelial ("transitional"), and glandular [ISBN 019-5134598, ISBN 978-0891896449]. It is worth reiterating that the specimen type will determine what cell type may be observed and whether it is abnormal, and that a “normal” specimen, in the absence of disease, is scanty cellular. In a typical voided clean catch urine, squamous cells are the most abundant. These cells are large, platelike with centrally placed nuclei and a low nuclear:cytoplasmic (N:C) ratio

- **Squamous cells** are large, platelike with centrally placed nuclei and a low N:C ratio and are derived chiefly from the urethra. It is worth noting that women may have squamous metaplasia up to and involving the bladder trigone and that these cells may cycle as under normal physiologic conditions as the estrogen and progesterone levels vary.

- **Urothelial cells**, as previously mentioned, are of 2 major types—superficial or basal. The former are relatively large, although smaller than squamous cells, with abundant cytoplasm and relatively large nuclei; not uncommonly, these cells may be bi-or multinucleated. The basal cells, which are usually uncommon in voided specimens, are smaller, have a higher N:C ratio, and also lack any nuclear atypia.

- **Glandular cells** are relatively uncommon and are usually small, have a lower N:C ratio, and may be bi-or multinucleated. Obviously, in catheterized specimens or other instrumented specimens, urothelial cells may be more prevalent. In voided specimens, the identification of clusters of urothelial cells is

### Cell counts

The determination of cell counts is of limited value in the examination of urinary specimens. At best, semiquantitative values may be reported (eg, 2+ neutrophils) but these do not correlate well with quantitative determinations even if they are performed. That said, the reporting of the presence of red blood cells (RBCs), the various types of white blood cells (WBCs) and epithelial cells (squamous, urothelial, renal tubular) is important. In addition, as noted elsewhere (Microbiologic examination, below), the reporting of bacterial culture results including the threshold for a significant bacterial count is subjective, institutionally driven, and therefore a bit of a quagmire.
9: Urine

9.1
- a. Benign, unstained squamous cells (bright field microscopy)
- b. Benign squamous cells, stained with a Papanicolaou stain (light microscopy)

9.2
- a. Benign squamous cell (bright field microscopy)
- b. Benign squamous cells (light microscopy), stained with Papanicolaou stain

9.3
- a. Benign, unstained urothelial cells (bright field microscopy)
- b. Benign urothelial cells, stained with a Papanicolaou stain (light microscopy); there are some RBCs & debris in the background

9.4
- a. A couple of benign urothelial cells (bright field microscopy) with debris in the background
- b. Benign squamous (arrows) & urothelial cells (light microscopy), stained with Papanicolaou stain
abnormal and may suggest stones, unreported instrumentation, or most significantly, urothelial carcinoma especially low grade. The identification of atypia, nuclear atypia in particular, may also be a sign of urothelial malignancy but this must be separated from degeneration. The identification of high grade urothelial carcinoma is well accepted but the recognition of low grade urothelial carcinoma is more controversial, although specific morphologic features have been identified [PMID 8062194, ISBN 072-1666029, PMID 14696141].

Glandular cells are rarely identified. While glandular cells are resident in the adjacent structures of the urethra (eg, prostate, bulbourethral glands), they do not exfoliate (as a general rule). More commonly but still rare are renal tubular cells that are shed. These are typically poorly preserved but are recognizable as glandular cells. Their recognition is important since it is a compelling indication of disease.

As noted above, it is also important to look at the background. For example, the identification of sperm especially in young females, should be treated as a critical value and immediately reported. Rarely, other cell types may be observed as in the case of seminal vesicle cells in men. Examples of malignancy include low grade urothelial carcinoma, high grade urothelial carcinoma, and squamous cell carcinoma. Finally, there are a number of causes of cellular atypia that may be confused with malignancy.

### Pitfalls: causes of cellular atypia

- Lithiasis (stones)
- Drugs: BCG, mitomycin C, thiopeta, cyclophosphamide
- Radiation
- Conduit: ileal loop, neo-bladder
Nonepithelial cells

RBCs may be observed in urine samples although they are often associated with disease including inflammatory, other nonneoplastic diseases (eg, glomerulonephritis, stones), or neoplastic \[9.11a\]; the identification of dysmorphic (eg, crenated) RBCs indicates renal disease \[9.12\] [ISBN 978-0930304874, ISBN 978-0891896203]. Their number may be semiquantitated. WBCs of all types may be observed but PMNs are most common \[9.11\]. Clusters of inflammatory cells may also be seen \[9.13, 9.14\] as well as histiocytes \[9.11b, 9.15\] and eosinophils \[9.16\]. Careful examination may help in the identification of malignant hematopoietic cells as in acute leukemia \[9.17\].
<table>
<thead>
<tr>
<th>Image</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.12</td>
<td>Crenated RBCs (bright field microscopy)</td>
</tr>
<tr>
<td>1.13</td>
<td>A cluster of lymphocytes (light microscopy; Papanicolaou stain)</td>
</tr>
<tr>
<td>1.14</td>
<td>A cluster of neutrophils (light microscopy; Papanicolaou stain)</td>
</tr>
<tr>
<td>1.15</td>
<td>A multinucleated histiocyte. Such cells may be seen in a variety of lesions. (light microscopy; Papanicolaou stain)</td>
</tr>
<tr>
<td>1.16</td>
<td>Eosinophilic cystitis; there are, in addition, RBCs, PMNs &amp; 1 urothelial cell (arrow; light microscopy; Papanicolaou stain)</td>
</tr>
<tr>
<td>1.17</td>
<td>A highly atypical hematopoietic cell from a patient with AML (arrow; light microscopy; Papanicolaou stain)</td>
</tr>
</tbody>
</table>
Microorganisms

A whole array of microorganisms may be identified in urine and the morphology is best done in concert with culture or other techniques used in the microbiology laboratory (eg, PCR). By far, the most common are bacteria and the Gram–bacilli, such as *E coli*. These are most often due to cystitis and associated with a prominent neutrophilic response **i9.18–i9.19**. Fungi may also be seen in urine typically *Candida* **i9.20, i9.21, i9.22, i9.23**. The morphologies of bacteria and fungi have been well described in various texts [ISBN 978-08918981031, ISBN 978-0930304874, ISBN 978-0891896449].

Similarly, evidence of viral infection may also be seen. Of particular importance is BK virus (a polyomavirus) that is usually associated with immunocompromised patients such as those who have had a kidney transplant. The infected cells are single and contain rounded nuclei that are very hyperchromatic **i9.22–i9.23**. The viral nature is best appreciated on Papanicolaou stained preparations. Importantly, these infected cells must be distinguished from nuclear degenerative changes, from whatever cause, and high grade urothelial carcinoma. Hence, the name “decoy cell,” which is often used for these virally transformed cells [ISBN 919-5134958, ISBN 978-0891896449].
A variety of parasites may also be seen, including *Trichomonas vaginalis* and *Schistosoma*; the former is often a vaginal contaminant and the latter found in specific patient populations where the flukes are endemic. Pediculosis (lice) and pinworms (*Enterobius vermicularis*) and are also contaminants that are not uncommonly encountered.
The recognition and identification of crystals are one of the most important components of a comprehensive urinalysis. While many crystals do not indicate significant disease, others do. Consequently, the morphologic distinction is critical. That said, the details of this analysis are beyond the scope of this text. Interested readers are referred to more focused texts on the subject. In addition, formal stone analysis is described below.

### Crystals

The recognition and identification of crystals are one of the most important components of a comprehensive urinalysis. While many crystals do not indicate significant disease, others do. Consequently, the morphologic distinction is critical. That said, the details of this analysis are beyond the scope of this text. Interested readers are referred to more focused texts on the subject.

### 19.5 Morphologic features of select commonly encountered crystals

<table>
<thead>
<tr>
<th>Type</th>
<th>Appearance</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate</td>
<td>Amorphous</td>
<td>None</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>Variable</td>
<td>None</td>
</tr>
<tr>
<td>Cystine</td>
<td>Flat hexagons</td>
<td>AR disorder</td>
</tr>
<tr>
<td>Oxalate</td>
<td>Oval, dumbbell, bipyramidal</td>
<td>May be pathologic</td>
</tr>
<tr>
<td>Struvite</td>
<td>Coffin lid, wedge shaped</td>
<td>May be pathologic “infectious” stones (triple phosphate, magnesium ammonium phosphate)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Usually rhomboid, other</td>
<td>May be pathologic hyperuricemia, tumor lysis</td>
</tr>
</tbody>
</table>

AR = autosomal recessive
Crystals typically form as a result of supersaturation, although this does not explain the whole story. Their morphologic identification may require other forms of microscopy (eg, polarized light) in order to make a definitive identification. Finally, examination of first morning urine is best.

An array of crystals is seen and some are illustrated in i9.28-i9.41.
Calcium phosphate crystals (bright field microscopy)

Amorphous urate crystals (bright field microscopy)

Ammonium urate crystals (bright field microscopy)

Uric acid crystals
- a-c Bright field microscopy
- d-e Light microscopy
- f-g Polarized light microscopy
In this chapter we will review some of the basic principles and tests in the evaluation of saliva, vitreous fluid and sweat. There will be an emphasis on chemical analysis and microbiologic testing. For a more complete and in depth review of the evaluation of these specimen types, the reader should refer to specialized texts.

Saliva

Saliva is produced by the salivary glands and is a filtrate of plasma. It consists predominantly (>99%) of water but also contains electrolytes, enzymes, mucus, hormones, antibacterial compounds and cellular elements. Normal saliva is clear, colorless, with a pH ranging from 6.0–7.4. It has a specific gravity between 1.002 and 1.012. The major salivary glands, including the parotid, submandibular and sublingual glands, are responsible for the majority of saliva production. It is estimated that humans produce 0.75–1.5 L of saliva a day with only minimal production during sleep.

The acini within the salivary glands are either serous, mucous or a mixture of the 2 types. The serous cells produce watery fluid that contains enzymes, such as amylase, that begin the digestion of starchy and fatty food substances. The mucous cells produce more viscous saliva that is richer in glycoproteins, which serves to lubricate the oral mucosa and protects it from trauma during speaking, swallowing and eating. It also contributes to the maintenance of oral hygiene. Patients with xerostomia, or dry mouth, have reduced production or flow of saliva, and the associated complications of hyposalivation. These include increased dental caries, oral candidiasis, burning or tingling in the mouth, mouth soreness and increased thirst.

Specimen collection, requirements & stability

The method of collection and transport of saliva specimens depends on the testing to be performed. For cytologic examination, fresh specimens are required for preparation of smears/cytospins or cell blocks as previously described. In addition, the use of oral brush collection techniques can produce more cellular specimens for oral cytology examination. The reader is referred to any of the specialized texts for in depth morphologic descriptions.

Saliva specimens for chemical analysis are preferably frozen prior to analysis. Freezing and centrifuging precipitates interfering glycoproteins and provides a less viscous sample for testing. Care should be taken to avoid contamination of saliva samples with blood from the oral cavity (eg, following aggressive brushing or flossing). Most biological analytes are present in higher concentrations in the blood; thus its presence in saliva may invalidate the measurement.
Time of collection should be noted if analytes that are subject to diurnal variation (eg, cortisol) are to be measured. The pH of saliva and serum differ and may affect the ratio of bound:free drug or hormone. Extremely acidic or basic saliva may compromise analytical measurements.

**Chemical analysis**

The primary areas of chemical analysis that utilize saliva as a sample type include endocrinology (hormones) and toxicology. It should be noted that saliva testing of these analytes is subject to numerous challenges. Most notably, a correlation between analyte concentrations in blood and saliva must exist in order for saliva testing to be a useful surrogate sample type. This direct relationship does not exist for all analytes. The small concentrations of most analytes found in saliva require assays with very low levels of detection. Reference values from healthy populations are limited and standardized assays and collection devices are not available.

Both hormone and drug concentrations measured in saliva are considered to be free of binding proteins and thus reflect the biologically active (unbound) concentration in blood.

**Cortisol**

Cortisol concentrations determined from saliva specimens mirror free cortisol concentrations in blood [PMID 1746296], since cortisol binding protein and albumin are not present in saliva. The lack of these binding proteins also eliminates the need for an extraction step prior to analysis. Salivary samples are frozen after collection and centrifuged prior to testing, which aids in precipitating the interfering glycoproteins found in this specimen type. Salivary cortisol is commonly detected using immunoassays with chromatographic and mass spectrometry methods becoming more widely available.

The loss of diurnal cortisol variation is indicative of Cushing syndrome, with the lack of a late night (midnight) cortisol nadir supporting other aspects of the clinical picture. Measurement of the late night cortisol value is the preferred test for Cushing syndrome and saliva collection is often more practical at this late timepoint in the outpatient setting. Two independent salivary cortisol measurements are recommended, with values >145 ng/dL (4 nmol/L) considered diagnostic for Cushing syndrome with 92%-100% sensitivity and 93%-100% specificity [PMID 18334580]. Salivary cortisol may also be more useful than urinary free cortisol in patients with mild Cushing syndrome [PMID 18057379, PMID 18334580]. Late night salivary cortisol assays are not sensitive enough to provide diagnostic information for suspected cases of Addison disease or hypocortisolism.

**Sex steroids**

The use of saliva for the measurement of sex steroids has been discussed but not widely adopted due to rapid fluctuations in this sample type [PMID 19176642]. Pulsatile patterns over short time frames have been observed for estradiol [PMID 16002538], progesterone [PMID 792642], testosterone, dehydroepiandrosterone and aldosterone [PMID 19176642]. Multiple samples would therefore be necessary to provide useful information for these analytes. Saliva concentrations have also proven to be of little value for thyroid and pituitary hormones [PMID 3326544].

**Toxicology**

Many exogenous analytes, such as drugs, can be detected in saliva. The lack of binding proteins present in the saliva allows for measurement of the pharmacologically active form of many drugs. However, pH greatly affects drug concentrations and extremely acidic or basic samples may provide erroneous results. The correlation between plasma and saliva concentrations will depend on the physicochemical properties of the drug in question. Sensitive methods are required to detect the small concentrations of drug found in this sample type. Immunoassays are often used as an initial screen with chromatographic and mass spectrometry methods used for confirmation. Toxicological applications of saliva testing include monitoring of therapeutic drug concentrations and detection of illicit or abused substances.

Monitoring of therapeutic drugs for arrhythmia, epilepsy, immunosuppression, cancer, viral and microbial infection and others have been described using saliva [PMID 15576290]. Appropriate measurements of therapeutic drug concentrations in saliva are dependent on the relationship between saliva and plasma for each drug. If the salivaplasma ratio deviates significantly from 1, saliva testing is often not effective [PMID 15576290]. Furthermore, there must be evidence of a correlation between saliva concentrations and clinical and/or pharmacological effects in order for testing to be clinically informative. Saliva testing may be particularly convenient for patient populations that are subjected to routine testing, such as monitoring of immunosuppressants following organ transplant.

Drugs of abuse are readily measured in saliva samples and relationships between saliva drug concentration and behavior or impairment have been described [PMID 1620213]. In cases where sample substitution or adulteration is suspected, saliva testing may be particularly beneficial. However, localized absorption of drugs via smoking, snorting or oral ingestion may confound interpretation of saliva concentrations. It should also be noted that similar to blood, the window of detection for drugs in saliva is short and therefore urine may provide more useful information regarding long term use [PMID 1640691]. Ethanol diffuses rapidly into saliva, as it is not ionized or protein bound, and is often found in higher concentrations in saliva than the blood [PMID 8215090]. Saliva testing may also be performed for amphetamines, cannabinoids, cocaine, opiates, methadone, barbiturates, benzodiazepines and even nicotine.
Microbiologic testing

Saliva is currently not a specimen that has clinical utility in testing for bacterial, mycobacterial, fungal, or parasitic agents of disease. Though some saliva tests do exist in the peer reviewed literature for organisms such as Borrelia burgdorferi and Helicobacter pylori, they are not conventionally accepted testing methods and are beyond the scope of this text. Saliva testing is currently used primarily in the field of human immunodeficiency virus (HIV), with evolving clinical applications for hepatitis C virus (HCV) and cytomegalovirus (CMV).

HIV

HIV testing from oral fluid is available as a point of care (POC) device and serves as a screen for HIV1 or HIV1 and HIV2 antibodies. These tests are typically performed in acute care settings by a physician or medical assistant, and the results are generally available in ~20 minutes. However, patients can also perform this testing at home using the same POC device (analogous to a home pregnancy test). These tests provide instructions to the patient as to how the test is performed and interpreted (including a toll free consultation service to provide resources for follow-up testing and local physician referrals).

Point of care HIV tests only serve as a screen for HIV infection and require follow-up confirmation testing, which must be ordered by a treating physician and performed at a qualified laboratory. The sensitivity and specificity of POC saliva tests for HIV have been shown to be comparable to blood based EIAs for antibody detection. In one study, 6 FDA approved POC HIV tests were shown to have nearly identical clinical agreement among them; essentially allowing for the laboratory to perform the assay that best fits its needs for product stability/storage, workflow, and other technical aspects. The testing algorithm for HIV is constantly evolving and inappropriate for inclusion in this text. It is imperative that the local health authority be contacted to determine the current state of the art algorithm necessary to achieve appropriate diagnostic evidence of infection. Consultation with local infectious disease/HIV experts is strongly recommended in all cases.

CMV

CMV acquired during pregnancy can result in significant birth defects, one of the most common being congenital hearing loss. Congenital infection with CMV may be entirely asymptomatic at birth, with hearing loss only being detected later during infancy. ~10%‑15% of children with congenital CMV develop hearing impairment.

The gold standard for diagnosing congenital CMV is rapid culture of saliva or urine. Many laboratories no longer perform viral culture, limiting the facilities in which this testing can be performed. CMV culture also requires specific cell culture reagents and considerable experience in viral culture and DFA staining, making it difficult to establish in the laboratory. CMV PCR testing from saliva has recently gained attention as a sensitive and convenient mechanism for testing for congenital CMV infections. One large multicenter study showed equivalent sensitivity for PCR vs rapid culture for both liquid and dried saliva samples. Currently, most major reference laboratories offer CMV PCR testing; however it is critical to confirm that the lab’s assay is validated for saliva specimens.

HCV

Salivary testing for HCV is currently not available in the United States; however, there are POC products currently in use in Europe. In 1 study, the saliva POC test showed 97.5% agreement with a standard EIA from serum specimens. This shows great promise and may become available in the future for US markets.

Fluids of the eye

Vitreous fluid

Vitreous is a viscous, clear, colorless fluid, comprised predominantly of water (>90%), that fills the interior of the eye. It contains inorganic salts, glucose, hyaluronic acid, ascorbic acid and type II collagen fibers. It is typically scanty cellular with only rare macrophages. Vitreous fluid is isolated, resulting in less susceptibility to biochemical changes and contamination. Under pathologic conditions, the vitreous fluid can become opacified due to accumulation of blood, inflammatory cells, tumor cells or amyloid.

Vitrectomy is the surgical removal of vitreous gel (vitreous humor) from the eye. It is performed for a number of indications, including repair of retinal detachments allowing access to the back of the eye, removal of hemorrhagic vitreous fluid, cytologic examination, chemical analysis and microbiologic studies. Commonly, 1.5‑2.0 mL of vitreous fluid can be removed. Chemical analysis is particularly useful during postmortem evaluation and can provide important information regarding the time of death or...
the presence of disease. Diagnostic vitrectomies or vitreous aspiration taps are performed for posterior uveitis unresponsive to therapy to rule out malignancy as well as when intraocular infection is considered the primary cause of the presence of inflammation. A major indication for vitreous aspiration is to evaluate for lymphoma \textsuperscript{10.1, 10.2} [PMID 11307054, PMID 23589676]. Flow cytometric analysis of vitreous fluid can provide additional supporting evidence for the presence of lymphoma.

Fine needle aspirations can be performed to sample localized intraocular lesions or tumors. The aspirated specimen is processed for smear/cytospin preparation and cell block preparation as previously discussed.

**Aqueous fluid**

Aqueous fluid is formed by the ciliary body behind the iris. It flows into the anterior chamber through the pupillary space. Aqueous fluid then travels into the angle structures and drains out of the eye \textsuperscript{10.2}.

The region in the anterior chamber at which the cornea and iris meet is known as the angle. Within the angle are some structures that make up the aqueous drainage system of the eye, including the ciliary body, the trabecular meshwork, and the canal of Schlemm.

As the aqueous fluid exits the angle, it passes through the trabecular meshwork that acts as a filter, then travels through a tiny channel in the sclera, the canal of Schlemm. The aqueous flows into minute channels, eventually being absorbed into the blood vessels of the eye.

The balance between production and drainage of aqueous fluid determines intraocular pressure of the eye. Glaucoma results when intraocular pressure rises from inability of the aqueous fluid to flow through the trabecular meshwork (open angle glaucoma) or narrowing of the angle that obstructs flow (angle closure glaucoma).

**Specimen collection, requirements & stability**

The time between collection and analysis should be as short as possible. While analyte stability may vary, a 4 hour delay in analysis did not affect glucose or lactate measurements in a study of 38 vitreous samples [PMID 19167848]. Glucose and lactate measurements are not affected by fluoride collection tubes [PMID 19167848].

The viscosity of vitreous fluid may hinder chemical analysis, particularly when automated chemistry analyzers are used for measurement. Pretreatment of the sample to liquefy it may be necessary. Hyaluronic acid present in the sample may be catalyzed by the addition of hyaluronidase [PMID 15027566] or heat. Ultrasonification and centrifugation have also been suggested, with comparable results [PMID 19729257, PMID 21511417]. Validation of the pretreatment technique and analytical method should be performed prior to testing.

The entirety of the ocular bulb should be gently aspirated in order to minimize analyte concentration gradients that may exist throughout the eye. Removal of the vitreous fluid, with as little disruption of the surrounding tissue, will provide the best specimen for further laboratory measurements.

It has been reported that concentration differences between the left and right eye may exist for analytes such as electrolytes [PMID 9608696]. However, other reports show no significant difference between the samples [PMID 15894848, PMID 19729257].

**Chemical analysis**

Chemical analysis of vitreous fluid may provide useful information in specific cases. Its uniquely isolated location delays the autolytic changes that are normally observed in blood postmortem. This makes vitreous fluid valuable in the diagnosis of metabolic diseases such as electrolyte imbalances and diabetes mellitus as well as the investigation of the postmortem interval. It can also provide clues in forensic investigations; however these are outside the scope...
of this text. A discussion of the more common chemical analyses performed on vitreous fluid is provided.

There are several critical issues that may affect the chemical analysis of vitreous fluid and must be appreciated prior to undertaking any laboratory investigation. Most analytical methods have been calibrated for serum and/or urine, and application of these methods to vitreous samples may not be appropriate [PMID 1678101]. In fact, no standardized methods exist for the measurement of chemical analytes in vitreous fluid [PMID 1678101]. Poor precision has also been reported for multiple analytes with this fluid type [PMID 19729257]. An earlier report revealed significant differences between analytical methods in vitreous fluid measurements for urea nitrogen, glucose, sodium, potassium and chloride [PMID 4031810]. Flame photometry, colorimetry and ion selective electrodes all performed differently in direct comparisons with this fluid type. Different preanalytical treatments of vitreous fluid samples can also affect chemical analysis [PMID 2151417]. Lastly, it is impossible to obtain reference intervals that describe the “normal” biochemical makeup of the postmortem state. Measurements must be related to similar clinical scenarios or, when possible, to antemortem values.

**Glucose & glucose metabolism**

Blood glucose concentrations decrease precipitously in the immediate postmortem period due to cellular and bacterial consumption. Vitreous fluid has very few cells present and is rarely contaminated with bacteria; therefore it is a preferred sample type for determining the antemortem glycemic state. Vitreous glucose concentrations decrease in the first 24 hours postmortem, but remain stable thereafter [PMID 1916748, PMID 1678101]. Historically, the combination of vitreous lactate and glucose concentrations were considered representative of the antemortem blood glucose concentration, assuming that lactate is produced during anaerobic metabolism of glucose [PMID 7076064]. A more recent study has indicated that the endogenous increase in lactate in the postmortem interval erroneously increases this blood glucose estimation, and vitreous glucose alone is a more appropriate measure of the antemortem glycemic state [PMID 1916748].

The biological decrease in glucose concentrations observed in both serum and vitreous fluid makes postmortem diagnosis of hypoglycemia difficult. Instead, vitreous is the preferred fluid to assess postmortem hyperglycemia. Clinical observations of living patients indicated that vitreous glucose concentrations are <1/2 of those found in the blood [PMID 8157178]. Glucose concentrations exceeding 10 mmol/L in the vitreous compartment may be indicative of non-diabetic hyperglycemia, diabetic ketoacidosis or hyperosmolar nonketotic hyperglycemia [PMID 1916748, PMID 2166346]; however, elevated values may also be found in other clinical situations (eg, asphyxia, congestive heart failure, hypothermia).

Ketoacidotic coma is one of the more frequent complications of uncontrolled diabetes mellitus and may be fatal. Therefore, postmortem diagnosis of ketoacidosis and the undiagnosed antemortem diabetic state are important. 3 endogenous ketone bodies (acetone, acetoacetic acid and β hydroxybutyrate) are produced by the liver as a source of energy in the absence of glucose. β hydroxybutyrate is a specific marker of ketoacidosis in the postmortem state [PMID 1916748, PMID 1639109] and vitreous concentrations correlate well with vitreous glucose concentrations [PMID 1639109]. A study of 453 cadavers assigned to diabetic and non-diabetic diagnostic groups reported that vitreous β hydroxybutyrate concentrations were statistically significantly higher in the diabetic group, indicating this measurement may be useful in cases of a negative or inconclusive autopsy [PMID 1639109]. Beyond diabetic ketoacidosis, ketone bodies may be found in a variety of other clinical situations including starvation, malnourishment, alcoholic ketoacidosis, hypothermia and infection.

Vitreous lactic acid concentrations increase postmortem due to glycolysis [PMID 1916748], which may confound the diagnosis of lactic acidosis at autopsy. However, elevated lactate concentrations may be also found in cases of diabetic or alcoholic ketoacidosis, renal or liver diseases, and toxicity associated with cyanide or iron. A recent study indicated that serum and urine methods for lactate measurement do not apply well to determining concentrations in vitreous samples [PMID 2151417]. The variation in measurement of vitreous lactate exceeded the analytical imprecision of the assay, regardless of the preanalytical treatment applied to the specimen.

Glycated protein concentrations may indicate antemortem glycemic control and aid in assessing diabetes postmortem. Vitreous fructosamine concentrations have correlated well with antemortem serum glucose concentrations [PMID 10460416, PMID 19290382]. Combined elevated vitreous fructosamine and glucose concentrations have been proposed to indicate undiagnosed antemortem hyperglycemia [PMID 19290382]. However, another study reported the sum of vitreous glucose and lactate was a better predictor of antemortem diabetes than fructosamine alone [PMID 11563732].

**Insulin & c-peptide**

Proinsulin is enzymatically cleaved to produce equimolar concentrations of insulin and c-peptide. This in vivo process allows for the use of c-peptide and the insulin: c-peptide ratio to differentiate between endogenous insulin production and exogenous administration of an insulin analogue. However, peptide hormones do not efficiently cross into the vitreous compartment; thus measurement of insulin and the precursor sequence c-peptide have not routinely yielded adequate results. Efforts to develop assays for the detection of insulin in vitreous fluid using mass spectrometry have only been documented recently [PMID 22102262, PMID 2279084] and are not available for routine clinical use.

**Electrolytes**

While vitreous fluid is considered a segregated fluid compartment, increased permeability of the retinal cell membrane and loss of active and selective membrane transport contribute to postmortem changes in analyte concentrations in this body fluid. Notably, potassium gradually and linearly diffuses into the vitreous fluid immediately postmortem. Increased potassium concentrations may