There are many preanalytical variables that can introduce error into laboratory test results. When unexpected results are seen in urine measurements, they should be interpreted in the context of other analytes and clinical results that measure similar aspects of renal function.

An example is blood and bacteria testing in routine urinalysis. A false-positive result for blood can be obtained when a specimen contains infection-causing bacteria. The microbial peroxidase activity can cause a false-positive reaction for blood. In this case, microscopic analysis will verify the presence of bacteria and possibly the lack of red blood cells. This will confirm that the response for blood on the reagent strip is inaccurate.

Another example is the effect of urine color on urinalysis reagent test pads. Abnormally colored urine due to medications, food dyes, or vitamins can alter the color change on dipstick test pads for tests other than color. This can be ruled out by doing a visual exam of the urine specimen prior to performing the dipstick.

For this reason, the College of American Pathologists (CAP) recommends that laboratories have a procedure for the correlation of microscopic and macroscopic results. In general, collection time, transport, and storage conditions can be examined to determine the causes of error.

There are some basic guidelines that should be followed for urinalysis and the storage and handling of urinalysis dipsticks. By following these precautions, inaccurate results can be minimized.

continued on page 2
Letter from the editor

This issue of LabNotes features the second in a two-part series on urine testing. While the first part (Vol. No. 200) focused on proper urine collection techniques, handling, and transport, here we will focus more on the preanalytical variables that can affect urine test results. We hope that you find this to be a valuable resource that will aid in avoiding any spurious results in your urine testing practices.

Also in this issue, we discuss the use of safety devices by healthcare workers. There are some interesting statistics on the needlestick injury rates that are occurring in hospitals and on some safety devices that are designed to prevent these injuries.

Lastly, please continue to check the BD Web site (www.bd.com/vacutainer) for upcoming Web-based seminars on issues related to blood and urine specimen collection and testing. We have already held Webinars on hemolysis and elevated potassiums, and we plan to continue providing future Webinars on topics of high interest and utility to our readers.

Many thanks to those of you who completed our reader survey on hemolysis in the last issue of LabNotes. We are always interested to know what challenges our customers are facing in the clinical lab, and we continue to welcome your comments and suggestions for future issues.

Regards,

Dr. Ana Stankovic

The Vacutainer® Brand and Trademark

Trademarks were developed to protect the consumer from confusion as to the source of products and services available in the marketplace. Trademarks identify and distinguish the source of goods or services of one party from those of another. Trademarks, otherwise known as brands, are intellectual property and are part of the assets or “good will” of a company.

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Preanalytical Variables

Color

The color of urine, which is normally colorless or one of the various shades of yellow, can be altered by medications, vitamins, dyes, or diet. If an unusual color is detected for the urine specimen, one of these conditions could be the cause. Once the cause is determined, it should be noted in the laboratory results.

Urine Testing…

continued from page 1

• Urinalysis testing should be conducted on unspun urine.
• Refrigerated specimens should come to room temperature before analysis because many of the enzymatic reactions on the reagent strips are temperature dependent.
• Follow the manufacturer’s directions for conditions of handling, levels of sensitivity, and interferences of tests.
• Reagent strips deteriorate when exposed to moisture, sunlight, heat, and volatile chemicals.
• Do not refrigerate or freeze the reagent strips. They should be kept at room temperature.
• Keep containers closed so the desiccant can do its job. Remove the strips as needed and do not transfer them into another type of container.
• Use urine dipsticks prior to the expiration date. Record the open date on the container and use the dipsticks within the “opened” expiration time.
• Monitor deterioration of the reagent test pads by matching a dry reagent strip to the negative pattern on the bottle to ensure the colors are stable.
• Don’t touch the reagent pads.
• Accurate timing is important in obtaining valid results. A timer or stopwatch should be used.
• Good lighting is important when reading the slips because sometimes reaction colors can be similar.
• Don’t allow urine to run between the test pads or the chemical reaction can be altered. Prevent this by running the edge of the strip along the tube lip when the dipstick is withdrawn from the tube. Follow up by blotting the edge of the strip on a paper towel. This will minimize excess urine on the reaction pads.
• While waiting to read the results, keep the dipstick horizontal to prevent excess urine from running between reagent pads.
• If automated analyzers are used to read the dipsticks, good laboratory practices should be followed. The instrument should be calibrated and maintained, and proper QC should be performed as described by the manufacturer and laboratory policies.

Typical urinalysis dipstick tests include: specific gravity, pH, protein, blood, nitrite, leukocyte esterase, glucose, ketones, bilirubin, and urobilinogen. Color and clarity can be measured either visually or by an analyzer. The following will be a review of some of the preanalytical variables that can affect these tests.

Preanalytical Variables

Color

The color of urine, which is normally colorless or one of the various shades of yellow, can be altered by medications, vitamins, dyes, or diet. If an unusual color is detected for the urine specimen, one of these conditions could be the cause. Once the cause is determined, it should be noted in the laboratory results.
Some abnormal urine colors and their possible causes are:
• Red – blood (or hemoglobin), laxatives such as senna, beets, and rhubarb
• Black – melanin in patients with melanoma
• Brown – bilirubin in patients with obstructive jaundice
• Black/brown – fava beans
• Green – medications, chlorophyll in mouthwash
• Blue – multivitamins, Vitamin E
• Green/blue - Pseudomonas infection

These abnormal urine colors can affect other dipstick results by causing a colorimetric reaction that may be misinterpreted by the instrument and give incorrect results. It is important that the tube be of a clear material when determining the color of the urine. Using good lighting and a white background helps to ensure the color is being read accurately and consistently. Color descriptions should be standardized.

Clarity
Another visual measurement is clarity. (A normal urine specimen is typically clear.) Urine clarity can be related to the handling conditions of the specimen. If a urine specimen is old and unpreserved, it can become cloudy from bacterial overgrowth. In turn, if a specimen has been stored in a refrigerator, amorphous urates or phosphates are found in alkaline urine. The collection time and storage conditions of the specimen should be reviewed to determine if cloudiness may be caused by storage conditions. Other causes of cloudy samples include talcum powder, mucus, crystals, leukocytes, epithelial cells, and fat.

Clear tubes are best for examining urine clarity. Result reporting should be standardized, with clear, hazy, cloudy, and turbid being the most commonly used descriptors.

Specific gravity, or the amount of dissolved particles in a solution, is another measurement performed on urine. Specific gravity is affected by the number, amount, and weight of solutes in the specimen. It serves as a measure of the kidney’s ability to dilute and concentrate urine. A normal random urine has a specific gravity range of 1.001–1.035.

Dehydration, sweating, diarrhea, radiopaque dyes, and antibiotics can cause high results because the ratio of dissolved particles in low volumes of solute will be elevated. A high fluid intake or consumption of diuretics can cause low measurements because of the low quantity of dissolved particles in a large volume of solute.

The pH test indicates whether a specimen is acidic (pH <7) or alkaline (pH >7). A normal urine pH ranges from 5-7 and is a useful tool for the laboratorian, often predicting what may be seen in subsequent microscopic examination. Certain crystals exist in either an acidic or alkaline environment. Some examples of these are uric acid or calcium oxalate crystals in acidic urine and calcium carbonate or magnesium phosphate in alkaline urine. Dilute and alkaline urines can dissolve casts and cells.

Bacterial overgrowth in a specimen standing at room temperature will produce a higher pH due to the conversion of urea into ammonia. Diets high in vegetables, citrus fruits, and dairy produce an alkaline pH. Lower pH levels may be seen in uncontrolled diabetes or may reflect a diet high in meat or cranberries. STARVATION and diarrhea can produce a more acidic urine. Lastly, mishandling the reagent strips by allowing runover from the protein reagent pad can cause false negative pH results.

Part 1 of this article can be found online
Volume 14, No.2
www.bd.com/labnotes

Related Industry Web site: www.cap.org

Q-Probes™ 2005 Urine Culture Contamination (QP052)

Participants from 127 institutions reviewed a total of 14,739 urine-culture specimens and submitted data related to microbiological results, patient gender, age, and site of specimen collection. If more than 2 isolates were found in quantities of ≥10,000 colony-forming units (CFU)/mL, the urine culture was classified as contaminated. The overall rate of urine-culture contamination and rates by gender, age, and collection site based on these criteria are listed below. The urine-culture contamination rate for patients less than two years old may not be comparable due to the low frequency of events.

Excerpted with permission from the College of American Pathology. To obtain this full Q-Probe document, contact College of American Pathologists directly by visiting their Web Site www.cap.org.

Aggregate Urine Culture Contamination Rates • Overall 17.8%

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www.bd.com/vacutainer
**Urine Testing…**
*continued from page 3*

**Protein** is a very important analyte measured in urine because it monitors the function of the kidneys. A normal urine specimen should not contain more than trace amounts of protein. Most reagent pads primarily measure albumin and, to a lesser extent, other proteins.

Residue of disinfectants in contaminated urine containers can cause false-positive protein results. Similar false positives may be seen after strenuous exercise and in highly alkaline urines. Urine specimens with a high specific gravity can produce trace results. Dilute specimens, fever, mental stress, mucus, and exposure to extreme heat or cold can produce false-negative protein results.

**Blood** is another measurement on the urine dipstick, detecting intact red blood cells, free hemoglobin, and myoglobin. A normal urine specimen is usually negative for blood. False positives can be caused by the presence of chlorine bleach, consumption of colored medications by the patient, or a microbial peroxidase reaction from bacterial presence.

False-positive-causing contaminants can also be introduced during time of collection, such as in specimens submitted by women during menstruation. High levels of a reducing substance such as ascorbic acid at >25 mg/dL can produce false-negative results. False-negative blood results can also occur when formalin is used as a preservative, when there is >10 mg/dL of nitrite, or when mixing is inadequate and red blood cells have settled in the tube.

**Nitrite** is also a test on urine dipsticks. A normal urine specimen is negative for nitrite. Some of the ways in which false-positive results can occur include bacterial overgrowth in specimens that have not been stored properly, colored medications, and dyes.

False-negative results can be seen with high levels of ascorbic acid, >25 mg/dL, or when dietary nitrate is missing due to starvation, IV feeding, or fasting. The conversion of nitrate to nitrite takes about 4 hours in the bladder. If this hasn’t occurred, results could be negative for the presence of nitrite. This can be seen in randomly collected specimens.

**Leukocyte esterase** is an indicator of white blood cells. It is typically negative in normal specimens. False positives can occur when collection containers have been contaminated with chlorine bleach or other oxidizing detergents. Other influences that can produce false-positive results include formalin as a preservative and vaginal discharge.

*continued on page 6*
Healthcare worker (HCW) safety is an important aspect of infection control. HCWs are at risk of occupational exposure to pathogens present in blood and body fluids. Although the use of universal precautions has significantly reduced this risk, accidental puncture of skin by needles, other instruments, or broken glass (“sharps”) still remains an important source of HCW acquired infections and necessitates further action in order to achieve the desired level of HCW safety.

Although more than 20 different infectious agents have been shown to be transmissible by exposure to blood and body fluids,1 most of the attention is currently focused on prevention of viral infections caused by human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Traditionally, the average transmission rates of infection following a needlestick injury from an infected patient are: 0.3% for HIV, 30 (23-62)% for HBV, and 1.8% for HCV.1,2 The major population at risk for percutaneous injuries (PI) are nurses.

It was estimated that the total US annual rate of HCW injuries involving contaminated sharps is close to 650,000.3 This estimate took into consideration the rate of PI underreporting, which can in certain settings be as high as 73%.3 Seventy-five percent of needlesticks that occur annually in hospital settings are preventable either by: eliminating unnecessary use (25%), using needles with safety features (29%), or using safer work practices (21%).3 Using these three approaches, 65 infections with HBV and 42 infections with HCV can be prevented; however, the number of HIV infections that would be avoided could not be validly estimated.3

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A great impact on HCW safety can be achieved through the replacement of conventional devices with safety-engineered products (e.g., shielded, retracted, or self-blunting conventional and winged set needles, plastic blood collection vacuum tubes, round-tipped scalpel blades, retracting-blade or shielded-blade scalpels, etc.). Data from the EPINet4,5 Multihospital Sharps Injury database showed that although conversion to safety devices was not proportional across different device categories, it resulted in a 51% decline of Pts (from 19.5 to 9.6 Pts per 100 occupied beds).6

Legislation has significantly increased the rate of conversion to safety devices. In the United States, the Needlestick Safety and Prevention Act of 2000 made the use of safety devices mandatory as of July 2001. As a result of that, healthcare employers in the United States must document annually in their exposure control plan that they have evaluated and implemented “safer medical devices designed to eliminate or minimize occupational exposure” to bloodborne diseases and have taken into consideration changes in sharps safety technology. During this process, input from nonmanagerial HCW is mandatory, as well as maintenance of a sharps injury log.

However, in order to achieve maximum effect, the safety features of the devices have to be engaged appropriately. When analyzing the injuries that occurred during the use of safety devices, it was shown that injuries occurred frequently either before the activation of the device (in 56.9% of cases) or during the activation (in 26.3% of cases).7 Because of that, use of passive needles that automatically allow engagement of safety features as soon as the needle is out of the blood vessel and in-vein activation devices will further reduce the number of Pts. BD (Becton, Dickinson and Company) released the first passive needle for blood collection in the spring of 2005, and the BD Vacutainer® Push Button Blood Collection Set was also released in 2005.

In recent years, major improvements have been made in the area of HCW safety. New and innovative safety devices, combined with effective HCW training, have ensured continued reduction of PI injuries and occupational exposure to pathogens, thus successfully lowering rates of HCW acquired infections. Conversion to passive safety devices, safety legislation, and discontinuation of unnecessary use of sharps will move this process even further.

References
Urine Testing…
continued from page 4

High specific gravity, some antibiotics, such as tetracycline, and large amounts of glucose or ascorbic acid can cause false-negative leukocyte esterase results.

**Glucose**, another urine dipstick test, is mainly used to monitor diabetes. Normal urines are negative for glucose. Some normal specimens have small amounts of glucose, that are below levels of sensitivity for the reagent strip.

Just like leukocyte esterase, false-positive glucose results are achieved when collection devices have been exposed to chlorine bleach or detergents. Improper storage of reagent strips, when exposed to air, have been noted to produce false-positive results.

Ascorbic acid, >50 mg/dL, is again a culprit in causing false-negative glucose results. Over time at room temperature, glucose will decrease due to glycolysis from bacteria. Tetracycline has been determined to cause false-negative glucose results, and refrigerated specimens that were not allowed to reach room temperature can produce false-negative results because the enzymatic reaction is affected.

**Ketone** bodies are a by-product of fat breakdown. Normal urines are negative for ketones. Increased ketones can be due to starvation or alcoholism. Also strenuous exercise, fever, fasting, vomiting, and high-protein diets can cause high ketone values. Urine specimens with a high specific gravity and low pH have been known to cause trace ketone results.

Acetone, one by-product of fat breakdown, evaporates rapidly if the uncovered specimen is left standing at room temperature. Specimens should, therefore, be tested immediately for ketones or refrigerated in a closed container. Some urinalysis preservatives can maintain ketone levels. It is important to check the manufacturer’s claims.

**Bilirubin** measures liver function and is usually negative in normal urine specimens. Colored medications, those in the yellow, orange, and red spectrum, can cause false-positive results.

Ascorbic acid, >25 mg/dL, causes false-negative results, as can specimens with high nitrite concentrations. Exposure to light causes bilirubin to degrade over time, leading to false-negative results. It is recommended that specimens submitted for bilirubin measurements be kept in a dark place or collected in an amber container.

**Urobilinogen** is another measurement of liver function. A normal urobilinogen result is <1 Ehrlich unit/dL. The same factors that affect bilirubin (exposure to light and colored medications) also affect urobilinogen. In addition, formalin, which is sometimes used as a preservative, will cause false-negative urobilinogen results, as can ascorbic acid or nitrite.

Standardization when processing urine specimens for microscopic sediment analysis has become a very important guideline as recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly known as NCCLS). This includes the use of engineered tubes, pipettes, and standardized calibrated slides. The best types of tubes for microscopic sediment analysis are clear, plastic tubes with conical bottoms. A cap or lid and volume graduations are valuable features. CLSI does not support the use of glass slides and cover slips due to the lack of sample volume standardization. The chambers on the specially designed slides are calibrated for a specific urine sediment volume that ensures standardization.

As part of specimen processing standardization, the ratio of the volume of urine required to fill the tube is compared with the volume of urine and sediment, which remain in the tube after centrifugation and decanting. Ensuring that these two are maintained is one of the keys to standardizing microscopic sediment analysis.

CLSI recommends centrifugation of urine specimens at 400 RCF (g). Most hospitals typically centrifuge for 5 minutes. Any variations in speed and time can change the cellular elements obtained in the sediment. All equipment used for sediment processing and analysis should be properly maintained. Centrifuges should be calibrated and microscopes should be quality-control and proficiency tested regularly to guarantee accurate testing.

Unpreserved specimens that have been unrefrigerated for more than 2 hours from time of collection should not be accepted for microscopic analysis due to the increase in bacterial overgrowth and the disintegration of cells and casts.

The urine becomes alkaline, causing red blood cells and white blood cells to lyse and casts to dissolve.

If a specimen has been refrigerated for storage, it should be allowed to come to room temperature and mixed well prior to analysis. Amorphous urates or phosphates develop in cold conditions and will affect the analysis. Contaminants that can be seen during sediment analysis include mucus, spermatozoa, fibers, talcum powder, and oil. It is important not to confuse these contaminants with cellular components. Stains can help in the identification of cells.

Flow cytometry is another method of examining the urine for microscopic elements. This method of microscopic analysis, however, is almost unaffected by preanalytical variables. The greatest factor that can affect flow cytometry test results is insufficient mixing of the specimen.

**Urine Culture and Sensitivity**

The microbiology lab also conducts clinical testing on urine. Preanalytical variables that could affect culture and sensitivity testing include:

- A contaminated collection container
- A leaky container
- A midstream clean catch specimen is less likely to produce contaminants as compared to a random urine. It has been documented that the contamination rate for females is double the rate for males. Generally, contamination has been defined as >10,000 CFU/mL of 2 or more organisms. Each facility should define their own level of contamination based on the patient population and the collection and transport methods authorized.

- When there is a delay in specimen transportation, specimens that are not handled immediately, are left unrefrigerated, or do not have a bacteriostatic preservative may contain bacterial overgrowth causing colony counts that may be almost impossible to read
- Extraneous sources of bacterial contamination include hands, skin, and clothing
- False-negative growth may be seen in specimens submitted from patients who are taking antibiotics

**Urine Chemistry**

Random testing of urine chemistry analytes is not usually considered valuable because of the lack of collection of these analytes at any given time in the bladder. Timed specimens provide the most valuable information for the concentration of a specific analyte.

Some of the preanalytical variables that affect these tests are redundant to those for urine dipstick chemistries. There are various factors, that can affect any one of...
these analytes. The most common influences are preservatives, diet, and medication. More specifically some of the drugs and foods that affect urine chemistry results are as follows:

- Sodium is influenced by diet. Increased sodium results can be caused by antibiotics, cough medicines, or laxatives. Decreased sodium results will be seen with diuretics.
- Potassium can also be influenced by diet. Increased measurements can be seen with diuretics, salicylates, or glucocorticoids.
- Chloride is falsely decreased by androgens, estrogens, methyl dopa, or cortisone. It is falsely increased by bicarbonates or corticosteroids.
- Creatinine is increased by gentamycin or heavy metal chemotherapeutic agents.
- Calcium shows increases by antacids, anticonvulsants, and some diuretics, while adrenocortico steroids and oral contraceptives cause decreases.
- Urine total protein is affected by heavy exercise, dehydration, food, and emotional stress. It can be increased with acetaminophen, antibiotics, and x-ray contrast media.
- Microalbumin is affected by dehydration or strenuous exercise.
- Glucose can be increased by lithium, estrogen, diuretics, chloramphenicol, and ascorbic acid.
- Uric acid is influenced by high levels of ascorbic acid, x-ray contrast media, alcohol, anti-inflammatory drugs, salicylate, and warfarin.
- Bilirubin is decreased by light and ascorbic acid and can be increased with antibiotics, diuretics, oral contraceptives, sulfonamides, and steroids.
- Amylase can be increased with aspirin, corticosteroids, codeine, and oral contraceptives.
- 5-HIAA is influenced by many types of foods, such as plums, pineapples, walnuts, avocados, tomatoes, bananas, and eggplants. It is recommended they not be eaten 3 days prior to testing.
- 5-HIAA can be increased by cough syrup. It can be decreased by heparin, methyl dopa, and tricyclic antidepressants.
- Porphyrins are affected by morphine, oral contraceptives, and sulfonamides. Some of these also apply to porphobilinogen.
- Catecholamines are influenced by chocolate, cocoa, coffee, tea, bananas, and vanilla. They are also affected by stress and exercise. They can be increased by lithium, insulin, tetracycline, and nitroglycerin. Catecholamines can be decreased with salicylates and imipramine. These same factors also affect VMA, a metabolite of catecholamines.

It is recommended that any drug known to interfere with a test be discontinued until a drug-free specimen can be produced. The most important that the total urine volume be collected and recorded during the time period in order to correctly calculate the analytic concentrations. Other guidelines that should be followed when performing urine chemistry testing are:

- Consideration should be given as to whether preserved or unpreserved specimens are used. Proliferation of bacteria could be a concern with unpreserved specimens.
- Potential interference with assay methods should be considered when chemical preservatives are used. Each institution should perform evaluations to make these determinations for their facility based on their populations and levels of clinical acceptance.

NCCLS GP16-A2 provides a table of common 24-hour urine preservatives corresponding to the chemistry tests. The most commonly cited methods for preservation were refrigeration, freezing, HCl acid, boric acid, and acetic acid. Specimens may need to be split if various tests that require different preservatives are requested. This can be accomplished by pooling the collections over time, then evenly distributing the specimen into multiple containers. Another option would be to collect multiple 24-hour specimens.

**Drugs of abuse** are tested on urine. The preanalytical variables that affect testing drugs of abuse in urine are listed below:

- In the US, 4% of urine samples are adulterated. Some of the common adulterants used are: water, bleach, eye drops, and vinegar. Commercial adulterants can be purchased.
- A common interference in urine drug testing is poppy seeds. They can cause false-positive opiate screens.
- Specimens must be refrigerated until tested.

Urine is a valuable specimen for the screening, diagnosis, and monitoring of diseases. It can also be used to monitor therapy. There are various collection methods for acquiring this specimen, each of which has its merits. By minimizing the preanalytical influences during specimen collection and handling, a suitable specimen can be obtained. Specimen handling is probably the most important step in obtaining a good urine specimen that can provide the most useful clinical information. It is also important to carefully record any details that may be beneficial in the interpretation of the urine results. After all, the results are only as good as the specimen collected.

**References**

Preanalytical Variables in Urine Testing

Letter from the Editor

Did You Know: Latex

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BD—Partnering for Health

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Industry News-Proteomics

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