Drug testing beyond the health care and criminal justice systems has increased throughout the last several decades. Common areas for drug testing now include the workplace (e.g., preemployment and random testing), the military, athletics, legal and criminal situations (e.g., postaccident testing, rehabilitation testing of ex-convicts), and health care (e.g., treatment, compliance monitoring, cause of death).

Urine, blood, hair, saliva, sweat, and nails (toenails and fingernails) are some biological specimens used to perform laboratory drug testing, and each provides different levels of specificity, sensitivity, and accuracy. Urine is most often the preferred test substance because of ease of collection and low cost of analysis. Concentrations of drugs and drug metabolites also tend to be high in the urine, allowing longer detection times than in other body fluids.

Workplace urine testing commonly includes five drugs of abuse (amphetamines, cannabinoids, cocaine, opiates, and PCP); however, several other substances can be abused (e.g., benzodiazepines), requiring screening for more drugs of abuse. Urine drug screens for alcohol, benzodiazepines, methadone, and tricyclic antidepressants are not uncommon.

Two types of drug screening tests are typically used with urine samples, immunoassay and gas chromatography-mass spectrometry (GC-MS). Immunoassays, which use antibodies to detect the presence of specific drugs or drug metabolites, are the most common method for the screening tests. Forms of immunoassay techniques include cloned enzyme donor immunoassay (CEDIA); enzyme-multiplied immunoassay technique (EMIT), a form of enzyme immunoassay; fluorescence polarization immunoassay (FPIA); immunoturbidimetric assay; and radioimmunoassay (RIA). In addition, immunoassay techniques are used in many home-testing kits or point-of-care screenings.

Positive results of immunoassay testing are always considered suspect until confirmed by a laboratory-based test for the specific drug (e.g., GC-MS or high-performance liquid chromatography). Gas chromatography-mass spectrometry is considered the standard for confirmatory testing. The method is able to detect small quantities of a substance and confirm the presence of a specific drug (e.g., morphine in an opiate screen). It is the most accurate, sensitive, and reliable method of testing for drugs; however, the test is time-consuming, requires a high level of expertise to perform, and is costly. For these reasons, GC-MS is usually performed only after a positive result is obtained from an immunoassay screening test.
In one of our previous lab experiments (See: “Diffusion and Osmosis”), we learned about forms of passive transport. “Passive” means that the energy of the materials themselves and the environment in which they are located are used to power their movement. Their movement from one place to another is based upon a concentration gradient. Osmosis, you will recall, is the passive transport of water in cells and tissues. Diffusion is the passive transport of substances other than water. As with other substances that enter the body, drugs are subject to the same processes. In the case of drugs, we refer to those processes as pharmacokinetics.

Once a drug has been taken into the bloodstream, the process of passive transport by diffusion ensures that the concentration of the drug will be the balanced in the cells, tissues and blood until something happens to remove the drug from the biosystem.

As blood is filtered through the kidneys of the body the amount of the drug present in the biosystem gradually decreases. The drug passes from the blood into the tubules of the kidney because of the existing concentration gradient between the blood and the tubules. The drug is then excreted in the urine.

The liver also helps reduce the amount of a drug that is present by metabolizing it and later passing the metabolites into the urine or the feces. Other routes, such as respiration (the basis for the Breathalyzer tests) and excretion through the skin, also assist in removal of many drugs and other substances from the biosystem.

**Half-life** is a term that refers to the duration of action of a particular drug in a biosystem. Half-life refers to the amount of time it takes for half of a certain amount of particular drug to leave the biosystem. It is a measurement that varies based upon the amount of the drug consumed and the particular method of analysis being used to determine the presence of the drug. Half-life measurement of a drug also depends upon whether the drug(s) in question is (are) water-soluble or lipid-soluble. Those drugs that are soluble in lipids will be retained in body tissues for a longer period of time than those drugs that are soluble in water or happen to be rapidly detoxified by the liver.

Here are some examples of half-lives for a few well-known drugs:

- Morphine (standard dosage) = 1-2 hours,
- Naproxen, Naproxen sodium = 12 hours,
- Aspirin = 2-4 hours,
- Acetaminophen, Paracetamol = 3-4 hours,
- Marijuana = 24-36 hours and
- Nicotine = 2 hours.

A urine test will not be able to indicate if a person has ever taken drugs in the distant past. It can only detect the immediate presence of the drug and its metabolites for drugs such as cocaine, THC, PCP, or others that might be in an individual’s urine given recent use of the drug.

Each test for these drugs has essentially the same procedure but uses different indicators, just as we did in our “Indicator Tests for Important Nutrients” laboratory exercise. A urine test can identify drugs within an individual’s body only if the individual:

1. Allows the testing to be performed,
2. has taken the drug or drugs within a certain period of time and
3. is not taking any specific over the counter (OTC) or prescription drugs which may interfere with the analysis.

Drug testing is commonly done on persons seeking employment in positions where alertness and responsibility are extremely important. Competitive sports may also require testing (for illegal drugs such as anabolic steroids). Drug testing is also used for animals participating in professional sports such as racing to make certain that their performance is not chemically enhanced. Drug testing is done, as well, in several aspects of law enforcement (DUI, probation performance, and forensic toxicology, among others).
PROCEDURE

1. Work in teams of two to perform this laboratory exercise and obtain these materials:
   - Two samples of artificial urine, “A” and “B” or “C” and “D.”
   - One microscope slide, which has two separate depressions in the surface.
   - Enzyme solution.
   - Anti-human antibody solution.
   - Reagent solution.

2. Test two separate samples, “A” and “B” or “C” and “D” according to these directions:
   - Place three drops of one sample into one depression on the slide and three drops of a different sample to the other depression on the slide.
   - Add one drop of enzyme solution to each depression.
   - Add one drop of anti-human antibody solution to each depression.
   - Add three drops of reagent solution to each depression.
   - Mix each sample with a separate, clean toothpick.
   - Observe and note any color change(s).
   - Record your results in Table 1.

Test results will be clear (not colored) if a drug is present (a positive result) or rose or pink colored if the drug is not present (a negative result).

In normal, drug-free urine, the enzyme donates energy to make the reagent solution bond to the anti-human antibody. This reaction results in a colored product and indicates that the drug is not present. If the drug is present, the drug interferes with the action of the enzyme and the reagent solution will be unable to react with the anti-human antibody. Hence, a colored product cannot appear and a noncolored result indicates the presence of the drug.

Different enzymes and reagents must be used to test for each separate type of drug just as we used different reagents to test for sugars, lipids, proteins and other drugs in previous lab exercises.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>RESPONSE</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Immunoassays

Immunoassay drug testing is based on the principle of competition between labeled and unlabeled antigen (drug) for binding sites on a specific antibody. Antibodies are protein substances with sites on their surfaces to which specific drugs or drug metabolites will bind. Several variants of immunoassays are usually employed in urinalysis—the most common being radioimmunoassays (RIA) and the enzyme immunoassays (EIA). The difference between these types of immunoassays is mainly in the indicator that is used. The EIA utilizes an enzyme as an indicator in the assay (the label), while RIA uses a radioactive indicator.

An enzyme immunoassay functions via enzymes which are coupled to a drug or antigen in the reagent. The antigen/enzyme particles compete with the drug in order to bind with the antibody. If no drug is present in the sample, the antigen/enzyme would bind to the antibody and the activity of the enzyme would be limited. If the drug is present in the sample, it would bind to the antibody, and the free enzyme available in the sample reacts with the substrate and forms some byproducts via catalyzation.

Drug Detection Times

Chemicals stay in your body from one hour to a lifetime. The length of time depends on the types and amounts of impurities absorbed by your body, your rate of metabolism and your general health. The amount of time that a drug/metabolite remains detectable in urine can vary, depending on the following factors:

- **Amount and Frequency of Use**: Single, isolated, small doses are generally detectable at a lower boundary. Heavy, chronic and long-term use typically result in detection periods near or at the upper boundary.
- **Metabolic Rate**: Individuals with slower body metabolism are prone to longer drug detection periods.
- **Body Mass**: In general, human metabolism slows with increased body mass, resulting in longer drug detection periods. In addition, THC (marijuana’s active ingredient), PCP and other lipid soluble drugs are known to accumulate in fatty tissue. Chronic users, physically inactive users, and individuals with a high percentage of body fat in relation to total body mass are prone to longer drug detection periods for these substances.
- **Age**: In general, human metabolism slows with age, resulting in longer drug detection periods.
- **Overall Health**: In general, human metabolism slows during periods of deteriorating health, resulting in longer drug detection periods.
- **Drug Tolerance**: Users typically metabolize a drug faster once a tolerance to the drug is established.
- **Urine pH**: Urine pH can impact drug detection periods. Typically, highly acidic urine results in shorter drug detection periods.

In a small percentage of cases, users may test positive longer than times shown—most notably in cases of long-term chronic abuse, in individuals with significant body mass and/or body fat, and in individuals with health related issues resulting in abnormally slow body metabolism.

Drug detection times for urine drug tests are expressed below in terms of approximate maximum detection durations.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>7-12 h</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>2 d</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>2 d</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>1-21 d</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>3-30 d</td>
</tr>
<tr>
<td>Cannabis (single use)</td>
<td>3 d</td>
</tr>
<tr>
<td>Cannabis (habitual use)</td>
<td>&gt;30 d</td>
</tr>
<tr>
<td>Cocaine</td>
<td>2-4 d</td>
</tr>
<tr>
<td>Codeine/Morphine</td>
<td>2 d</td>
</tr>
<tr>
<td>Heroin</td>
<td>2 d</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>2-4 d</td>
</tr>
<tr>
<td>PCP</td>
<td>8 d</td>
</tr>
</tbody>
</table>

Table 2. Urine Test detection times for commonly tested drugs