Principles of Drug Testing Technology

Immunoassays

Employing a lateral flow strip in conjunction with the principle of immunoassay testing is the basic form of rapid, onsite donor specimen screening for the possible presence of a drug or a class of drugs.

Typically a lateral flow strip mobilses the sample (analyte) by capillary action along a path, whereby various reagents are encountered in the correct order to facilitate the test for a particular drug. Such immunoassay tests are usually qualitative, meaning they are reported as a positive or negative result only.

Immunoassays are highly sensitive biochemical tests, specifically tuned to measure the concentration of a particular substance present in a liquid (*e.g*. portion of a biological sample). The specificity of such tests is a result of employing purified antibodies and antigens (the drug) as reagents, in the manner described.

Antibodies are proteins produced by β-lymphocytes (immune cells) in response to stimulation by foreign substances (antigens). Antibodies bind to the antigen that is responsible their production, so by measuring the formation of such complexes *via* an indicator reaction it is possible to make a determination for the presence of a particular drug.

Antibodies are usually harvested from sheep or rabbits and developed against specific classes of drugs. They recognise the antigen (drug) by their shape which, due to the similar functional groups of related molecules, can be the reason for some false positives.

Immunoassay tests work by binding an indicator (tag) to the target drug, which may be an an enzyme, fluorophore or particle. The type of tag employed influences by the method of detection for a particular test.

Determination of the test result is based on competitive binding between the antibodies and free drug or tagged drug. If the quantity of the drug is equal to, or greater than, the cutoff level it inhibits binding of the tagged drug to the antibody which results in a positive result.

A positive immunoassay test only provides an assumption that the outcome of a test is positive since the drugs detected by immunoassay are limited by the tests specificity and the cutoff levels. Since both of these may vary depending on the manufacturer and particular reagents (antibodies) employed a positive result cannot be taken as definitive, which gives rise to the

need for confirmatory testing.

GC-MS/LC-MS

Following an initial positive result derived from a screening test the sample will commonly be sent for confirmatory testing at a laboratory. These tests are usually undertaken using Gas Chromatography-Mass Spectrometry (GC-MS) or High Performance Liquid Chromatography (HPLC), both of which provide highly specific and accurate results.

The principle of both test methods, although slightly different, is essentially the same. Chromotography works by separating the many components that may be present in a sample by creating a partition between mobile (*e.g*. liquid or gas) and stationary phases.

The sample to be tested is mobilised by the fluid through a column which is either formed from coated fused silica (GC-MS) or inert packing material (LC-MS). Over a period of time and suitable distance the different molecules present in the sample, which all differ in size, shape and weight, become separated.

At the end of the column it is therefore possible to introduce the individual components into a mass spectrometer, which measures the weight and fragmentation pattern of each molecule. Such a spectra provide a unique fingerprint for each individual moiety, thus allowing for extremely specific detection, aiding in the confirmatory process.

In conclusion it is important to remember that even with two test procedures (initial and confirmatory testing), the number of positives is limited to the specificity and sensitivity of the initial immunoassay, which highlights the importance of selecting quality tests to begin with.