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Comparison of Drug Confirmation Methods (Part 1)

Dr. Joseph E. Graas, Scientific Director
Dr. Edward Moore, Medical Director

A confirmatory test, by definition, is a test that is performed by a completely different methodology than the original test. This confirmatory test must separate the compounds into individual components that are essentially free from contamination in order to positively identify the compound. A chromatography separation technique and an analytical identification is performed using standards with a defined concentration to identify analytes.

The toxicology testing laboratory performs tests under the direction of the treatment clinic. The arrangement between the clinic and the lab will dictate what level of testing that should be done, and this decision, quite commonly, will depend on the cost of the test. However this arrangement is made, the testing will typically start with a screening method of the urine sample, which is usually an immunoassay based technique. Normal samples are directly reported with no further testing necessary. Abnormal samples that have one or more class abnormal results will need a secondary test to determine the validity or correctness of the screen. The initial test can be followed up with a second screen test to verify the first value and minimize any screening anomalies. A second test that is an immunoassay based test, even from another manufacturer with a different methodology, is still a verification test and cannot be considered a confirmatory test. The following are the most prevalent types of drug confirmation in urine:

Thin Layer Chromatography (TLC)

In this methodology the drugs are removed from the urine by a column filled with a specific absorbent, and then eluted with an organic reagent in a small amount of solvent. This solvent is then spotted on a chromatographic plate which can contain 10 samples spaced out at approximately 1 centimeter apart. Standards and controls are spotted and treated like patient samples. The plate is then placed into a special solvent in a closed container that slowly wicks up the plate and separates out the drugs in the sample. It is at this point that the individual drugs are separated out of the sample. A chromogenic reagent is sprayed on the plate and reacts with the individual drugs. Each drug appears on the plate in a specific color and location. These drugs are detected by comparison with the standards and controls. The visual observation of location and color of the spot by a trained individual will then be called a positive detection of the target drug.

The beneficial performance characteristics of this method are that TLC has good sensitivity, it is not quantitative and is acceptable for non-legal analytical cases. It is also very economical. A drawback to this method is it cannot separate and detect compounds that are close in molecular weight and molecular structure (e.g. morphine and hydromorphone, codeine and hydrocodone).

Gas Chromatography Mass Spectrometry (GC/MS) and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)

The drugs from the identified samples are extracted from the urine by a series of solvents and then may be derivatized to increase the volatility for gas phase (GC) separation, or may be treated with reagents to increase the ionization as in liquid phase separation (HPLC). The concentrated and purified samples are injected into the chromatograph and separated on chromatographic media in the gas phase or in the liquid phase. The separated peaks enter into the mass spectrometer via an interface, and are then analyzed by ion separation of the fragmented masses in a quadrupole spectrometer. The fragmented pattern of each drug gives a unique pattern that is highly reproducible which results in the positive identification of the specific drug. This fragmentation pattern is a characteristic of the molecule and yields information that is the "gold standard" of identification.

This process is quite sophisticated. This methodology has a high level of sensitivity and the results are quantitative when compared to isotopic analogues. The results are considered "state of the art" and, when done with a proper chain of custody, are extremely defensible in court. The equipment is very expensive, demands a knowledgeable technician and is quite labor intensive. It is, however, considered to be the best analytical procedure for the identification of drugs of abuse, poisons, and metabolic intermediates.