

Testing for drugs of abuse

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Select a system for drug testing

- 1- onsite analysis or analysis by an outside laboratory
- 2- choice of laboratory for confirmatory test or all drug testing

Onsite testing

- simplified chain of custody, control and a greater sense of confidentiality, and immediate results.
- Chain of custody is a critical factor in a valid drug program.
- Confidence in the program is derived from knowing that the specimen tested actually belongs to the person who gave it.
- Onsite testing reduces the number of people who handle a specimen, which reduces the potential for mistakes.

- All specimens that give positive results in the onsite tests be confirmed by an alternate method, i.e., one of the chromatographic methods such as gas chromatography-mass spectrometry.
- An outside laboratory should be used for this procedure.

Selecting a laboratory

- **Information:** Find out from Federal and State agencies if the laboratory has been licensed in any government programs and how the laboratory performed.
- Only California, New York, and Pennsylvania have proficiency testing programs for drug testing laboratories.

- **Standard operating procedures:** Review the laboratory's manual for standard operating procedures.
- The manual should include a detailed description of every step for specimen handling and analytical methods.
- Each page should be dated and signed to show that it is continually updated as the laboratory modifies procedures.

- **Chain of custody:** Examine documentation on chain-of-custody procedures from the time the specimens are collected until results are reported.
- Special handling procedures should be in effect for employee drug testing specimens.

- Who has access to stored specimens and why? How are they stored?
- Positive specimens should be stored frozen in a secure place, and there should be a way to identify everyone who has had access to them.
- The laboratory should also be able to track exactly where each specimen was from the time it entered the laboratory until it was stored.

Analytical methods

- The specific methods chosen by a laboratory will depend on a number of factors, including cost, workload (number of specimens), turnaround time, sensitivity required, and reliability.
- In nearly all applications of detection, a confirmation analysis is essential for all specimens screened positive.
- A different type of analytical methodology should be used for the confirmation analysis.
- Analytical methods used in most laboratories for the detection of drugs in body fluids can be classified into two main categories immunoassays and chromatography generally used respectively for screening and confirmation.

IMMUNOASSAYS

- Immunoassays are 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.

- Two types of immunoassays are usually employed in urinalysis at this time:
- 1-The radioimmunoassay (RIA) and
- 2-The enzyme immunoassay (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.

- Because antihodies often cross-react with related drugs, and sometimes even with unrelated compounds, confirmation of positive immunoassay results with an independent procedure is imperative for definitive identification.

Radioimmunoassay

- RIA can detect very small concentrations of drug with sensitivity ranges on the order of 1-5 nanograms per milliliter (ng/ml). The required sample volume is small and sample preparation is minimal.
- Some of the disadvantages of this technique are associated with the use of radioactive substances and the high cost of reagents and instrumentation.
- Turnaround time is long--from 1 to 5 hours.

Enzyme immunoassay

- The most frequently used EIA method is the EMIT system .
- Some of the advantages of EMIT include:
- (1) a short analysis time, (2) an easily measured nonradioactive endpoint that is simply measured, and (3) no necessary separation of bound and free fractions as in RIA.
- The EMIT, however, is generally less sensitive than RIA but still has moderate to good sensitivity and specificity.

CHROMATOGRAPHY METHODS

- Chromatography is a method of analysis in which the various components in a biological specimen can be separated by a partitioning process.
- Chromatographic separations to resolve mixtures of drugs and metabolites require (1) a stationary (fixed) phase, which may be a solid or a liquid on an inert support having a large surface area, and (2) a mobile (moving) phase of liquid or gas.

- With a chromatographic method, substances are carried by the mobile phase through a column or across a plate, where the stationary phase interacts with the specimen to cause separation of the various components.
- After separation, a detection method distinguishes the components for identification and measurement.

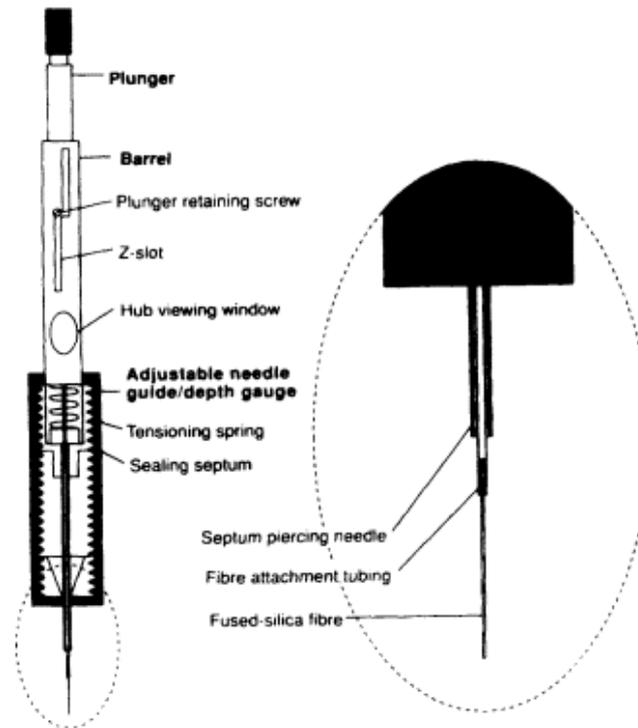
- Separation of the components of biological mixtures containing substances of various molecular types is based on the time spent by each component in each phase of the chromatographic system.
- Several different types of chromatographic techniques are used in laboratories for drug analysis.

- These various techniques offer different degrees of resolving power (the ability to separate one component from another) and often utilized in combination, depending on the drugs in question.
- While other chromatographic techniques are available, thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and high-pressure liquid chromatography (HPLC) are the most commonly used ones.
- The combination of GLC with mass spectrometry (GC/MS) provides the most specific type of analytical tool currently used in analysis.

- Extraction of biological samples is necessary for all chromatographic techniques for drugs .
- It is usually not required for the immunologic methods.
- Liquid-liquid extraction is the most commonly used method.
- This procedure involves the mixing of the sample in water with a
- water-insoluble organic solvent. If the drug of interest is more soluble in the organic phase, most of the drug is extracted from the water phase into the solvent.
- The solvent is then evaporated to dryness, and the residue is redissolved in a small amount of solvent and reserved for further testing.
- This is called direct extraction.

- Liquid-solid extraction with resin or charcoal, and more recently other solid-phase extraction techniques, provide the necessary isolation of drug or drug metabolite from the biological sample and provide a relatively clean sample for analysis by GLC, HPLC, TLC, or related procedures.

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Thin-Layer Chromatography (TLC)

- Advantages of using TLC are (1) low cost of equipment, (2) rapid analysis, and (3) ability to detect more than one drug or metabolite per analysis.
- Relatively small amounts of drugs can be detected, usually as low as of 0.5-1.0 micrograms per milliliter.

- The recent development of highperformance thin-layer chromatography (HPTLC) plates has enhanced the capability of thin-layer chromatography.
- In HPTLC, silica gel particle size and the thickness of the layer on the plate are reduced, allowing for the separation of drugs in much shorter distances. Increased sensitivity is also gained because the applied spots are smaller than those for regular TLC.

- Some of the disadvantages of TLC are that it provides only fair specificity and sensitivity and results that are highly dependent on the technician's skill.
- It does require practice to recognize patterns of drugs and/or their metabolites by the visualized colored spots.
- These problems are minimized by commercial systems that attempt to standardize the elements of extraction, application, and visualization. One such system, manufactured by Analytical Systems, Laguna Hills, CA, is called the Toxi-Lab system.

Gas-Liquid Chromatography *(GLC)*

- Gas-liquid chromatography is widely used in drug analysis as a confirmation method as well as a primary screening method under some conditions.
- It utilizes an inert gas, such as nitrogen or helium, as the moving phase to transport a vaporized sample of a drug through a glass column containing a stationary liquid phase. The drug is identified and quantified by a detector at the far end of the column.

- Several types of detectors are available to provide the selectivity and sensitivity needed to properly detect and identify drugs of interest as they emerge from the column.
- Popular detectors are the electron capture detector (ECD), the flame ionization detector (FID), and the nitrogenphosphorous detector (NPD).

- Each of these detectors has its own characteristics of sensitivity and specificity.
- The NPD detector is particularly suitable for nitrogen containing compounds such as phencyclidine or cocaine.
- The FID is of more general applicability, but is less sensitive than the NPD or the ECD detector.

High-Performance Liquid Chromatography (HPLC)

- It employs a column through which the drug passes while undergoing equilibration between two liquid phases, rather than a gas and liquid phase as in the case of GLC.
- Again, the characteristic of the drug molecule that is measured is the time it takes for the drug to traverse the column at a given solvent flow rate.

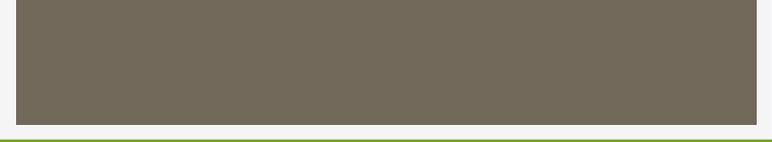
- Detectors are ultraviolet, fluorescent, or electrochemical in nature.
- HPLC has the advantage that polar drugs requiring derivatization on GLC systems can be assayed directly on HPLC.
- Its disadvantages are similar to those of GLC,
although specimen preparation may be simpler.

Gas Chromatography/Mass Spectrometry (GC/MS)

- GC/MS is generally considered to be the most conclusive method of confirming the presence of a drug.
- The major factors that have limited the use of GC/MS have been its comparatively high cost and complexity.
- Fortunately, GC/MS instrument manufacturers have recently introduced lower Priced systems that are easier to operate and this should result in significantly lower fees for GC/MS analyses.

SCREENING PROCEDURES

- Alternatively, thin-layer chromatography is often used as an initial screen when the ability to screen inexpensively for a large number of drugs is more important than the degree of sensitivity.
- Due to the level of subjectivity involved in the interpretation of TLC assays, however, it is important to confirm any presumptive positives with a highly specific method such as GC/MS.



Thank You