EVALUATION OF TOXICOLOGY TESTS RESULTS-
CHARACTERIZATION AND CONFIRMATION OF ANALYTICAL METHODS

Toxicology Laboratories frequently are asked, ‘is the testing accurate?’ This is likely a spillover from news media attention to workplace drug testing, and challenges clinical toxicologists to review their analytical procedures and laboratory protocols. Each laboratory procedure should be constructed in such a manner that the purpose for the test is defined, understood by the user, and reflected in the laboratory report. The report not only should list the result, but also state the degree of certainty with which the result is reported.

**Characterization of Analytical Methods**

Many analytical methods are used in toxicology, including colorimetric assays, immunoassays (enzyme multiplied immunoassay technique, radioimmunoassay, fluorescence polarization immunoassay), thin layer chromatography, gas chromatography, high pressure liquid chromatography and gas chromatography/mass spectrometry. Not all of these methods give unequivocal identification of a given analyte; some may detect a specific drug or metabolite while others focus on groups of drugs or metabolites. Therefore, all the methods should be fully characterized to permit proper interpretation of results. Some characteristics of analytical methods include:

**Accuracy** - the concordance between the experimental and true values as defined mathematically

**Precision** - reproducibility of the results of quantitative measurements

**Specificity** - the response by the method to a particular analyte sought

**Selectivity** - the ability to respond more readily to one target analyte than another

**Sensitivity** - the minimum concentration of analyte that is detected with high probability and clearly distinguished from background noise/signal

**Definitions**

**Screening Test** - a single or a series of initial tests designed to distinguish negatives from presumptive positives

**Confirmatory Test** - a second test used to definitively identify an analyte

**False Positive** - the report of an analyte that is not present

**False Negative** - the failure to report an analyte that is present above a threshold

**Unconfirmed Positive** - a positive screening result that is not further analyzed using a confirmation test
Confirmation of Tests

The American Academy of Forensic Sciences, Toxicology Section, has considered the necessity for test confirmation in forensic toxicology leading to the following statement:

“Confirmation of results is essential in forensic toxicology. Positive results of toxicological screening tests regardless of the method used and positive toxicological analysis results obtained by immunoassay methods should either be adequately confirmed before the results are used for forensic purposes, or be clearly designated as ‘unconfirmed’, results.

Analysis methods used for attempted confirmation of presumptive results must be appropriately sensitive and specific or unequivocally selective for the analyte(s) in question, and must be based upon different chemical or physical principles than the initial analysis method(s).

This requires testing a new aliquot of the original specimen using a different technique that has better specificity and greater or equal sensitivity to that of the initial test. However, in clinical toxicology, where rapid turnaround time is required for effective support of a diagnosis of suspected drug overdose, it may not be possible to meet these requirements. Presumptive positives are routinely reported, but interpretation by the physician is done within the context of both clinical and laboratory findings. Nonetheless, laboratories should consider implementing the additional measures to improve the certainty of laboratory tests.

Examples of the Need for Confirmation

1. The tricyclic antidepressant drugs and metabolites such as imipramine, desipramine, amitriptyline, nortriptyline, doxepin, and nordoxepin have close GC retention times. High concentrations may cause overlap and misidentification of peaks.

2. Quinine and quinidine are isomers and would have the same retention times in TLC or GC procedures.

3. When assaying propoxyphene, the thermolability of norpropxyphene may produce additional confusing GC peaks.

4. Immunoassays for the amphetamines cross react with phentermine and sympathomimetic amines such as ephedrine and phenylpropanolamine.

5. Immunoassays may exhibit cross reactivity to endogenous substances which may vary from batch to batch; reports of cross reactivity with ibuprofen and other non-steroidal anti-inflammatory drugs have been noted in the literature.

6. TLC commonly has co-migrating or closely migrating compounds, which have similar color characteristics.
Enhancing Reliability

A variety of approaches to additional testing may be applied to facilitate confirmation and enhance certainty.

1. A repeat of the original test, for example, an immunoassay, by reanalysis of the same sample by the same immunoassay. This serves as a check of instrument function and assay precision only. Repeat testing using a fresh aliquot may alleviate concerns about sample mix-up, it does not qualify as confirmation.

2. Confirmation testing of a new aliquot using a different technique, e.g., EIA followed by TLC, or TLC followed by a second TLC, or EIA followed by GC. This reanalysis increases the certainty since the alternate methodology is based on a different analytical principle.

3. Confirmation testing of a new aliquot using a highly definitive technique such as GC/MS. This may be done by various modes, such as selected ion monitoring utilizing three ions or full spectrum analysis. Notwithstanding claims that GC/MS is the ‘gold standard’, caution is required particularly when differentiating structurally similar compounds.

Reports

Reporting results that have been confirmed should be so stated, e.g., “methamphetamine, positive, confirmed.” Results that are not fully confirmed also should be clearly stated, e.g., “amphetamines, positive, unconfirmed, or amphetamines, presumptive positive.” An appropriate statement should accompany unconfirmed results indicating that such results should be used for diagnostic purposes only and should not be used for job related or other legal or administrative purposes.

Conclusion

The purpose of this article is to draw attention to the need to consider confirmation testing as a means to increase the certainty and reliability of an analytical result. Confirmation testing, a standard of practice in forensic toxicology, should be performed in clinical toxicology whenever possible. It is important that a laboratory defines and documents the limits of its procedures, and prepares reports that accurately state whether a positive result is a confirmed positive or an unconfirmed presumptive positive so that the clinician can properly understand and evaluate test results.

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