References


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Changes in Immunoglobulin Control Sera

To the Editor:

In our recent article on Immunochemical Determination of Human Immunoglobulins [Clin. Chem. 25, 526 (1979)] we noted errors in the Kallestad antisera lot numbers, which should read as follows:

Kallestad Antisera IgG, Lot A204L051
Kallestad Antisera IgA, Lot A202J0691
Kallestad Antisera IgM, Lot A206H0395

We are advised by the company that Kallestad Reference Serum Lot AR055K1211-1B is no longer available. We are currently using Kallestad Reference Serum Lot R180K1011-10 for IgG and IgA and Lot R180L101 for IgM. The new reference sera have been assigned calibration values by the company based on their comparison of radial immunodiffusion to their own laser nephelometry; we have observed a slight bias using these new assigned values when compared to our kinetic turbidimetric method.

To correct for the bias, divide the assigned IgG reference serum value by 1.1 to correct for the Kallestad RID assay. Divide the assigned IgM reference serum value by 1.27 to correct for the RID assay. The IgG reference serum value shows a 5% bias; we in our laboratories decided not to correct for this. If the user wishes to correct for the IgG bias, divide the assigned value by 1.05.

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Use of an Internal Standard in a Single-Solvent Thin-Layer Chromatographic Analysis for Drugs of Abuse in Urine

To the Editor:

Analysis for drugs in urine by thin-layer chromatography (TLC) has traditionally been treated as a purely qualitative screen. The toxicologist uses controls and standards to check the efficiency of his testing, which can indicate the quality of the drug group they are associated with, but do not yield a true picture of each urine individually.

To fill this gap in quality control of mass drug screens, I use an internal standard in each urine screened for drugs. The internal standard should prefer other drugs to be analyzed and should not be normally present in detectable amounts in the urine to be screened.

Scopolamine-HBr fits these criteria. Its RF is different from that of every drug tested in a normal urine drug screen in the solvent system we use, with the sole exception of phenobarbital. Although scopolamine and phenobarbital have the same RF in this system, they do not interfere because the phenobarbital spot disappears before the scopolamine is made visible.

Even after dosage with it, scopolamine is not present in quantities detected by TLC. All the scopolamine preparations on the market contain less than 1 mg of scopolamine-HBr (1). Because only 1% of scopolamine is excreted in the urine (2) this drug cannot be seen in the urine drug screen.

During a year, we performed an average of 4800 urine drug screens per week. The following procedure was used:

An internal standard was prepared (scopolamine-HBr, 40 mg/L). Urine, 19 mL buffered to pH 9.5, was combined with 1 mL of internal standard to give a 2.0 mg/L concentration of scopolamine. The urine was extracted with 25 mL chloroform/isopropanol (9/1 by vol). The organic layer was evaporated, and the residue reconstituted with a few drops of methanol and spotted on either a Brinkman/MN silica gel G or EM silica gel precoated plastic and glass TLC plates, without fluorescent indicator.

The TLC plates were placed in a glass tank containing Drug Screen Development II Solvent (3) (ethyl acetate/chloroform/methanol/conc ammonium hydroxide, 50/30/12/1.5 by vol). The solvent was allowed to migrate 12 cm. The plate was then removed from the tank, heated, sprayed, and the spots identified as described by Davidow and Quame (4).

Drugs detected in the urines were compared to standards and controls run on each plate. If the internal standard was not visible in a sample, the run was repeated. Of the 4800 samples analyzed each week, about 2.5% had to be repeated for this reason. Most such repeated runs revealed drugs missed on the initial screen. Most of the urines screened came from methadone maintenance programs, so most were assumed to have at least one drug present.

The 2.0 mg/L concentration for the internal standard was chosen very carefully. It is the minimum limit of detectability by this TLC procedure. If the internal standard was not visible, a fault in procedure or technician error was indicated.

The results of this study show there are inaccuracies that are overlooked in large batches of drug screens. These omissions can be corrected by use of an internal standard in each urine, which gives the toxicologist a semiquantitative tool for maintaining the quality assurance of his testing procedures.

Although scopolamine-HBr is a useful internal standard, toxicologists may want to consider multiple internal standards for TLC runs. A few examples would be a basic, neutral, and acidic extractable group of chemicals not found in urine, not interfering in RF values, and visible in the particular TLC procedure being used.

References


4. Davidow, B., and Quame, B., A TLC screening procedure for detection of drug