Development of a Multiplex Toxicology Panel of Immunoassays

WILLIAM LINK, RENATO DEL ROSARIO, JOEL HARDY, DAVID KING, RAISA LERNER, RANDY SWEET and KUNQUAN YAO

Bio-Rad Laboratories Inc, Hercules, CA 94547, USA

AIMS: To develop a fully automated, multiplex panel for the semi-quantitative and simultaneous assay of drugs in urine samples for the BioPlexTM2200 system. The panel consists of assays for the following drugs (current cutoff values in ng/mL are in parentheses): opiates (300), amphetamine (500), methamphetamine (500), MDMA (500), THC (50), cocaine (benzoylecgonine; 150), benzodiazepines (300), barbiturates (300), methadone (300), PCP (25), and tricyclic antidepressants (300). The panel includes a creatinine to test for specimen adulteration (<20mg/dL) and substitution (<5mg/dL).

METHODS: The entire panel is performed using one reagent pack consisting of three reagents. Bead Reagent is a suspension of 13 sets of microspheres with magnetic cores, each set labeled with a drug-protein conjugate and a unique dye for identification by a laser in the BioPlexTM2200 detector module. Antibody Reagent is a mixture of antibodies specific to the panel analytes, and Conjugate Reagent is a secondary antibody labeled with phycoerythrin (PE). During the first incubation, drug in the sample and drug immobilized on beads compete for limited binding sites on antibodies present in solution. Antibody not bound to beads is removed during a wash step, and Conjugate is added and allowed to bind antibody bound to drug immobilized on beads. After a final wash step, the bead suspension is transferred to a detector that quantitates the PE fluorescence associated with each bead set. Relative fluorescence of samples is converted to drug level from a stored calibration curve. Since many of the analytes in the panel are classes of drugs (e.g. opiates, barbiturates), the assays use antibodies that cross-react to varying degrees with the important members of a given class. The ng/mL values are based on calibration against a single drug within a class and results are used to determine if the specimen is positive relative to a cutoff value. An internal standard is used to correct for slight fluctuations in detector performance. All assay steps are fully-automated on the BioPlexTM2200 instrument.

RESULTS: Users can select up to 12 assays from a single analysis. Users can select an "amphetamine screen" which gives a single combined result based on the amphetamine and methamphetamine assays, or can report them separately. Custom panels, combinations of assays defined by the user, can also be run using the eFlex™ software. The first specimen in a run requires approximately 14 minutes to process before all of its results (up to 12) are reported. Results for subsequent samples are reported every 36 seconds, providing up to 1200 results per hour after initial processing. Dynamic range of the creatinine assay is 1-40mg/dL and is intended to identify specimens that have been adulterated. Preliminary precision studies using specimens at or near the cutoff (20 replicates/sample, two runs/day for 8 days) indicated within run precision of ng/mL values of 3.7-6.8%, and total precision of 5.9-7.9% (ANOVA). Calibration was performed only at the beginning of the 8-day study using five calibrators containing all drugs, plus creatinine.

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CONCLUSIONS: The BioPlexTM2200 is capable of screening urine samples for a wide range of drugs simultaneously, from a single small urine sample within 14 minutes. The multiplex design minimizes reagent handling and allows high throughput of results. The panel is currently in development.

KEYWORDS: *Multiplex, Immunoassay, Screening, Automation, Drugs*

Corresponding author: bill_link@bio-rad.comAM-p-24