

## Suspension Bead Array (SBA) technology as a presumptive tool in testing for amphetamine-type substances and designer analogues

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To our knowledge, no trials exist in available literature describing the use of suspension bead array (SBA) technology for forensic toxicology applications. Based on Luminex xMAP technology, target compounds, protein conjugates, or class-specific antibodies can be coated on the surface of spectrally addressable 5.6 µm polystyrene microspheres. As the beads are internally labelled with a ratio of two spectrally distinct fluorophores, the bead lots are assignable to different drugs and/or drug class-specific antibodies. Mixtures of different bead suspensions can then be used in the same well of a 96-well filtration plate format, allowing for multiplex analysis with theoretically the same, if not improved, sensitivity and selectivity of ELISA. For end detection, the reacted beads are measured for spectral address by a red laser, and the surface excitation fluorescence and subsequent analyte concentration is assessed using a green laser (Bio-Plex Suspension Array System; Bio-Rad Laboratories, Inc., USA).

From a competitive assay model utilizing bovine serum albumin (BSA)-drug conjugates, monoclonal drug-specific antibodies raised in mice, and a fluorescent-labelled anti-mouse antibody, early developmental trials using flow cytometry have demonstrated a bead-based immunoassay that exhibits adequate linearity over a concentration curve of toxicological relevance (0–1000 ng/mL amphetamine and/or methamphetamine; replicate CVs < 12%). As well, the percent decrease in fluorescence intensity due to competition also exhibits the capability to reproducibly detect drug concentrations at or below 62.5 ng/mL. Of additional significance, early assay trials exhibit specificity comparable to commercial ELISA assays: the amphetamine model assayed positive for MDA (72% relative response, CV < 1%); the methamphetamine model assayed positive for MDMA, MDEA, and ephedrine (110%, 25%, and 41% respectively, CVs < 5%). While these results relate to a phosphate buffered saline matrix (pH 7.4), studies have also demonstrated potential applicability to oral fluid screening.

While multiplex efforts to combine the amphetamine and methamphetamine assays exhibited some degree of non-specific protein binding using the Bio-Plex instrumentation, the merits of both individual assay models are demonstrated, and by multiplexing may allow for a complementary general amphetamines drug screen. The results to date show that additional research and development on the use of multiplex suspension bead array technology could be of great benefit to the working toxicology laboratory, as the ability to multiplex could lead to increased laboratory throughput, reduced sample consumption, and increased confidence in presumptive screening results.

**KEYWORDS:** *suspension bead array, immunoassay, amphetamine-type substances, saliva, methamphetamine, MDMA*

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