Multiplex Approach for an Immunological Detection of Drug Abuse: A Validation Study

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AIMS AND OBJECTIVES

The aim of this study was to develop a multiplex immunoassay on a miniaturized platform for nine different drugs. Therefore each reagent undergoes a stringent quality control e.g. antibodies used has to be validated with at least 2 independent methods.

MATERIALS AND METHODS

For validation Western Blot analysis and ELISA were performed. A competitive ELISA was established allowing the quantification of the drugs in sera. Appropriate controls were included for background subtraction and determination of unspecific signals. The miniaturized assay will be done on a microarray, which is produced with a non-contact spotter.

RESULTS

For 4 out of 9 selected drugs specific antibodies could be obtained and a competitive ELISA established for quantification. Validated antibodies are characterized by no cross-reactivity to serum and no binding to other compounds. Serum samples with spiked drugs or samples from the LKA Berlin were analyzed. Each sample was performed in triplicates and each experiment was done twice at least. Limits of quantification meet the requirements of the GTFCh. No cross-reactivity or matrix effects were observed with the validated antibodies. First multiplex and spotting experiments were done with a satisfying result.

CONCLUSION

The presented approach enables a sensitive and reliable detection method for drug abuse. The validation studies are continued for the remaining drugs.

PROCEDURE OF VALIDATION STUDY

A validation study includes following individual steps and different methods: Quality control (QC) of the antibodies, establishment of a standard/calibration curve and verification of the method via measuring samples provided by the LKA Berlin. The following figures give a brief overview of the progress of the immunoassay. The illicit drugs used in this study are: Amphetamine, Methamphetamine, MDMA, Cocaine, Benzoylecgonine, PCP, Morphine, Methadone and THC.

I) ESTABLISHMENT OF A STANDARD CURVE

Competitive ELISA in 96-MTP Format and on Microarrays

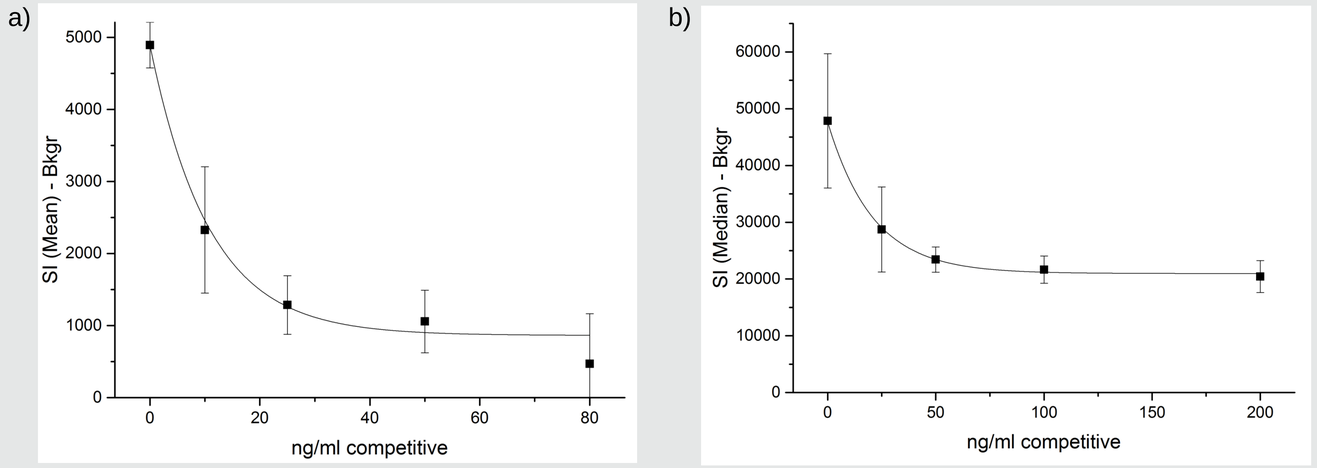

Fig. 1: A competitive assay with MDMA in a) a 96-MTP and b) on a microarray for generation of a calibration curve is shown. The microarray was produced with a non-contact spotter. MDMA-BSA was immobilized and incubated with increasing amounts of free MDMA. With increasing concentrations of free MDMA the signal decreased until a plateau was reached. The linear range of the calibration curve will be used for quantification of serum samples. Both platforms provided similar results. In both cases the linear range of the calibration curve ranged from 0 to 20 ng/ml and should be extended.

Summary of Antibody Validation

Tab. 1: The present results for QC and establishment of a standard curve with various methods are summarized. QC was performed with Western Blot analyses and direct ELISA. The standard curves were generated with competitive ELISA in 96-MTP and on microarray (MA). An antibody is validated when no unspecific binding with serum, other drugs or antibodies and assay components occurred.

Drug	QC	ELISA	MA
Amphetamine	✗	✗	✗
Methamphetamine	✗	✗	✗
MDMA	✓	✓	✓
Cocaine	✗	✗	✗
Benzoylecgonine	✓	✓	✗
PCP	✗	✗	✗
Morphine	✗	✗	✗
Methadone	✓	✓	✗
THC	✓	✓	✗

II) Assay - Validation

Initial Experiments for Multiplexing

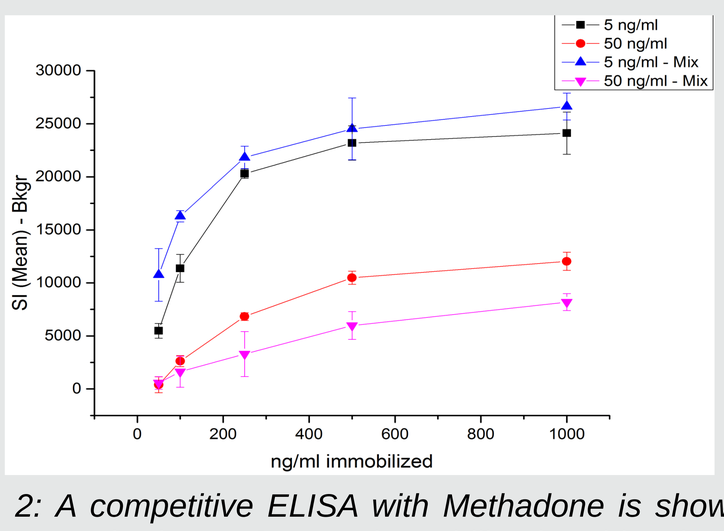

Fig. 2: A competitive ELISA with Methadone is shown. Methadone-BSA was immobilized and incubated with 2 different amounts of free drug. The influence of the presence of 9 drugs (blue and pink) of the same concentration was analyzed. Only minor differences to the solitary presence of Methadone (black and red) were observed.

Quantification of LKA Samples

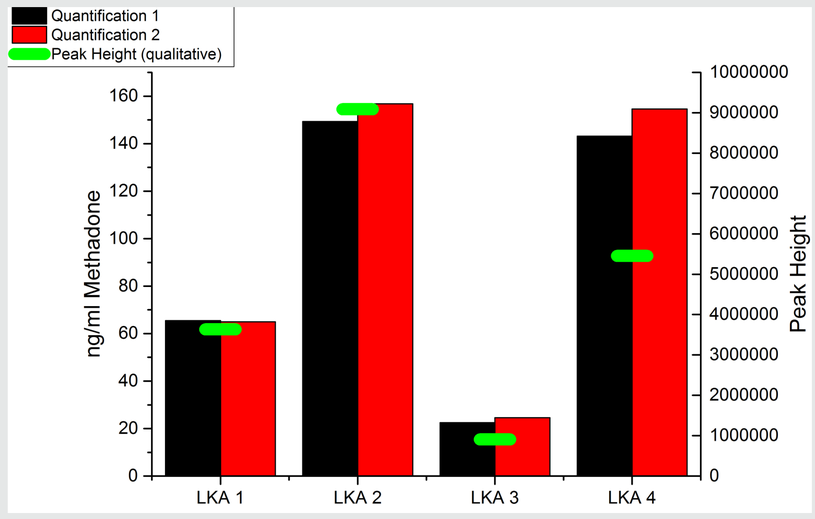

Fig. 3: Two independent quantifications of four Methadone samples is depicted. Methadone concentrations were calculated with a calibration curve and compared with qualitative LC/MS measurements. With both methods comparable results were achieved.

III) Summary

- For 4 out of 9 drugs specific antibodies validated and competitive assays established
- Minor signal differences by administration of a mixture of drugs or antibodies
- First successful quantifications of LKA samples with good correlations to reference data
- Successful miniaturization from 96-MTP to Microarray

Next Steps:

- Improvement of linearity of standard curves
- Extending multiplexing
- Quantification of further LKA samples

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