



Quality control of antibodies for assay development

Sarah Schumacher^{1,2} and Harald Seitz¹

¹ Fraunhofer Institute for Cell Therapy and Immunology – Bioanalytics und Bioprocesses, Am Mühlenberg 13, 14476 Potsdam, Germany

² Humboldt University Berlin, Department of Biology, Invalidenstr. 110, 10115 Berlin, Germany

Antibodies are used as powerful tools in basic research, for example, in biomarker identification, and in various forms for diagnostics, for example, identification of allergies or autoimmune diseases. Due to their robustness and ease of handling, immunoassays are favourite methods for investigation of various biological or medical questions. Nevertheless in many cases, additional analyses such as mass spectrometry are used to validate or confirm the results of immunoassays. To minimize the workload and to increase confidence in immunoassays, there are urgent needs for antibodies which are both highly specific and well validated. Unfortunately many commercially available antibodies are neither well characterized nor fully tested for cross-reactivities. Adequate quality control and validation of an antibody is time-consuming and can be frustrating. Such validation needs to be performed for every assay/application. However, where an antibody validation is successful, a highly specific and stable reagent will be on hand. This article describes the validation processes of antibodies, including some often neglected factors, as well as unspecific binding to other sample compounds in a multiparameter diagnostic assay. The validation consists of different immunological methods, with important assay controls, and is performed in relation to the development of a diagnostic test.

Introduction

Since the invention of *in vitro* production of monoclonal antibodies by Köhler and Milstein in 1975 [1], they have become favoured tools in basic research as well as in diagnostics. Their target specific binding and their robustness and stability according to the ambient temperature enable easy and rapid detection of a variety of different analytes. But besides the well-known pregnancy or allergy tests, there are only a few immunoassays which are routinely used and most deliver only qualitative statements. When looking more closely into the topic, many users report unspecific binding of the antibodies [2,3]. Another issue found in publications is poor reproducibility [3,4]. These characteristics make an extensive quality control and validation procedure for every specific application necessary. Unfortunately little information can be

found in the literature or is provided by suppliers of commercial antibodies [2,3]. Nevertheless within recent years, efforts have been made to improve the knowledge base of antibodies. Initiatives such as Biocompare (<http://www.biocompare.com>), Antibodypedia (<http://www.antibodypedia.com>) or Antibody Resource (<http://www.antibodyresource.com>) and academic projects such as the Human Proteome Atlas (<http://www.proteinatlas.org>) offer information concerning antibody type, reactivity and applications. The data provided could be a good starting point to avoid unnecessary and cost-intensive experiments. We believe that deliberate antibody validation and quality control is essential and a prerequisite to develop a reliable immunoassay with the potential to be deployed in diagnostics.

To emphasize the importance of antibody quality control, we have chosen the detection of drug abuse as an example. The drugs under investigation were amphetamine (PubChem CID 5826),

Corresponding author: Seitz, H. (harald.seitz@izi-bb.fraunhofer.de)

methamphetamine (PubChem CID 10836), MDMA (3,4-methylenedioxy-methamphetamine, PubChem CID 1615), THC (tetrahydrocannabinol, PubChem CID 16078), PCP (phencyclidine, PubChem CID 6468), methadone (PubChem CID 4095), morphine (PubChem CID 5288826), cocaine (PubChem CID 446220) and benzoylecgonine (PubChem CID 448223). This scenario is medically relevant and represents a difficult case of antibody validation due to the small size and structural similarity of the different analyte molecules. Starting with a set of nine different drugs to be implemented in one assay, the corresponding antibodies were validated by ELISA (enzyme-linked immunosorbent assay), Western blot analysis and on protein microarrays. A first screening of the antibodies was performed by direct ELISA and Western blot and later compared to findings on protein microarrays. We considered an antibody as validated if the following conditions were met: Specific binding to the target and no detectable cross-reactivity to chemically or structurally related molecules.

- No unspecific binding to assay compounds, for example, biological medium or blocking solution.
- Stable antigen–antibody reaction, as shown by technical and biological replicates with CVs < 20% (GTFCh – Society of Toxicological and Forensic Chemistry, ICH – International Conference on Harmonisation).

Here we outline the significance of antibody quality control and highlight some issues which are often overlooked, but which may influence the performance of an assay.

Material and methods

Reagents and equipment

For immobilization, BSA–drug conjugates from Fitzgerald Industries International USA (Amphetamine–BSA 80-IA22; Methamphetamine–BSA 80-IM59; MDMA–BSA 80-1044; THC–BSA 80-IT63; PCP–BSA 80-IP10; Methadone–BSA 80-IM55, Morphine–BSA 80-IM50; Cocaine–BSA 80-1034; Benzoylecgonine–BSA 80-IB31) were used. On the basis of the ratio of BSA to drug molecules provided by the manufacturer, the relative drug concentration of the conjugates was calculated. In the competitive assays, pure drugs dissolved in methanol (LGC Standards, UK) were used. As sample medium, undiluted human serum (UTAK, USA) was employed. The drug specific antibodies were purchased from Acris Antibodies, Germany (anti-amphetamine antibody AM31389PU-N; anti-THC antibody BM2701), Fitzgerald Industries International, USA (anti-cocaine antibody 10-1030) and Abcam, UK (anti-PCP antibody ab20457; anti-morphine antibody ab23357) and chosen such that all were detected with the same fluorescent labeled secondary anti-mouse antibody (anti-mouse IgG Alexa Fluor 555, Invitrogen, USA; A-21422). For dilutions and washing steps, PBS-T (phosphate buffered saline with 0.05% Tween 20) and a carbonate buffer (28.6 mM Na₂CO₃, 72.13 mM NaHCO₃, pH 9.56) were used. Unless noted otherwise BSA–drug conjugates and antibodies were used from the same batch to avoid variations.

For ELISA experiments, black 96-well plates (Nunc, Fisher Scientific, USA) were used. Western blotting was performed on nitrocellulose membranes (GE-Healthcare, UK) and for protein microarrays epoxy slides were produced in-house. The microarrays were produced by a non-contact spotting system with a piezo-nozzle (M2-Automation, Germany). The nozzle diameter was

100 μm and parameters of voltage, frequency, pulse duration and pressure were adjusted, such that a 120 pL droplet was formed. In total, 10 droplets of each sample were applied to the surface. As read-out systems for fluorescence detection, a Typhoon-Scanner (GE-Healthcare, UK; Western blot), microplate reader (BMG Labtech, Germany) and Axon Scanner (Molecular Devices, USA) were used.

Immunoassays

For Western blot analysis, 12%-SDS-PAGE was performed. The BSA–drug conjugates were diluted in PBS-T and 300 ng of each was applied to the gel. Following electrophoresis the samples were transferred to a nitrocellulose membrane using a semi-dry protocol. The transfer was controlled by a reversible Ponceau-S (AppliChem, Germany) staining. Blotting and blocking were performed at room temperature (RT) and the antibody incubation at 4 °C. During each incubation step the membrane was gently shaken. Unless stated otherwise, antibodies were diluted 1:1000 in 1%-milk powder. After each incubation step, the membrane was washed briefly 3 × with PBS-T. After a blocking step with 10%-milk powder (BioRad, USA) for one hour, incubation with a primary antibody was performed for 90 min. Finally, the membrane was incubated for 60 min with the fluorescent labeled secondary antibody and scanned.

For ELISAs and protein microarrays the BSA–drugs conjugates were diluted in carbonate buffer. In the case of ELISAs the MTP was incubated for 2 h at RT. Blocking was performed for 60 min with 2% BSA or undiluted serum. If not otherwise specified the antibodies were applied in a 1:1000 dilution in PBS-T or undiluted serum.

Protein microarrays were vacuum sealed and stored overnight at 4 °C after spotting. For the incubation steps, a Whatman slide holder (GE Healthcare, UK) with 16 incubation chambers was used. After immobilization, free binding sites were blocked for 60 min with either a 2% BSA solution, undiluted blank serum or an ethanolamine solution (Sigma, USA). Unless noted otherwise, the antibodies were diluted 1:100 in PBS-T or undiluted serum.

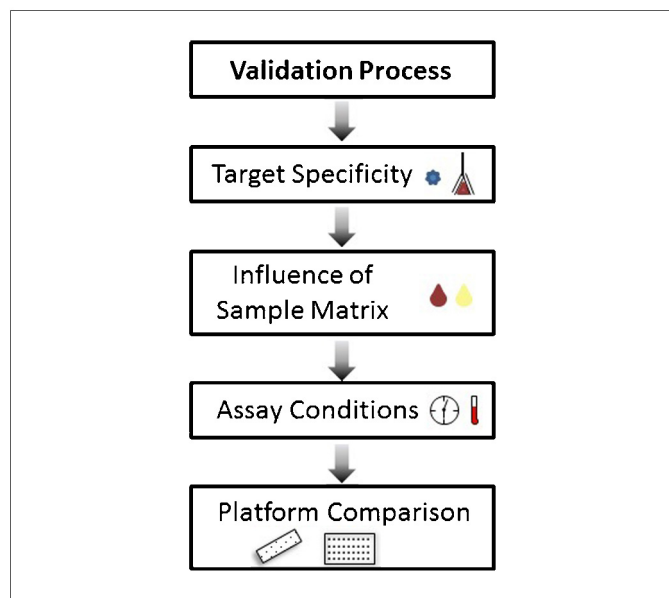
For ELISAs and protein microarrays primary antibodies were incubated for 90 min and the secondary antibody was applied for 60 min before reading. For both methods 3 replicates of each sample were analyzed, after each step, 3 short washes with 1xPBS-T were performed and all steps were done at RT on a shaker.

Data analysis

The mean (or median) of the replicates of each sample was calculated. The buffer was set as background signal and subtracted from the sample signals. For ELISAs the mean of the signals was used and for microarray analysis the median was calculated of the spots [5]. The signal intensities of the included negative controls BSA and blank serum should be at a similar level to the buffer if no cross-reactivity occurred. This means no unspecific binding to assay compounds, for example, sample medium, drugs or buffer was detectable. For analysis of the microarray data, GenePix 7 software (Molecular Devices, USA) was used.

Results

In the following section, effects which occurred during antibody validation are described. The validation process consisted of

**FIGURE 1**

A flow sheet of the antibody validation process. The steps were performed consecutively. The process is independent of whether a primary target specific or a secondary detection antibody was being investigated.

several steps to ensure the specificity of the antibodies, exclude possible cross-reactivities, optimize the experiments and test the comparability of the results on different platforms. Fig. 1 shows a flow sheet which depicts the process valid for every antibody used within this study. This follows a general schema routinely used in many academic and industrial laboratories. Firstly, each antibody was tested for its specificity in which it was incubated with molecules chemically related to the target. This was tested with Western blot and ELISA. Next, the influence of the sample matrix was investigated. This is an important step for application in diagnostic tests. In this study the experiment was run with the antibody diluted in parallel either in serum or buffer. Furthermore the assay conditions had to be tested carefully in regard to immobilization and blocking efficiency. Finally, the assay conditions depend on the chosen platform. Blocking solutions, immobilization amount and incubation time may differ between, for example, microtiterplates (MTPs) and modified glass slides. Altogether a total of 26 antibodies were tested targeting 9 different drugs. Although all antibodies were described as specific by the suppliers, only 5 were identified which fulfilled all the described criteria, meaning that approximately 80% of the antibodies failed during the validation process. Unfortunately this is a common issue. Egelhofer *et al.* [6] observed a similar result, in which the specificity of over 200 antibodies was tested and nearly 50% of their antibody panel was excluded on this basis. This underlines the importance of antibody validation.

Cross-reactivity to chemically related target compounds

One of the best known effects is the binding of the investigated antibody to molecules of the same class as the target. This so called unspecific binding is normally based on structural or chemical similarities of the analytes. In the case of illicit drugs, cross-reactivities often arise between the starting product (e.g. cocaine) and its derivatives or metabolites (e.g. benzoylecgonine). Fig. 2a and b show representative ELISA experiments which illustrate this

issue. These experiments include a single determination of each signal. In principle a Western blot under native conditions was performed. Future screening of drug abuse also will take place under native terms. To that with the 9 BSA–drug conjugates, positive control (primary antibody) and negative controls (BSA, undiluted serum) 8 different antibodies per MTP instead of one per membrane could be tested. The anti-cocaine antibody under test recognizes its target cocaine as well as its main metabolite benzoylecgonine (Fig. 2a). As shown, the drugs were detected with comparable signal intensity. Another example is the group of amphetamines. Again the target (amphetamine) as well as MDMA and methamphetamine were detected with nearly identical signal intensities (Fig. 2b) by the anti-amphetamine antibody. In general, no differentiation between these drugs was possible. As a positive example, an ELISA for THC is shown (Fig. 2c). Only the antigen THC and the positive control (1st AB) gave a clear signal. No cross-reactivities with other drugs, BSA or serum occurred. Furthermore the positive control, which was the anti-THC antibody, delivered a strong signal.

Influence of sample medium

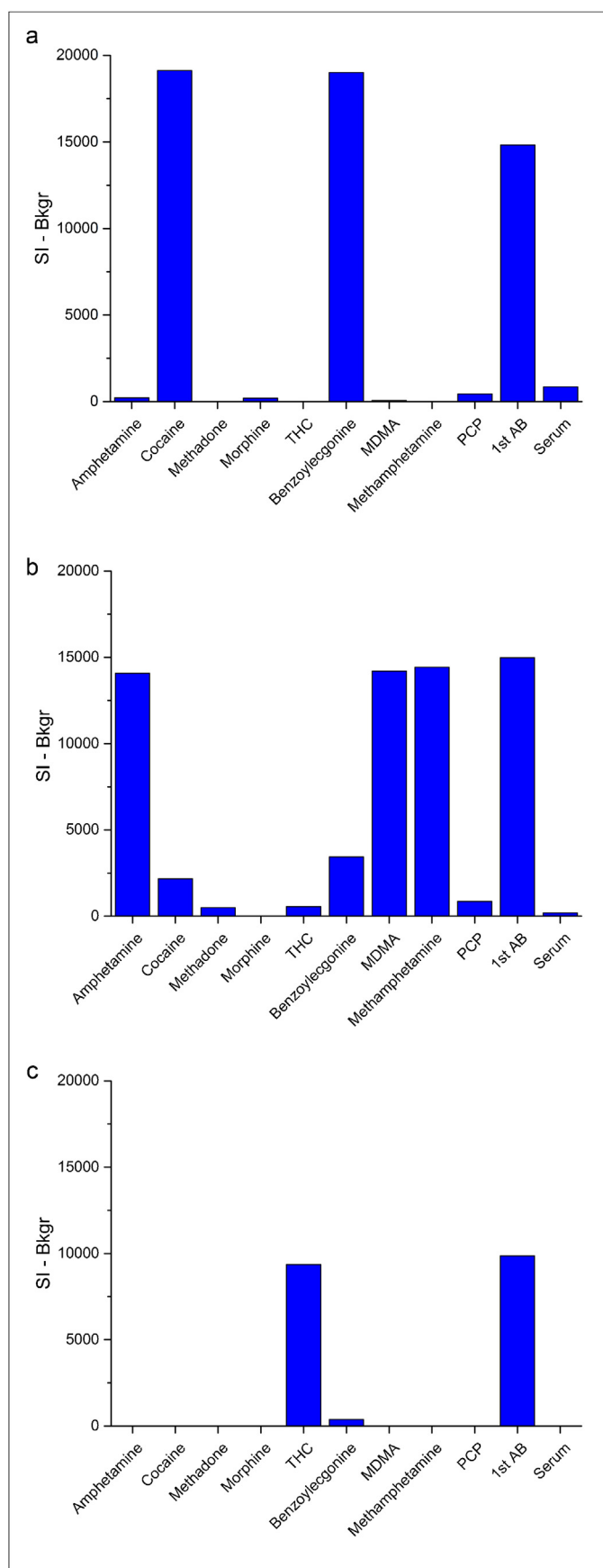
Another important factor is the sample medium. Antibodies which are specific in a system which uses buffer (e.g. PBS) might behave differently in biological fluids such as serum or urine. This could be due to proteins present in the samples which cross-react with the primary antibody. To rule out such cross-reactivity, measurements performed in serum and in buffer were compared (Fig. 3). For a better comparison in both measurements free binding sites were blocked with 2% BSA.

The anti-PCP antibody showed a good sensitivity in an ELISA when diluted in 2% BSA solution. A linear range up to approximately 200 ng/mL was observed; at higher concentrations saturation was reached. When the antibody was diluted in serum, an enormous decrease in signal intensity occurred: the PCP–BSA conjugate could not be detected in a serum sample. This led to the rejection of the antibody because it was not applicable in a diagnostic environment.

Choice of assay conditions

After the elimination of possible cross-reactivities, the assay conditions must be chosen carefully. One essential point is the selection of the blocking solution, which in turn depends on the platform. Different platforms often represent different immobilization strategies and surface chemistries, and as a result there can be differences in the choice of blocking solution. In Fig. 4 the impact of different blocking reagents on the background signal is depicted. Signal intensities of 3 different blocking solutions are compared on microarrays, an application which is still evolving in the diagnostic field. The samples shown represent negative controls, which should produce no signals if cross-reactivities of the primary antibodies had already been eliminated.

For all samples on the microarray, quite low signal intensities were observed. Each blocking solution showed the highest unspecific binding if undiluted serum was immobilized. In general the best results were obtained when the surface was blocked with ethanolamine. Blocking with 2% BSA was successful, but showed the highest signal for serum. If undiluted serum was used as the blocking reagent, each negative control was measurable with higher signal intensities, compared to the other two blocking

**FIGURE 2**

ELISAs for (a) cocaine, (b) amphetamine and (c) THC. Nine different drug-BSA conjugates and the corresponding antibody (1st AB, positive control)

solutions. In general unspecific binding of the secondary antibody could be the cause of the signals detected on the microarray.

Finally consideration must be paid to whether a target is presented in its native or denatured state. Due to conformational changes, an antibody might be not able to bind the epitope. This is often recorded for proteins and peptides. Although small molecules like drugs cannot denature, this effect was documented for an anti-morphine antibody (Fig. 5). This antibody worked specifically for morphine in a Western blot analysis, but failed during the further validation process in ELISA experiments.

Influence of platform choice

An often ignored factor is the dependency of antibody specificity on the choice of platform. Immunoassays can be performed on paper sticks, plain microtiterplates, membranes or functionalized glass surfaces. Fig. 5 shows an example with an anti-morphine antibody analyzed by Western blot and ELISA.

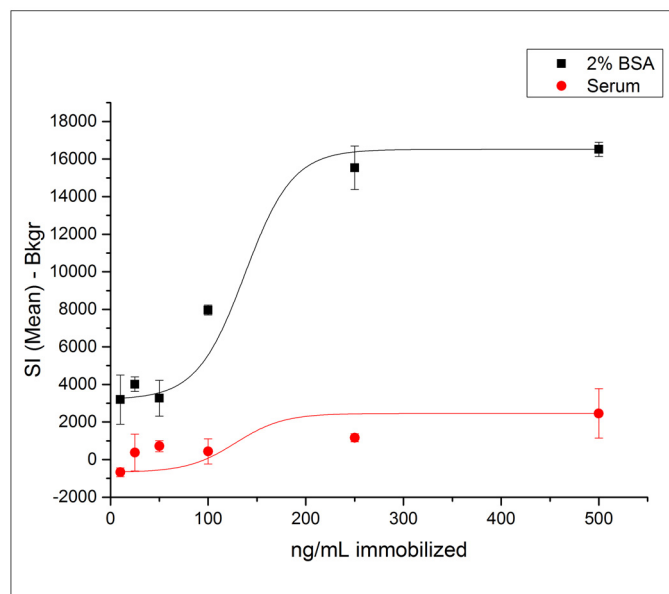
In reference to the Western blot (Fig. 5a) the anti-morphine antibody produced a specific signal for morphine-BSA (lane 3) and a slight cross-reactivity with serum (lane 11). No signals for other drug-BSA conjugates were visible. The conjugates were always visible as a smear, mainly caused by the effects of the conjugation reaction and associated aggregation of the BSA. In contrast, the ELISA experiment (Fig. 5b) showed a different result: no specific signal for morphine-BSA could be obtained and only weak signal intensities were measurable for other samples, such as MDMA or Benzoyllecgonine. From these results the anti-morphine antibody was excluded from further analyses.

Discussion

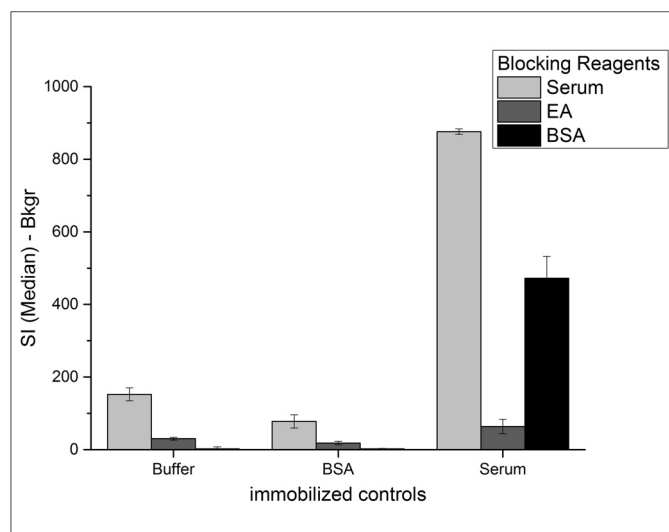
Cross-reactivity to chemically related compounds

One of the most reported scenarios is cross-reactivity of an antibody with chemically or structurally closely related molecules. In the case of illicit drugs, this effect is accounted for functional groups and/or the hapten based antibody production. First, the amino groups are quite prominent in these small molecules and are often located at an easily accessible position. Moreover, the occurrence of aromatic rings is another characteristic of illicit drugs. These common features suggest that there will be cross-reactivities. The named examples showed this clearly. Benzoyllecgonine is the main metabolite of cocaine [7] and originates from the cleavage of the methyl ester group. As an example Fig. 6 illustrates these small differences of cocaine and its main metabolite benzoyllecgonin. Amphetamine and methamphetamine differ only by a methylene group, which could be one reason for the cross-reactivities of the closely related compounds. Cross-reactivity to related compounds is a well-known problem. Castaneto *et al.* [8] reported such a case for the detection of designer piperazines (synthetic drugs). A similar result was published by Shen *et al.* [9] who observed high cross-reactivity between striatin B and striatal B which differ by an aldehyde group. These findings correspond quite well with those shown for the anti-cocaine

were immobilized at a concentration of 500 ng each. Undiluted serum was included as a control. Free binding sites were blocked with 2% BSA. Incubation with the primary antibody (1:1000) was followed by detection with a fluorescent labeled secondary antibody (1:1000). Signal intensities normalised by the signal of BSA are plotted.

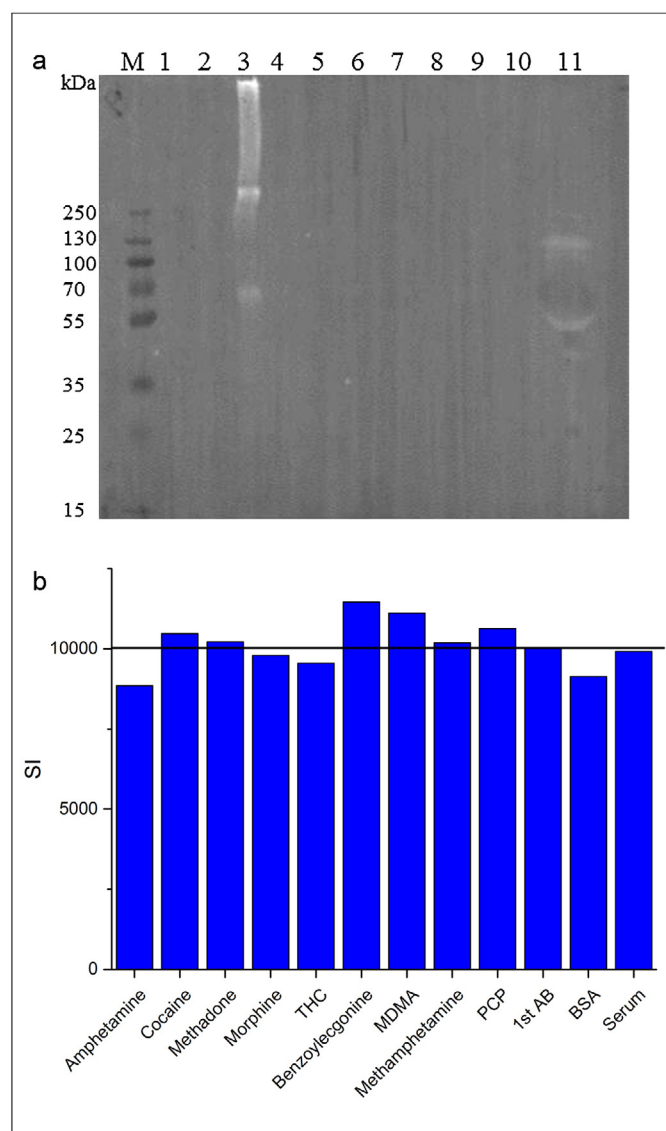
**FIGURE 3**

An ELISA for PCP is shown. PCP-BSA conjugate was immobilized in a concentration series of 10 ng to 500 ng. Free binding sites were blocked with 2% BSA. Incubation with the primary anti-PCP antibody (1:500), either in 2% BSA (black) or undiluted serum (red) followed and detection was performed with a fluorescent labeled secondary antibody (1:1000).

**FIGURE 4**

Results of a representative microarray for the negative controls with different blocking solutions is shown. The carbonate buffer, pure BSA and undiluted serum were immobilized. Free binding sites were blocked with 3 different solutions, namely undiluted serum (light grey), 2% BSA (black) and ethanolamine (EA, dark grey), and incubated with a fluorescent labeled secondary antibody (1:1000). The microarray was analyzed using GenePix Pro7. The slide surface in close proximity of the spot was set as the background by the software.

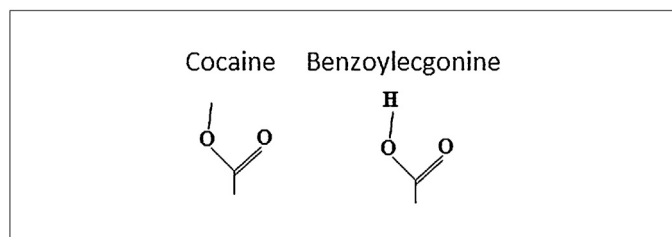
and anti-amphetamine antibody. Another origin of unspecific binding could lie in the method of production of the antibodies [10]. The host is immunized with a hapten, for example, in the case of the primary antibodies used in these experiments the drug coupled to BSA, due to the low immunogenicity of the small molecules. A crucial factor is the coupling site of the carrier molecule. Most important therefore is the accessibility of the

**FIGURE 5**

(a) A Western blot of morphine-BSA is shown. Nine different drug-BSA conjugates, BSA (300 ng each) and serum (1:10) were separated on a 12% SDS-PAGE and blotted. Free binding sites were blocked with 10% milk powder. Incubation with the primary anti-morphine antibody (1:1000) was followed by detection with a fluorescent labeled secondary antibody (1:1000). (M) Marker; (1) Amphetamine-BSA; (2) Methadone-BSA; (3) Morphine-BSA; (4) Cocaine-BSA; (5) THC-BSA; (6) Benzoyllecgonine-BSA; (7) MDMA-BSA; (8) Methamphetamine-BSA; (9) PCP-BSA; (10) BSA; (11) Serum (1:10). (b) An ELISA for morphine is shown. Nine different drug-BSA conjugates, the anti-morphine antibody (1st AB) and BSA were immobilized at a concentration of 500 ng each. Undiluted serum was included as a control. Free binding sites were blocked with 2% BSA. Incubation with the primary anti-morphine antibody (1:1000) was followed by detection with a fluorescent labeled secondary antibody (1:1000). The signal intensities are plotted; black bar marks the background signal of the carbonate buffer.

functional groups which are characteristic for the specific drug; if these are inaccessible, antibody production could lead to antibodies binding to the carrier molecule or the backbone structure of drugs, that is the aromatic rings. In general during antibody production a careful screening is mandatory to eliminate unspecific binder.

Because of the elaborate and uncertain *in vivo* antibody production, there is great interest in alternatives including the

**FIGURE 6**

The functional groups of cocaine and benzoylecgonine are shown. These chemical structures illustrate the small variations a specific antibody has to detect.

production of recombinant antibodies [11,12] or aptamers [13]. Recombinant antibodies are isolated from phage or yeast display libraries and aptamers are designed by an *in vitro* selection. Both do not need animal testing. Furthermore recombinantly produced binders can be genetically modified to optimize their performance.

Influence of sample medium

One important fact to keep in mind is the influence of the sample medium used in an immunoassay. A detection which works in a buffer system may fail when using a biological sample, for example, serum, as that shown in Fig. 3. Du *et al.* [14] described this issue when comparing measurements in buffer and in urine. It is mandatory, therefore, to perform the experiments within the medium, including relevant controls [15]. This can be caused by unspecific binding of the antibody to, for example, serum compounds. A major reason could be that other IgGs are present in the sample. During the incubation of the drug-specific antibody with a human sample, binding to other antibodies can occur which inhibits the binding to the drug. Furthermore the quantity of serum proteins, such as albumin, globulins or other carrier proteins, could hinder binding of the antibody to its target. Some publications [4,16] stated that a dilution or precipitation of the sample medium could overcome this problem. Within this study the reverse effect could be observed with serum. We assume that dilution of serum could increase accessibility of antibodies present in the sample and thereby increase cross-reactivity.

Choice of assay conditions

As already mentioned, assay conditions including the blocking reagent have to be checked carefully for every experimental set-up. Fig. 4 depicts how the background signals vary if different blocking solutions are used. Unfortunately this as well as other assay conditions have to be tested individually for each platform and analyte [17,18].

Influence of platform choice

It is very challenging to compare the same approach on different platforms [4]. As shown in Fig. 5, different results could be obtained. One reason could be the way compounds are immobilized on a

surface, for example, whether the molecules have a particular orientation which determines how the antigens are presented to the antibodies. A control to determine whether the antigens are still in their native state must be performed when, for example, the samples are spotted with a micro-dispensing device and stored for several days or weeks. Due to this and the indistinct bands in Western blot ELISA replaced the initial validation of the primary antibodies. Nevertheless Western blot is still a valuable tool for checking the quality of proteins and antibodies. Furthermore different platforms may have various surface chemistries. Thus the amount of immobilized antigens, blocking and washing conditions should be tested for each approach [19].

Conclusion

In general many parameters have to be tested during the course of an antibody validation procedure. The performance of an immunoassay depends on the specificity of the antibody to its antigen, the selected platform, sample medium and the assay conditions. Each individual point can be critical for the outcome of an immunoassay. Furthermore before a validation study is started, it should take into consideration whether a specific molecule or a substance class are to be detected. This is of particular importance when performing a multiparameter analysis. As shown in Fig. 2, the antibodies could be used for the detection of the groups of amphetamines or cocaine and its metabolites, but they are not useable for specific detection of the single molecule which is mandatory for a multiparameter approach. Consequently, the efforts which have to be put into validation of an antibody therefore depend on the final application. Nevertheless antibodies are powerful tools in basic research and diagnostics, which is why it is worth the effort to validate an antibody accurately. The data shows that it is quite feasible to establish an immunoassay on a single platform such ELISA. Finally, guidelines need to be established which provide essential characteristics of an antibody and whether a validation has taken place [2]. Comparable guidelines already exist for microarray (MIAME – Minimal Information about a Microarray Experiment) and proteomics (MIAPE – Minimal Information about a Proteomics Experiment) experiments [20,21]. Freely accessible databases and universal standards, and with that harmonized data, would improve the comparability of immunoassays. There are initiatives present like the previously mentioned Human Proteome Atlas but it has to go one step further. Where quality control of the antibodies has been performed, the data should be accessible, for example, in the supplementary data of a publication. This is already put into practice by the platform F1000Research (<http://f1000research.com>). All these points would improve the reliability of immunoassays and more sensitive applications could enter the routine work in basic research and diagnostics.

References

- [1] Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495–7.
- [2] Reiss PD, Min D, Leung MY. Working towards a consensus for antibody validation. *F1000Research* 2014;3:266.

- [3] Prassas I, Diamandis EP. Translational researchers beware! Unreliable commercial immunoassays (ELISAs) can jeopardize your research. *Clin Chem Lab Med* 2015;52:765–6.
- [4] Voskuil J. Commercial antibodies and their validation. *F1000Research* 2014;3:232.
- [5] Wellhausen R, Seitz H. Facing current quantification challenges in protein microarrays. *J Biomed Biotechnol* 2012;83:1347.
- [6] Egelhofer TA, Minoda A, Klugman S, Lee K, Kolasinska-Zwierz P, Alekseyenko AA, et al. An assessment of histone-modification antibody quality. *Nat Struct Mol Biol* 2011;18:91–3.
- [7] Jatlow P. Cocaine: analysis, pharmacokinetics, and metabolic disposition. *Yale J Biol Med* 1988;61:105–13.
- [8] Castaneto M, Barnes A, Concheiro M, Klette K, Martin T, Huestis M. Biochip array technology immunoassay performance and quantitative confirmation of designer piperazines for urine workplace drug testing. *Anal Bioanal Chem* 2015;407:4639–48.
- [9] Shen T, Hof LM, Hausmann H, Stadler M, Zorn H. Development of an enzyme linked immunosorbent assay for detection of cyathane diterpenoids. *BMC Biotechnol* 2014;14:98.
- [10] Swortwood MJ, Hearn WL, DeCaprio AP. Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Test Anal* 2014;6:716–27.
- [11] Kavanagh O, Elliott C, Campbell K. Progress in the development of immunoanalytical methods incorporating recombinant antibodies to small molecular weight biotoxins. *Anal Bioanal Chem* 2015;407:2749–70.
- [12] Bradbury AM, Pluckthun A. Antibodies: validate recombinants once. *Nature* 2015;520:295.
- [13] Bunka DHJ, Stockley PG. Aptamers come of age – at last. *Nat Rev Microbiol* 2006;4:588–96.
- [14] Du H, Wu M, Yang W, Yuan G, Sun Y, Lu Y, et al. Development of miniaturized competitive immunoassays on a protein chip as a screening tool for drugs. *Clin Chem* 2005;51:368–75.
- [15] Alvarez JC, Duverneuil C, Zouaoui K, Abe E, Charlier P, de la Grandmaison GL, et al. Evaluation of the first immunoassay for the semi-quantitative measurement of meprobamate in human whole blood or plasma using biochip array technology. *Clin Chim Acta* 2012;413:273–7.
- [16] Tort N, Salvador JP, Marco MP. Multiplexed immunoassay to detect anabolic androgenic steroids in human serum. *Anal Bioanal Chem* 2012;403:1361–71.
- [17] Cretich M, Damin F, Chiari M. Protein microarray technology: how far off is routine diagnostics? *Analyst* 2014;139:528–42.
- [18] Lee JR, Magee DM, Gaster RS, LaBaer J, Wang SX. Emerging protein array technologies for proteomics. *Expert Rev Proteomics* 2013;10:65–75.
- [19] Köhler K, Seitz H. Validation processes of protein biomarkers in serum—a cross platform comparison. *Sensors* 2012;12:12710–28.
- [20] Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat Genet* 2001;29:365–71.
- [21] Martínez-Bartolomé S, Binz P, Albar J. The minimal information about a proteomics experiment (MIAPE) from the proteomics standards initiative. *Methods Mol Biol* 2014;1072:765–80.