Improving Immunoassay Performance by Antibody Engineering

Most hormones and proteins as well as many drugs are currently determined by immunoassay. Clinicians generally trust that the results they get are correct, but this is not always the case. Several recent studies have described examples of nonspecific interferences producing misleading results. Many of the interfering factors have been known for decades and thoroughly discussed in this and other journals (1–4); it therefore may seem surprising that gross errors caused by these are not recognized. Overreliance on automation by laboratorians, who may never have learned the basics of immunoassay technology, may have contributed to a situation in which assay problems causing serious clinical misinterpretations remain unrecognized (5). It therefore is highly desirable that false immunoassay results be minimized by improvements in assay design. The approach described by Warren et al. (6) in this issue is an interesting and potentially useful way to achieve this goal.

The specificity of the antigen–antibody reaction is astounding; it is possible to measure picomolar concentrations of proteins, peptides, and haptens in the presence of other journals. These methods are based on the development of sensitive nonisotopic detection methods such as time-resolved fluorometry and luminometry in combination with two-site immunoassay methods. Such assays are now available on a wide array of automated analyzers. To increase throughput, many of these analyzers use assay times in the range 7–30 min, and even shorter incubation times are used in some point-of-care analyzers (8). In these systems, the immunochemical reaction does not reach equilibrium, but imprecision is usually not a serious problem thanks to exact control of temperature and incubation time in combination with efficient mixing. However, with decreasing detection limits, shorter incubation times, and one-step rather than two-step incubation, the impact of nonspecific interference tends to increase (3, 9).

Nonspecific interference is produced by several mechanisms, which may decrease results in one assay but increase results in another. The most common interference in sandwich assays is produced by heterophilic antibodies, i.e., naturally occurring human antibodies to immunoglobulins of animal origin (1–4). Because of the high degree of homology between immunoglobulins from various species, a heterophilic antibody can react with antibodies from, e.g., mice and rabbits. In sandwich assays, these antibodies mimic the specific antigen by forming a bridge between the capture and detection antibodies, causing a false-positive result. Immunoassay manufacturers reduce this problem by including in the assay buffer native or aggregated animal immunoglobulins. These reduce, but do not totally eliminate, the effects of all heterophilic antibodies (4, 10).

Interference caused by heterophilic antibodies can, in theory, be eliminated by removing the constant domains of either one or both of the antibodies used in the assay. In the study described in this issue, Warren et al. (6) used genetic engineering to produce a single-chain antibody fragment (scFv); when this fragment was used in an assay, the assay was much less affected by nonspecific interference than was an assay of comparable design that used the parent antibody. The authors also engineered a peptide that was biotinylated by the Escherichia coli host used to express the antibody fragment. The scFv was captured on a streptavidin-coated solid phase, facilitating optimal orientation of the binder. Theoretically, this should increase the binding capacity of the solid phase in comparison with physical adsorption of the intact antibody. Such an effect was not reported, but the main goal was to reduce interference. This was achieved, and the authors will use the new scFv-based carcinoembryonic antigen assay for clinical purposes. Interestingly, the scFv was very stable, which has not always been the case with engineered antibody fragments.

Can we expect that engineered antibodies and artificial binding reagents will be used in commercial assays? This is possibly already the case: assay manufacturers seldom disclose the characteristics of their reagents. The various steps used to produce scFv are now well known, and other, even more sophisticated tricks for improving the properties of more or less artificial binders have been described (11–13). Thus, antibody affinity can be improved by in vitro maturation, i.e., mutation of the complementarity-determining regions of scFv or Fab fragments. A substantial increase in affinity has been achieved by this approach (11), but the reported affinities have not been higher than those of the best native monoclonal antibodies. Another approach is to prepare monoclonal antibodies in animals other than mice and rats, which are closely related and produce similar antibody responses. Such antibodies can also be produced in other animals, e.g., rabbits (12) and dromedaries (13).

Dromedaries and llamas produce antibodies containing a single heavy chain, which in spite of this may have very high affinity. They have also been shown to recognize pits and groves in proteins, e.g., the catalytic sites of enzymes displaying specificities not obtainable with antibodies from commonly used laboratory animals (13). An interesting aspect of these single-chain antibodies is that phage libraries expressing the binding region can be produced by cloning antibody-coding mRNA isolated from peripheral blood lymphocytes and lymph node biopsies. Thus, monoclonal antibody fragments can be expressed in E. coli, eliminating the need to produce hybridomas. It will be interesting to see whether these antibody fragments are less prone to interference than mouse antibodies and fragments derived from these.

Although antibodies, whether natural or engineered, probably will remain the binders of choice in clinical assays, they are not the only options. Completely artificial
binders consisting of peptides or oligonucleotides can now be produced. Phage-displayed peptide libraries have been widely used to produce agents for cancer therapy (14), but they can also be used as binders in sandwich assays. A highly sensitive immunopeptidometric assay for enzymatically active prostate-specific antigen (PSA) was recently established by use of a capture antibody to PSA and a fusion protein of a PSA-specific peptide with glutathione S-transferase for detection. The peptide most likely recognizes the catalytic site in intact PSA as it does not recognize PSA inactivated by partial cleavage of certain peptide bonds (15). Thus, its specificity is similar to that displayed by single-chain dromedary antibodies. DNA- and RNA-based aptamers are another type of binder with potential clinical application (16). These can also be selected from random libraries and can have very high affinity. Theoretically, heterophilic antibodies should not affect peptide- and aptamer-based binders; thus, they might provide a solution to this problem. However, it would not be surprising if they were affected by other factors occurring in serum.

The study of Warren et al. (6) is an interesting example of how genetic engineering can be used to improve the performance of an immunoassay, and we can expect that this approach will be increasingly used in the future. The repertoire of methods provided by genetic engineering provides virtually unlimited possibilities to modify existing binders and to produce totally new ones. To date, these techniques have been used mainly to produce antibodies or antibody fragments for therapeutic purposes, but as these methods become more widely available, they may also give us better reagents for clinical diagnostics. It is a challenge for clinical chemists to adopt these methods and contribute to the development of more useful and reliable assays.

References

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