Homogeneous assays: a paradigm shift in immunodiagnostics

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Immunoassay methods are either heterogeneous or homogeneous and rely on the interaction between an antibody and antigen. Historically, diagnostic immunoassays assays have relied on heterogeneous assay formats for analyte detection.

In such formats, at least one component of the assay such as an antigen or an antibody is immobilised on a solid support. For instance, the solid support may be a tube, glass bead or a microtitre well. The analytical components are added in several stages, with numerous washing and incubations steps. Often, these assays incorporate enzymatic tags such as horseradish peroxidase or alkaline phosphatase that allow the detection of analytes at low concentrations due to signal amplification conferred by the enzyme.

Heterogeneous assay formats are inherently slow due to the limiting rates of diffusion between solution and solid phases. The most commonly used heterogeneous assay format is the enzyme-linked immunosorbent assay (ELISA), which can take up to 3 hours to perform. This format has been used both in competitive and non-competitive assay design for the detection of small molecules as well as large analytes such as antibodies and antigens, either with calorimetric, chemiluminescence or other forms of detection. In the clinical laboratory, ELISA tests remain batch procedures. The tests are performed on a schedule that meet the lab needs to process a large number of samples efficiently rather than the doctor's or the patient's need for a rapid result.

In contrast to heterogeneous assays, homogeneous assays require no separation steps such as filtration or washing. Add analyte, incubate and measure are the only steps required whereby the reaction occurs completely in solution and is independent of a solid support. In any homogeneous assay, all components of the assay are present during measurements and the results can be obtained within minutes. Homogeneous assays can also be easily automated on a dedicated instrument or on one that is used for other chemistries. With this technique, speciality immunoassays and routine chemistries can be run on the same instrument.

Homogeneous assays are generally limited to measuring small molecules, such as those of drugs, and have been very successful commercially. These include the CEDIA™ (cloned enzyme donor immunoassay) assays by Boehringer Mannheim [1] and, more recently, the Hithunter™ line of assays by DiscoverRX Corporation (now a part of GE Healthcare). Over 25 assays have been developed for therapeutic drug monitoring and drugs of abuse, with levels of sensitivity reaching 33.7 pg/mL for a Vitamin B12 assay. This assay format is based on the inhibition of the natural complementation between a ß-galactosidase enzyme and a polypeptide enzyme fragment. The presence of free analyte in a sample competitively binds to the antibody attached to the polypeptide enzyme fragment, thereby removing the inhibition caused by the antibody and allowing the natural complementation process to occur. The higher the concentration of free analyte, the more enzyme activity occurs. There are numerous other examples of homogeneous assays for small molecule detection such as the Roche homogeneous LDL-Cholesterol assay, but these are not based on the immunoassay principle [2].

Whilst the detection of small organic molecules is now well established using the homogeneous assay format, there are a few examples where homogeneous assays have been successfully applied for the detection of large analytes. Bayer has developed and markets the latex turbidimetric assay technology. This technology platform is an adaptation from the particle agglutination test. Latex beads are coated with test antibodies. In the presence of an analyte, cross-linking occurs, mediated by analyte specific antigen present in the reaction mixture. Over a period of time, this leads to an increase in the turbidity of the reaction mixture, which is then measured. This technology is bead based, requires proprietary instrumentation and has limited levels of sensitivity.

More recently, Dade Behring has been developing the LOCI [3] (Luminescent Oxygen Channelling Immunoassay) technology system. This technology is also bead based, requiring proprietary

instrumentation. It is based on two latex bead reagents and a biotinylated analyte receptor. One of the bead reagents is coated with streptavidin and contains a sensitive dye. The second bead reagent is coated with an antibody (sandwich format) or an analog (competitive format) and contains a chemiluminescent dye. During an assay, the three reactants combine to form a bead-aggregated immunocomplex and a chemiluminescent reaction is triggered.

Panbio Ltd, based in Brisbane, is pioneering the development of a novel homogeneous assay platform technology suitable for the detection of large molecular analytes such as antigens and antibodies utilising enzymatic signal amplification with calorimetric detection. This is based on recent advances in the engineering and construction of *in vivo* reporter technologies known as Forced Enzyme Complementation [4] (FEC).

The principle of FEC is based on two artificial enzyme fragments with low affinity for each other attached to protein interactors such as an antibody or an antigen that are analyte specific (Figure 1). In the presence of an analyte, the two enzyme fragments are brought together through the specific protein interaction, thereby restoring enzymatic activity and producing a colour change that is visually detectable. Fluorescence and chemiluminescence detection is also possible. This homogeneous assay format allows assays to be conducted either in the field for point of care applications or in the clinical laboratory. This technology could utilise microtitre plates in combination with existing instrumentation, as used for ELISA detection, without the need for expensive and dedicated instrumentation as required for other proprietary technologies and other forms of detection such as fluorescence and chemiluminescence.

Homogeneous immunoassays that are fast, sensitive and robust and that amenable for processing on simple non-proprietary instrumentation is a trend to watch for in the coming years. This will facilitate the consolidation of clinical assays on a single instrument, thereby causing a paradigm shift in immunodiagnostics.

References

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Figure 1. The IgM antibody from a patient's sample cross-links the antibody specific reagent (anti-human IgM) with the disease specific antigen, bringing the two enzyme fragments together and resulting in enzymatic activity.