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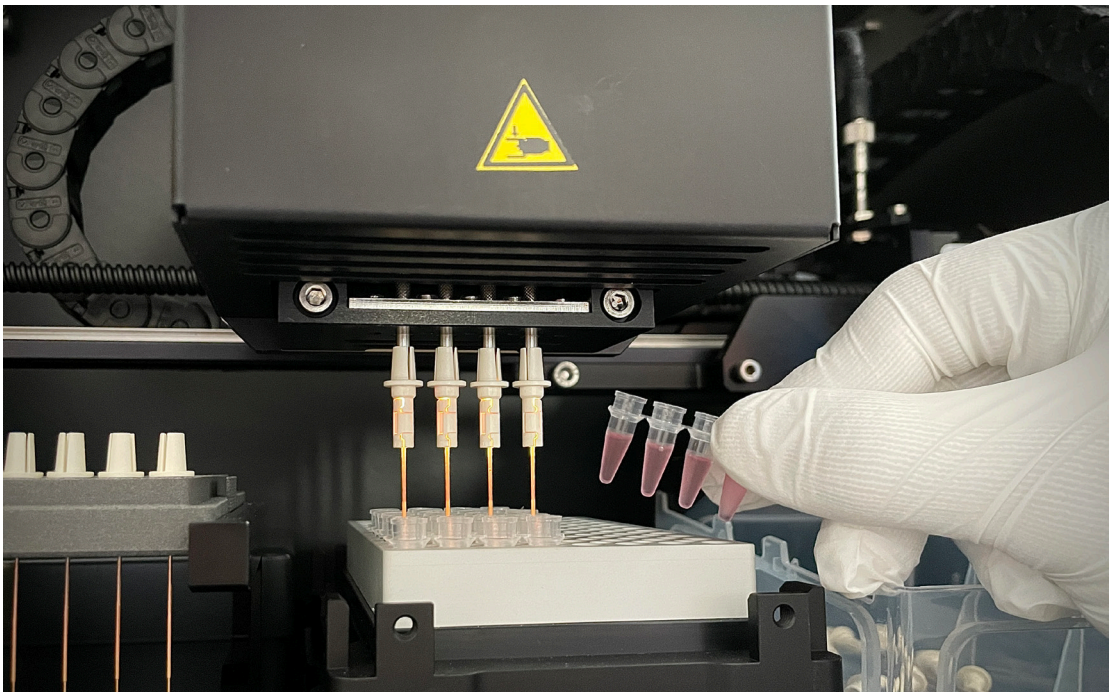
Translated contribution from IPA Journal 03/2022



New technology for the development of molecular markers

Protein assays can be carried out even faster in the future

Georg Johnen, Thomas Brüning

The detection of specific proteins is the focus of many methods for the diagnosis of occupational diseases. The widely used ELISA is a reliable and precise method for protein detection, but it can be very time-consuming. A novel method is now being evaluated at the IPA that can significantly speed up the measurement of proteins and thus also the development of new ELISAs.

Molecular markers

In almost all diseases, changes occur that are not only visible or noticeable externally, but also show effects on the microscopic and sub-microscopic, i.e., molecular, level. In most cases, molecular changes even occur much earlier, before a disease becomes clinically manifest. These molecular changes are usually also found in various bodily fluids such as blood, urine or saliva, so that they can often be detected there as biomarkers (molecular markers). Molecular markers can therefore be used for a wide range of medical issues. These markers are used, for example, in diagnosis, early detection, prognosis, determining the best individual treatment or monitoring the course of therapy of a disease. The challenge is to first find the suitable biomarkers for a disease from a large number of candidates and then to develop a sufficiently sensitive method to reliably determine them.

Proteins in diagnostics

Besides metabolic products such as blood sugar, cholesterol, uric acid, etc., proteins are considered the classic biomarkers. From the detection of a SARS-CoV-2 infection to allergens and the early detection of cancer, detection methods, so-called assays, are used that are based on the specific determination of proteins. As a rule, so-called immunoassays are used for this purpose, in which antibodies are used that precisely recognise and bind to their target protein (antigen). In the case of the ELISA method, a detected protein is made visible by a colour reaction catalysed by an enzyme bound to a detection antibody (Fig. 1).

In brief

Proteins serve as important molecular markers for the diagnosis of diseases such as COVID-19 or cancer.

Precise detection methods for proteins are comparatively time-consuming, and a new development requires a very resource-intensive optimisation.

New methods such as the so-called FO-SPR speed up both the development work and the actual measurements.

Bottleneck assay development

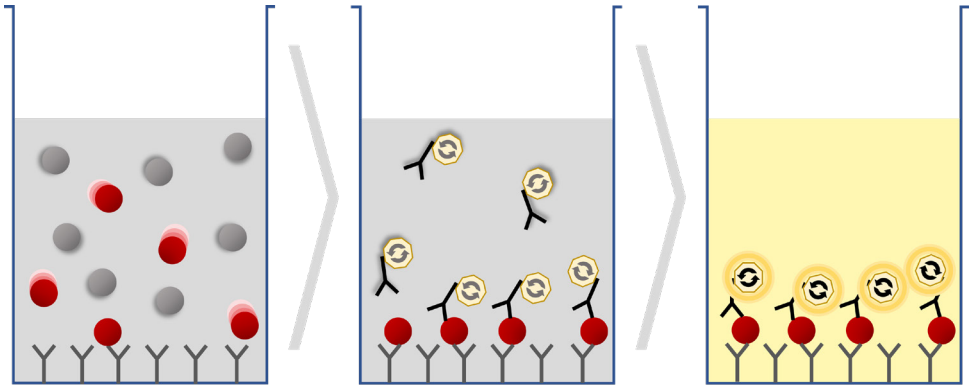
ELISAs and similar immunoassays usually allow an exact determination of the amount of protein in a sample, but they are rather slow to perform - depending on the degree of optimisation, they take one to two days. The development of new immunoassays is also correspondingly time-consuming. This often requires many months.

In the search for new disease markers, there are often dozens of new candidate proteins for which assay development would potentially be worthwhile. Sometimes, however, it is only late in the development phase that it becomes clear whether a marker or its assay is actually suitable for detecting a disease in patient samples. This bottleneck of many candidates to be tested and slow processing must be overcome, either by faster processes or by parallel processing.

New method saves time

Surface Plasmon Resonance (SPR) is an effect that has been known for a long time, but has hardly been used in routine diagnostics. The principle is based on the reflection of light in a prism covered with a thin metal layer. If molecules bind to this metal layer, a change in the wavelength of the light can be measured (Fig. 2). A colour reaction as in an ELISA is not necessary.

In the 1980s, its use in immunoassays was described for the first time (Liedberg et al. 1983). However, the SPR devices used in the past were usually large, expensive and, above all, required a lot of sample material. In recent years, the working group of Prof. J. Lammertyn at the Catholic University of Leuven has further developed SPR into the so-called FO-SPR (FO = *fibre optic*). With the help of FO-SPR, a measurement can now take place on a thin glass fibre that is simply immersed in a sample (Lu et al. 2017).

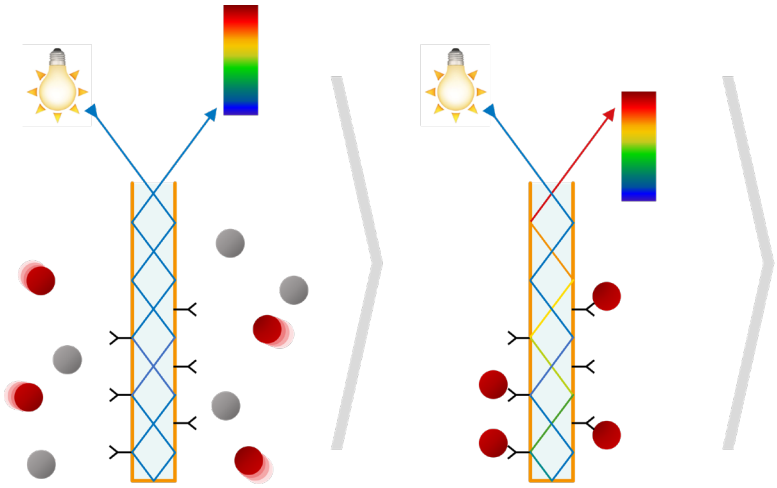


Target molecules (red) are specifically captured by antibodies anchored in a reaction vessel.

Trapped molecules (e.g., cancer markers) are bound by a second antibody, which is linked to an enzyme.

Addition of a colourless reagent which is converted into a dye by the enzyme according to the amount of cancer marker.

Fig. 1
Principle of the ELISA. Marker detection by an enzymatic color reaction.



On the gold-coated glass fibre are antibodies that specifically bind a target molecule.

The bound molecules (e.g. cancermarkers) shift the wavelength of the irradiated light.

A spectrometer transforms the displacement of the wavelength into a curve that reflects the binding and quantity of the marker in real time.

Fig. 2
Principle of SPR. In SPR, the wavelength of the irradiated light shifts when molecules are attracted to the metal surface of a coated light conductor (glass fibre or prism).

Parallel to this, various work steps are automated. In summary, the new method offers the following advantages:

- Speed through measurement in real time: First results already in minutes, maximum duration of a sample run one to two hours
- Minimal sample consumption: savings on valuable samples
- Multiplexing: several target proteins can be determined in parallel in the same sample
- Compact, robust device: can basically also be used for point-of-care tests
- Cost-efficient: few consumables, less hands-on time

Numerous application possibilities

Since ELISAs are proven, established methods that are feasible in all clinical laboratories, FO-SPR can initially be used as a complement rather than an alternative to ELISA. With it, the development of new immunoassays can be significantly accelerated and the bottleneck in the search for new biomarkers, e.g., for post-COVID or occupationally-related tumours such as mesothelioma, lung cancer and bladder cancer, would be eliminated. However, the new methodology also goes beyond the possibilities of a classic immunoassay (see web links to the method).

SPR is particularly suitable for determining how strongly two molecules bind to each other (measurement of so-called binding kinetics). This allows, for example, statements to be made about the effectiveness of vaccines against omicron variants of SARS-CoV-2. An automated FO-SPR device can also be used to “fish” disease-specific cells and so-called vesicles from patient samples in order to then examine them with further diagnostic procedures, such as DNA or RNA analyses.

Conclusion

Innovative methods not only improve existing ones, but can also open up new horizons for more complex and flexible diagnostics. Safety and health at work is subject to constant change. In the future, we can expect to see an increasing number of new diseases, for example as a result of climate change, for which such technologies can provide analytical solutions.

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Literature

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Lu J, Spasic D, Delport F, Van Stappen T, Detrez I, Daems D, Vermeire S, Gils A, Lammertyn J. Immunoassay for Detection of Infliximab in Whole Blood Using a Fiber-Optic Surface Plasmon Resonance Biosensor. Anal Chem 2017; 89:3664-3671

Web links to the method:

www.biw.kuleuven.be/biosyst/mebios/biosensors-group

foxbiosystems.com/fo-spr-applications-fox biosystems