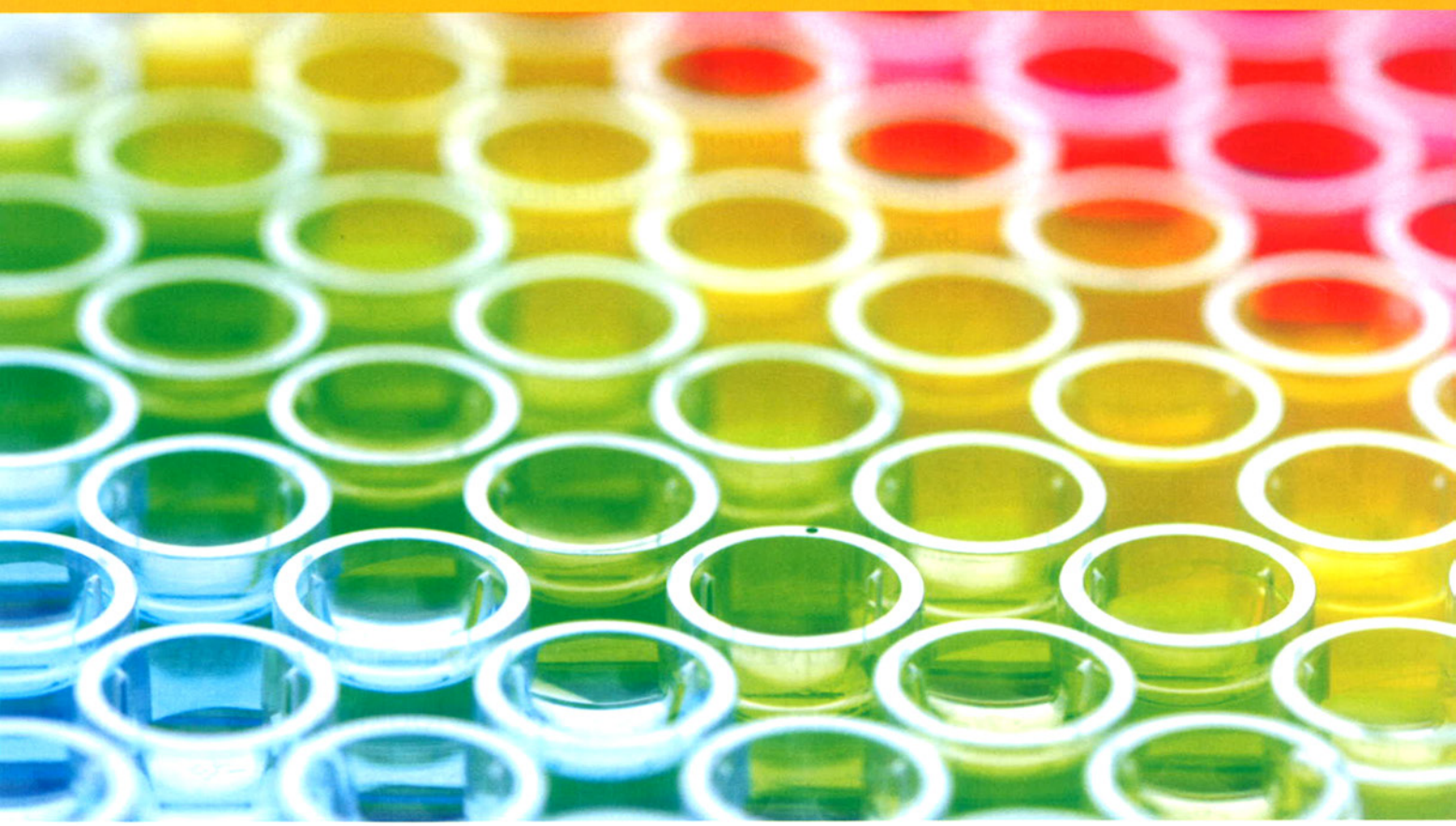
CELL-BASED IMMUNOASSAYS
THE FAST ROUTE FROM RESEARCH PARAMETER TO DIAGNOSTIC TEST

By Dr. Jacqueline Gosink,
EUROIMMUN AG, Germany



▷INTRODUCTION

An innovative technology based on the classic indirect immunofluorescence test (IIFT) is driving the rapid introduction of newly identified autoantibody parameters into routine laboratory diagnostics. In the new assay method, transfected cells are used for the first time as antigen substrates in IIFT to provide monospecific detection of antibodies. Cell-based IIFT systems represent an effective alternative to ELISA and immunoblot, especially for applications where these methods cannot, for various reasons, be deployed. The novel technology has already enriched various diagnostic areas, in particular autoimmune disease diagnostics.

▷A NEW TECHNOLOGY

In cell-based immunoassays, the DNA coding for a particular antigen is inserted into a plasmid, which is then introduced into cultured cells by transfection *(figure 1)*. The transfected cells expressing the target antigen are employed directly as a substrate for antibody detection by classic indirect immunofluorescence.

The IIFT procedure consists of two simple incubation steps *(figure 1)*. During the first incubation, specific antibodies from patient samples bind to the corresponding antigens in the substrate. In the second step, the bound antibodies are detected using a fluorescein-labelled secondary antibody. Results are visualised by fluorescence microscopy. Compared to tissue substrates traditionally used in IIFT, which contain a myriad of different antigens and sometimes require specialist knowledge to interpret, transfected cells are easy to evaluate.

▷SIGNIFICANT ADVANTAGES

In contrast to ELISA and immunoblot, which are based on purified antigens coupled to a solid phase, cell-based assays employ antigens expressed in vivo. This means that the technology is suitable in particular for antigens that are difficult to isolate or purify. For example, if an antigen is embedded in a membrane, the aggressive methods required to isolate it can also

destroy it. In other instances an antigen may contain unknown or conformational epitopes, which can only be preserved by purifying the antigen in its native state using complicated and time-consuming procedures. The use of in vivo expressed antigens circumvents these hurdles and enables the diagnostic application of assay parameters that might otherwise languish in research laboratories.

A further advantage of cell-based assays is that they are relatively fast and easy to develop. So when a new target antigen is identified by research groups, a corresponding cell-based IIFT system can be developed and brought to market much faster than immunoassays based on purified antigens. Thus, serological diagnostics and ultimately patients benefit much sooner from advances in antigen characterisation.

> "The use of in vivo expressed antigens circumvents these hurdles and enables the diagnostic application of assay parameters that might otherwise languish in research laboratories"

In some cases antibody binding can be boosted by modifying the target antigen. Where beneficial, the antigens used in cell-based assays are specially designed and/or adapted by molecular biological techniques to enhance their performance. For example, by selecting only relevant antibody-binding epitopes and deleting protein regions that cause unspecific reactions, the sensitivity and specificity of a test can, in some cases, be significantly increased.

▷COMPREHENSIVE, STANDARDISED ANALYSIS

Often it is advantageous to produce a detailed patient antibody profile to aid disease diagnosis and exclude other causes. To facilitate this, new cell-based substrates can be analysed side by side with classic tissue sections using proven BIOCHIP Mosaic technology. BIOCHIP Mosaics consist of miniature sections of different substrates positioned next to each other in the test fields of a microscope slide *(figure 2)*. The substrates are incubated in parallel, which reduces workload and enhances reproducibility. Hence, an extensive antibody analysis can be obtained quickly and reliably.

Further standardisation is achieved by choosing a microscope that is tailored to the requirements of immunofluorescence. State-of-the-art IIFT microscopes incorporate a controlled LED, which ensures highly reproducible results while providing cost-effectiveness through a long life span and low power consumption. The efficiency and convenience of IIFT can be further boosted by automation. Virtually the entire IIFT procedure, from sample dilution and incubation to evaluation and archiving of results, can be automated using specialised devices and software.

▷AUTOIMMUNE DIAGNOSTICS

The detection of characteristic autoantibodies is a central element in the diagnosis of many autoimmune diseases. Autoantibody analysis is also frequently employed to monitor the activity of an autoimmune disease or a patient's response to treatment. Below are some examples of how cell-based IIFT systems are now being used to support the diagnosis of autoimmune diseases of the skin, nervous system, gastrointestinal tract and kidneys.

▷AUTOIMMUNE BULLOUS DERMATOSES

Autoimmune bullous dermatoses are a group of severe blistering diseases, which are characterised by circulating autoantibodies directed against various structural proteins of the skin. Autoantibodies against the desmosomal proteins desmoglein 1 and desmoglein 3 occur in patients with pemphigus diseases, notably pemphigus foliaceus and pemphigus vulgaris. Two new IIFT systems based on transfected cells expressing tailored recombinant antigens allow these autoantibodies to be determined with ease *(figure 3)*. In clinical studies the assays yielded sensitivities of 100% for pemphigus vulgaris and 90% for pemphigus foliaceus at specificities of over 99%. Moreover, this analysis allowed a prima vista differentiation between these two disease forms.

FIG 1
PRINCIPLE OF IIFT USING CELLS TRANSFECTED WITH PLASMIDS EXPRESSING NOVEL ANTIGENS

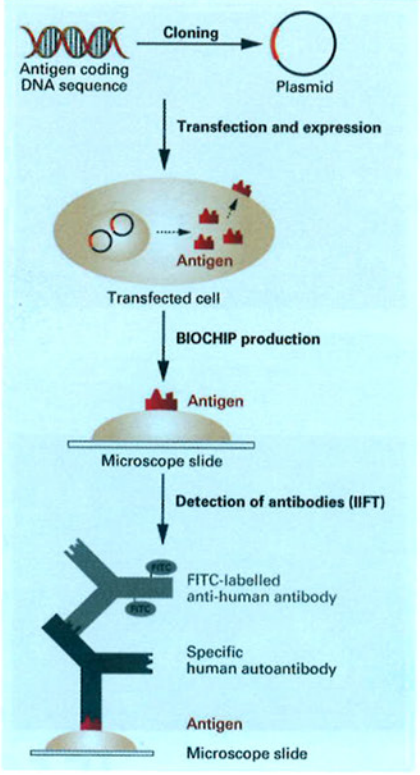
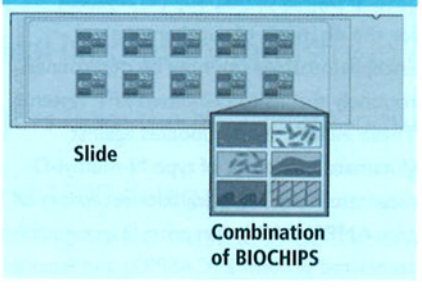


FIG 2
BIOCHIP MOSAIC



Autoantibodies against the glycoprotein BP230 occur in bullous pemphigoid and pemphigoid gestationis. Their determination enriches the diagnosis of these diseases by an additional, independent parameter, supplementing existing markers such as anti-BP180. A new cell-based immunoassay for determination of these autoantibodies yielded a sensitivity of 44-55% and a specificity of 100% for bullous pemphigoid in clinical studies.

BIOCHIP Mosaics combining the new cell-based substrates with classic tissue sections provide the most wide ranging analysis of autoantibodies occurring in autoimmune epidermal blistering diseases in a single test, and thus represent a powerful diagnostic tool for dermatologists. →

FIG 3
AUTOANTIBODIES AGAINST DESMOGLEIN 1 DETECTED BY CELL-BASED IIFT

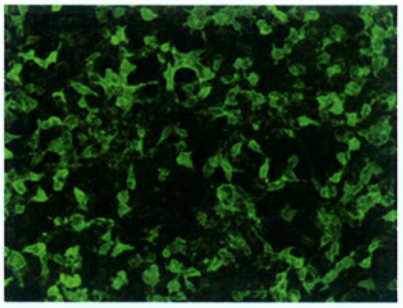
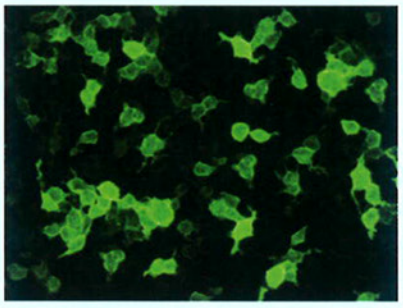


FIG 4
AUTOANTIBODIES AGAINST NMDA RECEPTORS DETECTED BY CELL-BASED IIFT



AUTOIMMUNE ENCEPHALOPATHIES

Several new autoantibody parameters for the diagnosis of autoimmune encephalopathies can now be determined routinely thanks to cell-based IIFT systems. These include autoantibodies against glutamate receptors of type N-methyl-D-aspartate (NMDA), glutamate receptors of type AMPA, GABA receptors B1, contactin-associated protein 2 (CASPR2) and leucine-rich glioma-inactivated protein 1 (LGI1).

The anti-NMDAR test system, in particular, has proven extremely valuable in diagnosing cases of autoimmune anti-NMDA receptor encephalitis *(figure 4)*. This considerably under-diagnosed disease manifests with severe psychotic symptoms. It frequently affects women with ovarian teratoma, but is also now increasingly identified in other patient groups. Early diagnosis is crucial since patients often improve with immunotherapy and tumour removal. In a clinical study the test demonstrated a very high efficiency for detection of anti-NMDAR autoantibodies, thus confirming its diagnostic value for neurologists.

CROHN'S DISEASE

Crohn's disease is a chronic inflammatory bowel disease, which has increased in incidence over the last 20 years. Autoantibodies against exocrine pancreas have long been known to be specific markers for Crohn's disease. But it is only recently that two autoantigenic targets have been identified, namely CUZD1 and GP2. This has paved the way for the development of cell-based IIFT systems to detect the corresponding autoantibodies.

In a comparative study the cell-based anti-CUZD1 and anti-GP2 assays proved to be more efficient for detecting anti-exocrine pancreas antibodies than traditionally used sections of human pancreas. More sera were identified with the cell-based substrates than with the tissue, and discrimination between positive and negative results was generally easier. In total, anti-CUZD1 and anti-GP2 autoantibodies were detected in 34 of 96 patients (35.4%) with Crohn's disease and in three of 39 patients with ulcerative colitis (7.7%), demonstrating the usefulness of these tests in the diagnosis of chronic inflammatory bowel diseases.

IDIOPATHIC MEMBRANOUS NEPHROPATHY

Autoantibodies against phospholipase A2 receptors (PLA2R) have recently been identified as triggers of the autoimmune reaction involved in idiopathic membranous nephropathy (IMN), a chronic inflammatory disease of the glomeruli which is accompanied by an increasing reduction in kidney function. They are highly specific for IMN and occur in the serum of 70% of patients. The method of choice (gold standard) for their detection is a new IIFT based on cells transfected with constructs expressing PLA2R, which provide highly sensitive and specific antibody detection.

Serological screening for anti-PLA2R autoantibodies supplements invasive diagnostic methods such as kidney puncture, histological examination or electron microscopy of kidney tissue and is also suitable for assessing the response to therapy.

CONCLUSION

Indirect immunofluorescence assays based on transfected cells represent a new technology for antibody diagnostics. A host of cell-based immunoassays based on freshly identified target antigens has already enriched the serological diagnosis of numerous autoimmune diseases. The assay technology is also employed in infectious disease diagnostics, for example, in the detection of antibodies against Crimean-Congo fever virus. Significantly, the new technology can be applied to virtually any antigen. Since new target antigens are being identified and characterised at a prodigious rate, cell-based immunoassays are certain to play an important role in serological diagnostics of the future.

REFERENCES

References available on request (magazine@informa.com)