Utility of Live Cell-Based Assays for Autoimmune Neurology Diagnostics

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Antibodies that recognize epitopes on the extracellular surface of integral membrane or membrane-associated proteins in the CNS (central nervous system) are associated with severe, treatable, autoantibody-mediated neurologic diseases. Twenty such antibodies have been discovered since 2004 (1). These human antibodies recognize conformationally native epitopes which has promoted the development of cell-based assays (CBAs) where mammalian cells are induced to express the native protein target on their cell surface. Patient serum, plasma, or CSF (cerebrospinal fluid) are then incubated with these cells and target-specific antibodies are identified by fluorescent secondary antibodies. Antibody levels are determined by end point titer microscopically or quantitatively by flow cytometry. A control antigen tested in parallel helps demonstrate antibody specificity (Fig. 1, A)

Before making contact with a patient sample, cells are either living or chemically modified. Live cells present the conformationally native antigen. However, maintenance of living cells requires a specialized laboratory and the test is labor-intensive. Fixation or dehydration of cells expressing the antigen means they can be stored for months and transported to laboratories worldwide. The downsides are the very real potential of modification of the native structure, loss of key epitopes and the creation of biologically irrelevant neo-epitopes, as well as the unmasking of cytosolic and nuclear targets that are not relevant for the detection of pathogenic antibodies. These diseases are severe, but often treatable, particularly if diagnosed early. Accurate antibody tests are useful diagnostic tools to support a clinical diagnosis, hence, to optimize patient outcomes, a major question is whether these chemical modifications impair the clinical utility of fixed tests?

A few studies have directly compared live and fixed cell-based assays on same patient samples which is necessary to differentiate test metric differences from cohort differences. In 2016, a large European study compared 66 aquaporin-4 (AQP4) antibody seropositive samples from10 centers (2). Live tests, performed in 3 European countries, were on average 11.5% more sensitive than the fixed tests performed in 5 different countries [mean sensitivity (range): 99.5% (98.5% to 100%) vs 88.9% (93.9% to 80.3%)]. Hence, firstly, it appears that the fixation or dehydration does matter to maintain human autoantibody reactivities and secondly, the wide range of test sensitivities using the same fixed assay at multiple centers

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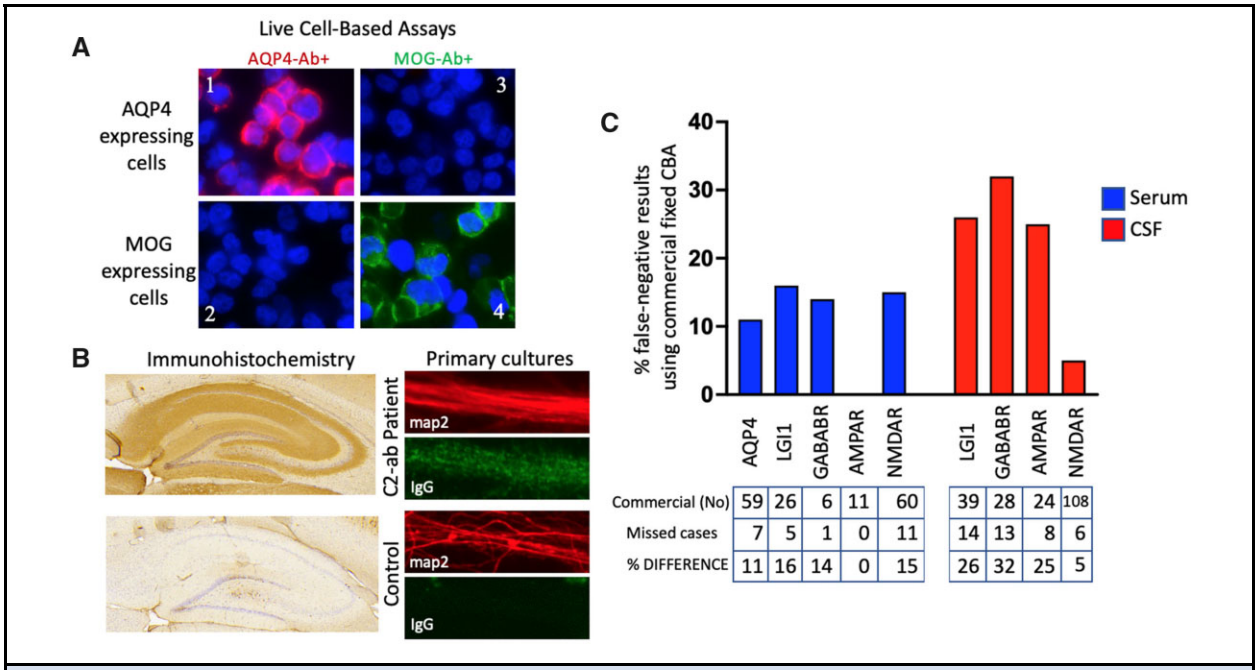

Fig. 1. (A), Live CBA showing a serum sample positive for aquaporin-4 antibodies (AQP4-Abs; red, 1) and negative on myelin oligodendrocyte glycoprotein (MOG)-expressing cells (2). A second serum sample is negative for AQP4-Abs (3) but positive for MOG-Abs (4 in green). Cell nuclei are labelled with dapi (blue); (B), Contactin-associated, protein-like 2 (CASPR2) antibody-positive serum shows a distinct hippocampal staining pattern on rodent central nervous system tissue and on primary neuronal cultures (patient IgG binding shown in green; neurons stained with antibodies to MAP2 (microtubule-associated protein 2) in red). This contrasts with healthy control serum which is negative on IHC and primary cultures; (C), Data from 3 studies demonstrate significant numbers of false-negative serum and cerebrospinal fluid (CSF) test results on commercial CBAs for multiple antibody targets in autoimmune neurology. Missed cases in (C) were positive by IHC, research CBAs, and supported by clinical data where available.

suggests a need for improved reproducibility of this platform between centers.

More recently two research groups have re-examined samples that had been tested on commercial fixed CBAs in their local clinical laboratory for antibodies associated with encephalitides. In one center, the fixed assay revealed 85/623 cases with a clear antibody result but 97 showed indeterminate results (3). The indeterminate results were largely due to excessive nonspecific labelling of the fixed cells, perhaps related to sera use at the recommended 1:10 dilution. In total, 88/97 indeterminate results were resolved by CBA and immunohistochemistry (Fig. 1, B): 7 seropositive and 81 seronegative. The authors state that the live CBAs were helpful for resolving cases with high background on fixed cells. They confirmed 81/85 cases identified in the clinical laboratory. Importantly, antibodies from an additional 15 patients' samples with false-negative results on the fixed commercial tests were identified as positive on live cells and by immunohistochemistry (IHC) using rat brain sections: ten *N*-methyl-D-aspartate receptor (NMDAR), one α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), three leucine-rich, glioma-inactivated 1 (LGI1), and one contactin-associated, protein-like 2 (CASPR2). The associated clinical phenotype

and response to immunotherapy, available in 12/15 cases, suggests these patients had immunotherapy responsive antibody-mediated diseases.

A second group identified 404/6213 samples that showed a positive stain by IHC (an example of IHC staining and primary cultures from a CASPR2-positive patient sample and healthy control sample is shown in Fig. 1, B). Of those, 163 (40%) were positive on a commercial fixed CBA (4). In the remaining 242 IHC positive/commercial fixed CBA negative samples, 21 (9%) were positive for antibodies not included in the commercial kit. However, mirroring the first study, another 21 (9%) samples were identified as positive by live CBA or in-house fixed CBA and IHC for antigens tested on the commercial kit: eleven LGI1, seven gamma-aminobutyric acid receptor 1 (GABABR), two AMPAR, and one NMDAR. Of note, a higher proportion of false-negative results reported on the commercial fixed CBA were from CSF.

In these rare diseases an average of 14% cases across 4 antigens were missed on commercial fixed CBAs in serum samples (Fig. 1, C). The data is more striking in the CSF with an average of 22% of cases missed with the false-negative frequency reaching 32% for GABABR autoantibodies.

In summary, these data highlight the alarming frequency of false-negative test results on commercial fixed antibody tests in autoimmune neurology. They highlight difficulties with background staining and uninterpretable tests results seen in routine clinical laboratories. Live cell-based assays resolve indeterminate fixed test results and immunohistochemistry is an important adjunct for specific antibody testing.

Nonstandard Abbreviations: CBA, cell-based assay; IHC, immunohistochemistry.

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