NEXT-LEVEL NEUROSCIENCE WITH SINGLE MOLECULE COUNTING (SMC®) TECHNOLOGY

Sponsored By

MERCK

MERCK

Biomarkers on Your Mind?

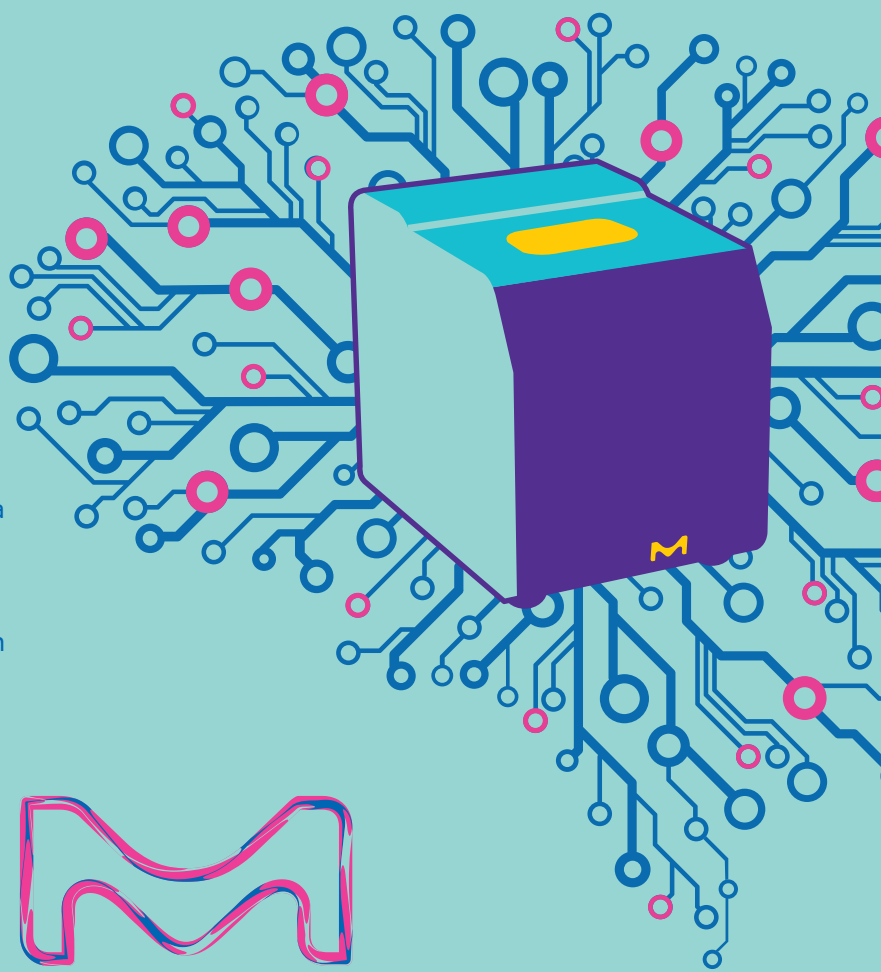
Explore with Ultrasensitive Neuro Immunoassays

Cerebrospinal fluid (CSF) biomarkers can present a challenge in neuroscience research because CSF samples are difficult to obtain. This creates a need for blood-based biomarkers. However, identifying them is limited by the sensitivity of standard immunoassays.

Ultrasensitive Single Molecule Counting (SMC®) immunoassay technology enables low-abundant biomarker measurement in a variety of biofluids such as serum, plasma, and CSF.

Our newest kits measure the following with unparalleled sensitivity and precision:

- NF-L
- SNAP-25
- TDP-43



Discover more at
SigmaAldrich.com/smc-neuro

For Research Use Only.
Not For Use In Diagnostic Procedures.

43266 08/2022

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Millipore®
Preparation, Separation, Filtration & Monitoring Products

ACCESSING THE BRAIN THROUGH BLOOD

Scientists conducting basic or clinical research leverage biomarkers found in body tissues and fluids to understand normal biology, disease states, and treatment responses. While this strategy proves fruitful in many research areas, researchers struggle to access biomarkers associated with the nervous system.

To study health and disease biomarkers, scientists often have to look beyond inaccessible brain tissue and collect cerebrospinal fluid (CSF). In CSF, biomarkers associated with brain health and neurodegeneration are plentiful, but the fluid is difficult to collect, requiring a painful and invasive lumbar puncture to extract a small sample volume.

To move away from biomarker identification in CSF, researchers explore circulating biomarkers in the blood. Blood is readily available in larger volumes, allowing researchers to track changes in biomarker levels over time. However, compared to their levels in CSF, neuroscience biomarkers are found in low abundance in the blood. As the popularity of blood-based biomarker analysis increases, scientists are seeking improved assays that reliably detect low-abundant analytes in plasma and serum samples.

Traditional Antibody Testing

The most popular biomarker detection methods employ antibodies that bind to molecules of interest in biological samples. Many researchers are familiar with the traditional enzyme-linked immunosorbent assay (ELISA), which uses sandwich-based capture and detection methods to measure analytes directly within multi-well plates. However, ELISA lacks the sensitivity needed to detect low-abundant neuroscience biomarkers, with a lower limit of quantification (LLOQ) typically greater than 10 pg/mL in blood. Most protein biomarkers in the blood can have concentrations below 1 pg/mL,[1] so detecting these analytes requires an assay with enhanced sensitivity compared to ELISA.

Great Sensitivity with Single Molecule Counting

New technologies continue to improve upon familiar assays to better serve scientists. Single Molecule Counting (SMC®) technology uses bead-based analyte capture coupled with a unique elution step to improve the sensitivity of single target analyses. These immunoassays start with a process that mirrors a bead-based ELISA, where beads coated with primary antibody are added to samples. Next, unbound proteins are washed away and secondary fluorescently-labeled antibodies are added to create a typical sandwich immunoassay complex. The following elution step chemically dissociates the analytes and detection antibodies from the capture antibodies. Researchers then remove the magnetic beads, transfer the eluate to a fresh plate, and detect antibody signal using the SMCxPRO® platform. This system uses a confocal laser that excites the free-floating fluorescent antibodies. The instrument then captures the resulting signals with a photodiode that digitally counts individual photons.

SMC® technology's unique elution step combined with the ultrasensitive protein detection platform decreases background noise and quantifies analytes at femtogram/mL concentrations with dynamic ranges of 4-5 logs,[2] allowing researchers to detect low-abundant biomarkers in serum and plasma samples. In a test comparing ELISA to a bead-based SMC® immunoassay, the SMC® assay was 1,250x fold more sensitive when quantifying an analyte in serum samples.[3] Additionally, the SMC® assay only required 1.6 µL of sample, while the ELISA needed more than 100 µL.

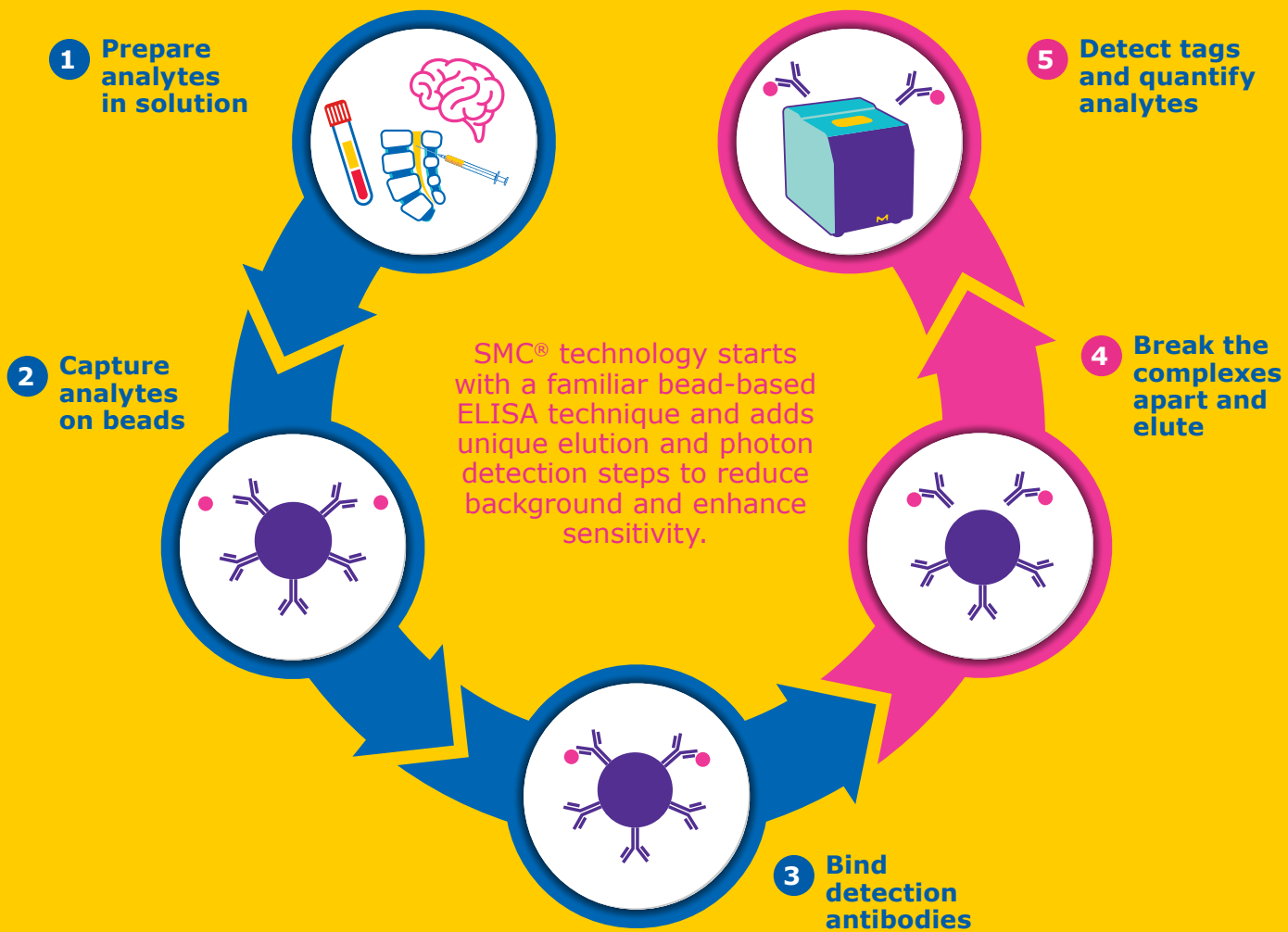
Researchers using SMC® technology for neuroscience research can use specialized assay kits to detect common biomarkers such as amyloid beta peptides in both CSF and blood-based samples. Alternatively, customized SMC® immunoassay development, troubleshooting, and testing to meet research needs are readily available. Using this technology, scientists can identify previously undetectable biomarkers and detect small yet important changes in protein levels.

See References on Page 7

For Research Use Only. Not For Use In Diagnostic Procedures.

Low-Abundant Biomarker Detection

With ultrasensitive Single Molecule Counting (SMC®) immunoassays, researchers analyze neuroscience biomarkers from multiple biospecimens, including blood-based samples.



The SMC® assay outperforms ELISA, enabling researchers to detect low-abundant biomarkers in blood-based samples.

IL-17A detection in mouse serum samples		
	ELISA	**SMC® Beads**
LLOQ (pg/mL)	62.5	0.05
Required Sample Volume (µL)	>100	1.6
Fold Sensitivity Improvement	-	1,250x
*LLOQ – lower limit of quantification		

A NEW LOOK INTO THE BRAIN

Quantifying biomarkers in cerebrospinal fluid (CSF) and blood has applications for numerous neuroscience research areas. For example, using immunoassays, scientists measure Tau to study Alzheimer's disease and frontotemporal dementia, alpha-synuclein for Parkinson's disease, and brain-derived neurotrophic factor (BDNF) for a number of conditions including epilepsy, Alzheimer's disease, and schizophrenia. In particular, researchers using Single Molecule Counting (SMC®) immunoassays for their research gain an unprecedented view into the brain by analyzing low-abundant biomarkers in the blood.

Tracking Glioma with Serum Biomarkers

Gliomas account for 80 percent of malignant tumors in the brain, and patients often fail to respond to radiation or chemotherapy treatment.[1] Currently, patients often undergo magnetic resonance imaging (MRI) scans followed by invasive histopathologic examination to establish an initial diagnosis. Once in treatment, they must have multiple imaging procedures and, occasionally, additional biopsies to assess their treatment responses.

To avoid the risks of brain biopsies and MRI scans, researchers from Seoul National University sought a novel, non-invasive glioma biomarker to determine prognosis.[2] They decided to track amyloid beta-42 (Aβ42) peptide, a biomarker related to glial cell proliferation found in the brain tissue, cerebrospinal fluid (CSF), and blood of glioma patients.[3-5] In a retrospective study, the researchers analyzed Aβ42 in human serum samples with varying grades of glioma using the SMCxPRO® instrument. They then compared Aβ42 levels to the clinical characteristics, histology, and outcomes.

The research team found that higher Aβ42 levels were associated with poorer patient outcomes, such as shorter progression-free survival. Aβ42 also indicates glial cell proliferation, as a high biomarker level correlated with a high Ki-67 index—a feature typically measured in histology to grade tumors and assess prognosis. Through these immunoassays, the scientists concluded that Aβ42 was a promising biomarker candidate for blood-based assessment of glioma.

Through these immunoassays, the scientists concluded that Aβ42 was a promising biomarker candidate for blood-based assessment of glioma.

Predicting Multiple Sclerosis Treatment Responses

Relapsing remitting multiple sclerosis (RRMS) is the most common MS disease course and is characterized by new or increasing neurologic symptom exacerbations followed by periods of remission or recovery. These exacerbations occur due to the damaging effects of inflammation on myelin surrounding nerve fibers, causing weakness, numbness, loss of muscle coordination, and problems with vision, speech, and bladder control. Interferon beta-1b injection is a common treatment for reducing RRMS symptom episodes, although some patients do not respond well to this immunomodulatory therapy.

Researchers from across the globe worked together to assess a potential biomarker for measuring interferon beta-1b treatment success. They analyzed serum samples with MS by measuring interleukin IL-17F at baseline and after six months of treatment. IL-17F is a cytokine released by Th17 helper cells, an immune cell type that contributes to aberrant immune responses in MS.[6] Using the SMC® IL-17F immunoassay kit, the researchers found that RRMS patients with high serum levels at the start of treatment had suboptimal responses to interferon beta-1b.[7] However, on its own, IL-17F did not tend to accurately predict patient responses to therapy or correlate with symptom relapse. Because the biology of the interferon beta is complex, the researchers concluded that they needed to measure IL-17F in combination with other biomarkers to find more sophisticated signatures of RRMS therapy response. A multiplex approach measuring multiple blood biomarkers may better capture the complexity of this autoimmune disorder.

See References on Page 7

For Research Use Only. Not For Use In Diagnostic Procedures.

MORE IS BETTER: COMBINING BIOMARKER IMMUNOASSAYS

While finding biomarkers in the blood gives scientists an advantage when studying the brain, measuring a single biomarker at a time sometimes offers an incomplete view of the complex nervous system. To have the best of both worlds, researchers combine single target, ultrasensitive Single Molecule Counting (SMC®) immunoassays with MILLIPLEX® multiplex immunoassays to better detect multiple biomarkers in tissue, cerebrospinal fluid, and blood.

Multiple Insights from a Single Experiment

Multiplex assays for screening biomarkers are becoming attractive replacements for traditional, single-target ELISAs. By quickly assessing related proteins in samples, researchers can identify promising candidates for further analysis.

MILLIPLEX® multiplex immunoassays come in a range of protein panels, allowing researchers to measure multiple markers at the same time in a single plate. In these assays, magnetic beads conjugated to capture antibodies bind analytes of interest in the same sample. Each antibody-bead pair associated with a different protein is distinguished by the ratios of two internal dyes. Upon detection, these signals combine to create unique fluorescent signatures for each antibody-bead set, allowing researchers to accurately measure the captured analytes.

Combining Multiplex and Sensitive Single Target Assays

Once researchers identify analytes of interest within a MILLIPLEX® panel, they can confirm their results and further explore individual biomarkers using ultrasensitive SMC® technology. SMC® immunoassays are especially useful when checking for low-abundant biomarkers in biofluids, which can help distinguish between healthy and disease state samples.

Once researchers identify analytes of interest within their MILLIPLEX® panel, they can confirm their results and further explore individual biomarkers using ultrasensitive SMC® technology.

Researchers explored the combined power of neuroscience MILLIPLEX® panels and single target analysis with SMC® immunoassays for detecting Alzheimer's disease (AD) biomarkers in human cerebrospinal fluid (CSF), plasma, and serum.[1] They used three MILLIPLEX® panels (Human Amyloid Beta and Tau (Cat. No. HNABTMAG-68K), Human Neuroscience Panel 1 (Cat. No. HNS-1MAG-95K), and Human Neuroscience Panel 2 (Cat. No. HNS2MAG-95K)) that measured different established and emerging neurodegenerative disease biomarkers in the CSF of samples with AD and non-AD controls. Notably, the scientists found differences in several biomarkers, including phosphorylated tau, amyloid beta 1-42 peptide (Aβ42), and neurogranin (NRGN). As Human Neuroscience Panel 2 is also verified for blood-based samples, the researchers tested this panel on AD and healthy-control plasma and serum samples and found differences in several protein levels between them, suggesting that these analytes had the potential to serve as blood-based biomarkers of AD.

Because some of these potential biomarkers were in low abundance in the blood samples, the researchers used ultrasensitive assays on the SMC® platform to more accurately measure them. They used two amyloid beta peptide kits for Aβ40 and Aβ42 and found differences in both proteins in CSF samples and an increase in plasma Aβ40 specifically compared to controls, suggesting that Aβ40 may serve as a promising AD blood biomarker.

These data show how important it is to monitor multiple biomarkers when studying a disease. Using multiplexed assays to quickly screen samples followed by targeted evaluation of encouraging candidates in various biofluids is a worthwhile approach for neuroscience researchers looking to move toward blood-based sample analysis.

See References on Page 7

For Research Use Only. Not For Use In Diagnostic Procedures.

References

Article 1: Accessing the Brain Through Blood

1) N. L. Anderson, N. G. Anderson, "The human plasma proteome: history, character, and diagnostic prospects," *Mol Cell Proteomics,* 1:845-67, 2002.
2) J. Hwang et al., "Quantitation of low abundant soluble biomarkers using high sensitivity Single Molecule Counting technology," *Methods,* 158:69-76, 2019.
3) "Improved performance with conversion from ELISA to an Erenna® immunoassay," *Merck,* application note.

Article 2: A New Look into the Brain

1) M.L. Goodenberger, R.B. Jenkins, "Genetics of adult glioma," *Cancer Genet,* 205:613–21, 2012.
2) K. Hwang et al., "Serum amyloid-β42 as a noninvasive biomarker for prognosis and histologic features of glioma," *Neuro-Oncol*, 23:vi13, 2021.
3) S.H. Park et al., "Suppressive effects of SuHeXiang Wan on amyloid-β42-induced extracellular signal-regulated kinase hyperactivation and glial cell proliferation in a transgenic *Drosophila* model of Alzheimer's disease," *Biol Pharm Bull,* 36:390-98, 2013.
4) A. Zayas-Santiago A et al., "Accumulation of amyloid beta in human glioblastomas," *Clin Exp Immunol,* 202:325-34, 2020.
5) P. Gagni et al., "Development of a high-sensitivity immunoassay for amyloid-beta 1–42 using a silicon microarray platform," *Biosens Bioelectron,* 47:490-95, 2013.
6) M. Mitsdoerffer, V. Kuchroo, "New pieces in the puzzle: how does interferon-beta really work in multiple sclerosis?" *Ann Neurol,* 65(5):487-88, 2009.
7) H.-P. Hartung et al., "Interleukin 17F level and interferon-β response in patients with multiple sclerosis," *JAMA Neurol,* 70:1017-21, 2013.

Article 3: More Is Better: Combining Biomarker Immunoassays

1) A. Saporita et al., "MILLIPLEX® multiplex and SMC® high-sensitivity immunoassay detection of Alzheimer's disease biomarkers in human cerebrospinal fluid, plasma, and serum," *Merck*, application note.

For Research Use Only. Not For Use In Diagnostic Procedures.

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Make More connections

with MILLIPLEX® multiplex neuroscience immunoassays

Neuroscience multiplex immunoassays give a complete picture of nervous system functions such as cognition, motor, and homeostatic control. MILLIPLEX® multiplex immunoassays, using trusted Luminex® xMAP® technology, enable a multivariate analysis of the processes that underlie neural development, homeostasis, and pathogenesis—all while conserving precious samples.

Make more connections in neuroscience research by exploring with:

- 13 Panels
- 55 Analytes
- Multiple Species
  - Human, Mouse, Rat

SigmaAldrich.com/milliplex-neuro

For Research Use Only.
Not For Use In Diagnostic Procedures.

43872 09/2022

MK_AN12306EN 43612
09/2022

Millipore®

Preparation, Separation, Filtration & Monitoring Products