Introduction & Research History

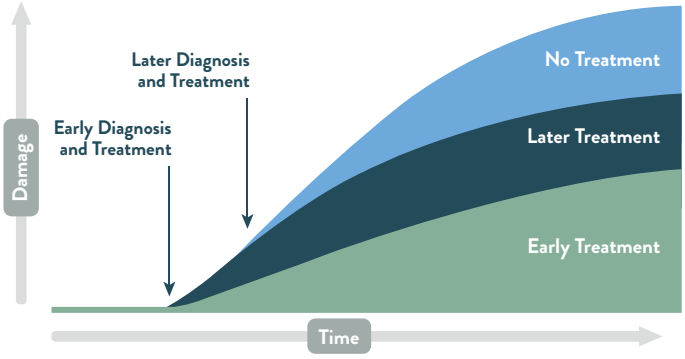
Overview of Isolate**MS**™ Multiple Sclerosis Test Platform

IQuity's IQIsolate technology is the product of over 12 years of research at Vanderbilt University Medical Center and over $4 million in NIH funding. The results of our research uncovered that differences in RNA expression patterns can be detected in blood samples from patients with a variety of human conditions spanning infection to more complex, inflammatory diseases. Building on this principle finding, IQuity's work has been focused on developing diagnostic tools that provide accurate, actionable information to healthcare providers at the earliest stages of disease. And, it is generally accepted that early diagnosis and early treatment of autoimmune disease leads to optimal patient outcomes.

IQuity offers gene expression-based panels for multiple sclerosis (MS), gastrointestinal disease (irritable bowel syndrome vs. inflammatory bowel disease-Crohn's or ulcerative colitis), and rheumatologic disorders (fibromyalgia, rheumatoid arthritis, and systemic lupus).

A diagnosis of multiple sclerosis relies on clinical symptoms and examination as outlined in the McDonald criteria, a set of guidelines used by neurologists and MS specialists to facilitate the diagnosis of MS. The referral of patients with symptoms suggestive of MS to neurologists or MS specialists is often delayed, leading to a diagnostic process that can last months to years and thus, delay treatment. An MS diagnosis is supported by magnetic resonance imaging (MRI) findings and other laboratory results including detection of oligoclonal bands (bands of immunoglobulins) found in the cerebral spinal fluid or evoked potential testing where providers measure the electrical activity of the brain in response to stimulation of specific sensory nerve pathways. Until now, there has been no simple blood-based test that can help providers rule-in or rule-out a diagnosis of MS. Misdiagnosis of MS has also been reported (in up to 35% of cases) and places additional burdens on both the patient and provider.

Central Nervous System Damage Progression



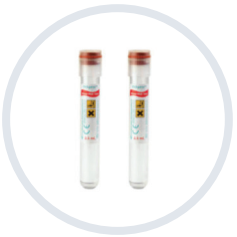
Capturing a snapshot of RNA uncovers the communication occurring inside the cells and tissues of the body. We have harnessed the power of cutting edge technologies like RNA sequencing to identify RNA targets that exhibit large changes in autoimmune disease. The reproducibility of current sequencing platforms using gold standard approaches including quantitative reverse transcription PCR (RT-qPCR) is quite high. We have identified and validated RNAs capable of distinguishing multiple sclerosis from healthy individuals and patients with other neurologic diseases with accuracy levels surpassing 90%.

Our research has focused on a new class of RNA – the long, noncoding RNAs (lncRNAs). Differences in lncRNA expression values found in the peripheral whole blood in autoimmune disease are greater than what we observed in previous studies that focused primarily on protein-coding genes. The use of lncRNAs as the foundation of our Isolate test panels enhances the confidence of disease determinations using our analytical approaches built upon machine learning.

Through clinical collaborations in the United States and Europe, we have recruited almost 650 subjects that were used to validate the RNA targets in our IsolateMS test. These datasets were used to build computational models capable of distinguishing disease and non-disease samples.

Early intervention with a disease-modifying therapy in MS is thought to give the best long-term prognosis.
Figure from *Brain health: Time matters in multiple sclerosis,* Prof. G. Giovanni et al. 2015

Overview of Discovery and Validation of IsolateMS Multiple Sclerosis Test Platform



Peripheral whole blood was collected into Paxgene Blood RNA tubes across multiple autoimmune diseases and related conditions

Whole genome RNA sequencing and data analysis

RT-qPCR validation of gene targets in expanded patient and control cohorts. Analysis of gene expression data using machine learning methods

External validation of research findings in an independent, clinical laboratory

Internal validation in IQuity's CLIA certified specialty diagnostic laboratory

1. Whole blood was collected in Paxgene tubes from healthy individuals, subjects at various stages of multiple sclerosis, and patients with other neurologic disorders commonly seen by neurologists (n=641). Subjects were recruited from seven sites in the United States and two sites in Europe.

2. RNA was isolated from these subjects and whole genome sequencing was performed on a subset of these patients to derive RNA targets of interest.

3. Once the sequencing data were analyzed, RNA targets that evidenced the highest differences across patient cohorts (i.e. healthy control vs. MS or MS vs. other neurologic diseases) were then analyzed in all recruited subjects using RT-qPCR. IQuity uses the Applied Biosystems® QuantStudio™ 12K Flex PCR machine for PCR studies.

4. IQuity can effectively discriminate multiple sclerosis from healthy subjects and patients with other neurologic disorders in a single computational cycle. Data generated by RT-qPCR serves as the input into machine learning classifiers capable of discriminating patient populations. Our research has derived novel approaches to manipulate the gene expression dataset inputs and 'tune' machine learning classifiers to optimize classification supervised and unsupervised clustering.

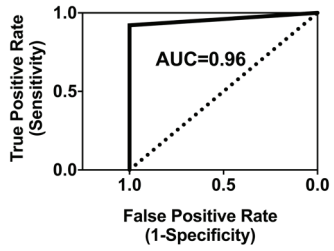
The Isolate**MS** algorithm has been validated using an independent group of subjects. IQuity's algorithm was tested using data from blood samples of healthy subjects, patients with multiple sclerosis, and inflammatory (i.e. optic neuritis, transverse myelitis, neuromyelitis optica) and non-inflammatory (i.e. Parkinson's and Alzheimer's) neurologic diseases.

Figure 1 shows the results obtained for the determination of multiple sclerosis (MS) versus controls that include other inflammatory neurologic diseases, and other non-inflammatory neurologic diseases in our validation study.

Significant Finding - IsolateMS Identifies MS in Patients with Clinically Isolated Syndrome (CIS)

Through a collaboration with the Accelerated Cure Project, IQuity was able to obtain blood specimens from patients with a clinically isolated syndrome (CIS) who later developed multiple sclerosis. A clinically isolated syndrome is the major clinical precursor to multiple sclerosis. Patients are often diagnosed with a CIS months to years before a diagnosis of MS can be made using the McDonald criteria. Our CIS blood samples were obtained years before a clinical diagnosis of MS was made and using our algorithm, we were able to make a positive MS determination at the time of a patient's CIS event in 95% of the patients we enrolled in the study. We conclude from this retrospective study that examining RNA levels in a patient's blood sample can accurately identify MS at the earliest clinical time points. Had our test been utilized at the time of a CIS diagnosis, the attending physician would have had additional information to confirm a suspected case of MS.

Figure 1



Accuracy: 94% False positive rate: 2%
Specificity: 98% False negative rate: 7.5%
Sensitivity: 92%

Patient Cohort -641

160 – Healthy Controls

114 – OND-NI (Other Neurological Diseases-noninflammatory),

98 – OND-I (Other Neurological Diseases-inflammatory)

269 – MS (All of the previous cohorts were included: CIS->MS; MS-NAIVE, and MS-ESTABLISHED (with and without meds).

5. In late 2015, IQuity contracted a validation of its assay system with an independent CAP-accredited, CLIA-certified lab. This validation process included evaluation of the gene targets used and clinical validation of blood samples from healthy subjects and MS patients through a process that was managed entirely by the independent lab. IQuity successfully replicated its research findings in their lab confirming the accuracy of the Isolate**MS** test panel.