

A Blood Test for Autism? The GERSTEL Twister Aids Scientist's Search

Autism Spectrum Disorder (ASD) is one of the world's most prevalent non-communicable disorders. It currently affects one in 68 children in the United States, according to the Centers for Disease Control and Prevention. This figure has been climbing at an alarming rate over the past decades. In 2002, for example, the disorder affected only 1 in 150 children.



Autism currently has no known cause. Researchers are looking at several possible reasons for autism's rise, ranging from ethnic and socioeconomic background, child rearing, genetics and harmful chemical exposures from the environment.

None of these possible factors has been singled out as the single cause, and many researchers believe a complex array of different factors may lead to autism. One theory: Genetic factors may influence a person's ability to fend off the effects

of certain toxins. Another: Toxins and other environmental exposures themselves may cause epigenetic modifications on the DNA that may be passed on to subsequent generations.

THE ENVIRONMENTAL CHEMICAL CHALLENGE

Studying chemical influences from the environment has been hampered by techniques available for analysis. Most chemical analysis cannot detect potentially harmful chemicals at the low concentrations they exist in the environment. This limitation has made it virtually impossible to determine whether or not environmental toxins or other chemicals may play a role in autism and other non-communicable diseases.

Professor H.M. "Skip" Kingston, a bio-analytical chemist at Duquesne University in Pittsburgh and his colleagues created [a new analytical method](#) employing GERSTEL technology that was sensitive enough to detect these chemicals in blood at low enough concentrations to be useful for studying links to autism and other disorders.

This method was extensively applied in a three-year clinical study involving healthy and autistic children in Pittsburgh. The research led to six peer-reviewed papers focused on the analytical, methodological and medical aspects.

The new method looks at blood serum concentrations of [persistent organic pollutants](#), also known as POPs. These organic compounds were identified by the Stockholm Convention, which was established by the United Nations Environmental Program to identify chemicals that could adversely impact human health. Initially, the [convention identified](#) 10 POPs: the pesticides aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene, and the industrial chemical polychlorinated biphenyls (PCBs). More recently the list has been expanded to include dozens of other POPs.

These Stockholm Convention chemicals were selected because of their longevity, environmental distribution, accumulation in fatty tissue, and toxicity to humans and wildlife. The convention was signed by more than 180 countries to restrict or eliminate production of these chemicals. As the detection instruments advanced and made trace-level analysis possible, quantifying the level of POPs in the environment and in humans has gained more research and regulatory

interest to determine their long-term roles in human and environmental health.

The popular 20-year-old solid phase micro-extraction (SPME) method wasn't compatible with immersion in human blood or plasma, as hormones and other circulating proteins interfered with SPME fibers.

When Kingston and his colleagues began searching for a link between the POPs and autism, they found that current analytical methods couldn't deliver the data they hoped for. "We sent reference standards to blood labs, and got nothing but wrong answers back." Kingston said.

Conducting targeted studies — which provide quantitative results on specific POPs concentrations and activity — was crucial to understanding the role between POPs and disease, Kingston said. "Physicians and caregivers need quantitative results because dose response is extremely important," he added. Up to this point, the analytical tools only answered the question, "what is there." For the first time, the caregivers will have the level of quantification and actionable quality of data that will answer the question, "how much." This, in turn, will help establish cause and effect relationship between the toxins and disease, and result in more effective health and disease management solutions.



A NEW, COMBINED TECHNIQUE: TWISTER AND D-ID

Kingston and his team combined the GERSTEL Twister Stir Bar Sorptive Extraction (SBSE) with Direct Isotope Dilution (D-ID) mass spectrometry quantification, which provided a new way to conduct targeted POP analysis.

The Twister bar had 50 to 1,000 times the absorptive capacity than the SPME fiber and was more amenable to different applications. By comparison, “we couldn’t get more than three uses out of the SPME fiber, due to irreversible fouling,” Kingston said. The new method also helped resolve a problem associated with semi-volatile and non-volatile chemicals. SPME fibers can work well in the headspace — the air space above samples. Extraction of volatile chemicals, like benzene, toluene and some solvents, is effective in the headspace. For less volatile organics, however, only GERSTEL Twister would work at trace levels of volatile, semi-volatile and non-volatile simultaneously, resulting in large improvements in efficiency.

D-ID was invented by Kingston and was adopted by the US Environmental Protection Agency as [EPA Method 6800](#). It is based on spiking known amounts of enriched stable isotopic analogs of test compounds into a sample. Equilibrium is obtained between the endogenous and spiked compounds before extraction. Knowing the isotopic abundance of both spiked and endogenous compounds means the analyst knows the amount of spike added, measures the new altered isotopic ratio, and directly calculates the concentrations of endogenous molecules in the samples. Each measurement becomes its own calibration and uses pure mathematic equations to calculate the concentration.



Pairing D-ID with the [GERSTEL SBSE Twister](#) and automation improved accuracy in analyzing POPs. The Combined technologies of Gerstel, Duquesne University and Applied Isotope Technologies (AIT) were required to enable method, hardware, and isotope reagent standards to come together to advance the technology. Compared to traditional quantification methods, D-ID improved quantification accuracy 250 percent, reduced sample preparation time, and improved reproducibility between samples. The SBSE Twister step improved extraction accuracy by 37 percent compared to standard SPME methods. Overall, the new method is 10 times more precise than standard methodology, Kingston said.

In essence, the Twister bar delivered two critical performance improvements. The higher adsorptive capacity improved signal to noise ratio. This, in turn, made it possible to achieve D-ID quantification at trace and ultra-trace levels. Clinically relevant POPs measurements for human samples require the lowest possible limit of quantitation, typically at parts-per-trillion levels. The second important performance improvement was in cost per analysis. The extended re-usability of the Twister Bar lowered to cost of tests to the point where they can be offered commercially.

A NEW LOOK AT AUTISM'S CHEMICAL CONNECTION

Kingston's team is using this new combined technique to determine the relationship between blood levels of POPs and autism and other diseases. The new technique meant "we could do hundreds of measurements and get great statistics on children with autism," he said. They are now comparing their results with physicians' visual and clinical diagnoses to see if they match.

"It's still in an early stage of research; however we believe we will be able to predict the severity of autism [in individual children], and measure its progress before brain damage has occurred. We may possibly quantify early indicators enabling medical professionals to prevent the disease from happening."

All this from a blood test and D-ID/Twister analysis.

Technically, the biggest challenge was pinpointing toxins. "Until now, there has been no standardized test that does this," Kingston said. "People hadn't been doing these tests routinely and papers that looked beyond volatile chemicals were not accurate. We've created a reliable, rugged, accurate and sensitive test to give doctors accurate and precise measurements."

THE TWISTER ADVANTAGE

The GERSTEL SBSE uses the same basic chemical principle as in SPME, but is coated with a thicker layer of polydimethylsiloxane, which enhances the capacity for binding non-polar, less volatile compounds simultaneously. Twisters can be rinsed off, so proteins and other compounds don't get baked on during thermal desorption (in Kingston's case, they used the GERSTEL [Thermal Desorption Unit](#)) before gas chromatography/mass spectrometry analysis. This provided the added capacity to analyze non-volatile organics and collect much higher volumes of data.

The Twister avoids the use of hazardous solvents required for some other extraction procedures, resulting in a greener technique. After Twister extraction, sample analysis can be automated and the bars can be reused 50 to 60 times, allowing the abundance of data collected by Kingston and his colleagues. The combined Twister/D-ID method routinely has a precision of 3 percent to 6 percent. This is a significant improvement over the 15 percent to 30 percent precision and accuracy normally seen using existing methods. The quality of data enables researchers to observe the effects of metabolism of chemicals, including POPs. "This allows the physician to follow trends in patients that he never would have been able to observe before," Kingston explains.



Professor Kingston believes the physicians will finally be able to examine the relationship between POPs, other chemicals and developmental disorders like autism. “We could do this in a completely automated fashion with the Twister and the GERSTEL system,” said Kingston. “We could do the extractions simultaneously, and do hundreds of measurements.”

ADDRESSING ADDITIONAL DATA VARIABILITY CONCERNS FOR SMOOTHER CLINICAL ADOPTION

In the clinical laboratories, data-quality variability is always an important factor. In order to assess instrument-to-instrument variability, Kingston’s group assessed ease of applicability and transferability of the D-ID-enabled Twister method between two different instrument systems used by two different analysts at different laboratories.

The set-up simply included identical samples and a relatively simple standard automated protocol which was implemented in both instruments without modification. Reproducible sample handling and introduction and higher automation provided by the GERSTEL TDU were critical factors in achieving outstanding repeatability between the two instruments.

The results of this experiment, which was published in a peer-reviewed paper, demonstrated the feasibility of easy and painless transferability of the method among labs. Kingston emphasized this is a unique performance level in mass spectrometry. In the past, the MS has found limited use in clinical labs due to RSD variability within and among instruments. The combined D-ID, Twister and TDU as an integrated quantitation tool, overcomes this limitation. “This is now ready for the clinical laboratory,” Kingston said.

BEYOND AUTISM

The technique promises to be applicable to other disorders where the role of toxins needs exploration, Kingston said. These include disorders like asthma, certain cancers, and diseases, like ADHD. “It may be possible to get toxins out of a mother before pregnancy. Then, we could prevent toxins from entering the food supply of a child. But you have to make measurements of toxins in the body, if you want to reduce them.”

Early next year, Applied Isotope Technologies (www.sidms.com) will offer D-ID enabled POPs tests as a commercial service to measure body toxin-burden (BTB) of Chinese patients as part of health improvement strategies for adults and children in China. Professor Kingston points out that the short-term and long-term adverse effects of BTB are concerns for everyone. An increasing number of medical research studies indicate exposure is a primary risk factor that accelerates the onset or progression of a large number of debilitating non-communicable diseases.

In addition to disease detection (and possible prevention), Kingston’s method is being adopted by the U.S. Environmental Protection Agency for testing and monitoring toxic components of hazardous waste and drinking water. The method will make it easier for determining the content of hazardous waste in order to properly manage its generation, treatment and disposal.

“We’ve brought this analysis to a new level of scientific readiness,” Kingston said. “Now, we can make measurements we’ve never made before and provide actionable data to the physicians.”



About GERSTEL

GERSTEL is a family owned company founded in 1967. Its focus is on developing and producing systems and solutions for chemical analysis. Our main emphasis is on automated sample preparation for Gas Chromatography / Mass Spectrometry (GC/MS) and Liquid Chromatography / Mass Spectrometry (LC/MS).

No other company has a bigger tool box of sample introduction and preparation techniques available (many of them patented). GERSTEL can provide complete “turn-key” solutions that include either a GC/MS or a LC/MS with integrated software control. The innovative MAESTRO software enables intuitive control and flexible, efficient operation for R&D or routine laboratories. Your daily analysis tasks are set up using just a few mouse-clicks.

GERSTEL is a German based company with a global reach. With offices in the U.S.A., Japan, Singapore and Switzerland, and a global network of distributors, GERSTEL is able to deliver and support our unique solutions worldwide.

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