Applied Isotope Technologies

Proposal to Develop Quantitative Dried Blood Spot (QDBS) Test Kits for Children with Autism

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AIT – Company At-a-Glance

- **Co-founder, Prof. Skip Kingston, invented an advanced measurement technology Platform and associated automation tools**
  - Privately funded technology company focused on delivering new solutions in many industries
  - Unblemished scientific record and large body of published peer reviewed scientific papers
  - Proven technology: accepted, recognized or adopted in the US (EPA, CDC), Canada (MOE) and many EU countries
  - Codified as a national EPA method and specifically referenced on EPA website: https://www.epa.gov/hw-sw846/sw-846-test-method-6800-elemental-and-molecular-speciated-isotope-dilution-mass
  - Inventor is a world-renowned scientist: https://www.duq.edu/academics/faculty/skip-kingston
  - Patented technology estate – globally protected
  - Core measurement technology, SID for the MS has been applied, adopted, recognized, acknowledged in many fields over the past two decades

TECHNOLOGICAL FOUNDATION:

Advanced Blood Collection and Testing for Accurate & Precise Analytical Data

AIT Solutions - Overarching Value and Benefits

***AIT's Patented Next-Generation Mass Spectrometry (MS) Technology Delivers*:**

SID - Definitive, actionable, legally defensible data and zero-false-positive results with the lowest error margins, and the highest accuracy and precision levels

Thor's Hammer™ technology boosts level of MS detection by 2 orders of magnitude lower than currently achievable sensitivity & quantitation

SID: Speciated Isotope Dilution (*a patented operating system for the MS*)

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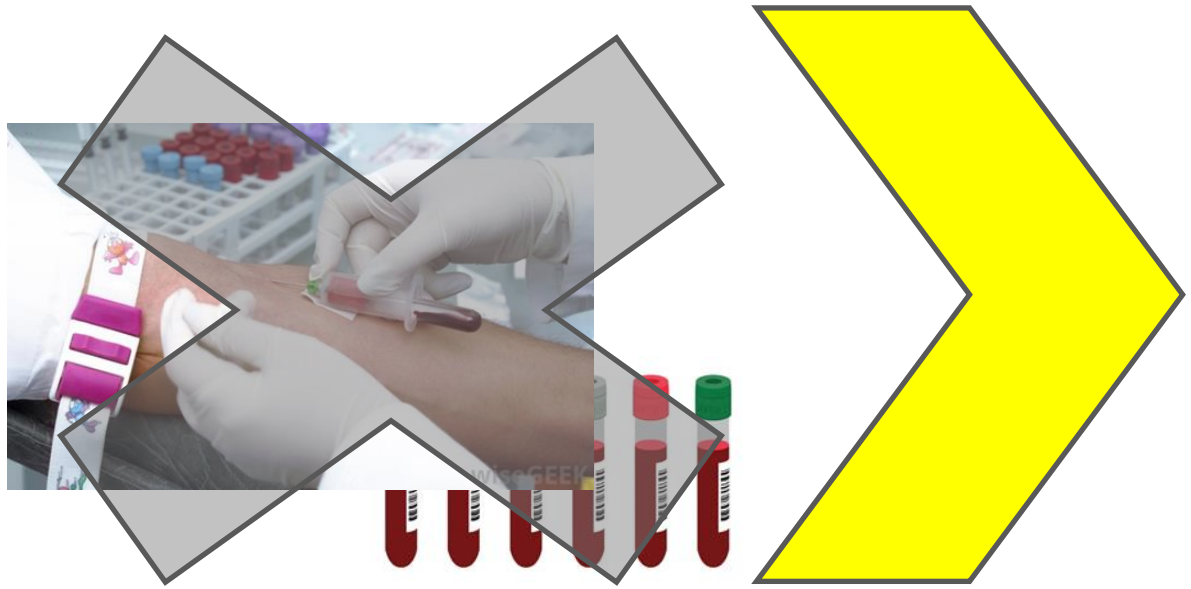
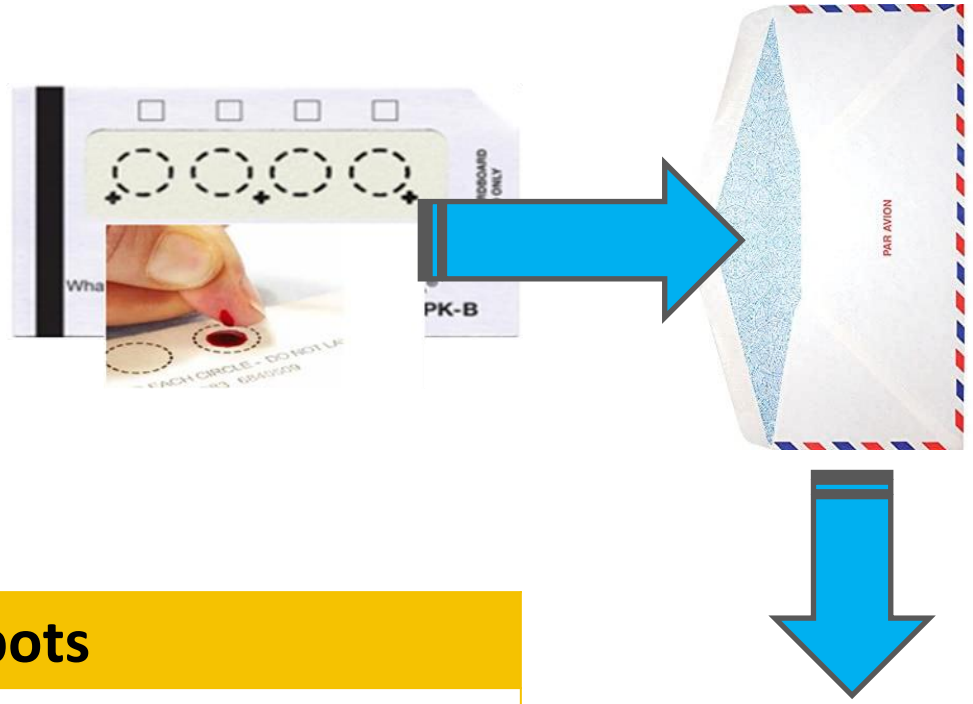
Quantitative DBS Will Transform Medical Testing





Blood Draw	Dried Blood Spots
5,000-6,000 cc of blood drawn to each tubes	20-30 cc applied on each spot
Invasive Intra-venous blood draw (1 or more)	Minimally invasive finger prick; no needles
Done by a phlebotomist in a clinic	Collected at home; sent by ordinary mail
Expensive, special-biohazard handling	Stable at room temp; long-term storage
Wasteful; most blood in tubes unused	Every drop of blood is used for testing

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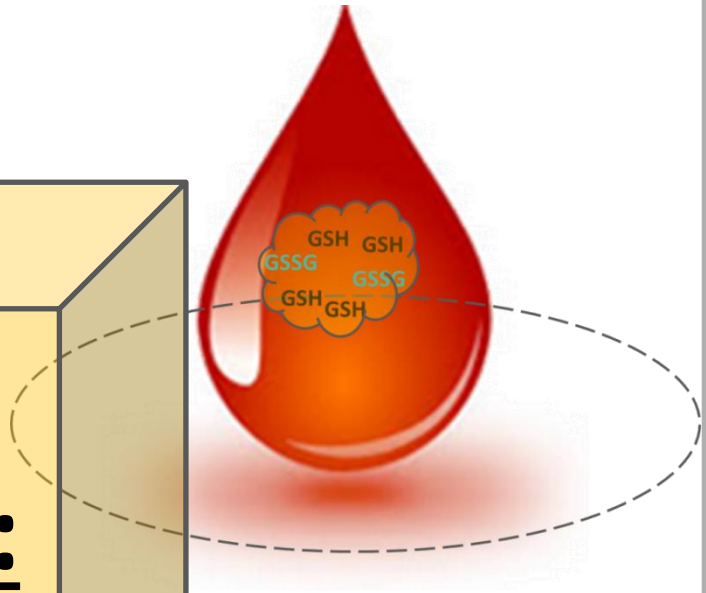
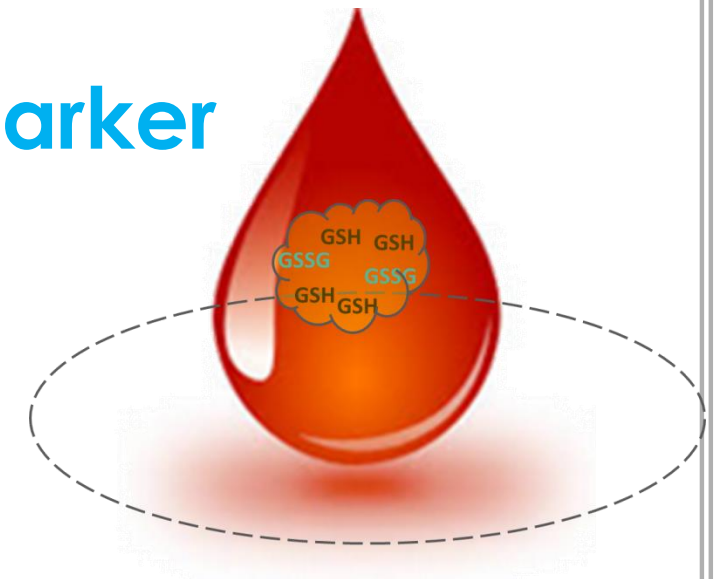
MAKING IMPOSSIBLE BIOMARKER TEST POSSIBLE:

Glutathione biomolecule is one of the most sought-after health indicators

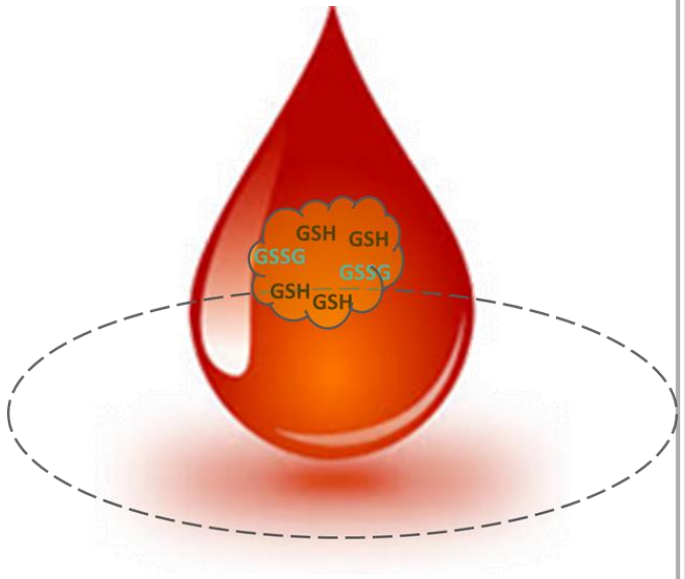


Glutathione (GSH): Important Blood Biomarker



- The most abundant blood molecule that has multiple critical functions in human body
- Key detoxifier and a modulator of the body's intra- and extra-cellular metabolic systems
- Unambiguous indicator of immune dysfunction
- The most sought-after biomarker among doctor's wish-list of tests for autism monitoring
- Must test for 2 variants – both needed to yield clinically important results
- Impossible with current technologies to do the test and obtain reliable, actionable data

Challenges in GSH Measurement



- GSH Test is a 4-point assay, including
  - Total (GSH-t)
  - Reduced (GSH-r)
  - Oxidized (GSSH)
  - Ratio: GSH/GSSG
- Ratio of the two GSH Measurements is highly informative about patient's health
- *Challenge:* very low abundance in blood
  - GSH-r is very difficult to measure
  - GSSG is currently impossible to measure because its abundance is 1-2 orders of magnitude lower than GSH (ultra-trace concentration)
- *Solution:* SID-Quantification with Thor's Hammer

TH(BOOSTER) + SID(OS) = Transformational MS Measurement Platform

- *Direct SID Quantification: MS Operating System (OS):*
  - *Advanced, patented MS quantification technology*
  - *Patent-pending solution to difficult or many impossible problems in blood testing*
- *Thor's Hammer*
  - *Is an MS signal booster that increases quantification by 2 orders of magnitude*
  - *Is an invention that has been reduced to practice*
  - *Is a highly selective technology for a targeted biomarker or a group of biomarkers*
  - *Is universally applicable to every sample that can be analyzed in an MS*
  - *Is not a protein molecular modification or chemical derivatization scheme*
  - *Is not a chemical enrichment/amplification manipulation that changes the protein*
  - *Is not an MS-data manipulation algorithm*
  - *Eliminates false positives – valuable attribute for clinical testing*

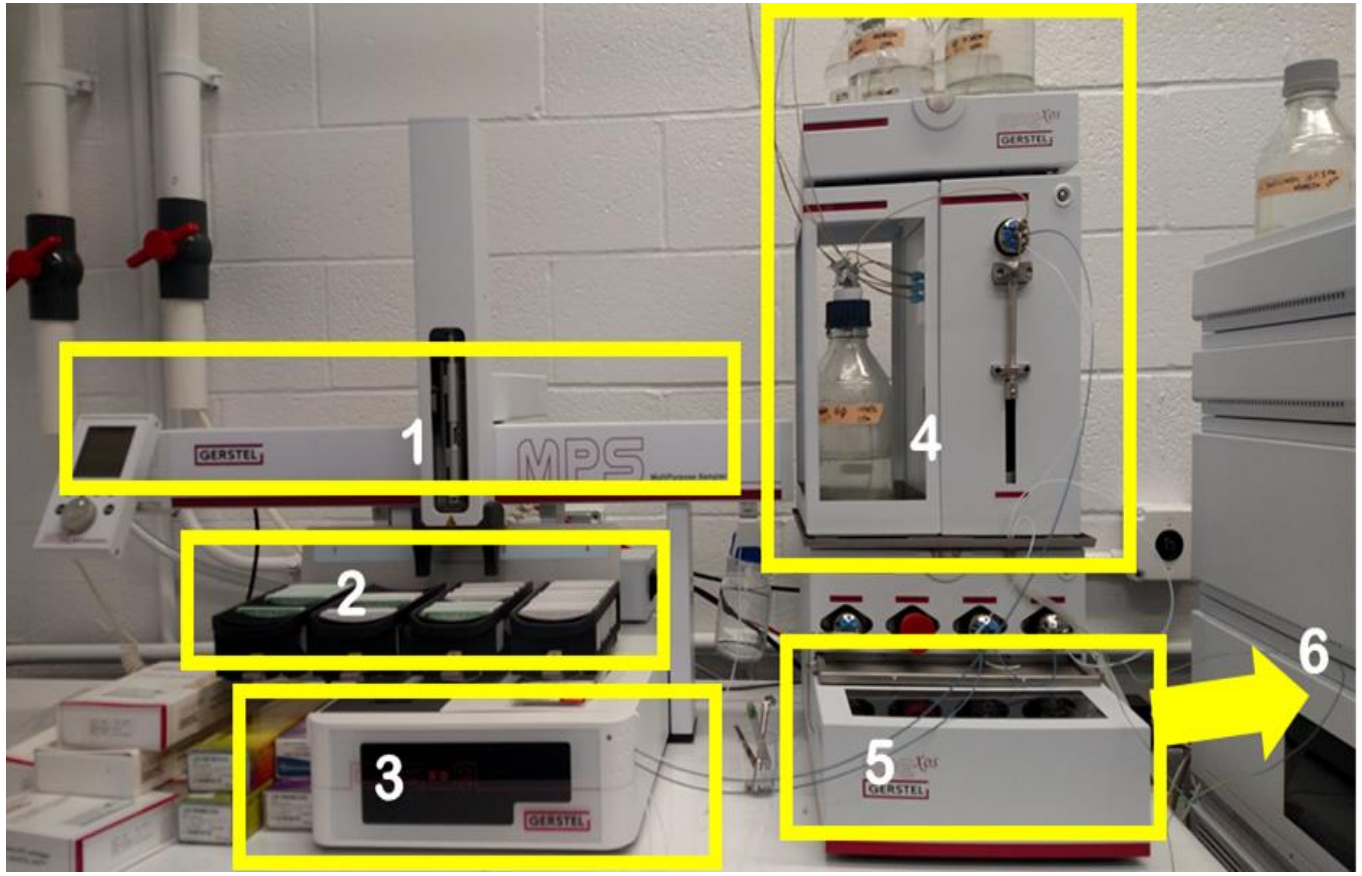
SID: Operating system for the MS

Uniquely-enabled Biomarker Tests on QDBS Cards

- Organic biomarkers –
  - Glutathione (GSH) Assay for immune system status
    - GSH (Reduced)
    - GSH (Oxidized)
    - GSH (Total)
    - GSH (Ratio: reduced/oxidized)
  - Methylmalonic Acid
  - Vitamin C, Vitamin B12
- Inorganic toxins, toxicants
  - Semi-quantitative scan: 50 metals (toxin burden surveillance)
  - Quantitative tests: Set of 20 electrolytes , immune regulators and other elements of biological significance
- Organic toxins, toxicants
  - Set of 15-20 volatile organic compounds (air pollutants), including forever chemicals, PFOAs and PFAS'

Automated Prototype QDBS System is Fully Operational and Easily Scalable

1. Multi-Purpose Sampling (MPS) Arm
2. MP Autosampler Card Rack
3. Desorption Chamber
4. High Pressure Dispenser
5. Auto Cartridge Exchanger
6. Liquid Chromatograph-MS



This automated prototype QDBS system is being used by Prof. Skip Kingston's Center of Excellence at Duquesne University, Pittsburgh, PA.

MEDICAL FOUNDATION AND GOALS OF AUTISM PROJECT:

Helping some of the children with autism change their otherwise irreversible trajectory within first 3 years of their life

Accumulating Data Suggests Environmental Interaction is a Causal Factor

- Emerging Paradigm: Children with autism experience a genotoxic influence from environmental interaction in the womb and after birth
- There are genetic vulnerabilities that impede performance of detoxification functions, such as
  - Heavy metals
  - Chronic exposure to volatile organic pollutants
  - Chemicals that disturbs normal biological processes such as methylation, sulfonation and the immune system
  - Several genotoxic, epigenetic and environmental impacts

Increased Evidence of Toxicity-induced Effects

- Children with autism have a problem of placing methyl and sulfur groups on fat soluble toxic chemicals, upsetting phase 1 and phase 2 detoxification processes
- This problem adversely affects the transformation of toxic chemicals into water-soluble products that can be normally eliminated from the body in urine and handled by the kidneys and liver; subsequently they remain in blood and are sequestered into body tissues
- Early intervention within the critical 3-year window of vulnerability of the developing nervous system is essential to change the trajectory of the spectrum

3-Phase Clinical ASD Program - Summary

- **Phase-I (DONE *)**: Demonstrated the role of environmental exposure and develop accurate biochemical tests that allow numerical assessment of the children with autism
- **Phase-II (DONE – Self Funded)**: Technologically demonstrated performance of Quantitative Dried Blood Spots that eliminate blood draw with syringe and needle
- **Phase-III (PROPOSAL - $3.8 M)**: Extend all discoveries, QDBS tests in a clinical study to demonstrate clinical utility under a safe, experiential therapy with Dr. Scott Faber's17 years of success; develop, validate and release a QDBS Kit for home blood collection and testing in a central laboratory; automate the entire laboratory workflow

** Funding provided by Heinz Endowments and Richard King Mellon Foundation.*

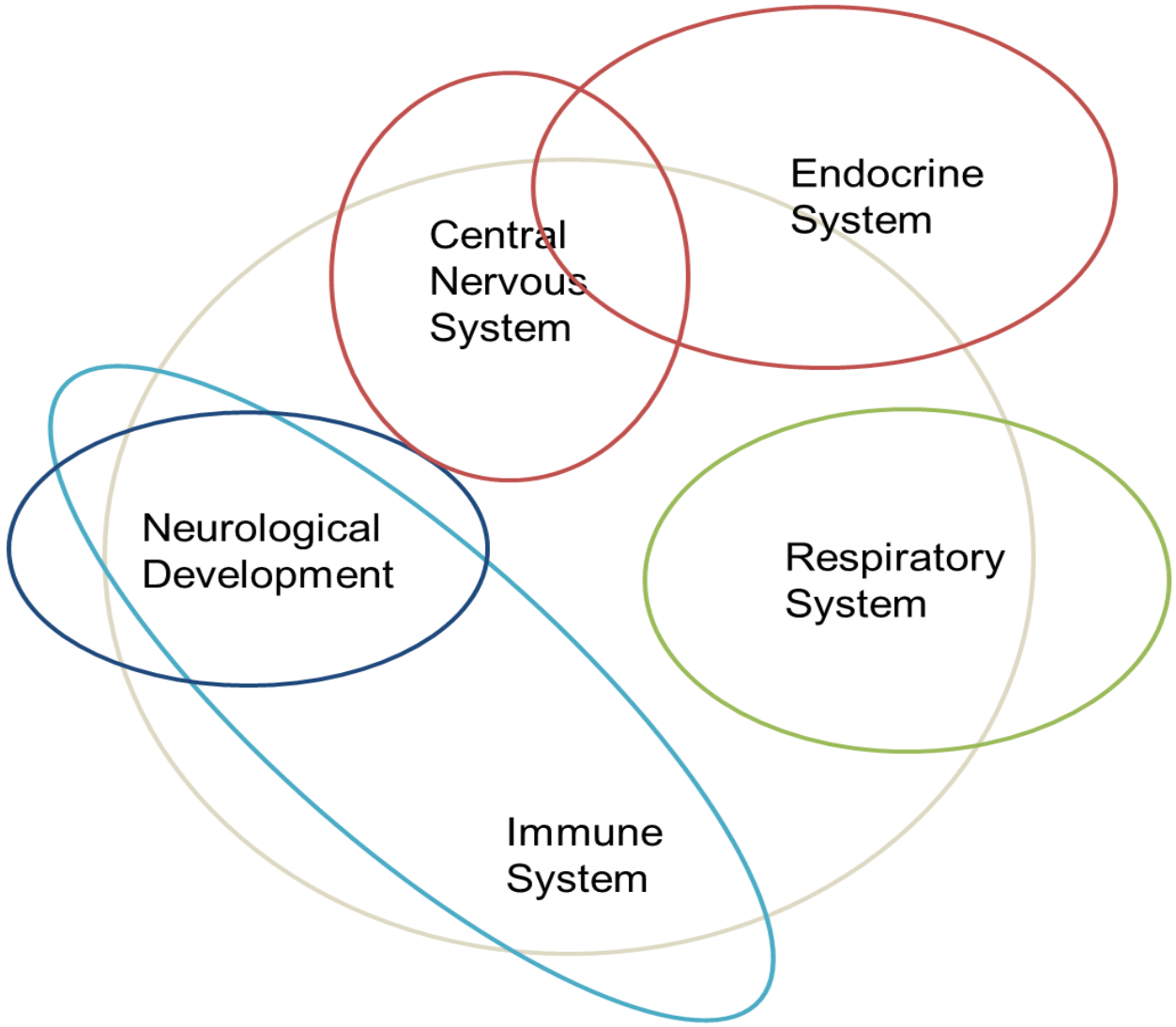
Overview - Scientific/Medical Foundation

- In 2011 a team of multidisciplinary scientists and medical professionals conducted a "Phase-I" double-blind, controlled study focused on Autism
- The team aimed to understand the impact of environmental exposure as a factor in autism progression
- Realizing there were no biomarker tests for autism assessment, the team developed new advanced test that would yield reliable, clinically acceptable and actionable data
- Using these tests, toxins in the blood and hair of children with autism and controls were quantified along with correlations with autism severity
- After the Phase-I study, the Team developed Quantitative Dried Blood Tests (QDBS) to overcome expensive and painful venous blood draw – an insurmountable barrier

Findings and Conclusions of our Phase-I Study

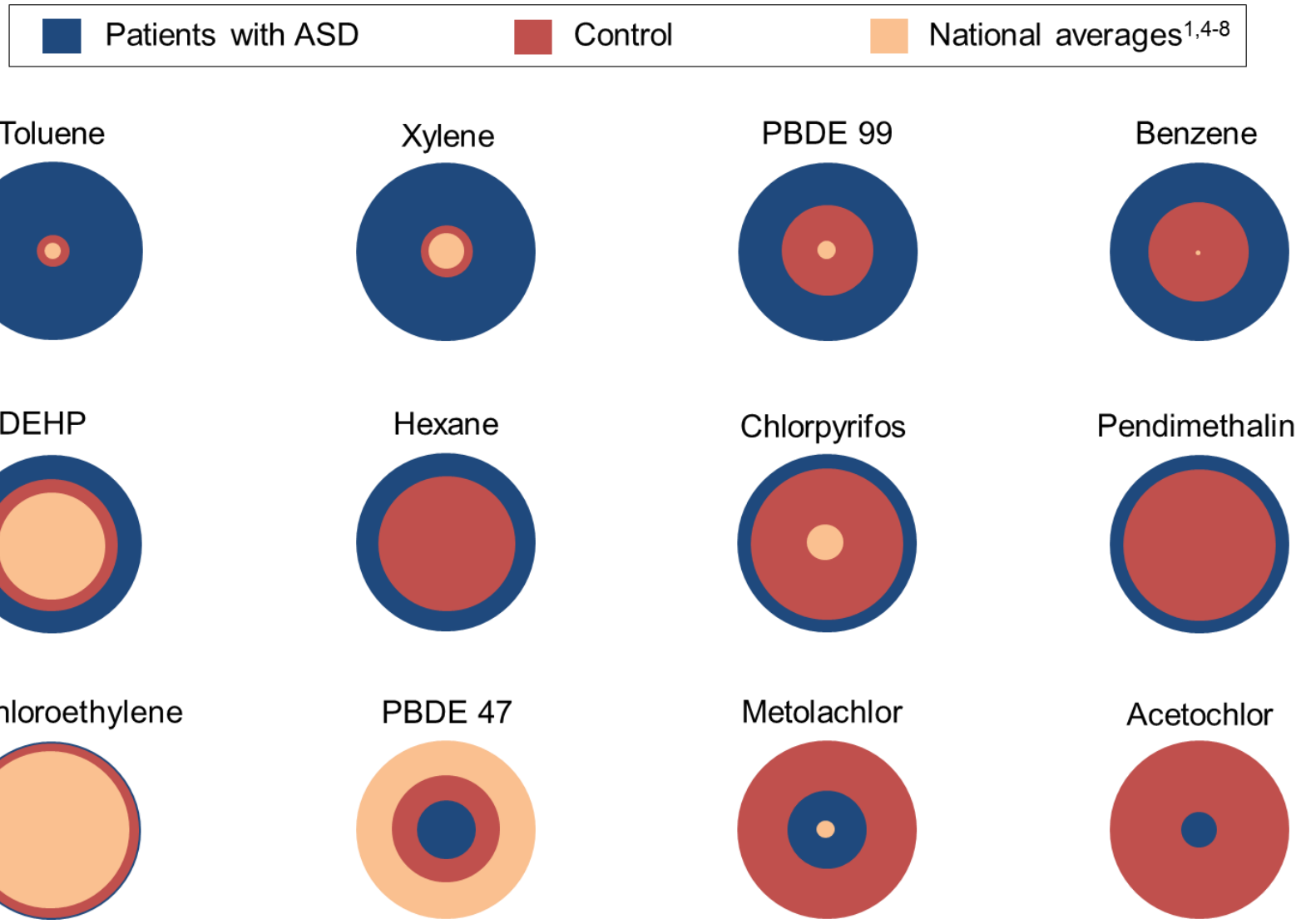
- The most compelling finding of our Phase-I blinded study was the unambiguous identification of a sub-set of autistic children whose improvements were independently revealed by the biomarker tests
- All findings were confirmed at the end of the study when samples were matched with children
- Biochemically established a greater metallothionein dysfunction, chemical detoxification disturbances, immune dysregulation and subsequent loss of immune tolerance
- Unique biomarker tests supported the presence of compensatory physiology in ASD children exposed to higher heavy metal and chemical stress
- Children living in more polluted regions had significantly higher aromatic pollutants, such as benzene, toluene and xylene
- Cumulative concentration of these toxins tracked closely with severity of autism when correlated with the standard ADOS results

Health Impact of Volatile Organic Pollutants Tested in Phase-I Study



- Automobile Exhaust
- Solvents
- Flame Retardants (PBDE)
- Aroclors (PCB)
- Chlorinated Pesticides
- Phthalate (plasticizers)
- Organophosphate Pesticides, Herbacides
- Toxic Metals (Pb, Hg)

Population Comparison of Average Measured Concentration Blood Toxicants Phase-I Study



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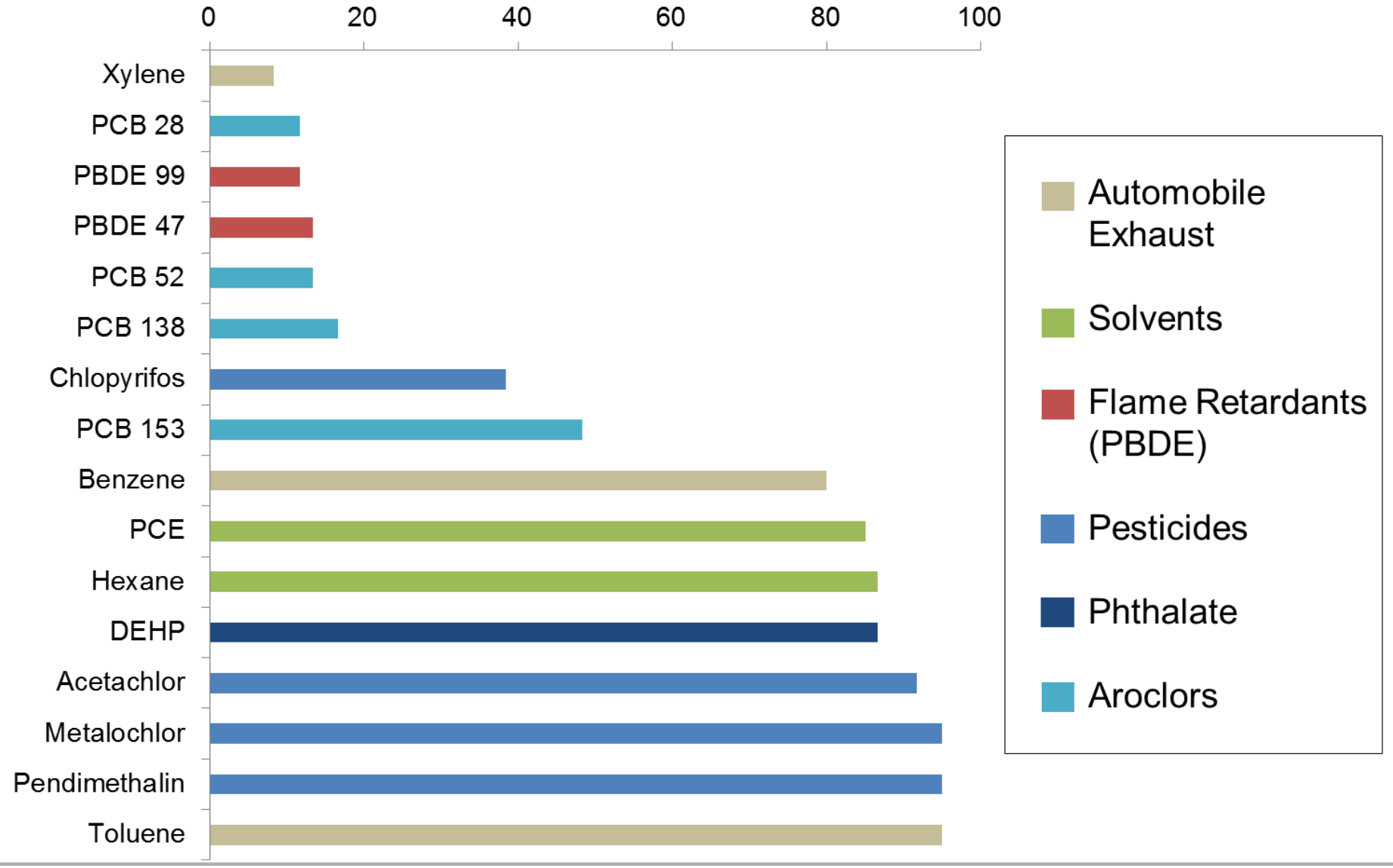
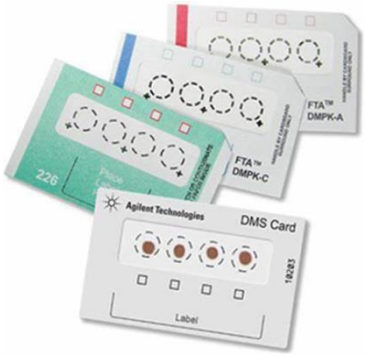
Percentage of Tested Children in Phase-I Study with Measured Levels of Each Volatile Organic Compound



Knocking Down Barriers to Accessible Testing



- Realized early there were no reliable biochemical tests
  - Invented and developed new, advanced, accurate tests
  - Automated much of the laboratory workflow
  - Published our methods, data and medical findings in peer-reviewed scientific journals, resulting in a dozen papers
- Removed the biggest barrier: blood draw by a phlebotomist
  - Painful, invasive, wasteful, expensive
  - Incredibly difficult to draw blood from autistic children
  - Must be refrigerated, must be packed and shipped in dry-ice
- Developed Quantitative Dried Blood Spots (10-yr effort)
  - Minimally invasive, simple to use, inexpensive
  - Requires only a drop of blood per spot – can be collected at home
  - Multiple tests can be done from a single spot
  - Dried blood on DBS cards are not considered bio-hazardous
  - After drop-collection, DBS cards can be sent by regular mail
  - Stored at room temperature and archived for look-back studies
- Regulatory: All tests are CLIA-waived (no FDA approval needed)

Testing with Dried Blood Spot (QDBS) Cards

At-home collection of a single-drop of blood on a QDBS card and sending from anywhere in the world by regular mail

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THANK YOU

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