Technologies and Applications

Background

AIT is a privately-funded company that developed an advanced, globally patented measurement Platform that incorporates transformational technologies *delivering significant improvements in sample preparation, automation and analysis.* The Platform *provides the most precise and accurate process* to measure vitally important toxins, organic and inorganic molecules, protein biomarkers and other compounds that are currently cannot be accurately detected and analyzed.

The Platform utilizes a universally versatile, advanced detector called mass spectrometer (MS) that can detect and measure virtually any sample: liquid, solid or gas. Potential applications of the MS in many fields, such as medical/clinical testing, drug development and other human health areas have been realized but the *MS limitations curtailed its wider adoption.* Enabling technologies of *the Platform that overcome these MS limitations are*:

1. **MS Operating System with D-SID[1]:** This technology enables unprecedented accuracy and precision, and facilitates development of endless number applications on the MS in all fields where critical testing performance is required. This is similar to PC programs, such as word processing and spreadsheet applications that cannot be used without the PC-OS like Windows. Tests already developed or targeted by AIT utilize the OS.

   D-SID has been recognized or adopted as a standard test by government agencies in the US, Canada and EU as the most accurate technology that is capable of delivering the highest level of accuracy and precision at the lowest detectable levels (highest sensitivity) with a mass spectrometer. The US EPA has codified AIT's technology as a national method in 2008 (designated as Method 6800) and expanded in 2013. The US NIST has relied on Method 6800 to assign true value to its Standard Reference Material No.2701. Canadian Ministry of Environment adopted D-SID for routine toxin testing throughout in Canada and published scientific papers with AIT scientists.

   Various government agencies and internationally respected organizations have confirmed that data produced by D-SID are "Definitive, Actionable and Legally-Defensible." These are three of the highest attributes that can be bestowed on a measurement technology.

2. **Advanced MS Measurement with Thor's Hammer**: This technology is the most powerful magnifier in the bioresearch laboratory: it opens a new world of biomarker discovery similar to Webb Telescope that allows discovery of individual stars that are not visible from earth. Thor's Hammer lowers the Lowest Limit Of Quantitation (LLOQ) of the MS by one to two orders of magnitude (10-100 times lower). There is huge potential for this technology as it will expand biomedical knowledge and lead to new therapies and testing. AIT plans to offer kits for bioscience researchers under a license requiring license fees and royalties from each discovery.

3. **Automation with Improved Solid-Phase Testing:** Almost all analytical detectors require some sample preparation steps. It has been long known that analytical testing process based on solid-phase (SP) versus liquid-phase technique is highly beneficial for many important reasons. SP methods provide shortened, streamlined, less expensive sample preparation and minimize sample losses that occur during the process. A key enabling aspect of this technology is the use of unique isotopic markers that are covalently or physically held in place using every kind of nonporous or porous solid phase material. The claims of this recent patent include D-SID enablement and thereby extending the Platform's total exclusivity for additional years.

4. **Direct Patient Access to Testing with QDBS[2]**: Promising future of Dried Blood Spot cards in sampling and testing for human health and disease has been anticipated merely for its potential to reduce the volume of blood needed (0.3mL vs. 4,000 mL) for testing. However, it has only been used for simple screening tests because of wide variability of results and measurement errors. AIT has removed this barrier by eliminating DBS data variability and rendering them Quantitative. This is covered by one of AIT's newer patents with claims that include D-SID enablement, thereby extending the Platform's total exclusivity beyond 2040. The compelling global implication in the medical field is described in the ensuing pages.

The disruptive and transformational impact of the Platform will be rapid, lasting and sustainable in the life sciences sector, particularly in biomarker-based medical testing, biomarker discovery and new drug development. Health and wellness assessment for environmental exposure will provide additional revenue growth as total body toxin testing, especially child-bearing-age women, and continue to be the foundation of all other testing.

[1] D-ID – Direct Speciated Isotope Dilution, an invention that enables significantly higher accurate/precise testing in the MS.

[2] QDBS – Quantitative Dried Blood Spots has porous layer encased in a card with holes on top to place the blood drop.

Applications in Healthcare: Biomarker-based Testing

Proteins are functional units in humans. Changes in their shape, molecular form or concentration reveal real-time condition of a person; therefore proteins are one of the most important indicators, or biomarkers, of health and disease. So far, measurement of blood protein biomarkers has been nearly impossible task due to two reasons. First reason is low abundance: more than more than 97% of human proteins known to be exist in such low concentrations that they are beyond limits of current detection/measurement tools and methods. For example, of the 50,000 to 100,000 proteins that are known to be present in the body less than 3,000 have been characterized[3]. So, more than 95% of known, observed or theorized blood proteins are yet to be measured because their concentrations are well below the limit of existing MS detectors.

The second reason is the current method of quantitation based on external calibration is non-optimal for the MS, as the method is prone to too many analytical errors that grow larger -and unsuitable- as concentration of the target biomarkers (i.e. 95% of all plasma proteins) becomes smaller. D-SID and Thor's Hammer technologies do not require any internal or external calibration for quantitation; they are completely different.

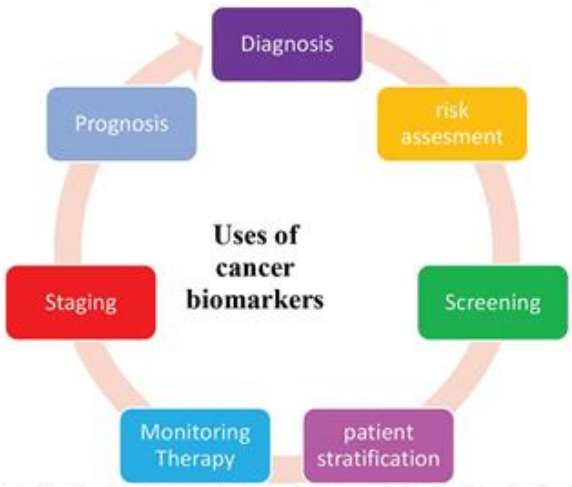
There is a long list of potential application opportunities in the life sciences sector where AIT's Platform will address critical needs. Potential for rapid, sustainable revenue and margin growth is unequalled.

Chemotherapy (CT), the gold standard in cancer treatment. This preference in the oncology field is expected to continue in the foreseeable future. Testing cancer patients represents the biggest opportunity with the shortest path to revenue generation because of the urgency and potential to extend lives.

Cancer Application: Stages in Chemotherapy where Biomarker Testing can make a difference

Chemotherapy (CT), the most widely used anti-cancer therapy, is an aggressive regimen. Today, nearly 60% of all cancer therapies involve CT which is projected to increase 50% by 2040[4]. Despite its popularity, adverse reactions are common because each person's tolerance to CT is different. A patient's intolerance to CT is sometimes inherent or resistance may be developed during therapy that is administered in sessions over weeks.

A British study[5] investigating post-CT deaths reported that (i) CT hastened death of 27% of cancer patients, (ii) post-CT, 25% of patients died within 30 days, (iii) 43% of patients suffered significant CT-related toxicity. A more recent study[6] found improvements in 30-day mortality with respect to breast and lung cancers. Overall, there are still tens of thousands of cancer patients who die within six months after CT, suffering from ailments associated with previous chemo and radiation therapy. Cancer patients would benefit from predictive testing, monitoring and prognostic biomarker testing.



There is no predictive way to know how each patient will tolerate CT before treatment. In addition, it is impossible to assess between sessions if a patient receiving CT is developing resistance and subsequently may suffer serious adverse effects, such as damage to the immune system and organs. Currently, physicians select and administer chemo-drugs, primarily by following guidelines and flow-charts based on accumulated data on the particular type of cancer of the patient.

Today, there is no clinical test that can predict how patients will tolerate CT, or monitor in real time their ability to withstand CT during therapy or assess post-CT their survival (prognosis). Each and every time, lack of reliable protein measurement tests has been pointed out by oncology publications as the biggest barrier to more effective, personalized chemotherapy.

[3] According to Human Plasma Proteome Database, of the estimated 20,043 proteins, only 10,500 were detected by the participating 18 labs, and of those, only 1,278 have been measured. That is less 3% of the 50,000 proteins estimated to exist in humans by leading research scientists.

[4] https://archive.cancerworld.net/news/more-than-50-percent-rise-in-chemotherapy-demand-by-2040/

[5] http://www.pharmatimes.com/news/chemotherapy_causes_death_in_more_than_25_of_cancer_patients_986391

[6] https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(16)30383-7/fulltext

After years of research and consultation with experts, AIT developed a list of well-characterized protein biomarkers that provide general or cancer specific tests from diagnosis to prognosis. Biological, medical and molecular characteristics of AIT's biomarkers are thoroughly studied and mechanisms of action in cancer are unambiguously demonstrated. All of that information is stored in publicly available databases and portals.

A critical protein, ERCC1[7], has been extensively studied by the oncologists over the last 3 decades and its potential has been articulated in publications. ERCC1 is a critical protein that takes part in the repair of DNA when it is damaged by the CT agent and therefore is a potentially reliable companion test for chemotherapy.

The other is a thiol-containing small blood bio-molecule, GSH[8] and its oxidized variant, GSSH that play key roles in regulation of the immune system, cell signaling. GSH-GSSG levels in blood have been studied and identified as a marker for chemo-resistance because of the ability of GSH to conjugate with the chemo-drug and transport into the cells. GSH and GSSG were thought to be impossible to measure until AIT has invented the first accurate GSH-GSSG test, validated it in a clinical study and published scientific papers that showed the association between chronic exposure to toxins and progression/severity of autism. [9]

Cancer testing for ERRC1 and GSH-GSSG will be a paired tandem-test that will be available as a QDBS kit for the hospital, doctor's office or home. Eventually, it will become a screening test sold in pharmacies.

In collaboration with a highly experienced scientist at Hillman Cancer Institute of University of Pittsburgh Medical Center (Pittsburgh, PA), cancer testing for the *first clinical trial was selected as ovarian cancer* where more 70% of the time, diagnosis occurs at stages III or IV when cancer has already metastasized and spread substantially. Ovarian cancer is the second deadliest cancer for women in the US. Less than 50% of patients will survive beyond 5 years after chemotherapy and 80% of the time, women experience a relapse.

Other Disease-specific Testing

There are more than a dozen diseases and novel payload delivery drugs where AIT's Platform will provide high-impact biomarker-based clinical testing. AIT's target list contains diseases that include dozens of well-characterized proteins with detailed understanding and demonstration of their mechanisms of action. Among them are inborn errors of metabolism, such as Lysozyme Storage Disorders (45 different conditions), neurotransmitters in cerebral spinal fluid (CSF) that affect the brain and neurological pathways, diseases associated with central nervous system (CNS), intra-cellular protein biomarkers, such as metallothioneins that regulate important cellular functions/signaling and proteins that are involved in the repair of DNA when it is damaged due to toxin exposure or chemotherapy treatment. AIT's list of disease candidates will grow as more proteins are discovered, measured and characterized and new discoveries are made, particularly by researchers who will use testing protocols using D-SID and Thor's Hammer.

Patient Equity through Wide Access to Testing:

AIT has developed a solid-phase technology using Quantitative Dry Blood Spots (QDBS) that produce data quality suitable for clinical testing. Presently, the factor that limits wider patient access to medical testing is the painful, invasive blood withdrawal with a needle and syringe. Blood must be drawn in special 6,000 mL tubes, today, by a phlebotomist at a hospital or clinic near a testing lab. These tubes have short shelf lives: a couple of weeks if refrigerated, 6-8 weeks if frozen. Many difficulties, transportation regulations – especially cross-borders, high cost of shipment to a distant location, high cost, fear of needle by a large portion of the public curtail wider acceptance and use of medical testing.

The most attractive feature of the Platform-enabled MS in metabolomics, proteomics, translational medicine and clinical diagnostics is that only micro-volume is sufficient for testing in most cases. This means a drop of blood from finger-stick will be adequate for most applications.

The QDBS, the sample carrier, has an absorbent filter with rapid wicking properties, and is encased in a sealed card. Its porous structure has AIT's unique molecular codes that combine with blood (typically about 0.03 mL, or 30

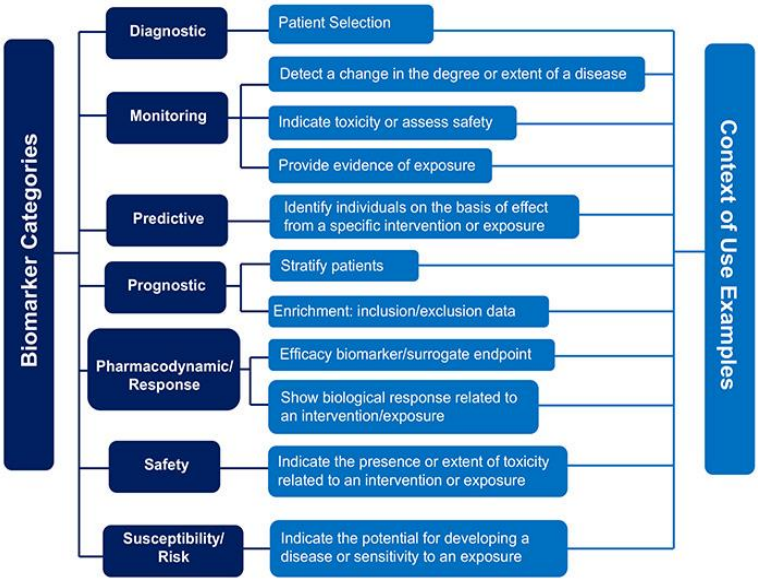
[7] ERCC1 – Excision Repair Cross Complementation 1: a protein which universally accepted by the scientists as a top cancer-testing biomarker when a reliable protein measurement test becomes available.
[8] GSH – Glutathione: a small but very important bio molecule inside and outside of cells, made of 3 amino acids,
[9] Two AIT patents include claims related to GSH-GSSH.

microliters), when applied by the patient after a simple finger prick. Dried blood on the QDBS is not considered bio-hazardous and therefore it can be sent inexpensively in an ordinary envelope via ordinary mail from anywhere in the world.

AIT's QDBS kits will be widely accessible and can be used by the patient at any geographical location. The QDBS cards can be stored at room temperature in a cabinet inexpensively for a long time. Archived cards can be used in look-back[10] testing to apply a newer biomarkers-test that becomes available in the future.

Biomarker Applications for Drug Development and Clinical Assessment

The following chart is based on BEST (Biomarkers, EndpointS and other Tools) – a glossary that capture distinctions between biomarkers and clinical assessments (https://www.ncbi.nlm.nih.gov/books/NBK326791/) and summarized by the US FDA as examples in contextual relevance[11]."



Susceptibility/risk Biomarker: indicates the potential for developing a disease or medical condition in an individual

Diagnostic Biomarker: detects or confirms presence of a disease or condition of interest

Monitoring Biomarker: serially measure to assess status of a disease or medical condition

Prognostic Biomarker: identify likelihood of a clinical event, disease recurrence or progression

Predictive Biomarker: identify individuals who are more likely to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent.

Pharmacodynamic/response Biomarker: show that a biological response has occurred due to a medical product or an environmental agent.

Safety Biomarker: measure before and after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity adversely.

The value of biomarker-testing for drug development has been proven in the pharmaceutical industry using qualitative and quasi-quantitative staining techniques that helped shorten development time for a class of oncology drugs. The US FDA is actively encourages use of biomarkers for drug development and, post-approval, for patient testing.

Drug development business will be developed through strategic partnerships with pharmaceutical companies under an exclusive license for each particular drug, generating license fees and royalties. AIT's Platform will be equally valuable for testing in most of the 7 categories identified by the FDA in the figure above.

Industrial and Regulatory

AIT's Platform has been used in many industrial and regulatory applications. AIT collaborated with Chevron and King Abdullah University, by applying its Platform to analyze analytically-challenging mercury species in crude oil. A scientific paper, co-authored and published with Chevron scientists, remains as a benchmark papers in the literature. In an ongoing collaboration, AIT used its Platform-enabled tests to analyze commercially valuable catalysts produced by ExxonMobil and certify compliance to EU regulations. A manuscript is being prepared for publication by Prof. Skip Kingston, AIT's co-founder, and ExxonMobil scientists. Pepsi Company collaboration was another example where AIT's platform has been applied to analyze an ingredient critical for preferred taste in the company's products. A scientific paper has been jointly prepared and published by PepsiCo scientists.

[10] Potentially, look-back studies can also provide valuable epidemiological data.
[11] FDA link: *https://www.fda.gov/drugs/biomarker-qualification-program/context-use*

Some foods and a number of supplements and nutraceuticals (over-the-counter drugs) contain harmful toxins. A large number of wellness pills and herbal remedies contain dozens of toxins that adversely affect humans even at very low concentrations. Multiple toxins are frequently found in daily ingested supplements can cause long-lasting health problems or permanent damage because together their action may be amplified as a "combinatorial" reaction. The potential for multi-generational adverse impact from multi-toxin chronic burden is a serious health concern that is not yet thoroughly investigated.

For over a decade, AIT has been active in creating and using its tests to assist all stakeholders, such as researchers, companies and regulators. At the federal level, AIT's co-founder and a leading scientist in the environmental field, Prof. Skip Kingston, created several methods that were codified as national methods by the US EPA and adopted by NIST and CDC. At the state level, AIT tested supplements for toxins sold for babies, kids and pregnant mothers on behalf of one of the US state attorneys general. As a result, several contaminated products were either pulled out of shelves or re-formulated. AIT worked with several supplement manufacturers to help improve their manufacturing and quality standards. Two of these companies, Kirkman Labs and Herbal Sciences, have either directly benefited from or avoided lawsuits because of the tests done and the advice provided to them by AIT. The importance of this application cannot be stressed, simply because there are no serious regulatory health safety standards or enforcement tools in this sector.

Environmental Testing and Compliance Monitoring

AIT and co-founder, Prof. Skip Kingston have spent more than a decade in actively participating with tests and creating methods based on its D-SID technology and published dozens of scientific papers. The US EPA has codified AIT's technology as a national method in 2008 (designated as Method 6800) and expanded in 2013. The US NIST has relied on Method 6800 to assign value to its Standard Reference Material No.2701. Canadian Ministry of Environment adopted D-SID for routine toxin testing throughout its labs in Canada.

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AIT assisted compliance testing for the EPA and state agencies. Scientific publications, presentations, lectures and one-on-one discussions with policymakers helped formulate policies governing the environment and public safety.

Homeland Defense – Actionable tests

Under a grant-award from the US Department of Defense, AIT applied its Platform to accurately measure in all five CBRNE categories to produce "actionable" data to support armed forces in the battlefield and at the home front. The classified 400 page confidential report has won for its excellence by the surgeon general of the US Air Force.

The strategic importance of this endeavor was to demonstrate the universal applicability of AIT technologies by accurately measuring fugitive agents in all five CBRNE categories of interest to the Department of Defense: Chemical (a couple of nerve agents), Biological (Yersinia Pestis, known as the Plague), Radiological (post-event testing), Nuclear (post-event testing) and Explosive (classified). The report includes conceptual testing framework to support in the theatre by a mobile lab or casualty assessment and management.

Summary

AIT has created a unique measurement Platform that integrated patented technologies that address critical needs in several sectors. AIT's testing and delivery solutions will be disruptive and transformational in each targeted area.

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