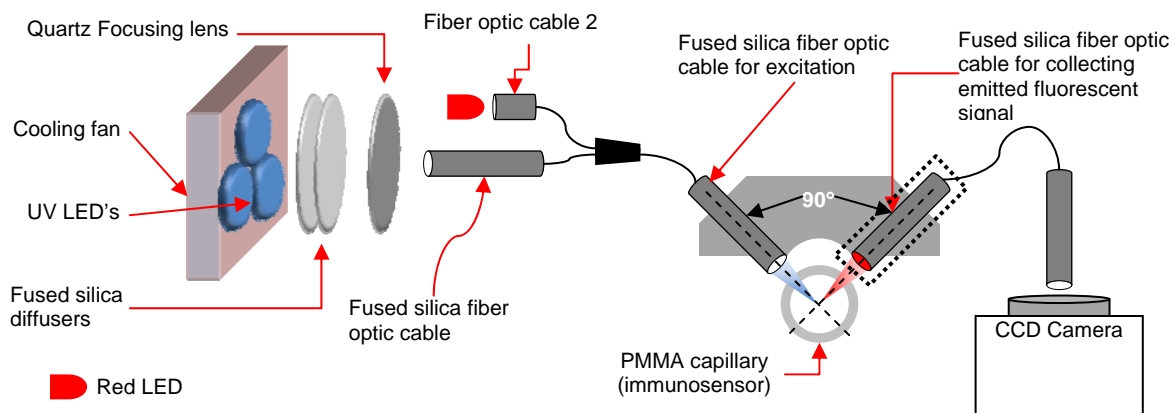




Quantum Dot ELISA Test (QLISA)



Executive Summary

1. Summary

The QLISA technology is a new *in vitro* diagnostic test for highly sensitive detection of inflammatory biomarkers and differentiation of IBD vs. IBS. QLISA uses quantum dots to measure inflammatory biomarkers and represents a superior alternative to ELISA technique by requiring much smaller sample volumes and simpler assay protocol while preserving ELISA's sensitivity. These unique features translate into significant practical advantages of using QLISA as a platform for *in vitro* diagnostics applications. The initial application of QLISA being developed at Drexel is a diagnostic test discriminating between IBS (irritable bowel syndrome) and IBD (irritable bowel disease). As many as 4 million people worldwide (1 million Americans) suffer from a form of IBD and more than 15 million have IBS, with estimated medical costs in the USA at over \$2 billion/yr when adjusted for loss of productivity. IBD refers to two chronic diseases of intestinal inflammation: ulcerative colitis and Crohn's disease. Diagnosing these diseases is an expensive and lengthy process requiring knowledge of medical history, blood tests, X-rays, endoscopy, tissue histopathology and testing of stool samples. As a result, early differentiation of IBS vs. IBD is difficult, which leads to unnecessary excessive testing of IBS patients and significant delays in much needed treatment of IBD patients. Thus, there is a critical need to develop a rapid, low cost, reliable diagnostic test to differentiate common intestinal symptoms of IBS from IBD, to monitor remission and relapses of IBD, and to evaluate success of or need to change medication.

The QLISA technology circumvents issues related to sampling and is envisioned for use in a clinical lab environment as a platform for a broad variety of *in vitro* diagnostic (IVD) tests, including IBD vs. IBS. It can also be used as a research tool in animal experiments and forensic setting where sample size is small. The QLISA Technology is available for licensing from Drexel University. See Paragraph 8 for contact information.

2. Product

What unmet medical need does the product address?

Differentiating IBD from IBS is critical not only for immediate selection of appropriate treatment but also for monitoring the future health of the patient. Specifically, ulcerative Colitis (UC), a form of IBD which affects the inner lining of colon, can progress to colorectal cancer. Compared to the general population, patients diagnosed with UC are at a higher risk for colorectal cancer, with increasing risk by 1% per year since the onset of the disease. A recent study indicates that patients diagnosed with Crohn's disease (CD), another form of IBD which can affect any part of the gastrointestinal tract, are equally at risk for colorectal cancer. IBS on the other hand, does not cause inflammation, does not lead to cancer, nor does it require frequent hospitalization and surgery. However, the symptoms of IBS and IBD are so similar that it is possible to misdiagnose IBS for IBD without using expensive, mostly invasive procedures. Conclusive assessment of IBD versus IBS may necessitate tests such as colonoscopy, barium enema, upper endoscopy, X-ray and sigmoidoscopy and usually takes many months to complete. These assessments with the exception of X-Ray are mostly invasive. There is, therefore, an unmet need for a technology to differentiate IBD from IBS in a cost effective and non-invasive manner at early stages of patient evaluation.

Presence of lactoferrin (Lf) and myeloperoxidase (MPO), another inflammatory marker, and their ratio has been shown to be sufficient in differentiating IBD from infectious diarrhea. Thus early detection of MPO would facilitate in differentiating IBD from IBS and also IBD from infectious diarrhea. Currently the best available method for Myeloperoxidase detection in stool samples is radioactive labeling. This method is time consuming, requires expensive labeling facilities and could expose patients to radiation risks. Radioactive labeling was used in a 2006 study of MPO to detect inflammation in IBS and collagenous colitis as the only reliable quantitative assay. This latest publication proves the need for a user friendly, fast and inexpensive detection assay platform. Enzyme Linked ImmunoSorbent Assay (ELISA), a widely popular method for detecting an antibody or antigen in a sample, has thus remained the most prevalent technique for detection of MPO when radioactive labeling is not practical. ELISA, though meets the sensitivity requirements for detecting MPO and Lf in the case of IBS/IBD differentiation, has limitations in several other fronts such as sample volume requirements, lengthy protocol, and short life time of the chromophore that is used to estimate the concentration of the analyte. Typically, ELISA requires at least 50-100 μ L of sample per well, along with standards and other reagents. The life time of the chromophore used in ELISA lasts merely 15 – 30 min, so the data must be collected within the first few minutes to ensure reliability.

What Technology is the Product based on?

The proposed product is an accurate, reliable, low-cost diagnostic kit capable of differentiating IBS from IBD based on Myeloperoxidase (MPO) and lactoferrin detection in stools. These inflammatory markers are over expressed in IBD, but not in IBS.

QLISA is a microcapillary sensor capable of detecting multiple antigens, based on the superior fluorescence of quantum dots (QDs) and a sandwich immunoassay on a specialty substrate. It can detect what is currently detected by ELISA using QDs, hence the name QLISA (Quantum dot Linked ImmunoSorbent Assay). It circumvents the limitations of ELISA while being comparable in sensitivity to ELISA.

1. QLISA utilizes pre-fabricated functionalized PMMA capillary substrates for capturing the antigen of interest.
2. QLISA Utilizes antibodies conjugated to fluorescent nanoparticles (quantum dots, Qdots) for detection and quantitation of the antigen.
3. The life time of Qdot (chromophore in QLISA) at the end of the assay is far greater than chromophores used in ELISA.
4. QLISA requires only 1 to 5 microL of sample including triplicates to achieve equivalent sensitivity level with ELISA.
5. An innovative way of calibration allows use of far less samples to determine a standard curve (very few dilutions are necessary). This further reduces the test costs and increases probability for immediate adoption.
6. Due to the small volume requirements the test can be done from trace amounts of stool rather than a standard stool sample and therefore routinely test the biomarkers, the response to treatment, any relapses etc.

It is the outstanding sensitivity in a small sample volume that make the product highly suitable for IVD tests such as IBS vs. IBD being developed at Drexel.

What Intellectual Property Rights protect the Technology?

QLISA is protected by two pending patent applications: a) US Patent Application 12/307,901: "Methods of quantitatively assessing inflammation with biosensing nanoparticles", related to Drexel's invention disclosure 06-637D and b) US Provisional Patent Application 61/146,928: "Apparatus and Methods of Detecting Inflammation Using Quantum Dots", described in Drexel's invention disclosure 07-0841D. Several new invention disclosures related to this technology have been submitted to Drexel's Office of Technology Commercialization.

3. Market Size and Potential

Both IBD and IBS present with overlapping and similar symptoms, and their conclusive differentiation is based largely on clinical signs and symptoms aided by expensive radiologic or endoscopic procedures. Both populations of patients experience significant reduction in their quality of life. In 2004 alone there were approximately 3.1 million ambulatory care visits directly associated with both medical conditions (9). The number of such patients is expected to increase annually. It is estimated that there are approximately 15 million patients with IBS in the US. The estimate of prevalence for IBD is between 1-1.5 million patients in the US. In the 7 major economic markets, the estimated total IBD and IBS population is 90 million people. Excluding IBD at an early stage without recourse to invasive and expensive endoscopies would help patients avoid intrusive tests and would save millions of dollars in health care costs.

The financial costs associated (direct and indirect) with IBS are in excess of \$200B annually in the US. For Crohn's Disease alone in 2004, it is estimated that approximately \$512M were spent for the non-treatment aspects of the disease (i.e. office visits, endoscopy, lab, pathology and radiology). Even a modest improvement in providing better diagnostic ability for IBS will lead to realizable medical savings and improve the quality of care that a patient receives.

4. Competitive Landscape and Advantage

Competitive ELISA technologies: BMA Biomedical: Calprotectin ELISA kit (\$720.00), Calpro: PhiCal - Calprotectin in stool extracts (Genova Diagnostics), Hycult Biotechnology: Human lactoferrin ELISA kit (\$870.00) and Human calprotectin ELISA kit (\$870.00), TechLab: IBDScreen - fecal lactoferrin (\$834.52).

What are the advantages of this technology?

Main factors that set QLISA apart from ELISA is the very small sample volume, test simplicity, speed, efficiency and reproducibility: 1.5 microL of sample is sufficient to detect 100 pMolar MPO in stool extract in the case of QLISA. ELISA requires 100 microL of stool extract at a sensitivity of 1-60 pMolar MPO in stool extract. This translates to cost savings in reagents as well as ability to use samples from rectal swab tests as opposed to full stool samples. Rectal swab samples can be obtained by gastroenterologist during manual rectal exam routinely performed during regular office visits when patient presents typical IBS/IBD symptoms. Conventional ELISA method for detecting MPO requires 12 hours processing time while QLISA requires only 2 hours and capability to reduce processing time to 30 min per sample. Unlike ELISA's complex multistep process, QLISA is a 2 step process.

Multiple innovations have been implemented in developing the QLISA protocol and prototype. These include: a) prefabricated substrates where antigens or antibodies of interest are covalently attached. This procedure increases the reliability of the protocol, its specificity and sensitivity and makes it possible to mass produce substrates that can be stored and used/distributed on demand. b) Use of fluorescent semiconductor nanoparticles that are conjugated to an antibody that binds specifically to the target biomolecule. Conventional methods require several dilutions of samples along with standards to estimate the concentration of the analytes, while the QLISA approach would require less dilution standards, saving time and reagent costs. The proposed prototype will utilize 'off-the-shelf' components that can be readily assembled requiring custom software development for automation, data acquisition and reporting. The technology is currently being validated using Invitrogen's Quantum Dots. However, fluorescent dots from other manufacturers could also be used. The assay is not relying on a particular QD to work.

5. Regulatory

The FDA will approve the QLISA assay in the same regulatory path as other clinical lab tests.

6. Development status

The following studies were completed so far: 1) Specificity of the assay using standard and animal samples from an experimental model of IBD. The results show negligible interference from other irrelevant biomolecules in the stool extract. 2) Detection limit of the assay: 100 pMolar of MPO in animal samples. 3) Device Sensitivity: 10 pM, more than adequate to determine the concentration of MPO in biological samples. 4) Implementation of low cost UV source (300 mW LED) to excite quantum dots. A research article with key findings of the experiments with animal samples of control and experimental IBD animal model was published and showed that QLISA is capable of differential diagnosis of IBS from IBD, reliably and reproducibly.

7. The Team

Elisabeth S. Papazoglou, Ph.D. is an Assistant Professor at the School of Biomedical Engineering at Drexel University and her research focuses on biomedical applications of nanotechnology. Her projects include nanobiosensor development as in QLISA or in using carbon nanopipettes as cellular electrodes and gold-nanoparticle assemblies to delay HIV infection. She has extensive experience in industry in product development of new materials and processes for pharmaceutical and cosmetic applications. In 2008 Dr. Papazoglou won the Drexel Translational Award for innovations with translational potential.

Sreekant Murthy, Ph.D. is a professor in the department internal medicine, Drexel University College of Medicine and Adjunct Professor of Biomedical Engineering, School of Biomedical Engineering, Drexel University. He is also vice provost for research compliance at Drexel University. In 1964, he attended Central College, Bangalore and received B.Sc., from University of Mysore. In 1968, he attended Govt. College of Pharmacy, Bangalore and received B. Pharmacy Degree from Bangalore University. In 1973, he received Doctor of Philosophy Degree from the Philadelphia College of Pharmacy currently known as University of Sciences Philadelphia. Dr. Murthy's research interests are in the area of Gastroenterology with focus on inflammatory diseases particularly focusing on inflammatory bowel diseases (Crohn's disease and ulcerative colitis). He also serves on the editorial boards of journals; he is a peer reviewer for many journals and funding agencies and consults extensively with industry in their anti-inflammatory drug discovery programs.

Sundar Babu Nadarajan Ph.D. is research faculty at the School of Biomedical Engineering, Science and Health Systems, Drexel University. Dr. Babu received his Master of Science degree in Applied Chemistry, 1994 from Madurai Kamaraj University, Madurai, Tamil Nadu, India and Ph.D. in Materials Science in 2000 from University Malaya, Kuala Lumpur, Malaysia. His research interests include Nano-optical biosensors, electrochemical biosensors, surface enhanced Raman spectroscopy, drug delivery vehicle design, transdermal drug delivery, and microfluidics.

Dr. James Reynolds, M.D. is Professor of Medicine and the June F. Klinghoffer Distinguished Chairman of the Department of Medicine at Drexel University College of Medicine in Philadelphia. Dr. Reynolds graduated from Florida State University and received his medical degree from the University of Florida. He completed his residency at Cornell University and a three-year fellowship at the Hospital of the University of Pennsylvania. Dr. Reynolds is a member of the editorial board of Digestive Diseases and Sciences and is a reviewer for a large number of journals. His primary clinical interests are in the early detection and prevention of cancer, complications of gastroesophageal reflux and gastrointestinal motility disorders.

8. Further information and licensing inquiries

Drexel University's School of Biomedical Engineering, Science and Health Systems is an integral part and a driver of the regional economy. The focus of the School of Biomedical Engineering on translational research resulted in several ground breaking biomedical innovations. It is the goal of Drexel University to license those technologies either to established corporations or start-up companies to move those innovations from bench to bedside.

For licensing information please contact:

Alexey Melishchuk, PhD, Associate Director, Licensing
Office for Technology Commercialization
Drexel University
3225 Arch Street, Ground Floor
Philadelphia, PA 19104

Tel: 215-895-0304

Fax: 215-895-0310

Email: amelishchuk@drexel.edu