COMMERCIALIZATION OF MOLECULAR IMPRESSION POLYMERS

AS A DIAGNOSTIC KIT AT POINT OF CARE

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Commercialization of Molecular Impression Polymers As A Diagnostic Kit At Point Of Care

ABSTRACT

By

Sushmita Venkatraman

Molecular Impression Polymers MIPs have been widely implemented as analytical tools for biotechnology and more recently as biosensors, drugs, and bioassays. MIPs in modern times can serve as antibody mimics called as “plastic antibodies”, and this has been positively exploited by Infigo Diagnostics. The Company has developed a patented lateral flow based immunoassay, which embeds plastic antibodies in the place of natural antibodies and provides qualitative and quantitative information of an analyte present in a sample. Infigos’ technology can provide an alternative to existing immunoassays due to several advantages which include greater stability, faster preparation, reusability and better cost advantages when compared to natural antibodies used in conventional tests. However, the limited selectivity and sensitivity of a MIP, and problems associated with the preparation of a MIP has challenged its use as effective biosensors. Recent studies indicate that MIPs can be successfully used for the detection double stranded DNA as well as detect point mutations in a DNA strand. Infigo Diagnostic’s technology targets the “point of care” market and the use of the technology can help physicians implement effective treatment strategies and promote successful decision making.
Introduction to STEP

The Science and Technology Entrepreneurship Program in Biotechnology at Case Western Reserve University is a two year Master’s program focusing on the commercialization of biotechnologies. The course comprises of 4 core courses namely a two-part biotechnology innovation course offered in the first and second semester, a Technology Feasibility course offered in the first semester, and Technology Venture Creation course offered in the second semester. The courses are very challenging and are found to inculcate the basic of entrepreneurship and commercialization in students. There are two additional courses that are to be chosen by the student during or after the completion of the core courses. The courses chosen by me included “Principles of Managerial and Financial Accounting” and “Enterprise Development” both from the Weatherhead School of Management.

During the course of the program, students are encouraged to undertake an internship in a field of study, and most students including myself were provided with the opportunity to intern in a healthcare incubator or a start-up field. In my perspective, the experience adds tremendous value to our program, as students are able to gain exposure to real business situations. Students are encouraged to write grants, business plans, and conduct market and financial research depending on the company and internship requirements. In December 2009, I joined BioEnterprise as a Business Development Associate.

In October 2010, I was offered an internship opportunity as business/commercialization analyst at the Medical Device Development Center (MDDC) at Austen BioInnovation Institute at Akron, (ABIA) a center of excellence for biomedicine and healthcare focused on patient centered
innovation and commercialization. At MDDC, I am involved with the commercialization of medical device technologies in the Akron region. My work mainly involves the analysis of market, competition and intellectual property landscape for novel medical device ideas. This comprehensive data will support the path of commercialization for novel medical devices.

BioEnterprise is a Cleveland based nonprofit company that helps accelerate the growth of bioscience companies in the region. BioEnterprise is partnered with the Cleveland Clinic, Summa Health systems, University Hospitals, Case Western Reserve University and the BioInnovation Institute in Akron, all of which help and support BioEnterprise’s mission.

BioEnterprise is a Cleveland based nonprofit company that helps accelerate the growth of bioscience companies in the region. At BioEnterprise, I was engaged in many projects with the senior consulting team. These projects included gathering data for primary and secondary market research for the assessment of healthcare technologies in the region, preparation of due diligence market reports, business plan writing, SWOT, and intellectual property, competition and financial analysis for startup medical device companies in the region.

During the summer of 2010, I embarked on the Infigo project. The project required comprehensive primary market research to identify and guide Infigo’s marketing strategy. My interests and involvement with this project under the mentorship of Bob Johnson with the assistance of Glen Gaughan motivated me to dive deeper and choose this as my thesis topic.
**Infigo Diagnostics LLC**

Infigo’s platform technology is a lateral flow immunochromatography assay embedded with “Plastic Antibodies”, which can detect target analytes in blood, serum and urine, similar to a pregnancy test. The value proposition of the device lies in the fact that the technology embeds molecular impression polymers (MIPS) in the place of natural antibodies in a lateral flow format device that captures and detects the analyte of interest. The plastic antibodies are found to be less expensive, more stable and easier to prepare for novel biomolecules when compared to natural antibodies.

The Company is based in Israel and is seeking to enter the US market. For this purpose, the Company is seeking suitable partners, collaborators and investors. The initial market identified by the Company was the US Vitamin B12 deficiency market. The Company has one patent application (PCT/IL2007/000851) that has claimed the use of MIPS in the detection of range of analytes in a lateral flow immunochromatographic format.

The technology is seeking FDA approval and thus cannot be sold in the US market yet. Infigo is an investment by the TargetTech incubator in IL. The City of Akron’s investment in TargetTech, has encouraged Infigo to seek for establishment in the US. BioEnterprise provides market research and strategy support.

The company’s current status depicts the challenges a typical startup company faces during its development. The Company is currently seeking venture capital for the development of the MIP technology for the recently identified market.
Infigo’s technology comprises of a rapid, sensitive diagnostic device which can qualitatively and quantitatively measure a target molecule in urine, blood plasma, and serum. The technology follows the principle of a lateral flow immunochromatography assay and the device is incorporated with plastic antibodies in the place of natural antibodies. (similar looking to a pregnancy test). These plastic antibodies are also called as Molecular Impression Polymers, which can be designed to function as natural antibodies; by binding to a target analyte/antigen and by providing quantitative and qualitative information of the analyte in approximately 15 minutes. The binding of the analyte to the plastic antibodies embedded on the device is detected by visual inspection or by using an instrument, such as an appropriate reader or scanner, which detects the color or fluorescence from the readout area.¹

Molecular Impression Polymers, (MIPs) also known as plastic antibodies are synthetic receptors that can bind selectively to a target molecule (template).² On removal of the template they are able to retain a “memory” for the molecule, thereby acting as artificial antibodies.² Although imprints against small molecule organic templates have been generated for decades now, recent focus has been on the development of imprints for successful binding of proteins and biomolecules. The rapid and inexpensive generation of MIPS, mimicking the shape of the template and replacing antibodies in its binding capacity has seen enormous potential in the field of biotechnology for various applications.³ An overview of the immune system and MIPS are provided in the following sections to provide a better understanding of the concepts involved in the proposed technology.
I. Basic Overview of the Immune System

A. The Immune system- Definition and Types.

The body’s remarkable defense mechanism against the foreign invasion of microbes, germs, bacteria and viruses is collectively termed as the immune system. The immune system creates both innate and acquired immune responses. While the body’s attack on these foreign cells is beneficial, a balance should be maintained such that it does not destroy its own cells.

The innate immune system defends the host from various types of infection by recognizing the causative organisms in a non-specific manner and by destroying them. Lysozymes, mast cells, interferons, complement, acute phase proteins, collectins and toll like receptors mediate innate immunity.

On the other hand, adaptive immunity occurs in response to specific antigen exposure, i.e. it shows memory. This “immune memory” helps the body fight known organisms rapidly with ease. The specificity does not cause the organism to attack its own cells. Acquired immunity includes the function of two types of important cells namely the lymphocytes and cytokines.

When a foreign organism attacks the body, it essentially passes through three lines of defense. The first line of defense occurs at body surfaces comprising of skin surfaces, gastrointestinal tract, urogenital tract and mammary glands etc. The second line of defense comprises of phagocytotic cells such as eosoniphils, macrophages, basophils, and antimicrobial proteins (interferons) that are secreted when the first line of defense has been compromised. The third line of defense is triggered when the first two lines of defense fail, and constitute the action of lymphocytes and antibodies.
Immunity can be acquired by both active and passive microorganisms, and by natural and passive means. Antibodies can be passed on naturally from mother to offspring (natural passive), or can be passively injected into the body from another individual who possess specific immunity to the particular pathogen (artificially passive).

Immunity can be acquired naturally via organisms that attack the body; leaving the body susceptible to fight the same infection effectively the next time around (naturally active). Also, live or attenuated viruses can be injected into the body through vaccination. Vaccines contain microorganisms that produce exotoxins, inactivated by chemical treatment, but will retain its antigenicity to produce an immune response or a memory when injected into the body. (artificially active). Examples are diphtheria and tetanus vaccines.

**Phagocytosis:** is a process by which cells engulf microbes, either by recognizing sugar resides on the microbes or by binding with opsonins which are coated on the surface of the microbes (opsonisation). The cells invaginate around the microbe to form a phagosome and kill the microbe.
Cells of the immune system

The important cells of the immune system and their functions are depicted in Table 1.

Table 1: Important cells of the immune system and functions.⁵ (Table is Summary from Optomerists Immunological Basics)

<table>
<thead>
<tr>
<th>1) Phagocytes</th>
<th>Neutrophils are the most abundant type of white blood cells and are the first responders to infection.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Neutrophils - Principal phagocytic cell of innate immunity</td>
<td>Macrophages participate in both innate and acquired immunity. They phagocytose cellular debris and pathogens, either as stationary or as mobile cells.</td>
</tr>
<tr>
<td>B. Macrophages</td>
<td>Eosinophils are white blood cells that are known to combat multicellular parasites and certain infections in vertebrates. They are responsible for the control mechanisms associated with allergy and asthma, along with mast cells.</td>
</tr>
<tr>
<td>2) Eosinophil- Principal defender against parasites</td>
<td>Basophils appear during allergies. Basophils bind to immunoglobulin IgE, which is responsible for the eliciting the immune response to allergies.</td>
</tr>
<tr>
<td>3) Basophil- Similar to eosinophils and mast cells.</td>
<td>Monocytes are secreted in response to inflammation signals, move to site of infection quickly and differentiate into macrophages and dendritic cells.</td>
</tr>
<tr>
<td>4) Monocytes- Mononuclear phagocytic cells</td>
<td>Lymphocytes are found commonly in lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus.</td>
</tr>
<tr>
<td>5) Lymphocytes</td>
<td>A. B cells</td>
</tr>
<tr>
<td></td>
<td>B. T cells</td>
</tr>
<tr>
<td></td>
<td>The Helper cells Th (Th1 and Th2)</td>
</tr>
<tr>
<td></td>
<td><strong>NK cells</strong></td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7</td>
<td><strong>Dendritic cells</strong></td>
</tr>
<tr>
<td>8</td>
<td><strong>Mast cells</strong></td>
</tr>
<tr>
<td>9</td>
<td><strong>Complements</strong></td>
</tr>
<tr>
<td>10</td>
<td><strong>Acute Phase Proteins</strong></td>
</tr>
<tr>
<td>11</td>
<td><strong>Interferons</strong></td>
</tr>
<tr>
<td>12</td>
<td><strong>MHC molecules</strong></td>
</tr>
</tbody>
</table>
C. Antigens

An antigen is a substance that illicits an antibody response against it, upon which it gets neutralized and destroyed. An antigen binds very specifically to an antibody and recent studies elucidate its binding capabilities to a MHC in order to be presented to a T cell receptor. Self-antigens are normally not reacted by the body’s immune system except in the case of autoimmune systems.

The term immunogen is different from an antigen in the sense that it is a molecule that can provoke an adaptive immune response if injected on its own. While an immunogen is able to induce an immune response, an antigen is able to combine with the products of an immune response once they are produced. There are three types of antigens.

A. Upon entry from the outside, exogenous antigens are presented by the antigen-presenting cells (APCs) to T helper cells (CD4+) by the use of class II histocompatibility molecules on their surface. Some T cells are specific for the peptide: MHC complex and secrete cytokines upon activation. Cytokines activate cytotoxic T lymphocytes (CTL), antibody-secreting B cells, macrophages, and other particles of the immune system.

B. As a result of normal cell metabolism, or viral and bacterial cell metabolism, Endogenous antigens are presented on the cell surface in the complex with MHC class I molecules. They are identified by cytotoxic CD8+ T cell, which secrete various toxins that cause apoptosis or lysis of the cells.

C. The immune system of patients with autoimmune diseases recognizes the body’s antigen as foreign and attacks the cells. These antigens are called as auto antigens. Tolerance is a mechanism of the human body to suppress the immune systems attack on its antigens.
central tolerance involves apoptosis when T cells and B cells attack normal cells, peripheral tolerance involves the removal of mature lymphocytes which are not tolerant to healthy cells.\textsuperscript{9}

D. The Structure and Function of an Antibody

**Figure 1:** Structure of an antibody\textsuperscript{7}

- B lymphocytes called as plasma cells secrete antibodies and are also called as immunoglobins.\textsuperscript{10} By definition, immunoglobins is a “family of globular proteins that comprise antibody molecules and molecules having patterns of molecular structure (antigenic determinants) in common with antibodies”.\textsuperscript{10} They range in size from 150 to 1000 KD.\textsuperscript{7}

- Antibodies bind to the epitope region of an antigen. Each arm of the antibody is called as fab region and contains a binding site, thus making the antibody a bivalent molecule.\textsuperscript{7} Various antibodies exhibit differences in the arms enabling them to bind specifically to one unique epitope, making the antibody very versatile and specific in response to antigens.\textsuperscript{7}

- The term immunoglobin arises as the five classes of antibodies contain a common structural domain.\textsuperscript{7} Two heavy chains (50KD) and two light chains (23KD) are held
together by disulphide bonds and non-covalent interactions, thereby representing a Y shaped molecule.\(^7\)

- The 5 classes of antibodies are named as IgG, IgM, IgA, IgD and IgE.\(^5\)

- The Fc domain of the antibody is formed by the carboxy-terminal of the heavy and light chains.\(^7\) This region is responsible for the activation of complement pathway and phagocytosis and The amino terminal of the two chains forms the antigen binding domain.\(^7\)

- Both the heavy and light chains are divided into constant and variable regions. The heavy chain has one variable and 3 constant regions each approximately 110 bp long.\(^10\) Different combinations of both the chains provide the antibody the ability to interact with large number of antigens of different chemical structures.\(^10\)

- The interactions between antibody and antigen is pivotal for the body’s natural biological mechanism and the specificity of the response is mediated by T cells and B cells through membrane associated receptors that bind antigen of single specificity.\(^5\) The variable regions of both the heavy and light chains bind together to form the antigen-binding domain.\(^7\)

- The two arms are connected by a hinge region which is rich in amino acids proline, threonine, and serine.\(^11\) This region provides the antibody the ability to interact with variety of antigen presenting cells.\(^11\)
The binding of an antigen causes the B lymphocytes to divide, and produces memory cells.\textsuperscript{5} These clones then produce antibodies that are able to recognize the antigen.\textsuperscript{5} These memory cells remain dormant until they encounter the same antigen again.\textsuperscript{5}

The V region contains areas of increased variability called hypervariable regions or complementarily determining regions, especially prevalent in residues 30, 55, and 95.\textsuperscript{10} This is called hypervariable regions or complementarily determining regions (CDRs), of approximately 10 residues.\textsuperscript{11} The V region of the heavy chain has three CDRs.\textsuperscript{10} The joining of the heavy and light chains forms a cleft (by the CDR) and will form the binding site for the immunoglobin.\textsuperscript{10}

Figure 2: Light and Heavy chains in an antibody.\textsuperscript{7}
**Table 2:** Different classes of Antibodies and the Function.\(^7\)

<table>
<thead>
<tr>
<th><strong>Type of Antibody</strong></th>
<th><strong>Function</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Most common antibody in the blood, (4 classes of IgG’s in the blood) coats microorganisms to speed up their uptake by phagocytic cells.</td>
</tr>
<tr>
<td>IgA</td>
<td>Concentrates in body fluids to guard the entrances of the body.</td>
</tr>
<tr>
<td>IgM</td>
<td>Effective activator of complement-best indirect killer of blood-borne bacteria.</td>
</tr>
<tr>
<td>IgD</td>
<td>Regulates B cell activation.</td>
</tr>
<tr>
<td>IgE</td>
<td>Attaches itself to specialized cells and triggers allergic response.</td>
</tr>
</tbody>
</table>

E. Monoclonal and Polyclonal Antibodies

**Table 3:** Differences between Monoclonal and Polyclonal antibodies.\(^10\)

<table>
<thead>
<tr>
<th><strong>Monoclonal Antibodies</strong></th>
<th><strong>Polyclonal Antibodies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies (MAbs1) are produced by a single B lymphocyte clone.</td>
<td>Polyclonal antibodies are obtained from different B cell resources and are a combination of immunoglobulin molecules secreted against a specific antigen, each identifying a different epitope</td>
</tr>
<tr>
<td>Monoclonal antibodies target a single epitope and have low cross reactivity.</td>
<td>The ability of the polyclonal antibodies to cross react with similar epitopes on antigens with lesser specificity, can serve as a basis to identify antigens.</td>
</tr>
<tr>
<td>MAb’s require the generation of hybridomas and the process can take up more than a year, thus becoming expensive. However, once the hybridoma is generated MAb’s can be generated as a constant and renewable source. However, MAb’s for consistency and homogeneity are superior to polyclonal antibodies. The concentration and purity is higher from this source.</td>
<td>PAbs can be generated much more rapidly, in different animals at a lesser expense and requiring lesser technical skill when compared to MAbs. PAbs have greater specificity, as they are produced by a large number of B clones each generating antibodies to a specific epitope. PAb’s can be obtained after several months of initiating an immunization. However using different animals will not necessarily produce exactly identical PAbs and the avidity can change as they are harnessed over time. The quantity will also depend on the size of an animal and lifespan.</td>
</tr>
</tbody>
</table>
The binding of an antibody to an antigen is very specific and follows the lock and key mechanism which is similar to an enzyme substrate reaction. The binding takes place between the antigenic determinant (epitope) and the variable region (complementarily determining region, CDR) of both the heavy and light immunoglobulin chains of the antibody. This interaction has led to the development of immunological assays that can detect antigens and antibodies which can be used to identify and diagnose certain disease states. There are 3 kinds of bonds that can be formed between an antigen and antibody namely, Vander Waals bonds, Hydrogen and Ionic bonds between the interacting molecules.

Two mechanisms have been postulated for antigen and antibody interactions

**The Lock and Key Model** - The antibody and antigen bind together without change of the structure of the antibody.

**Mutual or Induced Fit Model** - In this, structural changes cause the binding of the antibody and antigen. Examples of structural changes are small side-chain rearrangements, movements of CDRs, or alterations in the rotation of variable light and heavy chains. The strength of these interactions is referred to as affinity if the binding sites are homogenous (e.g. monoclonal antibody) and avidity if the antigen binds to a variety of paratopes (e.g. polyclonal antibodies).
The association equation is depicted as

\[ \text{Ag} + \text{Ab} \rightleftharpoons \text{Ag-Ab}; \]

\[ k_1 = \text{forward (assoc) rate constant} \]
\[ k_{-1} = \text{reverse (dissoc) rate constant} \]

whereby \( k_1/k_{-1} = K_a \)

\[ K_a = [\text{Ag-Ab}] \quad \text{value of } K_a \text{ depends on } k_1; \]

\[ [\text{Ag}] [\text{Ab}] = 10^4 \text{ to } 10^{12} \text{ L/Mol} \]

Greater the value of \( K \), stronger is the binding between the antigen and the antibody.

For small haptens, \( k_1 \) is high. For large protein Ag’s, \( k_1 \) is lower. \(^{11}\)

Although the interactions between an antibody and antigen are specific in nature, sometimes antibodies are found to cross-react with antigens, i.e they bind to structurally similar antigens. \(^{10}\)

The ability of antibodies to selectively bind a specific epitope present on a chemical, carbohydrate, protein, or nucleic acid and has been extensively studied and used for various clinical applications. \(^{11}\)

Applications include simple qualitative and/or quantitative analyses to

- Test the presence of an epitope of interest in a solution. \(^{10}\)
- To test for the **purification** of an antigen, antigen- associated molecules, or cells expressing an antigen; \(^{10}\)
Techniques that use antibodies to **mediate and/or modulate physiological effects** for research, diagnostic, or therapeutic purposes.  

G. **Tests for Antibody- Antigen Interaction**

The antibody and antigen interaction can be used for many diagnostic tests. A summary of the principle of important tests is depicted in the table below.  

---

10
Table 4: Different types of antibody/antigen interaction tests.\textsuperscript{12}

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Agglutination tests</td>
<td>Blood tests, which are conducted to detect different blood groups, is an example of agglutination test, and can be both quantitative and qualitative. In this test, the antigen clumps on interacting with the antibody, and this interaction can act as an indicator of various blood groups.</td>
</tr>
<tr>
<td>2) Coombs test</td>
<td>This test method involves incubating the red blood cells with the serum sample, washing out any unbound antibodies and then adding a second anti-immunoglobulin reagent to cross-link the cells. This test can verify whether a serum sample has antibodies directed against a particular red blood cell.</td>
</tr>
<tr>
<td>3) Radial Immunodiffusion</td>
<td>An antibody incorporated in an agar medium is made to react with an antigen, which diffuses into the agar medium to form a precipitin ring. This happens when the point of equivalence is reached and the diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. By running different concentrations of a standard antigen, standard curve can be generated from which one can quantify the amount of an antigen in an unknown sample.</td>
</tr>
<tr>
<td>4) Immuno Electrophoresis</td>
<td>An agar gel is used to place a mixture of antigens in the form of a well, and using electrophoresis antigens are separated, based on their charge. After electrophoresis, a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an antigen/antibody reaction takes place.</td>
</tr>
<tr>
<td>5) ELISA tests</td>
<td>Enzyme Linked Immunosorbert Assays (ELISA) are based on the measurement of an enzymatic reaction associated with immune complexes. An enzyme may be linked to either the antigen or the antibody. By using known amounts of a standard unlabeled antigen a standard curve relating radioactivity (cpm) (Enzyme) bound versus amount of antigen can be generated. From this standard curve, determine the amount of an antigen in an unknown sample can be determined.</td>
</tr>
<tr>
<td>6) Non Competitive RIA/ELISA for Ag or Ab</td>
<td>Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. An antigen is used for the detection of antibody in the unknown sample. The amount of labeled second antibody bound is related to the amount of antibody in the unknown sample. This assay is commonly employed for the measurement of antibodies of the IgE class directed against particular allergens by using a known allergen as antigen and anti-IgE antibodies as the labeled reagent</td>
</tr>
<tr>
<td>7) Immunofluorescence</td>
<td>In immunofluorescence an antibody labeled with a fluorescent molecule is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.</td>
</tr>
<tr>
<td>8) Direct IF</td>
<td>In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the fluorochrome. In indirect immunofluorescence, the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome.</td>
</tr>
</tbody>
</table>

\textsuperscript{12}References: [12]
H. The problem with Interfering Antibodies

While immunoassays based on antibody antigen interaction remain the largest segment in the *invitro* diagnostic market, most assays battle with the problem of interference from circulating antibodies in the patient’s sera. Detection of analytes such as steroids, thyroid, cardiac enzyme are all affected by the interfering antibodies and while lab assays have been modified to minimize this, the inherent cause of the problem is due to the intrinsic and unpredictable nature of the antibodies. This can be attributed to the following:

- Heterogeneity of the Antibodies.

- Immunoglobin class switching may also take place within a patient during immune response, for instance IgM may be converted into IgA.

- The contact surface between the antibody and the epitope of an antigen is called paratope. The three dimensional shape of the epitope and its complementary paratope influences the interaction between the antigen and antibody. Since the complementarily of the electron cloud and the overall configuration of the outer electrons influences the reaction, molecules such as water can also potentially alter the interaction as well as “interfere” with the reaction.

- Interfering antibodies can interfere in 3 ways. 
  1. Imitating an antigen and mimicking its binding and function at the combining site.
  2. Recognizing and binding the antigen itself
  3. By causing a hindrance between the binding site of the antigen and antibody.

Nowadays assays are designed such that they can neutralize and block the interfering antibodies.
It can be seen that the problem of interference also exists using plastic antibodies, especially when an interferent displaces the analyte conjugated with an enzyme from the site of MIP, instead of the target analyte, and this can produce erroneous results in quantitative and qualitative information. This is a significant problem which needs to be addressed in this field.

II. INTRODUCTION TO THE MIPS TECHNOLOGY

A. MIPS- An overview

Molecular Impression Polymers, (MIPs) also known as plastic antibodies are synthetic receptors that can bind selectively to a target molecule (template). On removal of the template they are able to retain a “memory” for the molecule, thereby acting as artificial antibodies. Although imprints against small molecule organic templates have been generated for decades now, recent focus has been on the development of imprints for successful binding of proteins and biomolecules. The rapid and inexpensive generation of MIPS, mimicking the shape of the template and replacing antibodies in its binding capacity has seen enormous potential in the field of biotechnology for various applications.

A template molecule is first allowed to interact, either through non-covalent or (reversibly formed) covalent bonds, with functionalized monomers (examples include methacrylate, acryl amide, or styrene derivatives). Formation of these interactions preassembles the monomers around the template. The mixture then is copolymerized in a suitable solvent (the “porogen”) with a large excess of cross linker (such as ethylene glycol dimethacrylate) to yield a rigid macro porous plastic. The bounded template is washed away, leaving behind its “shape”, which is capable of
rebinding to the template. During MIP synthesis, selectivity is introduced when the template molecule is designed to mimic the analyte, and the cavities formed are chemically complimentary to the target analyte allowing multiple interactions between the MIP and the functional groups of the analyte.

**Figure 3**: Formation of a MIP

**B. Advantages of MIPS**

- MIPS bind to protein templates with binding constants similar to natural receptors such as antibodies.
- Compared to natural antibodies, MIPS are capable of withstanding harsh conditions such as high temperature, pressure, extreme pH, and organic solvents that often denature natural antibodies. (Refer table 1)

**C. Disadvantages of MIPS**

- The water-soluble nature of proteins may not be compatible with the organic solvents used for MIP preparation. An aqueous environment affects competitive reaction between analytes and conjugate analytes. Moreover, cross reactivity of structural analogues is higher.
- It is difficult to remove the template from the MIP after the polymerization reaction.
- The binding affinity and the selectivity of MIPs is lower than the natural antibodies.
The high cross linking in MIPS can limit the access of the target analyte to the site thereby limiting sensitivity. The affinity and selectivity of MIP depends on

- size of target

- and shape of the imprinted cavity, rebinding interactions

- functional groups on target analyte

Proteins have a large number of functional groups that can interact with functional monomers, and this makes the procedure of selecting the imprinting protocol difficult as some cases use strong/dissociable (e.g. methacrylic acid) and other cases use weak/neutral binding monomers (e.g. acrylamide) for the template recognition.

- Absence of a general procedure for MIP preparation

- Difficulty in integrating them with a transducer

- Difficulty in transforming the binding event into an electric signal
Table 5: Differences between MIPs and Antibodies.\textsuperscript{16}

<table>
<thead>
<tr>
<th>MIPS</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can be used in both aqueous environment and</td>
<td>Restricted to aqueous solutions</td>
</tr>
<tr>
<td>organic media.</td>
<td></td>
</tr>
<tr>
<td>Synthetic- No use of animals</td>
<td>Biological- involving animal welfares issues</td>
</tr>
<tr>
<td>High chemical, physical and thermal stability</td>
<td>Very fragile</td>
</tr>
<tr>
<td>Very stable for a long period at simple and</td>
<td>Requires Lyophilisation, controlled storage and</td>
</tr>
<tr>
<td>reproducibility can be achieved</td>
<td>deteoriates upon long storage period</td>
</tr>
<tr>
<td>Preparation-fast and simple</td>
<td>Preparation- time consuming and elaborate</td>
</tr>
<tr>
<td>Can be prepared against small molecules</td>
<td>Small non immunogenic molecules need derivitization to produce immune response</td>
</tr>
<tr>
<td>Simple to integrate into device production</td>
<td>Integration into device is very difficult due to</td>
</tr>
<tr>
<td>process</td>
<td>environment conditions</td>
</tr>
<tr>
<td>No limitation on nature of target sites</td>
<td>Targets must be biocompatible</td>
</tr>
</tbody>
</table>

The Biochemistry Of Binding: Proteins and MIPS.

In the formation of MIPS, the polymers can be targeted to recognize\textsuperscript{15}

- The sequence (or part) of the protein.
- The shape of a protein.
Formation of MIPS Using Protein Sequence

Kempe in 1995 demonstrated the formation of MIPS using a sequence of a peptide.

- In this method, double bonds are introduced into silica surfaces and are used as anchorage points for the polymer.
- The surfaces are derivatised with 3-(trimethoxysilyl)-propyl-methacrylate.
- For example ribonuclease is used as a template protein and is mixed with metal ions and with the functional monomer \( N-(4\text{-vinyl-benzyl})\text{-iminodiacetic acid (VBIDA).} \)
- Imidazole groups (Im) of the histidines exposed on the surface of the protein formed complexes with the metal chelating functional monomers, creating complementary cavities with ‘anchoring points’ for the recognition of the protein.
The binding of the protein with the silica surface is shown in the figure above. However, this method is more useful for proteins with histidine on its surface, as metal chelating groups provide strong anchoring points for non-specific interactions, which in real samples might seriously impede specific recognition.

**The Epitope Approach**

![Diagram of epitope binding](image)

**Figure 5**: Formation of MIPS using epitope approach.

- MIPS can be generated using the natural binding capability of an antibody to an epitope of an antigen.
- Rachkov and co-workers used a short peptide sequence of about 3-4 peptides (Oxytocin) which were exposed at the protein surface for the MIP preparation using the relevant polymer mixture. (methacrylic acid (MAA)) and ethylene glycol di-methacrylate (EGDMA).
- The resulting MIP was designed to bind to the entire protein upon its interaction.
Shape recognition protein with polyacrylamide gels (PAA)\textsuperscript{15}

- The PAA gels, made of acrylamide and \(N, N'-\text{methylene- bisacrylamide}\), were mixed at different ratios and were imprinted with ribonuclease (RNase) hemoglobin, lysozyme, myoglobin and human growth factor (HGF) peptide.

- The biocompatibility and neutrality feature of PAA minimizes non-specific interactions with the proteins.

- The polymers had high recognition abilities and were very specific in binding with the relevant proteins.

- PAA-imprinted polymers were able to discriminate between two homologous proteins that differ only in 20 residues of a total sequence of 153 residues.

- This method was found to be better than the metal chelating method. The main limitation of PAA gels is the softness and the limited mechanical strength of such material. Strategies such as use of Chitosan beads can be used to overcome this.

Immobilizing the template on a surface\textsuperscript{15}

- The template molecule can be supported on a solid, and the MIP is polymerized in close contact with the template-carrying surface.

- After polymerization, the support is dissolved by harsh chemical treatment.

- Advantage of this method includes using a template bound onto the surface. For example, the solid surface can be used to support a template, which is insoluble in the polymerization mix. Moreover, template immobilization minimizes protein aggregation and promotes homogenous binding.
The biochemistry of binding when comparing MIPs and antibodies is similar as it involves Vander Waal forces, hydrogen bonding etc. However, nature has 20 amino acids, while chemists can engineer many different chemical groups into the polymers, potentially giving them the ability to more finely tune the interaction.

III. OVERVIEW OF BIOMARKERS

Biomarkers are defined as “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids that can serve as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.” 17

A molecular alteration of a cell on DNA, RNA or protein level is referred to as a molecular biomarker. Molecular biomarkers enable the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease.17

A biomarker is valid if

- A test system to measure a biomarker is well established.
- There is evidence for its clinical significance.
Uses of biomarkers

- Delineation of events between exposure and disease
- Identification of early events in the natural history of a disease
- Identification of mechanisms by which exposure and disease may be related
- Reduction in misclassification of exposures or risk factors and disease
- Establishment of variability in a group of people having the same disease
- To study the effect of a drug
- To identify new drugs/drug screening
- To serve as surrogate end points in clinical trials

A. History of Biomarkers

Table 6: Important biomarker events with time since 1847

<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1847</td>
<td>First Lab test for a protein cancer biomarker; Bence Jones protein in urine.</td>
</tr>
<tr>
<td>1960</td>
<td>The term “Biomarker” was defined in connection with metabolites and biochemical abnormalities associated with several diseases.</td>
</tr>
<tr>
<td>1967</td>
<td>An improved test for myocardial infarction based on a biomarker- Serum creatine phosphokinase</td>
</tr>
<tr>
<td>1971</td>
<td>Report of carcinoembryonic antigen (CEA) as biomarker for cancer</td>
</tr>
<tr>
<td>1987</td>
<td>Troponin I as a biomarker for myocardial infarction</td>
</tr>
<tr>
<td>1990</td>
<td>Mass spectroscopy for the study of Biomarkers</td>
</tr>
<tr>
<td>1995</td>
<td>Proteomics for the study of biomarkers</td>
</tr>
<tr>
<td>1999</td>
<td>Metabolomics for the study of biomarkers</td>
</tr>
<tr>
<td>2000</td>
<td>Sequencing of human genome opened way to study gene biomarkers</td>
</tr>
<tr>
<td>2005</td>
<td>Discovery and application becomes a major activity in biotech and pharma industries.</td>
</tr>
</tbody>
</table>
B. Types of biomarkers

**Biomarkers of exposure**: Biomarkers of exposure estimate the actual internal exposure of a disease rather than relying on time or history of exposure.\(^\text{17}\) The use of biomarkers improves the sensitivity and specificity of the measurement of the exposures or risk factors.\(^\text{17}\) This measurement of precision of the risk factor is improved.

**Diagnostics biomarkers**: Biomarkers used in the early detection of a disease and also to study the progression of a disease.\(^\text{19}\)

**Biomarkers of disease**: A Biomarkers are not the only determinant of a disease but could plays a relevant role in the progression of a disease and remains strongly related to the disease.\(^\text{19}\) The biomarker could also be related to an exposure that has already been identified or could represent an alteration caused by the exposure that results in the disease.

**Biomarkers for drug discovery**: Biomarkers can also bind to the target drug and can be determined for drug discovery.\(^\text{20}\) Besides this they can also be used to test the efficacy of a drug as well as the toxicity of the drug.\(^\text{20}\)

**Predictive biomarkers**: Biomarkers are used for predicting the risk of a disease, at a presymptomatic stage.\(^\text{17}\)

**Surrogate biomarkers**: Biomarkers can act as substitute end points of a clinical trial.\(^\text{19}\)

Biomarkers used for screening or diagnosis also often represent surrogate manifestations of the disease. The potential uses of this class of biomarkers include:
➢ Identification of individuals destined to become affected or who are in the “preclinical” stages of the illness.\(^\text{19}\)

➢ Reduction in disease heterogeneity in clinical trials or epidemiologic studies.\(^\text{19}\)

➢ Reflection of the natural history of disease encompassing the phases of induction, latency and detection.\(^\text{19}\)

➢ Target for a clinical trial.\(^\text{19}\)

**Table 7:** Advantages and Disadvantages using biomarkers \(^{17}\)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective assessment</td>
<td>Timing is critical</td>
</tr>
<tr>
<td>Precision of measurement</td>
<td>Expensive (costs for analyses)</td>
</tr>
<tr>
<td>Reliable; validity can be established</td>
<td>Storage (longevity of samples)</td>
</tr>
<tr>
<td>Less biased than questionnaires</td>
<td>Disease mechanisms often studied</td>
</tr>
<tr>
<td>Homogeneity of risk or disease</td>
<td>Normal range difficult to establish</td>
</tr>
</tbody>
</table>

**Table 8:** Different Biomarkers and Applications \(^{17}\)

<table>
<thead>
<tr>
<th>Term</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predisposition Biomarker</td>
<td>To identify the predisposition of the disease</td>
</tr>
<tr>
<td>Screening biomarkers</td>
<td>To identify those suffering from a disease</td>
</tr>
<tr>
<td>Staging biomarkers</td>
<td>To determine the stage of progression of a disease</td>
</tr>
<tr>
<td>Prognostic biomarker</td>
<td>To assess disease progression and outcome</td>
</tr>
<tr>
<td>Recurrence monitoring biomarkers</td>
<td>To identify the recurrence of a disease.</td>
</tr>
</tbody>
</table>

**Development and Validation of Biomarkers Arrays** \(^{21}\)

The use of arrays has become very significant due to the knowledge of the complete genome and the availability of large EST sequences. Latest technology by Affymetrix promises to monitor the entire genome on a chip. The oligonucleotides corresponding to the genes whose expression has to be analyzed are attached in an ordered fashion to a solid support such as a nylon membrane. Latest
technology in automation and miniaturization makes it possible to produce arrays with several thousand genes per square centimeter, which accounts for a significant part of the human genome. Each “feature” of an array contains picomoles ($10^{-12}$ moles) of a specific DNA sequence, known as *probes* (or *reporters*). Macroarrays have spot sizes greater than 300 microns and can be easily viewed with imaging techniques, whereas microarrays need specialized robotic tools and imaging equipment for visualization as spot sizes are less than 200 microns.

The sample containing RNA to be measured is labeled with a fluorescent or radioactive marker and then hybridized with the arrays. The intensity of the hybridization signal is a measure of the relative abundance of the corresponding mRNA in the sample. The expression profile or transcriptome refers to the complete collection of mRNAs present. Thus comparing the hybridization signals for different mRNA samples allows changes in mRNA levels to be determined under the conditions tested for all the genes represented on the arrays. This involves complex bioinformatics and thereby using this technology, thousands of genes can be viewed simultaneously for both gene discovery and gene expression studies.

![Figure 6: DNA array with spots and probes.](image-url)
Proteomics

The study of the entire proteome is called proteomics. Proteomics is used to discover and validate the use of biomarkers. Techniques classified under proteomics include western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA) or mass spectrometry.²²

 Challenges in biomarker discovery and development.²³ (Summary from Anderson 2005)

Proteomics has led to the detection of over thousand different proteins, yet there still a lack of biomarkers that have been discovered, approved and used in the clinical setting over the last few years. (Almost zero today). The reasons for the slow growth in this field can be attributed to heterogeneity-of a disease process and variability between different people in a population, which make it very difficult to evaluate an abnormal protein in a pool of normal proteins. The problem is referred to as “Needle in Haystack problem”. This makes it difficult as the level of abnormality can be very small in many cases. This makes is extremely difficult to identify a single abnormal marker. Recent research has shown that a panel of independent biomarkers can signify the onset of a disease with good reproducibility of results rather than using a single marker alone. The main difficulty lies in identifying new biomarkers in the blood and this is due to the characteristics of the complex proteome and the limited capabilities of current proteome strategies.

 Challenges of the plasma proteome (Summary from Anderson 2005)

The plasma proteome is very complex and contains an average of $10^6$ molecules arising from 30,000 genes most of which are present in low levels. While the most common protein albumin can be detected easily due to its very high concentration in the blood, other potentially valuable markers, found in extremely low concentrations is very difficult to measure.
Discovery of biomarkers can use two types of proteomics analysis

(1) Complete analyses that can enable an investigator observe all differences in multiple samples.

(2) Targeted analysis (to measure one or more hypothesis-generated candidates).

Complete analysis would allow the direct selection of optimal biomarker proteins at the outset. Three different proteomics platforms are used namely multidimensional chromatography of proteins followed by 2-D electrophoresis and mass spectrometry (MS) identification of resolved proteins; tryptic digestion and multidimensional chromatography of peptides followed by MS identification; and tryptic digestion and multidimensional chromatography of peptides from low-molecular weight plasma components followed by MS identification.

When these datasets are searched for “candidate biomarkers” for which plasma concentration normal values exist, not all proteins are biomarkers are detected uniformly with same consistency and specificity by all 3 methods.

Targeted analysis has been used for many years and is hypothesis based, where an investigator hypothesizes that a biomarker can cause a particular disease using the knowledge of literature and experiments. Most assays include immunoassays that rely on the specificity of the antibody to detect extremely small proteins. It becomes insignificant to test one marker at a time as there are at least tens of thousands of candidate protein forms, and at least hundreds of disease entities. The procedure is long, expensive and makes it difficult for the investigator to arrive at a significant conclusion.

While a panel may have advantages as stated above, the production of separate assays for each of the marker becomes a problem. This requires increased sample requirement, and bringing them
together as a prototype panel for application as they are typically developed in separate laboratories.

Hybrid approaches can be implemented to overcome these limitations, by focusing on intelligent preselected candidate biomarker proteins and by using targeted proteomics; i.e. combining the multiprotein view of traditional proteomics and specificity of immunoassays. Multiplexed immunoassays and mass spectroscopy can best serve this purpose.

**Principle of Mass Spectroscopy.** (Summary from Anderson 2005)

Mass Spec is a complex automated procedure used for the quantitative measurement of drugs, hormones, drug metabolites.

- The sample is subjected to some form of high-throughput prefractionation (e.g. solid phase extraction; SPE).

  ⇒ The samples are then subjected to reverse phase chromatography for separation.

  ⇒ The resulting protein stream is introduced through an ionizing spray interface into a triple-quadrupole MS (TQMS).

  ⇒ The first mass analyzer (MS1) only passes the analyte under detection. (by using the mass to charge ratios).

  ⇒ The analyte is then fragmented in a collision chamber.

  ⇒ The fragmented analyte is passed to a second mass analyzer (MS2) which will choose to pass only a specific fragment, thus providing very high specificity.

  ⇒ The signal can be detected that traces a peak in the chromatogram at the expected retention time corresponding to the selected analyte, and integrating this peak gives a measure of the quantity of the analyte.
**Limitation of targeted proteomics** (Summary from Anderson 2005)

While the high specificity of mass spectroscopy can differentiate and detect the target analyte from many samples better than immunoassays, it is not useful for proteins larger than about 10 kDa. The higher mass cannot be adequately resolved by current MS systems as they are not fragmented efficiently. Besides, these labeled internal fragments used in the procedure are very expensive.

For novel biomarkers, production of an antibody is a cumbersome and expensive task. The costs involved in the production and commercialization of an immunoassay based diagnostic test is about $2–4 million for each biomarker. It often becomes necessary to first conduct quantitative mass spectroscopy to evaluate candidate biomarkers prior to investment in immunoassays and use it as a reference to standardize immunoassays.

The plastic antibodies are designed with known information about the sequence of the peptides, and studies have not exploited its features to discover novel biomarkers in a sample. However, once the biomarker has been discovered, the plastic antibody has potential advantages of the use of antibodies to detect its qualitative and quantitative information (Refer Table 3).
D.MIPS for genetic material

In a study by Masaya Ogiso, MIPS for target sequence in a dsDNA were formed in a gel matrix. It was found that target ds DNA was captured by the MIPs in the gel, and this was observed from the deviations from the linear relationship between the migration distances of the DNA standard size markers in the polyacrylamide gel and those in the MIP gel, while the non-target ds DNA maintained a linear relationship. The MIP was formed in the polymerization matrix by polymerization in the presence of the DNA template. The monomer used in the process was 2-vinyl-4,6 di amino-1,3,5-triazine (VDAT) for double stranded DNA. The functional group of VDAT interacted with the A.T pair of the DNA. While the migration of dDNA in a polyacrylamide gel depends on the size of the DNA fragments, the migration of dDNA in a MIP gel depends on both the size and the capture effects of the MIP in the gel. As the migration of the dsDNA is prevented; the fragment size of the strand in the MIPGE was higher than the fragment size in the PAGE while the size of the non-target DNA strand was the same.

In addition, the sequence selectivity of dsDNA was confirmed as MIPS could successfully identify between the target and base pair substitutes, except for MIP could not distinguish between A.T base pair from a T.A base pair. The study by Masaya Osio confirmed that MIP gel electrophoresis could be used to identify target DNA sequences in a sample. The advantages of this method included non-labeled native DNA samples which can be analyzed directly by this system. In addition, MIPGE is more cost effective than using DNA arrays, microfabricated chips. The sample and the gel is simple to prepare and the design is very flexible.
In another study by Shea, a molecularly imprinted synthetic receptor \(^{(\text{Shea}, 2002)}\) or 9-ethyladenine (9-EA) and for other nucleotide base analogues were attempted.\(^{26}\) In the case of 9-EA, the network polymer has an affinity for adenine and its derivatives with an average association constant \((K_a)\) of 75,000 M\(^{-1}\) in CHCl\(_3\).\(^{26}\)

When a 9-EA imprinted polymer was used as the chromatographic support, adenine eluted at 27 minutes using 92.5/5.0/2.5 CH\(_3\)CN/H\(_2\)O/CH\(_3\)CO\(_2\)H as the mobile phase, while cytosine, guanine and thymine derivatives all eluted close to the void volume (2.0 min).\(^{26}\)

Based on Shea’s papers on the construction of MIPS for DNA, it can be concluded that for the imprinting process is very different and requires significant changes. This is attributed to the fact that oligonucleotides are water soluble, and the usual polymers used for imprinting procedure cannot be used. The choice of solvents will have to not interfere with the interactions between template (the oligo) and functional monomer, as the interaction may get affected.

The Shea lab identified that the imprinting of a single nucleotide base could be extended to the imprinting of a short DNA fragment and several polymer formulations were examined to develop adenine receptors.

**The potential challenges are**

A. Binding studies done in 10mM K\(_3\)PO\(_4\) buffer solutions showed decreased binding of the analyte to the polymers most likely due to a change in the solvation and conformation of the polymer and hence the microenvironment of the binding sites in a MIP.\(^{26}\)
B. The pH and/or the ionic strength of the uptake solutions in the rebinding studies have a significant effect on the binding of the analyte to the polymer, and the uptake studies are best performed in water alone.  

C. The DNA fragment is very small for the construction of the MIPS that it is significantly bound to error.  

DNA imprinting is still a new field and the potential for DNA MIPS is very high if successful imprinting and techniques are established. Identification of target DNA sequences can enable the very early detection of genetic disorders, changes in the chromatin structures and mutations. The biggest advantage of DNA imprints is that if the require selectivity is established, it has the potential to replace the use of DNA arrays and gene on chips (for detection of DNA sequences) as these procedures are complex and expensive.  


IV. DESCRIPTION OF INFIGO’S TECHNOLOGY

Infigo’s technology comprises of a rapid, sensitive diagnostic device which can qualitatively and quantitatively measure a target molecule in urine, blood plasma, and serum. The technology follows the principle of a lateral flow immunochromatography assay and is incorporated with plastic antibodies in the place of natural antibodies. (similar looking to a pregnancy test) The technology can be used to predict the quantity of an analyte in real time in approximately 15 minutes.\textsuperscript{27} The binding of the analyte to the plastic antibodies embedded on the device is detected by visual inspection or by using an instrument, such as an appropriate reader or scanner, which detects the color or fluorescence from the readout area.\textsuperscript{27}

The technology can be used for a variety of applications including \textbf{point of care (POC) diagnosis} of analytes in a hospital setting, biological fluids filtering, drug screening, contaminant screening in health food and environmental industries, and recently in the field of companion diagnostics. For instance, Infigo’s technology can be used to detect Methyl Malonic Acid (MMA) to detect vitamin B12 deficiency.\textsuperscript{27} Currently a simple, reliable and generally accepted method for determination of vitamin B12 deficiency is not available. It is hypothesized that the use of MIPS in the diagnostic device would lead to an improvement in the detection of target molecules with greater sensitivity and specificity as current sensor devices using natural antibodies or enzymes often lack storage or operational stability, besides being expensive and difficult to produce. Antibodies and enzymes can be easily denatured due to heat, organic solvent, pressure, excessive salts, and other denaturants.\textsuperscript{28}
Infigo’s technology uses synthesized MIPS as the detecting agents and permits \textit{the use of an inexpensive, simple, analysis (both single and double displacement principle) of target molecules}.\textsuperscript{1} The device specifically favors the detection of those targets which are both small and those that are deemed as non-ideal candidates for antibody production.\textsuperscript{27} The advantages of Infigo’s technology include specificity of detection, stability of MIPS, and controlled synthesis of MIPS for several target molecules.\textsuperscript{1} Example of target molecules may include a wide range of different types of proteins, vitamins, hormones, and enzymes that are classified as biomarkers of diseases. Also included are natural or synthetic toxins, drugs and metabolites.

The analytes are usually found in liquid sample which includes water, blood, oil and process fluids. The sample can be used directly or can be subjected to pretreatment (separating plasma from blood, dilution, centrifugation, concentration, distillation etc) to modify its character for more effective detection of the analyte. Buffers can also be used.

MIPS can be developed both by covalent imprinting (where the monomers are covalently attached to the analyte) and by non-covalent imprinting (where target non-covalently interacts with the monomers).\textsuperscript{28} The end result would consist of the synthesized polymer having the capacity to bind to the target monomer.\textsuperscript{28} The molecular imprinted polymer comprises a polymer polymerized from diethylaminoethyl methacrylate monomer cross-linked with ethylene glycol dimethacrylate (EGDMA), in the presence of methylmalonic acid (MMA) and toluene as a porogen.\textsuperscript{28}
Description of the device

**Figure 7**: Infigo’s lateral flow device comprises of the following components

A. **Sample Pad**: The liquid sample containing the target analyte is applied in this zone. This zone is made up of porous material and efficiently absorbs the sample liquid rapidly. Materials such as polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile and polytetrafluoroethylene are used.

B. **MIP-conjugate zone**: The liquid sample flows into this zone via capillary action through a solid support made up of nitrocellulose. This zone contains the MIP bound with the analyte conjugate. This analyte conjugate differs from the analyte in two ways.
i. *Dissociation constant (K\textsubscript{d})*

The lower the K\textsubscript{d} value, higher the affinity. In this case, the analyte conjugate is designed in such a way that it has a higher K\textsubscript{d} value than the analyte, which makes analyte-conjugate less attracted to the MIPs than the analyte itself\textsuperscript{1}. The analyte displaces the analyte conjugate from the MIP zone, and does so in an amount directly proportional to the concentration of the analyte.

ii. *Chromophore attachment*

A chromophore called reporter is a compound that has the ability to produce light when it comes in contact with a particular substance. The chromophore/reporter attached to the analyte conjugate (Biotin) has the ability to produce a visible color when it is bound to its binding element (Streptavidin)\textsuperscript{1}. Biotin is a reporter molecule which is colored or produces a color when it participates in a chemical reaction. When biotin binds to streptavidin present in another zone in the device, it produces a color which is either viewed with the naked eye, under UV light, or under a scanner\textsuperscript{1}. The signal produced in the reaction will depict the concentration of the analyte. The single (and double) displacement diagnostic device invented by Infigo thereby determines qualitative and quantitative information of the analyte in the sample.

C. *Results zone*\textsuperscript{1}: The displaced analyte analog bound to the reporter flows downstream in the path of liquid flow to the results zone. The zone comprises of a binding element and binds to the displaced analyte conjugate bound with the reporter, thereby generating a signal.
D. Reference zone: This zone acts as a reference point in determining the presence of the analyte in the tested sample and is next to the results window. Next to it is comprised of several indicator lines of binding element, which is impregnated with precise amounts of the reporter conjugate. These lines will exhibit a range of color intensities, which are proportional to the known amount of the reporter conjugate bound to them. Comparison of the intensity of the signal in the results window with the intensity of the lines at the adjacent reference window enables good estimation of the amount of the analyte in the tested sample.

E. Positive control zone: This zone is used to test if the working of the device.

F. Absorbent zone comprising a pad of absorbent material that has the capacity to absorb excess liquid.

Working of the technology. (Summarized from Patent Application)

Single Displacement Method- Principle

Figure 8: Single Displacement Imprinted Polymer receptor analysis
The liquid sample containing the analyte is introduced into the sample pad of the device and is made to migrate through the device by capillary action.

The analyte binds to MIPs synthesized to the analyte-conjugate with biotin (reporter molecule) in the MIP zone and is bound by it.

As the sample containing the target analyte is introduced, it binds to the MIPS zone and displaces the already bound analyte conjugate with biotin.

The displaced analyte conjugate migrates through the solid support to the results zone.

This complex binds to the reporter conjugate binding element (Streptavidin) and produces a signal, which positively indicates an amount of analyte in the sample.

The sensitivity of the device is a function of the relative affinity of the target analyte to the MIP and the intensity of the signal produced by the reporting system. Optimizing and characterizing the sensitivity and dose-response characteristics of this device can enable best results.

**Figure 9: Double Displacement Principle**
- In this particular modification, the sample consisting of the analyte moves along the device via capillary action.
- The analyte displaces the first analyte conjugate with biotin from the MIP specific sites.
- In the zone bound by the reporter binding element, a second analyte reporter conjugate with biotin derivatives bound to the binding element are found. These elements have a lower affinity to the binding site than the analyte conjugate bound to biotin displaced in the previous step. The second analyte reporter conjugate is displaced from the binding element by the first analyte reporter conjugate. The displacement takes place in a dose dependent manner, which is directly proportional to the concentration of the specific analyte in the sample.
- The displaced second analyte reporter conjugate with biotin derivatives reach the second biotin-binding element at the results window, where they are captured. The binding process produces a signal which is visualized.
- The device may optionally include a reference zone, a positive control zone and an absorbent zone to absorb excess liquid.
- Also, the device can be designed to measure the intensity of the signal in the results zone and compare it with the intensity of the signal in a reference zone to determine the concentration of the analyte in the sample. The results zone includes a scale like thermometer. The presence and concentration of analyte in the liquid is determined by the area covered by the second binding agent: reporter-conjugate along the second binding area of reporter-conjugate binding element as viewed in the results window.
Figure 10: Visualization process in Infigo’s technology

In the Vertical visualization method, the reporter-conjugate binding element is placed vertical to the sample flow path to capture and binds to the free-flowing reporter conjugate molecules in a random order. The greater the amount of the reporter conjugate (which is a direct representation of the amount of the analyte in the tested sample) which binds to the binding element, the stronger the visual signal obtained. In the horizontal visualization method, the reporter-conjugate binding element is horizontally placed at the path of the sample flow and binds to the analyte conjugate.

Advantages of Double Displacement.¹

- As biotin is a relatively small molecule, combining biotin with the analyte conjugate molecule will not interfere with its binding to the analyte-specific MIP. The binding of biotin to the various biotin-binding elements is very rapid and strong, and therefore contributes to the overall performance of the device.
• The binding of biotin to a reporter overcomes the inability or difficulties in binding a reporter molecule to certain analytes, which may become a limiting factor. Also, a wide range of reporters can be used to bind to the target.

• For most target molecules, which are similar in structure and nature, it is sufficient to produce the specific MIP and the target analyte conjugated to biotin. This makes the time to market of new products shorter and reduces development and production costs.
V. MARKET FOR INFIGO

Infigo Diagnostics was formed to specifically cater the Point of Care diagnostics market.

Infigo’s technology is centered on providing an efficient, rapid, inexpensive POC diagnostic device, which can detect biomarkers both quantitatively and qualitatively. The market for POC is summarized below.

A. Point of care market (POC)

POC at the hospital setting is defined as a test that provides results instantly, which can help clinical specialists make treatment decisions faster in order to improve patient healthcare. 29 Typical areas include blood gas and blood sugar determination, and today the spectrum of POC testing has widely expanded to use of cardiac markers to indicate onset of stroke and heart attack, detection of infectious diseases, drugs of abuse, measurement of cholesterol, detection of coagulation and to indicate the presence of deadly viruses like HIV to name a few. 30

In general, lab tests take anywhere between 3 hours to 4 days to process, obtain and deliver results to patients thereby constantly creating a need for a rapid and efficient test that could enable healthcare specialists make quick decisions. 31 This could in turn dramatically change a patient’s treatment strategy. In addition, with the revolution of proteomics and genomics that has led to the invention of novel biomarkers, it is now not only possible to diagnose a particular indication, but it is now possible treat it accordingly. The speed of diagnosis in any hospital setting can thus directly contribute to improved patient healthcare through data supported decisions.

Today the spectrum of POC medical devices has extended to integrated platforms of analysis used in core labs and hand held miniature devices that can both address different needs and has
changed the way of healthcare management. POC devices have also been the main driver for more patient centered primary care facilities, one-stop clinics that are preferred to lengthy stay and consolidation of hospital facilities. POC has also extended to bedside, ward side, satellite lab side, central lab, and offsite and core labs. Additionally, POC testing also promotes home based testing as patients can now purchase tests, interpret values and thereby can inculcate better personalized healthcare management strategies.

The type of decisions can that can be influenced by POC results are

- Can it rule out an indication?
- Can it provide an earlier indication of the disease that can help change a treatment decision to achieve a better outcome?
- Can it indicate whether a condition can be improved or maintained?
- Can it improve patient compliance?
- Can the results reduce the length of stay in a hospital?

The main advantages of Infigo’s technology in a POC setting include

- **Saving time** (transport into lab and culturing is no longer necessary),
- **Reduced utilization of resources** such as better use of staff times
- **Easy availability** of diagnostic procedures in primary care settings and in physician’s office; this reduces patient wait times and anxiety. Example the diagnosis of ectopic pregnancy takes days for patient to find our results resulting in increase in anxiety levels.
- **Improved outcomes**; the quicker the results are displayed in ER and ICU setting, more effective is the treatment strategy process.
• **Simple to use:** Infigo’s technology in a point of care setting is very simple, and can be conducted by personnel who have no detailed training for diagnoses or in lab medicine thus eliminating additional training costs substantially.

Due to improved outcomes as shown above, the popularity of POC tests have been on the rise with discovery of novel techniques for detection of analytes, faster interpretation times and higher sensitivity and specificity of analytes during testing. The demand for efficient POC tests in ED, Patient clinic, ICU, Outpatient clinic, surgical room, home care and at the physician clinic is of tremendous value.  

**The Market for POC devices**

Frost and Sullivan estimates that the (US Point of care testing markets, 2007) POC market was approximately $35 billion in 2009, and the US alone contributes to $2.1 Billion to this market.  

It is also estimated that the total POC market is expected to reach 3.9 billion dollars in 2016.  

This corresponds to a growth rate of 9.2%. It is predicted that the US POCT market will grow more rapidly compared to centralized labs, with the transfer of many tests to the POC setting. 

The following table provides a list of all current POCT applications and biomarkers.
Figure 11: Total market for POC

![Total POCT Market: Revenue Forecast (U.S.) 2006-2016](chart1)

*Note: All figures are rounded; the base year is 2009. Source: Frost & Sullivan*

**Figure 11: Total market for POC**

Total POCT Market: Percent of Revenues by Segment (U.S.), 2009

![Total POCT Market: Percent of Revenues by Segment](chart2)

*U.S. POCT Market, 2009: $2.1 Billion*

*Note: All figures are rounded; the base year is 2009. Source: Frost & Sullivan*

**Figure 12: Key markets in the POC spectrum**
B. Market Drivers for Infigo

A. There are a number of growing companies entering the POC diagnostics industry. Infigo’s establishment as a start-up company eventually looking to be licensed by a key market player will provide value to the industry. The unique method to detect analytes will facilitate Infigo’s licensing agreements to top key players, who will need this technology to serve their market needs better and more effectively.

B. There is a key trend of a shift from lab tests to tests conducted in the point of care setting. Novel research is being conducted to identify biomarkers that can help diagnose a chronic condition rapidly which will enable physicians to promote better patient management strategies. New opportunities arise everyday in the POC platform; for example the break out of H1N1 and other endemic diseases provide opportunities to Infigo especially if they have the ability to synthesize a MIP rapidly. This will save the time required to find a novel antibody for the biomarker.

C. The increase in focus on remote data management capabilities for point of care devices, in other words the real time measurement of analytes, with improved connectivity solutions and remote access monitoring can be incorporated into Infigo’s technology. This can help the way test results are stored and accessed and can minimize errors.

D. Competetion for Infigo

A. Key players in the POC industry, engaged in sophisticated systems with accurate specificity and sensitivity could hinder the growth of a start up company. Inverness Medical has the top market share with 27%, owning POC tests for almost all known biomarkers.
Figure 13: The key players in the POC market.\textsuperscript{30}

B. Central Lab tests

Most physicians still rely on tests performed in the central lab and are reluctant to change the trend from the clinical/central lab to a POC setting. Sophisticated systems are being developed in the lab to minimize wait times for patients. For example POC tests that detect glucose have an error rate of 20+ or – the standard value, whereas the lab tests produce an error of 8+ or – the correct value.\textsuperscript{34} This can limit the use of POC test in very critical settings.
**E. Vitamin B12 deficiency diagnostics**

Vitamin B12 is found in protein rich non-vegetarian food and plays a key role in maintaining the functions of the brain and nervous system.\(^{35}\) It is also essential for DNA synthesis in cells.\(^{36}\) It is required for normal red blood cell formation and tissue and cellular repair.\(^{35}\)

Vitamin B12 is considered safe and non-toxic and toxic or adverse effects have been associated with large intakes of vitamin B12 from food or supplements in healthy people.\(^{35}\) The body stores 3 to 5 years worth of B12 in the liver, thus making the diagnosis of a vitamin B12 deficiency and the associated symptoms very difficult as it may take several months to years to manifest in adults.\(^{35}\)

Vitamin B12 deficiency usually occurs in individuals who are strict vegetarians or those individuals who have a stomach or intestinal disorder due to which the body is unable to absorb Vitamin B12.\(^{35}\) This deficiency may lead to dementia, reduced cognitive function, movement disorders, neuropathy and nerve damage, megaloblastic anemia and numbness.\(^{35}\) Vitamin B12 deficiencies in pregnant women may lead to premature birth and neural tube birth defects, such as spina bifida, in the child.\(^{37}\)

1) **CBC test.**\(^{38}\) A CBC is a group of tests ordered for those patients who are suspected of anemia for deficiencies of vitamins, minerals and blood group cells. CBC is a routine test that is suggested to be taken by everyone. Very rarely, a specific vitamin B12 deficiency test is conducted, and is usually indicated from CBC test results. A person suspected of vitamin B12 deficiencies might have low levels of hemoglobin and RBC’s may be abnormally large.

2) **Testing for RBC folate deficiency** \(^{38}\): This test is found to more clinically relevant than measuring serum RBC.
3) **Causative Antibody Detection**\(^{38}\): Intrinsic Factor Binding Antibody- This protein is found to interfere with B12 binding and is detected by this test. A parietal cell antibody is produced against parietal cells that produce intrinsic factor and is seen in patients with pernicious anemia. Treatments include lifelong supply of vitamin B12 supplements.

**Market Potential**

Infigo can target pregnant mothers especially, vegetarian mothers since they have higher chances of having Vitamin B12 deficiency. Home diagnostic tests would be most useful at this stage as mothers are concerned about their diet.

**Vegans in the US**

It is estimated that approximately 92% of the vegans have vitamin B12 deficiency.\(^{39}\) In 2002 a poll conducted by Time magazine stated that 4% of the American population considered themselves vegetarian and 0.2% were vegans.\(^{40}\) The present American population is 307 million\(^{41}\), which implies that approximately **614,424** vegans are there in the US alone.\(^{42}\)

**Vegan Mothers in the US**

There are 4.2 million births per year in the United States. Assuming that 0.2% of the mothers are vegans, a market size of **8562** can be estimated.\(^{43}\)

**Pernicious anemia**

Pernicious anemia is a disease caused due to the lack of vitamin B12 in the diet, affecting 399,455 people in the US.\(^{43}\) This disease usually requires lifelong treatment with vitamin B12 pills or shots.\(^{43}\) This indicates that these patients require lifelong monitoring of vitamin B12 content.
Bariatric surgery patients

Patients who undergo bariatric surgeries like gastric bypass surgery and duodenal switch have an incapability to absorb vitamin B12. These patients may have to receive B12 shots, depending on the type of surgery. The doctors can use Infigo’s diagnostic test to assess vitamin B12 absorption by the patient’s body after surgery. Based on the result, the physician can determine if the patient needs vitamin B12 shots.

In 2004, a total of 121,055 surgeries were performed which is a nine fold (804%) increase when compared to 13,386 surgeries performed in 1998. Out of the total procedures performed, 78% of the patients were privately insured. Most patients were women accounting to a total of 82% of the surgeries.

Transverse myelitis patients

These patients are initially screened for vitamin B12 deficiency before the physician makes a decision on the treatment. About 33,000 Americans have some type of disability from this disorder and 1400 new cases are diagnosed each year.

The need of a diagnostic test:

The psychiatric disturbances associated with the disease such as depression, mood swings and personality changes are vague symptoms of vitamin B12 deficiency and can be easily overlooked, especially as the serum concentration of vitamin B12 can lie within the reference range. Also, the diagnosis of cobalamin deficiency has been found to be poorly sensitive and specific, and the recent focus is to use biomarkers markers such as serum levels of MMA and homocysteine. (Elevated levels of MMA and homocysteine depict cobalmin deficiency as
cobalmin is required for their conversation process to succinyl CoA and methionine respectively.) However, the diagnostic performance of a test launched for this purpose must be compared to the established gold standard for measuring cobalamin deficiency. This is particularly problematic, since there is currently no established gold standard, making the confirmation of a diagnosis of subclinical disease problematic. At present, lab tests such as CBC tests exist which can detect vitamin B12 deficiency in patients periodically.

Moreover in January 2004, the Axis-Shield HoloTC RIA developed an radioimmunoassay for holo-TC in vitro diagnostic assay that is used for quantitative measurement of the fraction of cobalamin (vitamin B12) bound to the carrier protein transcobalamin in the human serum or plasma. The device has been approved by the FDA as a 510 K class II device. The ELISA test is currently being developed by the company will be released in 2011. With respect to home settings, even while vitamin B12 tests conducted at home by patients can add value, especially for pregnant patients to monitor levels periodically and eliminating visits to physician office on a regular basis, it can be seen that there is no urgent need to constantly measure vitamin B12 as compared to measuring glucose and Coumadin at home. The effects of deficiency are not immediate, and need not be conducted on a day-day basis.

The fact that the market size is small, lab tests exist, there are companies in this space with improved immunoassays, no gold standard for comparison, and most importantly no immediate need to detect drastic vitamin B12 level changes at home or hospitals (ER, ICU etc) eliminates the need for Infigo to consider using their technology for detection of MMA at point of care both at home and hospitals.
F. Company’s Strategy in the POC market

Infigo’s technology is centered on providing an efficient, rapid, inexpensive POC diagnostic device, which can detect biomarkers both quantitatively and qualitatively. At present, many POC diagnostic tests provide qualitative information instantly, and a central lab or a sophisticated POC device test is often required to identify the concentration of the biomarker present in a sample. For example, the B-HCG can be detected qualitatively in pregnancy tests but require central labs to measure it quantitatively. Example detection of quantity of B-HCG in a sample is determined using a lab test.

Infigo can approach the market in 3 ways.

Approach 1: Enter existing market space and provide a cheaper diagnostic kit.

Infigo’s technology can enable its penetration into any existing market space and significantly compete in this space if

- The company can efficiently synthesize the MIP with the required specificity and sensitivity for the biomarker and thus can replace the use of antibodies in existing diagnostic tests. Examples include a D-Dimer detection test with greater sensitivity and specificity to detect pulmonary embolism.

- If the cost of the diagnostic kit provides cost savings to the end user, i.e. if it can be priced lesser than existing tests. For example the test for Coumadin is expensive. Infigo’s synthesis of a diagnostic test for half the cost of existing Coumadin test, can add to potential cost savings by companies, hospitals and insurance companies.
**Approach 2:** Infigo can look for unmet needs in the market space, and provide a rapid test to detect conditions at POC for already existing FDA approved biomarkers.

Infigo’s technology has the ability to overcome the challenges faced by current point of care diagnostic technologies i.e identifying antibodies for novel biomarkers that can effectively detect target analytes at POC platform.

For certain diagnostic procedures, the biggest challenge is to synthesize an antibody for a known and investigated biomarker in a POC platform with the required sensitivity and specificity. In many cases, the biomarker is already approved by the FDA, lab tests are conducted but a diagnostic test is unavailable in the market due to inability to produce an effective antibody that can be used in the POC platform. Infigo can therefore synthesize MIPS for known and FDA approved biomarkers and could collaborate with investigators who are in need of an efficient diagnostic test to identify these biomarkers at POC.

**Approach 3: Infigo can find a novel biomarker, gain FDA approval and synthesize a MIP for the novel biomarker.**

Although this approach provides Infigo several advantages including owning the rights to a particular biomarker and becoming the first and key player in this market, the clinical costs involved with seeking regulatory approval of the biomarker will make it cumbersome for the start-up company.

Examples include identifying a panel of biomarkers that can accurately detect stroke in an ambulance or ER settings, which can be extremely useful to physicians in the immediate and accurate decision making procedure for effective treatment strategies. Currently physicians rely on time taking MRI scans for this cause.
However, Infigo will not only have to identify the panel of biomarkers, but will need to seek regulatory approval for

A) The panel of biomarkers

B) Interpretation of results,

C) The diagnostics test to detect this panel in a POC setting.

Apart from this, synthesis of MIPS for this panel will also have to be undertaken by the company. These steps will obstruct the growth of the company.

Infigo is a start-up company with limited funds and this approach will most likely not be feasible for the company to pursue at this point of time.
G. Intellectual property

**Patent status**

The company has filed for one international patent.

<table>
<thead>
<tr>
<th>Name</th>
<th>Patent Number</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Diagnostic Devices Based On Molecular Imprinted Polymers</td>
<td>PCT/IL2007/000851</td>
<td>17.01.2008</td>
</tr>
<tr>
<td>Inventors</td>
<td>Raphael Levi, Ido Margalit, Yarden Dloomy,</td>
<td>Infigo Diagnostics</td>
</tr>
</tbody>
</table>

**Patent’s novelty**

It should be noted that the concept of MIPS to detect proteins and concept of lateral flow devices (U.S. Pat. Nos. 5,075,078; 5,096,837; 5,354,692 and 5,229,073) is not novel; and the medical device’s novelty lies in the “application of MIPS to a lateral flow device”.

**Patent Protection**

The above patent is claimed to safeguards the company’s key platform technology and its products on five levels:

- Conjugation of reporter molecules to templates = New chemical Entity
- Creation of MIP to specific templates
- Assembly of MIP on lateral flow devices
A unique and innovative signal amplification method

A unique and innovative visual quantification method

The claims of the patent specifically indicate that the use of MIPS for biological molecules incorporated into a lateral flow device for its detection as claimed by this patent cannot be developed. However, lateral flow devices incorporating natural antibodies for a biomolecule, (as in a standard immunochromatography or its modifications) can still be developed. Additionally, Claim 33 in the patent application claims the detection vitamin B12 using the diagnostic device. However, this does not necessarily mean that Infigo’s device has the only rights to detect Vitamin B12. The company has the rights to detect Vitamin B12 using plastic antibodies in a lateral flow format at point of care, and the patent does not stop another company with a novel diagnostic method to detect vitamin B12.

The value of the intellectual property is essentially focused on the use of plastic antibodies over natural antibodies.

**Freedom to operate**

As of March 2010, the company obtained the freedom to operate, as the patents whose claims may be impeded by Infigo would expire. The following patents were identified that are closely connected to Infigo, however Infigo’s patent does not impede the claims as provided in table x and thus has the freedom to operate.
Table 10: Related patents and analysis

<table>
<thead>
<tr>
<th>Patent</th>
<th>Patent Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US Patent Nos. 5,602,040; 5,656,503; 6,228,660 and 5,622,871</strong> (‘the ‘040 Patent Family Members’) on LFI by Inverness Medical</td>
<td>The Infigo process does not use a “label molecule, which produces the signal which is visualized.</td>
</tr>
<tr>
<td><strong>US Patent 5798273,5622871,6228660</strong> LFI by Inverness Medical</td>
<td>The Infigo process does not use natural antibodies in the lateral flow immunoassay, but uses MIPS</td>
</tr>
<tr>
<td><strong>US Patent No. 5,895,750</strong> Immunoassay for the detection of ligands by Abbot</td>
<td>Infigo process does not use antibiotin antibody for detection of target ligand, and uses streptavidin.</td>
</tr>
<tr>
<td><strong>US Patent No. 7,238,538</strong> Chromatographic assay device and methods by ZBX corporation</td>
<td>The Infigo process does not use reservoir containing a labeled reagent that can be used to detect analyte ligand complex.</td>
</tr>
<tr>
<td>Patents by Mosbach <strong>US Patent No. 5,872,198</strong> Molecularly imprinted beaded polymers and stabilized suspension polymerization of the same in perfluorocarbon liquids</td>
<td>The Infigo process does not use a stabilizer and can be practiced without an acrylic monomer.</td>
</tr>
<tr>
<td><strong>9, US Patent No. 5,821,311</strong> Stabilizers, polymers, and emulsions useful for molecular imprinting technology</td>
<td>The Infigo process does not use the recited stabilizers, suspensions, or polymers.</td>
</tr>
<tr>
<td><strong>US Patent No. 6,316,235</strong> Preparation and use of magnetically susceptible polymer particles</td>
<td>The Infigo process does not use magnetically susceptible polymer particles.</td>
</tr>
<tr>
<td><strong>US Patent No. 5,959,050</strong> Supports useful for molecular imprinting technology</td>
<td>The Infigo process does not require a fluorocarbon-containing liquid or a fluorocarbon-containing copolymer.</td>
</tr>
<tr>
<td>Patent No.</td>
<td>Title</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>US Patent No. 6,316,235</td>
<td>Molecularly imprinted beaded polymers and stabilized suspension polymerization of the same in perfluorocarbon liquids</td>
</tr>
<tr>
<td>US Patent No. 5,959,050</td>
<td>Supports useful for molecular imprinting technology</td>
</tr>
<tr>
<td>US20080248961</td>
<td>Materials for screening of combinatorial libraries</td>
</tr>
<tr>
<td>US Patent No. 6,255,461</td>
<td>Artificial antibodies to corticosteroids prepared by molecular imprinting</td>
</tr>
<tr>
<td>US Patent No. 6,127,154 (Mosbach)</td>
<td>Methods for direct synthesis of compounds having complementary structure to a desired molecular entity and use thereof</td>
</tr>
<tr>
<td>US Patent No. 6,187,598</td>
<td>Capillary immunoassay and device therefor comprising mobilizable particulate labeled reagents</td>
</tr>
</tbody>
</table>
Additional IP

Even while Infigo’s technology is well protected by its patent, the device provides lesser value compared to when the company identifies and patents a novel biomarker to diagnose a particular condition. For instance, Infigo’s technology can be used to detect cholesterol at point of care; however the value of using the device for this will be significantly lesser as compared to Infigo identifying a novel biomarker for the detection of stroke at point of care. (The detection of stroke is complex and till date no biomarker has been validated for its detection at point of care, attempts have been made but failed due to insufficient data provided). This is due to the fact that Infigo becomes one among several market players to detect cholesterol at point of care.

To summarize Infigo’s patent value, additional and maximum value can be acquired if

A. The company identifies a novel biomarker and patents its use for diagnosing a particular health defect/ condition.

B. The method of detecting the biomarker using MIPs at point of care is also patented.

G. Regulation by the FDA.

According to FDA’s classification, Infigo’s device would qualify as class II 510K medical device, based on its safety profile as it is found to be equivalent in function to a predicate medical device. The average approval would take about 75 days, and could take a maximum of 150 days depending on the nature of the invention.
The total maximum costs involved in obtaining FDA approval could cost approximately $100,000 depending on the nature and risks involved with the misinterpretation of results provided by the diagnostic test. Although many POC devices expect to be classified as 510 K class II device, FDA could classify the device as PMA Class III, if Infigo seeks approval for a novel biomarker to be used at a POC setting. In general, regulatory processes for diagnostic devices should ensure that they contribute to improvement and protection of patients’ health. With the increasing number of diagnostic devices entering the market every year, the regulatory pathway can become very stringent and cautious. There are about 1000 genetic tests that have been developed in labs but are not subjected to FDA approval.

ADVAMED states that devices should be classified by calculating the risk associated with the misinterpretation of results to patients’ health. The proposal put forth by AdvaMed claims tests that detect same biomarkers should be exempt from FDA regulation. This would not only make the process of market entry easier, but will encourage innovation in this field.

In 1988, the Congress passed the Clinical Laboratory Improvement Amendments CLIA “to establish quality standards for all laboratories testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed.”

The FDA characterizes In vitro into 3 categories based on the potential for risk to public health.

a. CLIA Waived Testing: These are non-critical tests, approved by FDA and can be used at home by the patient. These tests are simple and accurate, risk free and the rate of erroneous decisions is low. Examples include glucose monitoring at POC.

b. Non CLIA Waived testing: There are 2 types of non waived testing
i. **Moderately complex testing:** These tests require training, however they are minimally scientific and technical and training has to be performed moderately. The operational steps can be controlled easily and minimal interpretation and judgment is required. Example cardiac troponin measurement.

ii. **Highly Complex Testing:** Tests which require specialized scientific and technical training and knowledge, training and experience to perform accurately, operational steps require close monitoring or control, and extensive independent interpretation and judgment are required.

A point of care committee chaired by the Clinical Laboratory Director includes the following people or their designees; Chief Medical Officer, Medical Nursing, Surgical Nursing, Ambulatory Administration, Quality Improvement, Purchasing, Risk Management and Medical Center Administration. The committee is formed exclusively to

A. Monitor all tests for compliance & proficiency

B. Assess POC technologies and safety.

C. Evaluate and approve tests to be established as point of care

D. Provide recommendations for approval.

The evaluation step ensures that the testing of a point of care device by clinical lab staff. This ensures that the testing complies with state and federal standards and meets regulatory approval. The procedures for the test are clear and written in a standard format. Quality control is established and is reviewed by the clinical lab regularly. A training program to ensure that testing personnel also meet regulatory approval is implemented. The clinical lab personnel provide documentation and tests results with Quality Control data to the POC committee.
Unless the POCT committee approves of the test, units and clinics may not be able to perform the test.

Infigo’s technology is easy to use, however depending on the analyte and the risk associated with mis-diagnosis of results, Infigo’s technology used in the point of care testing for various analytes could either be CLIA Waived or Non CLIA waived. Example if Infigo uses its technology for glucose monitoring at home setting, it can be considered CLIA waived, however if the test is used to detect troponin in the emergency setting, then it will be considered as valuable.

H. Reimbursement

Many insurance plans do not include clinical laboratory tests among the scope of benefits, and for those that do cover lab tests; all POC tests may not be included.

Under Medicare, clinical laboratory tests are classified as “diagnostic laboratory tests,” and are grouped along with X rays and other tests. Existing CPT codes for lab tests are assigned to new POCT technologies, which may cost much more than a lab test. Currently, the costs associated a POC test is almost equal to the reimbursement cost, and this limit a physician’s ability to derive positive revenue from using these tests. Therefore there is a pressure to produce efficient tests at a lower price, so that hospitals and physicians not only breakeven but also are able to gain profits. However, since the accuracy of a POC test is lesser when compared to a lab test, the pressure is on the POC test manufacturer to improve accuracy and reduce the costs of production. This can be a great challenge to the launch of Infigo’s diagnostic test in the present US market.
POC tests are classified under the clinical lab benefit provision under Medicare. Also most tests get reimbursed only when they are performed in the lab with appropriate CLIA amendments. A non waived test is not covered if performed by a lab that holds only a CLIA waiver.

In general POC tests are covered only if they are deemed as “reasonable and necessary”. For example Medicare contractors denied coverage for home prothrombin-time testing by claiming that these tests were not “reasonable and necessary.” On the other hand, glucose monitoring at home is reimbursed by most insurance plans, as the cost savings is significant. Cost savings is calculated when it the testing is compared to the effects of hospitalization for a acute diabetic patient if testing was absent.

Different plans have different types of coverage’s for POC testing. In general, under the Medicare inpatient Prospective Payment, hospitals are paid one fee that covers all services under a DRG code. This indicates that all procedures, including diagnostics (except patient fees) is covered under one payment. In other words, the number of POC tests conducted within this DRG will not make a difference, as it is not reimbursed separately. The DRG is determined by the main reason that the patient is admitted in the hospital (for example if a diabetic patient is admitted for pneumonia, payment would be the same for either 1 or 25 tests conducted.) This would be a problem for the hospital as they do not get reimbursed separately for each POC test and will want to purchase only those tests that are less expensive and can facilitate lesser usage.

Point of care testing that can be implemented at home may or may not be reimbursed depending on the health plan as well as nature of the test. Pregnancy tests are not reimbursed at home but may be reimbursed my Medicare it the hospital setting. The POC test to detect prostate cancer is not reimbursed at home and needs to be paid by the patient.
J. Other Markets

Companion diagnostics

The discovery and validation of several biomarkers has given rise to companion diagnostics; defined “as assays used in determining the efficacy and dosage of a drug in a patient to ensure safe and therapeutic response.” The field of companion diagnostics is becoming common and will eventually become the norm. Recently pharmaceutical companies have increased the awareness that in order to save money and produce effective drugs, drugs will have to become more personalized. Once the use of biomarkers is identified, question of whether it is a prognostic (related to disease outcome) or a predictive biomarker (related to treatment outcome) and impact on the clinical development strategy will have to be studied.

There are 2 kinds of tests; tests that have been developed after a drug has come into the market and tests that have been developed in conjunction with the drug. The use of Infigo’s technology to rapidly detect biomarkers in companion diagnostics can be further investigated. Evaluation of a biomarker for companion diagnostics include the knowledge of

- Function of the marker
- Prognostic (related to disease outcome) or predictive biomarker (related to treatment outcome)
- The impact of the biomarker in clinical development strategy.
- Use of the biomarker in the clinical trial setting.
- The particular stage in the patient’s treatment regime that the biomarker should be used.
- Approval status by the FDA.
• Will the use of biomarkers be reimbursed by payers?

The use of HER2, is likely to be relevant to drug mechanisms of action as predictors of drug efficacy of the drug herceptin.63

Conclusion

At the inception, Infigo targeted the Vitamin B12 deficiency market, however, a comprehensive POC market analysis indicates that the market for Vitamin B12 is very small and the need for a diagnostic kit is questionable. While exploring market opportunities for this device, some high potential applications have surfaced such as drug level monitoring, medical toxicity, and detection of cancer diagnostics, environmental analyte detection, and companion diagnostics. A market analysis indicates that technology can find best value in the detecting of conditions/diseases for which biomarkers are yet to be discovered. In this way, Infigo could not only enjoy the rights of the biomarker but also have privilege of being the first to entrant in this market. However, this can again be very challenging, as the process of discovery and validation of biomarkers involves a complex combination of proteomics and array technologies. In addition, Infigo Diagnostics would have to follow an extremely tedious pathway for regulatory approval, taking up much of the initial time and investment, a pathway unfavorable for a start-up company.
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