The term “molecular diagnostics” refers to laboratory tests used to identify a disease, or the predisposition to a disease, by analyzing DNA- (deoxyribonucleic acid) or RNA (ribonucleic acid) or their proteins, in humans or, in the case of infections, in microbes. Its scope includes the clinical testing devices, as well as their reagents and supplies, that are utilized in hospitals, clinics, commercial laboratories, reference laboratories, research institutes and doctors offices to detect cells and proteins for the purpose of diagnosis and monitoring disease. Molecular diagnostic technologies have been and will continue to play an important role in the practice of medicine, public health, pharmaceutical industry, forensics and biological warfare in the 21st century. Some of these technologies include nucleic acid amplification like the polymerase chain reaction (PCR), fluorescent in situ hybridization (FISH), peptide nucleic acids (PNA), electrochemical detection of DNA, biochips, nanotechnology and proteomic technologies. Many applications of molecular diagnostics are for infections but are now increasing in the areas of genetic disorders, pre-implantation screening and cancer.

Molecular Diagnostics and Beyond: Putting Molecular Testing in Action

Dr Lisa F. P. Ng and Associate Professor Raymond Lin Tzer Pin

The science of medical diagnostics has advanced significantly during the past decade. Traditionally, diagnosis has been overshadowed by new drug discoveries, but the expansion of molecular diagnostics is creating new careers for researchers in many fields. It was suggested that the fastest-growing, most profitable area in laboratory medicine at the moment is molecular diagnostics. Clearly, much of this improvement has come as a result of new knowledge of the human genome and related proteins, which form the foundation of cell and molecular biology, and disease at the molecular level. Genomics, sequencing and technologies allowing the study of functional genomics have changed everything in the diagnostics field. Like genomics and functional genomics, molecular diagnostics depends heavily on a strong network of scientists (molecular biologists, chemists, biostatisticians and even engineers) to understand and capitalise on the data-rich field to bring a test from the research and development phase and release it into the fast growing market. The situation wasn’t so promising more than 15 years ago when techniques were laborious and tedious, relying on methods like Southern blotting (a molecular technique in which DNA molecules separated on a gel are transferred to a solid support so that specific fragments can be detected using known labelled probes) and frequently involving the use of radioactive reagents. It could easily take up to three days for the complete assay before results were obtained.

The birth of molecular diagnostics has been a little ambiguous and for a long time, some people have dated it to the elucidation of the double helical structure of DNA by Watson and Crick (Watson and Crick, 1953) as that revolutionary discovery provided scientists with the molecular tool that paved the way for molecular biology research. However, it took scientists many more years to use that knowledge to have a significant impact on clinical medicine.

There are approximately five to six areas of molecular diagnostics carried out by hospital, reference laboratories and research institutes...
worldwide. Not every laboratory will perform all of them as different laboratories have their own expertise and interest. In general, they all use the same molecular tools and technologies, and they can be classified as:

1. **Infectious diseases molecular testing**
   This involves the design of DNA probes that are directed against the specific sequences of viruses, bacteria, or parasites.

2. **Molecular oncology testing**
   This looks at individual cancer markers and involves detecting any derangements or alterations in either the DNA sequence or gene expression that could be found in a tumor and nowhere else in the body.

3. **Inherited diseases molecular testing**
   This looks at inherited disease (genetic diseases) where one should be able to locate mutations in the body inherited from the parents (parent).

4. **Identity testing — DNA fingerprinting**
   This looks at benign variations in DNA sequences that exist between people in a population and has many applications, such as, forensic testing to solve crimes, paternity testing, monitoring bone marrow transplants as it can be used to distinguish donor from recipient cells.

5. **Tissue type testing — HLA (Human Leukocyte Antigen) typing or histocompatibility testing**
   This is a blood test that measures substances called antigens on the surface of body cells and tissues. Checking the antigens can tell if donor tissue is safe (compatible) for transplant to another person. In some cases, a tissue type test may be done to see whether a person has a chance for developing certain diseases that cause the body to attack its own cells, such autoimmune diseases.

6. **Pharmacogenetics testing**
   This is probably the newest area which looks at benign variants in the human genome in genes that are involved in drug metabolism and alter subtly how a drug is metabolised. Such tests will be useful prior to initiation of therapy in order to choose the best or most appropriate therapy for the patient. This is one key technologies in what is popularly referred to as “personalized medicine”.

   One of the earliest FDA (US Food and Drug Administration) approved molecular diagnostics tests was in 1985, which was a nucleic
acid-based test for Legionnaire’s disease. Following that, most of the other tests that were licensed in the 1980s and 1990s were targeted mainly at infectious diseases, namely, HIV (human immunodeficiency virus), HPV (human papilloma virus), hepatitis B and C. In the area of infectious diseases, diagnosis traditionally involved trying to detect and identify a microbe that is infecting the patient using classical microbiological techniques such as viral and bacterial culture, and the use of antibodies. These methods are not only long and tedious, but they may not detect or be able to identify the target organisms or their subtypes of interest. A good example is the detection of HPV. There are about sixty to eighty subtypes of HPV that have subtle differences in their DNA sequence. The classical microbiological methods are unable to distinguish them because the viruses look the same from the “outside”. But with molecular techniques such as PCR, specific genomic sequence variants could be detected. This has important clinical implications because some of the subtypes cause benign disease, while others cause invasive carcinoma of the cervix.

In addition, the use of molecular diagnostics technologies has enabled the accurate quantitation of the viral load in systemic viral diseases. It is now possible not just to identify the microbe, but also detect how much of it is there. For example, following kidney or bone marrow transplant, doctors routinely monitor the presence and level of cytomegalovirus in the blood, in order to know when to start antiviral treatment. Another example is the monitoring of HIV viral load in infected patients to help doctors determine the best combination therapy.

Molecular diagnostics technologies have also paved the way for the detection of several bacteria and viruses that are difficult to culture, and some not “culturable” at all, and thus delay the initiation of therapy. An example is the tuberculosis bacillus, which can take two to eight weeks to grow in culture, but which can be detected and reported in the same day using molecular methods.

In the area of infectious diseases, cutting edge technologies that include some kind of microarray, or “DNA chip” (containing different DNA probes all laid down on a solid support that can be tested together in parallel rather than one at a time) would be possible in the very near future. This new technology is miniaturised and can allow detection of multiple pathogens at one time.

Molecular diagnostics are also rapidly expanding for the detection and risk assessment of cancer (oncology). Usually many laboratory methods are used in examining cancerous tissue, including histology, immunohistochemistry, conventional and real-time PCR and FISH analysis,
New lab-on-a-chip device being developed at the National University Hospital.

and the tests often complement each other. Examples of molecular tests are: minimal residual disease (MRD) testing to check whether a patient with leukaemia has responded to treatment; and BRCA-1 mutation screening for familial forms of breast cancer.

In addition to diagnostics and prognostics, molecular diagnostics are now being used to discover patients’ responses to treatments in the area of pharmacogenetics. For example, a commercial DNA microarray chip is now available to help doctors determine whether the patients are ultra-rapid metabolisers of certain drugs (e.g. for treatment of severe depression, schizophrenia etc.) and choose the best medicine, and avoid adverse drug reactions.

It is important to mention that cost is a practical issue as molecular diagnostics tests are often expensive. However, the tests are worthwhile performing if they result in faster diagnostic results, more targeted therapies and shorter hospitalization, which in their turn reduce health care costs. With the gradual automation of the tests, prices will clearly drop. It was mentioned that 70% – 80% of a person’s entire healthcare expenditures over their whole life occur in the last 4 – 6 months of life. As a result, more attention is being given to catching a disease condition early before it gets out of hand and becomes a potentially fatal illness. At the moment, biomolecular screening, diagnostic, and treatment products are being developed to catch diseases states early so that they can be dealt with before they become life threatening and expensive to treat.

From the public health perspective, better infectious disease detection capability is vital, as earlier detection would allow time to allocate adequate and appropriate resources to prevent or manage outbreaks of infection like influenza.
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