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# Advanced techniques in the field of Immunology and immunological tests

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#### Abstract

Immunity is a very prime factor related to health for the sustainability of life. Environmental factors are responsible to modulate the immunity. Each person has identical and different type of immunity. It is found that even twins have the different immune system. Immunology is the branch of biomedical sciences concerned with all aspects of immune system in all multicellular organisms. Physiological functioning of immune system and Immunological disorders i.e. hypersensitive, allergies, transplant rejection, virus attack etc are studied in immunology. Various techniques are required for specific and accurate results and to understand the behavior of the factors involved in a particular type of immunity. Flow cytometry is one of the basic important techniques widely used in the study of immune system. Large number of research papers show data obtained and analyzed from Flow Cytometry. Mass cytomerty is applied in the research of immunology which is basically a mass spectrometry technique. It is based on inductively coupled plasma mass spectrometry. Fluorescence microscopy (FM) is used to locate a component within the cell. The objective of this technique is to relate the results to the structure and function of the immune system. In this paper few important emerging technologies, which are empowering the research and development work in the field of immunology have been discussed in detail.

Keywords: Immunology, Antibodies, Technology, Mass cytometry, Imaging microscope.

#### 1. Introduction

Human health is a complex factor which requires a lot of care and supporting systems in which without technical tools and devices it is very difficult to work in the present scenario. Several factors are responsible for the human health. Strong Immune system provides strength to protect the human body from harmful viruses such as SARS-CoV-2 (COVID), HIV and hepatitis C etc. If immune system is weak then our body feel frequent infections, faces tummy troubles, feel tiredness, have a cold again and again. Generally, there are three main types of immunity i.e. innate, passive and adaptive. From the starting of covid-19 pandemic, studying of immune system of the people using quick and accurate techniques was considered as the prime factor for the safety and security of the people. Large number of peoples were facing problems related to weak immune system during and post covid-19 because covid-19 and its variant affect the immune system of the body.

Immunology is one of the very important factors to be responsible for the immune system of the living being. Immunology is related to the biomedical sciences but for specific and accurate result, the use of relevant technique is required. Observation of immune system requires techniques and clinically tested proper methods which requires advanced technological support. Quantitative approach to study immunology was discussed in detail<sup>[1]</sup> using theoretical tools required for the research. Physical concepts of immune function had been reviewed. Studies are there to assess the technological advancement with improved sensitivity<sup>[2]</sup>. Although most of the emerging technologies and traditional methods used for the human health related tests are discussed in detail<sup>[3]</sup> but few important techniques and methods required for immunology is lacking in terms of detailed study. Due to scientific development, new advanced technologies are coming on the basis of new sophisticated skills. Mass cytometry is used to study qualitative and quantitative properties of the cells. It also based on important technique which is time of mass flight mass spectrometry. Physical

Correspondence: Hariom Dwivedi Department of Physics, Isabella Thoburn College, Lucknow- (U.P.) India. characteristics of immune systems are studied under immunology.

#### 2. Methodology and discussion

This review paper deals with recent advances in the field of immunology giving account of important techniques by the literature review. Artificial antibodies are produced, which matches with the substance or the virus under observation, to be used in immunological tests. Antibodies produced artificially, come into contact with their matching substance available in the sample, then antibodies bind with them giving signal during the tests.

## 3. Recent technology in the immunology

Immune system is directly related to human health and hence the health of our society resulting the growth and development of the nation. It also covers broad area of research because immunity changes with person to person as well as on surrounding environment. Immunological tests are used to identify the presence of antibodies produced against the effect of an antigen. Sometimes antibodies are used to check presence of antigen in the human body. The amount of IgG, IgM and IgA available in the blood of the human body used to diagnose the immune system. These tests are done using sophisticated devices equipped with latest technologies. Keeping in mind these factors, several types of techniques are used in the study of immune system in which few important techniques based on physical principles are discussed below:

# 3.1 Flow cytometry

Flow cytometers (FC) are automated instruments which are used to study quantitative properties of single cells. Properties like cell size, cell granularity, the amount of cell components, amount of messenger RNA for a particular gene etc. are determined accurately by FC. It uses laser beam technology in which laser beam is focused on cells and scattered light is observed and analyzed. At a time, large number of cells are tested. At present, computers are used to process the data and then storing of the data generated. In this process light is absorbed and then light of cell characteristic is emitted and analyzed. Sometimes, spectral overlap is observed which becomes difficult to analyze and this also limits its uses. It is used to analyze the functions of the cells giving information about the immune system.

# **3.2 Spectrometers**

Mass cytometry is advance technology than the flow cytometry because mass cytometry uses discrete isotopes in form of reporting system. Mass spectrometry technique which is based on inductively coupled plasma mass spectrometry is known as mass cytometry<sup>[4]</sup>. It also depends on time-of-flight mass spectrometry. Properties of the cells are determined through this technique. In this method, antibodies are conjugated with isotopically pure elements. Then these antibodies are used to label cellular proteins. In the next step, Cells are nebulized for which different nebulizers play an important role. Then, these are sent through an argon plasma. The argon plasma ionizes the metal-conjugated antibodies. Further the metal signals are analyzed through a time-of-flight mass spectrometer. Number of cells analyzed at a time is very less hence it is a slow process in comparison to flow cytometry. The

advantage of this device is that it differentiates the isotopes very accurately without any overlap in the information. Due to this, it reveals more information about the specific cell. Some more types of important spectrometers used in the study of immune system are Raman spectrometer, Xray spectrometer, Nuclear Magnetic Resonance (NMR) spectrometer, Augur electron spectrometer, Fluorescence spectrometer, portable spectrometer etc.

## 3.3 Immunoassay analyzer and system

One highly sensitive immunoassay is the enzyme-linked immunospot (ELISpot) which measures the frequency of cytokine-secreting cells. Protein secreting cells can be quantified through this technique which are analyzed by medical experts to study the immune system. Enzyme linked immunosorbent assay (ELISA) technique is also used in immunology to test the antibodies and particular type of proteins. ELISA readers use special kind of plates with sensor technology to give instant information by using a substrate as a sensing material which changes color with enzyme.

# 3.4 Fluorescence imaging microscope

Microscopes are the instruments which are used to visualize cells providing information about the immune system of the body. There are a large range of microscopes in which fluorescence microscopes and electron microscope are used widely.

These microscopes use light filters for the emission of specific wavelength of light. Light of specific lower wavelength is used to excite the fluorophores which emit light of larger wavelength with the release of the energy as a result of reaching the fluorophores in ground state. Which are visualized using high-tech microscopes. Fluorescence imaging microscope delivers excellent images without delay.

Research and development work in the field of quantum dot<sup>[5]</sup> is showing large scope in the field of such types of analysis.

# **3.5 Immunoblotting Equipment**

So many types of immunoblotting equipments are used in immunological analysis such as UV transilluminator, Gel Combs, Electrophoresis power supply, Gel image capture system, western blot processor etc. In which most commonly used Immunoblotting technique is western blotting. This is a widely used technique in research and clinical tests. Most of the accessories are based on the sensing techniques. Immunoblotting technique is used for the identification of target protein from a large number of species of unrelated proteins using antibodies and sometimes using specific ligands in the related techniques. With the advancement of technology, equipments are available having automated systems controlled with the help of software. These automated equipments are available with multiple powerful features which analyses the samples very fast and very accurately. Electrophoresis equipment such as the electrode assembly, transfer systems, blotting membranes, buffer tank are used in these accessories. Antibodies have important role in this analysis. During processing, protein transfer from gel to subsequent membranes are done very carefully. In this a blot membrane is used having protein band showing color intensity on the basis of which specific proteins are analyzed.

World Wide Journal of Multidisciplinary Research and Development

#### **3.6 Protein purification systems**

Immunoprecipitation (IP) based techniques are used in the separation of specific protein. In this technique artificial antibodies are used against the protein under observation. In IP the immuno word refers to antibodies and precipitation word refers to pulling down the substance from given solution. Analysis of isolated proteins are done using Nuclear Magnetic Resonance, mass spectrometry and western blot technique.

#### **3.7 Spectrophotometers**

In the study of immunity, sometimes it is required to find out types of molecules available in the cells for which spectrophotometers are preferred to use. Various types of spectrophotometers are available to be used to study the proteins and other molecules by measuring the concentration of a particular molecule in the cells. These absorption spectrophotometer, are atomic spectrofluorometer, Infrared spectrophotometer, visible spectrophotometer, UV spectrophotometer, double beam spectrophotometer etc. These devices provide information about the sample by measuring the wavelength of light absorbed by the samples which provides inherent information about the sample.

### 3.8 Immuno chemistry Analyser

Immunochemical techniques <sup>[6]</sup> used in the study of immunology to get information about the immune system during the clinical tests and also for research. Human viral infections are detected using these techniques in clinical tests and data is used for the treatment, research and development work for further improvement. Apart from above techniques and devices, physical statistics and dynamics is also used in the analysis of immunological problems<sup>[7]</sup>.

#### 4. Conclusions

There is a large range of devices based on wide range of techniques used to study the variety of immune systems which is the result of advancement in the physical science because most of them are using physical principles. Mass cytometry, spectrophotometer are the techniques which are commonly used to study immune system. Technological development and theoretical quantitative concepts have made research and development work very convenient, accurate and fast.

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