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Serological Investigation in Viral Infection. A Review

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ABSTRACT:

The conventional basis for the serologic prognosis of viral contamination is a demonstration of seroconversion or a huge increase in circulating homologous viral antibody over the path of infection. Conventional methods include neutralization, supplement fixation, hemagglutination-

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inhibition, indirect hemagglutination, and indirect immunofluorescence tests. Although these strategies are dependable, each suffers from boundaries that consist of procedural complexity, want for titrating reagents and serially diluting specimens, occasional fake-high quality or false-terrible results, and shortage of interlaboratory standardization. Because of those issues, advanced methods, and new strategies for serologic diagnosis were evolved and investigated. Many appear superior to conventional strategies in sensitivity, specificity, price, the time required for completion, and potential for automation. The advent of hybridoma generation has furnished a first-rate possibility to enhance serologic reagents for the analysis of viral disease. Also, IgM-particular antibody tests for fast and early prognosis of many viral infections are being reevaluated to dispose of the interfering effects of a rheumatoid issue and antinuclear antibodies. Many of the new methodologies employ immunofluorescence assay or enzyme immunoassay for detection of particular IgM antibody, and latex agglutination, further to immunofluorescence and enzyme immunoassays, to stumble on particular IgG antibody. Simplified kits using those strategies are now becoming available commercially. The main aim of my study is to assess the importance of serological investigations in viral infections.

INTRODUCTION:

Serology is a scientific study that assesses serum and other body fluids for the presence of antigen or antibody. One of the components taking part in the reaction is antibody. They generally refer to the identification of antibodies within the serum. Such antibodies are generally in opposition to the microorganisms or toward other proteins or towards themselves (an autoimmune disease). Testing of viral nucleic acid and serological testing enhance blood safety (AlMutairi*et al.,* 2016). HIV, Hepatitis B, Hepatitis C are at greater risk of blood safety (Tigabu, Engda and Mekonnen, 2019)(Pratha, AshwathaPratha and Geetha, 2017). Viral infections of nonhuman primates also play an important role (Wachtman and Mansfield, 2012). They are effectively applicable throughout the procedure of rising viral outbreaks and provide various advantages while in comparison with alternative diagnostic methods (Albadaw, 2018). Surveillance on the type of virulence factors is harboured by the organism (Priyadharsini*et al.*, 2018b). There is a need for antibiotic surveillance and alternate therapeutic measures (Priyadharsini*et al.*, 2018a). Timely investigation of the causative agent will prevent the unwarranted use of antibiotic and drug resistance.

The traditional foundation for serological diagnosis of viral contamination is a demonstration of seroconversion or increase in circulating homologous, viral antibody (Mozayani and Noziglia, 2007). There are various limitations in the serological method of investigation (Turgeon, 2013). A serological analysis can be made with the aid of the detection of growing titres of antibody between acute and convalescent stages of contamination, or the detection of IgM(Stevens and Miller, 2016). In the majority of cases, unusual viral infections can be recognized with the aid of serology. Rapidly emerging infectious disease outbreaks are an exceptional strain on laboratories to develop and put in force sensitive and particular diagnostic checks for patient management and infection control in a well-timed manner (Chan *et al.*, 2017). Hospital-acquired infections are due to their propensity of accumulating and exhibiting great challenges for physicians (Priyadharsini*et al.*, 2018a). The specimen used for route detection and virus isolation could be very important. Newer pathogens are emerging day by day which is a major threat in treating

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hospitalised patients (Ashwin and Muralidharan, 2015). Acinetobacter Baumanni improves patient care in the hospital environment (SmilineGirija and Others, 2019). An effective result of disease would be of greater diagnostic importance than the ones from different sites (AlMutairi*et al.*, 2016). For instance, in the case of herpes simplex encephalitis, a result from the CSF of the brain could be much more significant than an effective result from an oral ulcer, due to the fact reactivation of oral herpes is common in the course of instances of strain (Studahl and Sköldenberg, 2017). Plaque accumulation and increase in oral microorganisms are the main factors of poor oral condition (Selvakumar and Np, 2017). Oral hygiene of patients is compromised (Shahana and Muralidharan, 2016). Orange peel oil can have good antibacterial activity (Vaishali and Geetha, 2018).

The isolation and identification of viruses are generally not done in the laboratory (Jerome, 2016). Serology plays a great role in the diagnosis. The development of antibody components of viruses is used in staging the disease. Intracanal medicaments are also helpful (Marickar, Geetha and Neelakantan, 2014). Naturally, some leaves are very helpful in treating some conditions. For example, Laurus Nobilis has many medicinal properties which can be used to treat cancer, joint pain, gastric problems (M, Geetha and Thangavelu, 2019).

By serological diagnosis, many common viral infections were diagnosed (Chan *et al.*, 2017). There are various new techniques which are the mainstay of viral diagnosis such as Radioimmunoassay, Enzyme-linked immunosorbent assay etc. They are diagnostic methods that detect antigens and antibodies in a sample. These tests may be performed to diagnose infections ('Prevalence and Trends of Common Transfusion Transmitted Infections Using Serological and Nucleic Acid Markers in Saudi Blood Donors', 2015). It also identifies if a person has immunity to certain diseases and determines an individual's blood type (Stevens and Miller, 2016).

Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Ariga*et al.*, 2018; Basha, Ganapathy and Venugopalan, 2018; Hannah *et al.*, 2018; Hussainy*et al.*, 2018; Jeevanandan and Govindaraju, 2018; Kannan and Venugopalan, 2018; Kumar and Antony, 2018; Manohar and Sharma, 2018; Menon *et al.*, 2018; Nandakumar and Nasim, 2018; Nandhini, Babu and Mohanraj, 2018; Ravinthar and Jayalakshmi, 2018; Seppan*et al.*, 2018; Teja, Ramesh and Priya, 2018; Duraisamy*et al.*, 2019; Gheena and Ezhilarasan, 2019; Hema Shree *et al.*, 2019; Rajakeerthi and Ms, 2019; Rajendran *et al.*, 2019; Sekar*et al.*, 2019; Sharma *et al.*, 2019; Siddique *et al.*, 2019; Janani, Palanivelu and Sandhya, 2020; Johnson *et al.*, 2020; Jose, Ajitha and Subbaiyan, 2020).

DEFINITION:

Serologic tests are blood assessments that diagnose antibodies of blood (Wachtman and Mansfield, 2012; Hati*et al.*, 2014). They can contain several laboratory strategies. Different forms of serologic tests are used to diagnose numerous disease conditions.

Serologic tests. have one aspect in common. They all focus on proteins that enable us to keep healthy by destroying foreign invaders which can make us fall sick (Institute and National Cancer Institute, 2020).

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IMPORTANCE OF SEROLOGICAL INVESTIGATION:

Serology is a mainstream diagnosis in viral infections. It detects a rising titre value of antigen for the detection of IgM in a primary infection (Yong *et al.*, 2020). Variations and differences are seen in the clinical presentation and in convalescence. They have a positive correlation with the antibody titre in the body. Antigens are substances that provoke a response from the immune system. They are generally too small to peer with bare eyes. They enter the human body via the mouth, damaged pores and skin or nasal passage (Stevens and Miller, 2016).

The immune system defends against these antigens by using antibodies. When serological tests are done, they test blood, they can perceive the type of antibodies and antigens and type of infection we acquired (Mozayani and Noziglia, 2007).

TECHNIQUES

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Pc, Marimuthu and Devadoss, 2018; Ramesh *et al.*, 2018; VijayashreePriyadharsini, SmilineGirija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai*et al.*, 2019; Sridharan *et al.*, 2019; VijayashreePriyadharsini, 2019; Chandrasekar *et al.*, 2020; Mathew *et al.*, 2020; R *et al.*, 2020; Samuel, 2021)

There are numerous techniques available. Some of the classical techniques include complement fixation tests, neutralisation tests, single radial haemolysis, immunofluorescence technique. There are newer techniques which have been developed and it includes Radioimmunoassay, latex Particle agglutination (LPA), Western blot, ELISA test (Vainionpää, Waris and Leinikki, 2015).

The complement fixation test is a blood test where sample of serum is exposed to particular antigen to determine whether antibodies to that particular antigen is present (Institute and National Cancer Institute, 2020).The main advantage of CFT includes sensitivity, can be read easily and quantitative results.CFT is time consuming , not standardised and need high technical skills (Sever *et al.*, 1965). Virus neutralisation test detects antibodies that are capable of inhibiting viral replication and it does not detect all antigen-antibody reactions. It only detects those antibodies that block virus replication. Single radial haemolysis is a technique which detects assay of antibody to virus haemagglutinin (Mason *et al.*, 2010).

ELISA [Enzyme-Linked Immunosorbent Assay] is a test that determines if the antibody present in our body is related to infectious conditions. There are various advantages to performing ELISA tests. It includes specificity and sensitivity. It is cheap and they have a long shelf life, easy to perform, easily available and used for a variety of infections (Solanke, Karmarkar and Mehta, 2015).

Western blot is a confirmatory test that separates blood proteins and detects specific proteins (Jansen van Vuren*et al.*, 2016). It is a very specific but sensitive test. The antigen used is a

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mixture of natural antigens or purified recombinant proteins (Lai *et al.*, 2020).Well trained techniques are required for this technique and is a semi quantitative test.

Radioimmunoassays are one of the most important techniques in determining biologically important molecules and their metabolites .It has high sensitivity, specificity and rapidity with which analysis are carried out (VanVunakis and Levine, 2019).Nucleic Acid Amplification Testing is also used which is very sensitive and does not require any viable organism (Vainionpää, Waris and Leinikki, 2015). Counterimmunoelectrophoresis evaluates binding of antibody to antigen.Clinically it detects Ags of Cryptococcus and Meningococcus in CSF (Volpe Chaves *et al.*, 2020).

CRITERIA FOR DIAGNOSING VIRAL INFECTION:

Prozone phenomenon is very important in the serological diagnosis of any viral infection, this can be avoided by serial dilution of the patient's serum and allowing them to react with the antigen. Prozone phenomenon will lead to false negative reactions. Antibody once produced will be demonstrated in the patient for a longer period. In some infections the antibodies are demonstrated even after five years of exposure. Deciding the critical value is very important in the differential diagnosis. Another important point is cross reaction between the species of the same genera. Identifying the type specific antigen is very important to avoid false positive reaction. The different classes of antibody appear at different intervals during the clinical course of the disease. They are used as a marker to understand the diagnosis and the prognosis of a disease. Screening of viral infections is done, * for diagnosing etiological agent of viral infection* Forgiving proper antiviral therapy * Identification of antigenic variation of viruses which help in vaccine preparation, control of outbreaks (Templeton, 2007). * Detection of the etiological agent of epidermis * Helps in surviving viral infections (Winter and Hegde, 2020).

During serological investigations, the trappers in the primary humoral response to antigen. The antibody first appears in IgM, followed by IgG (Turgeon, 2013). In reinfection cases, IgM remains the same or increases slightly whereas IgG increases rapidly and more than in primary infection. Some assays such as CFT and HAI, detect total antibody which mainly comprises IgG. EIAs and radioimmunoassay are more sensitive tests. EIAs offer more sensitivity, specificity and reproducibility. The specificity and sensitivity of assay depend on the antigen (‘Surgical Pathology and Diagnostic Cytology of Viral Infections’, 2016; Patnaik and Tefferi, 2016). DNA was molecularly screened (Girija, Jayaseelan and Arumugam, 2018). A. Baumanni assess druggability, immunogenicity and toxigenicity (Smiline, Vijayashree and Paramasivam, 2018)(Girija*et al.*, 2019).

For diagnosing primary infection criteria includes, * widespread rise in titre of IgG - however significant increase in value is difficult to detect and it depends on the assay. The most important problem is that diagnosis is typically retrospective because by the point convalescent serum is taken, affected men or women have possibly recovered (Patnaik and Tefferi, 2016). * Presence of IgM - EIA, RIA and IF are used to detect IgM. This gives a fast method of prognosis [There are numerous assays with IgM assays, collectively with the interference of rheumatoid element reinfection via virus and unexplained endurance of IgM after primary infection (Ma *et al.*, 2019).

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* Seroconversion- defined as changing from previously antibody-negative state to positive state
* High titre of IgG - unrelated manner of serological prognosis since cut off value is hard (Solanke, Karmarkar and Mehta, 2015).

For diagnosing re-infection, * It is difficult to differentiate reinfection. Or reactivation from several infections (Pankuweit and Klingel, 2020). Under most instances, it is difficult to distinguish between contamination and recontamination. In the present day, a large rise in antibody titre is determined by recontamination at the same time as IgM is commonly low or absent in instances of re-infection or reactivation (Burton, 2001).

SEROLOGICAL REACTIONS:

Serological reactions are those Ag - Ab reactions which are used for the detection of unknown Ag or Ab. In virology, it can be a viral diagnosis or direct diagnosis which is for detection for viral antigens and serological or indirect diagnosis which is used for detection of specific antiviral antibodies (de Bont*et al.*, 2020).

In direct serological testing, they use Ag - Abreaction in the laboratory to identify unknown antigens such as microorganisms (Hati*et al.*, 2014). In indirect serological testing, they use Ag - Ab reactions in the laboratory to initially diagnose disease by detecting antibodies in a person ‘s serum produced against disease antigen (Abras*et al.*, 2016).

POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

Qualitative detection of total specific antiviral antibodies, Quantification of total specific antiviral antibodies, detection of antibody classes and determination of specific antibody avidity (Haynes, 2012).
There are various possibilities of viral diagnosis. If testing shows no antibodies, it indicates that the person doesn't have an infection. It can be false-positive which means a test result wrongly indicates that particular conditions are present. It can be false negative also (Wahed, 2019).

Cross-reactivity between antigens occurs when an antibody raised against specific antigen has a high affinity towards antigen (Chan *et al.*, 2017). This generally occurs when two antigens have similar structural regions that antibody recognises(‘Surgical Pathology and Diagnostic Cytology of Viral Infections’, 2016). It is the ability of an individual antibody combining site to react with more than one antigenic determinant. It has an epitope which is structurally similar to one on immunizing antigen. In some cases, the cross-reactivity can be destructive, and an immune response to one pathogen can interfere with or lower the immune response to a different pathogen (Burton, 2001).

Specificity is the ability of an individual antibody combining site to react with only one antigenic determinant. There is a high amount of specificity in antigen-antibody reactions. Antibodies can distinguish differences in the primary structure or isomeric forms of an antigen (Burton, 2001; Turgeon, 2013).

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The window period determines specific disease, especially infectious disease and it is the time period from exposure to infection to when the body produces enough antibodies to be detected by standard tests (Vainionpää, Waris and Leinikki, 2015). In antibody-based testing, the window period is dependent on the time taken for seroconversion.The length of window period time varies depending on the test used.

APPLICATIONS OF SEROLOGICAL DIAGNOSIS

In microbiology, serologic checks are used to decide if someone has antibodies in opposition to a selected pathogen or to locate antigens related to a pathogen in someone's sample. Serologic assessments are especially beneficial for organisms which are hard to culture through ordinary laboratory techniques, like Treponema pallidum (the causative agent of syphilis), or viruses (Marangoni, no date).

The presence of antibodies against a pathogen in a person's blood suggests that they have been uncovered to that pathogen. Most serologic checks certainly have one of the varieties of antibodies: immunoglobulin M (IgM) and immunoglobulin G (IgG) (Turgeon, 2013). IgM is produced in high quantities rapidly after a person is uncovered to the pathogen, and production declines fast thereafter. IgG is also produced on the first publicity, but no longer as quickly as IgM. On next exposures, the antibodies produced are mainly IgG, and that they continue to be in the movement for an extended time frame (Turgeon, 2013; 'Surgical Pathology and Diagnostic Cytology of Viral Infections', 2016).

This influences the interpretation of serology effects: a tremendous result for IgM shows that a person is currently or these days inflamed, while a fine result for IgG and a negative result for IgM suggests that the character might also have been infected or immunized in the past (Vainionpää, Waris and Leinikki, 2015). Antibody testing for infectious illnesses is regularly done in two stages: initial phase (acute section) and after the recovery phase. (convalescent phase).

Blood typing is typically performed by the use of serologic methods. The antigens on patients' red blood cells, which decide their blood type, are diagnosed using reagents that include antibodies, referred to as antisera (Institute and National Cancer Institute, 2020). When the antibodies bind to red blood cells that express the corresponding antigen, they motivate red blood cells to clump together (agglutinate), which may be recognized visually (Fierz, no date). The person's blood group antibodies can also be recognized by adding plasma to cells that express the corresponding antigen and observing at the agglutination reactions (Institute and National Cancer Institute, 2020).

Other serologic methods utilized in transfusion remedy include crossmatching and the direct and oblique antiglobulin tests. Crossmatching is achieved before a blood transfusion to make sure that the donor blood is compatible (Burton, 2001). It includes the recipient's plasma to the donor blood cells and observing for agglutination reactions. The direct antiglobulin test is accomplished to detect if antibodies are bound to red blood cells inside the patient body, that is abnormal and might occur in conditions like autoimmune hemolytic anaemia, a hemolytic disorder of the newborn and transfusion reactions (AlMutairi*et al.*, 2016). The indirect antiglobulin test is used to screen for antibodies that could cause transfusion reactions and

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identify certain blood group antigens.

During the SARS - CoV epidemic a wide variety of serological assays have been established. Most of the assays showed high sensitivity and specificity whereas some studies reported cross reactive antibodies to antigens (Meyer, Drosten and Müller, 2014). COVID -19 is similar to the common cold. N-6 Adenosine methylation can be used as a tool in cardiovascular diseases (Paramasivam, Priyadharsini and Raghunandhakumar, 2020). Patients with diabetes are at high risk for COVID-19. Apigenin which is a common dietary flavonoid affects blood reduction (Shahzan*et al.*, 2019). A Serotype is a group of related microorganisms differentiated by a common group of antigens. Serotypes determine species and subspecies (Lebani, no date). Serology tests are simple, quick and cost -effective. During epidemic large numbers of patients can be tested quickly ;giving an indication of incidence of disease in a given population (Doneley, 2006).

ADVANTAGES

There are various advantages of serological diagnosis. It is directly used by health workers and provides immediate results. The reaction can also be assessed manually with naked eye.There is potential for patient participation in the process. It provides learning opportunities (Benabderrahim, Feki and Khairallah, 2017). It interrupts the clinical significance of positive culture. It identifies a new isolate when the antibody is demonstrated against a particular antigen. There are rapid diagnosis and prognostic markers. It is more specific and sensitive (‘Surgical Pathology and Diagnostic Cytology of Viral Infections’, 2016). It measures antibody in the late phase of illness when both NAAT and culture is negative (Zhang *et al.*, 2012).It is a prognostic marker.It also measures severity or stage of disease.

The usefulness of serological diagnosis depends on individual viruses Foreg, viruses such as rubella and hepatitis A, the onset of clinical symptoms, coincide with the development of antibodies (Winter and Hegde, 2020).The detection of IgM or rise in titre of IgG in the serum of a patient would indicate active disease. However many viruses often produce clinical disease before the appearance of antibodies such as respiratory and diarrhoeal viruses (Lebani, no date; Benabderrahim, Feki and Khairallah, 2017). So in this, any serological diagnosis would be retrospective (AlMutairi*et al.*, 2016). There are viral diseases which produce clinical symptoms 9 months or years after seroconversion e.g. HIV. In the case of these viruses, the mere presence of antibodies is sufficient to make a definite diagnosis (Tigabu, Engda and Mekonnen, 2019).

DISADVANTAGE

There are various problems with serological investigations. A long period required for diagnosis for acute and convalescent sera. Mixed local infections may not produce detectable humoral immune responses (31). The immunocompromised patient often gives a reduced or absent humoral immune response. Patients with infectious mononucleosis and those with connective tissue diseases react specifically giving false-positive results. Patients given blood or blood products may give false-positive results due to transfer of antibody (AlMutairi*et al.*, 2016). The lab can be expensive which makes continuous monitoring difficult. Detection of macromolecules

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microbial antigens generally require relatively large microbial burden, which may limit assay sensitivity (Abras*et al.*, 2016).

CONCLUSION

Serology remains the mainstream of diagnosis of infections in routine diagnostic laboratories, especially for viral infections. It plays less role in other areas of virological testing, such as epidemiological research and antiviral resistance testing. It has made investigations available and affordable to everyone. Many methods are not dependent on automation. It is easy, simple and doesn't need much experience. The diagnostic valve is more important and should be in correlation with the clinical findings. The significant titre value should be re-evaluated periodically to avoid false positive reactions and it should be determined based on the epidemiological status of the disease. However, serology will be the mainstream of diagnosis for many viral infections and it will continue to play an important role in the 21st century. As far as COVID 19 is concerned clinical validation of serological reactions are yet to be evolved. It is not possible to give a mandate in a short period of time, it will take a minimum of 2 to 3 years.

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