RETROSPECTIVE ESTIMATION FOR QUALITATIVE DETECTION OF COVID -19 (CORONA VIRUS) IN BLOOD SAMPLES THROUGH DOT ELISA

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ABSTRACT - In this present pandemic situation across the world wide, India is not deprived of it, like other nation we are also facing the mobilized problem to find out the vaccination for the corona virus. But before its remedy to be found out, one more challenge is there to estimate the patients with corona positive. For a nation with dense population like India our first approach shall be towards the maximum testing of people for concerned virus. In this context the present article is emphasized on, includes previous literature some retrospective estimation of virus detection (plant, animals) techniques with the help of immunological parameters has been observed. Even though so many immuno-techniques are popularly using now days for rapid detection of number of serological tests, but Dot ELISA can be the cheapest, fast and cost effective tool for the qualitative detection of corona virus. This tool will reduce time, labor energy and expenditure up to maximum limit, thereby can make even the lower class people of India able to avail the testing facility for this pandemic disease.

KEYWORDS: Dot ELISA, COVID-19, SEROLOGY

I. INTRODUCTION

Recently identified novel coronavirus of zoonotic origin (2019-nCoV) is officially classified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Alexander et al (2020)**,** ICTV (2020). Corona Virus Disease (COVID-19) is genetically similar to SARS coronavirus and bat SARS-like coronaviruses causing acute respiratory disease via human-to-human transmission Zhu et al (2020), WHO (2019). Full-genome sequencing and phylogenic analysis indicated that 2019-nCoV is a distinct clade from the beta coronaviruses associated with human severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) Yamaoka et al (2016). COVID-19 is an acute resolved disease with a 2% fatality rate due to massive alveolar damage and progressive respiratory failure. Huang et al (2020), Chan et al (2020).

Serology assays like traditional reverse transcription-polymerase chain reaction (RT-PCR) methodology developed for SARS and MERS are time consuming, resource intensive, complex because proper animal models, protocols, and specific antibodies had to be developed. Gallagher (2020). To develop a sensitive and reliable assay for routine laboratory diagnosis, a rapid dot enzyme-linked immunosorbent assay (dot-ELISA) method can be developed for testing of infected patient by COVID -19. Based on anti COVID-19 rabbit antiserum the Dot ELISA expected to be highly reliable sensitive and specific toward COVID-19 diagnosis. Prabha et al (2017).

II. DETECTION AND SEROLOGY

Coronavirus has only 4 structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Alexender et al (2020).

In the case of COVID-19, the spike protein appears to be the primary protein interacting with host cells. Hence, the spike protein is likely the protein to which antibodies are raised, but this is not clear at this time.
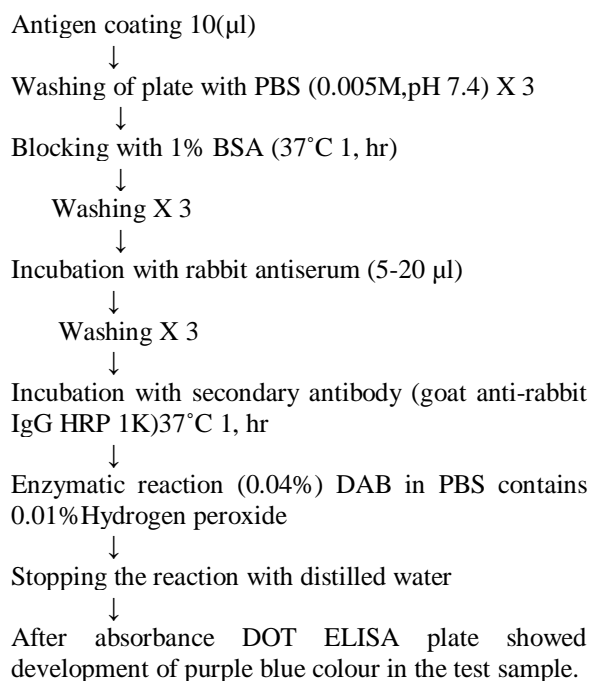
Immunofluorescent assay (IFA) shows whether a patient has antibodies to a pathogen by displaying a fluorescent signal when patient antibodies interact with virus proteins. Enzyme-linked immunosorbent assays (ELISAs) are more rapid serology tests that provide a readout of antigen- antibody interactions. Essentially, patient antibodies are "sandwiched" between the viral protein of interest and reporter antibodies, so that any active patient antibodies are detected.

The accuracy of two blotting media, polyvinylidene fluoride membrane (PVDF membrane) and nitrocellulose filter membrane (NC membrane), was compared. In order to facilitate the on-site diagnosis. Wang et al (2012) The Development and Application of a Dot-ELISA Assay for Diagnosis of Southern Rice Black-Streaked Dwarf Disease in the Field. Prabha (2018)
ELISA strips are supplied having three well defined zones:

• **Negative control zone** that is blocked with an inert protein.

• **Test zone** having an antibody immobilized on it and then blocked with an inert protein.

• **Positive control zone** having the antibody immobilized on it, blocked with inert protein and has a specific antigen bound to the immobilized antibody.

Antigen coating 10(µl)
↓
Washing of plate with PBS (0.005M,pH 7.4) X 3
↓
Blocking with 1% BSA (37˚C 1, hr)
↓
Washing X 3
↓
Incubation with rabbit antiserum (5-20 µl)
↓
Washing X 3
↓
Incubation with secondary antibody (goat anti-rabbit IgG HRP 1K)37˚C 1, hr
↓
Enzymatic reaction (0.04%) DAB in PBS contains 0.01%Hydrogen peroxide
↓
Stopping the reaction with distilled water
↓
After absorbance DOT ELISA plate showed development of purple blue colour in the test sample.

III. DISCUSSION

Throat swab samples are collected from upper respiratory tract for nucleic acid detection is not standardized, risky for medical staff leading to incompleteness and inaccuracy in updating new cases, as well as delayed diagnosis. Insufficient supply of assay kits creates a demand for fast-performing serologic assay to handle an epidemic of the current scale. Xie et al (2020), Xiang et al (2020). Hopefully, we will begin to understand the viral proteins and immune response to COVID-19 in the coming weeks. Companies and researchers are now working to produce antibodies and antigens of COVID-19. Native Antigen Company, press note (2020). If we will succeed in doing the same DOT ELISA could help in enhancement of rapid qualitative detection of virus with cost effectiveness and reliability at national level.

IV. CONCIUSION

Detection of corona virus is possible with low and cost effective method by utilizing the immunoassay technique with authentic value and quatitative results.

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