COMPARATIVE STUDIES ON THE DIAGNOSTIC TECHNIQUES OF SARS-CoV AND SARS-CoV-2

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Abstract

Diagnostic tests for identification of people infected with severe acute respiratory syndrome coronaviruses is crucial in the control and prevention of SARS-CoVs. Towards the ending of the year 2019, a novel coronavirus that resembles SARS-CoV called COVID-19 that causes respiratory disease appeared in China and later spread subsequently through more than 215 countries including Australia, Japan, Italy, Germany, South Africa, United Kingdom and the Unites States and has continue to spread worldwide. The genome of SARS-CoV is 29,727 nucleotides in length, and the genome organization is similar to that of other COVID-19. Generally, viral infection have been diagnosed in the laboratory through detection of viral antigens, nucleic acid, specific antibodies and by isolation and electron microscopy. Coronaviruses were detected usually from respiratory samples and blood by ELISA, Immunoflourescence test, Immunoblot and polymerase chain reaction. For epidemiological purposes the virus have to be cultured prior to other techniques in order to obtain good results. However, cultural technique is not recommended for COVID-19. The highest yield for the detection of SARS-CoVs results from real time polymerase chain reaction (RT-PCR). Sensitivity and specificity of IgG-IgM rapid diagnostic test (RDT) kits were acceptable as they are speedy, easy to use and cheap procedures which contribute to its application in mass testing. However, it cannot be employed instead of RT-PCR to detect COVID-19, but can be optional and complementary test. Development of rRT-PCR/IgG-IgM combined test kit could be helpful to ease diagnosis and the disease management, therefore more efforts are needed to investigate alternative diagnostics for coronaviruses.

INTRODUCTION

Coronaviruses are viruses from the family coronaviridae and subfamily ortho coronaviridae that usually cause mild and severe respiratory distress in man and other animals. Three types of viruses causes severe diseases to humans; SARS-CoV that cause severe acute respiratory syndrome, MERS-CoV that cause Middle East respiratory syndrome and the currently discovered SARS-CoV-2 (David and Steven, 1996; CDC, 2020; NIM, 2020).

Viral respiratory illness commonly known as severe acute respiratory syndrome is caused by SARS associated coronavirus (SARS-CoV) generally called SARS virus. It is a recently recognized febrile respiratory illness that first appeared in Guangdong Province, southern part of People's Republic of China in November 2002 (Sampathkumar et al., 2003; Kang et al., 2005; Maschinen et al., 2005), has since spread to more than two dozen countries in North America, South America, Europe, and Asia. The SARS global outbreak contained (CDC, 2017) has resulted in a cumulative total of 8422 cases, with 916 deaths reported from 29 countries during the outbreak (Sampathkumar et al., 2003; WHO, 2003; Maschinen et al., 2005). A global case-fatality ratio of 11% was recorded (WHO, 2003). World Health Organization (WHO) announced that the last chain of human transmission was broken on 5 July 2003. Since 2004, there have not been any known cases of SARS reported anywhere in the world (CDC, 2017). (Cheng, et al., 2020)

The incubation period of SARS is generally between 2– 12 days (Maschinen et al., 2005) which was estimated to be 6.4 days with mean incubation time of 3-5. The disease usually presents with fever, chills, dry cough, malaise, headache, myalgia and dyspnoea; sore throat, rhinorrhoea, vomiting and diarrhoea, symptoms may mimic other respiratory diseases such as influenza, pneumonia or bronchitis (Hui et al., 2004).

Detection of SARS CoV using real time polymerase chain reaction (RT-PCR) was not reliable at early stage of the disease (Kang et al., 2005), whereas serologic confirmation takes more than 2 weeks to achieve good result. Serum quantitative assessment of SARS-CoV RNA with RT-PCR constitutes only about 80% result at early days of infection (Hui et al., 2004).

At the end of the year 2019, a novel coronavirus was identified in China presenting with respiratory distress that mimic pneumonia (Mcintosh and Martin, 2020) that later disseminated into Asia, Australia, Africa, and European regions, and subsequently spread worldwide into different countries including Japan, Italy, Germany, South Africa, Singapore, United Kingdom and the Unites States (Elflein, 2020;Wu et al., 2020). The virus was initially called 2019-nCoV-2 and later renamed as severe acute respiratory syndrome virus type 2 (SARS-CoV-2) and the disease called corona virus disease 2019 (COVID-19) in February 2019 by the WHO (McIntosh and Martin, 2020) and subsequently declared as world emergency. COVID-19 has the ability to spread rapidly having impact socioeconomically and medically around the Globe (CDC, 2019; FDA, 2020). The disease affect commonly the middle age, older individuals and immunosuppressed, however, the later have the likelihood of severe disease. It has incubation period of 2-14 days following exposure, most cases occurring 4-5 days (McIntosh and Martin, 2020) with most patients being asymptomatic at the initial stage (Fuk-Woo et al., 2020; Shi et al., 2020). Many individuals present with high fever, fatigue, dry cough and myalgia; dyspnoea and hypoxia that progress to acute respiratory distress syndrome (ARDS) results, and subsequently organs failure (Wu et al., 2020). Currently (as at May 9), COVID-19 accounts for over 4.8 million cases, more than 316, 000 deaths, over 1.85 million recoveries and 2.5 estimated infection rate in about 216 countries and territories worldwide (ECDC, 2020a; WHO, 2020). Higher cases (over 1.87 million) with 165,995 deaths were reported in the European region and the lowest incidence being reported in Oceanian region with as low as 8,440 cases, about 126 deaths and over 8,000 recoveries). About 589.9 cases per million cases and 40 death per million population have been reported Globally (CDC, 2020; ECDC, 2020; Elflein, 2020; Roser et al., 2020; WHO, 2020).

Generally, viral infection can be diagnosed in the laboratory through detection of viruses from nasal swab, pharyngeal swab, broncho-alveolar fluids, sputum and or bronchial aspirates and blood by Electron microscopy, viral antigens, nucleic acid, specific antibodies and by isolation (James, 2017; Huang et al., 2020). Detection of nucleic acid is achieved by polymeraase chain reaction (PCR), i.e nucleic acid amplification test (NAAT) whereas antigen and antibody are detected by serologic techniques such as Enzyme Immunoassay - ELISA, Serum neutrallisation assay, Western blotting, Immunodiffusion, Immunoflourescence, haemagglutination inhibition assay and Class specific antibody assay (Reller & Weinstein, 2000; James, 2017). Similar specimen and procedures apply to common respiratory viruses such as adenoviruses, parainfluenza virus, avian influenza, MERS-CoV respiratory syncytial virus, influenza virus including COVID-19 and SARS-CoV (Huang et al., 2020). SARS-CoVs were known to spread very fast, hence the need to have a method of detection that is easier, faster, reliable and economically adaptable more than the currently used techniques especially at point of care.

LABORATORY DIAGNOSIS OF THE CORONA VIRUSES

SAMPLING

Samples for the diagnosis of both SARS-CoV and COVID-19 were usually taken from the upper respiratory tract, either the nasopharynx, nasal mid turbine (deep nasal swab), the anterior nare or nasopharyngeal wash. They can also be taken from the lower respiratory tract through bronchoalveolar leavage, pleural fluid, tracheal aspirate, or lung biopsy; sputum is also used (Roos, 2003; CDC, 2020; Corman et al., 2020). For epidemiological purposes, the peak detection for SARS-CoV usually occur at the first 2 week for respiratory samples, rectal swab or stool can be taken 2-3 weeks and at week 4, urine can be collected. The rectal swab,

sputum and stool can be collected in transport media whereas urine and blood samples in sterrile containers (Chan et al., 2004).

VIRAL DETECTION

Coronaviruses can be detected by ELISA, Immunoflourescence test, Immunoblot and PCR (Roos, 2003; Chan et al., 2004; Maschinen et al., 2005; ECDC, 2020; WHO, 2020). For epidemiological purposes the virus have to be cultured prior to other techniques in order to obtain good results (WHO, 2003; Ren et al., 2004). However, cultural technique is not recommended for COVID-19 (WHO, 2020). The main techniques currently in use for both SARS-CoV and 2019-nCoV are the RT-PCR and ELISA (Leung et al., 2004; Ren et al., 2004; CDC, 2020; Wu et al., 2020) . Analysis of blood proteins has provided a helping tool for identification of biomarkers for evaluation SARS-CoVs although they are not essential diagnostics at point of care (Ren et al., 2004; Kang et al., 2005).

Culture

Viruses can be detected in specimen from patients suspected of SARS-CoVs by inoculation of cell cultures and growing the virus, which must be identified through either molecular or serologic techniques (WHO, 2003). Positive cultures indicate the presence of virus whereas negative result do not mean the virus is not present unless RT-PCR shows negative result (WHO, 2003). However, viral culture for COVID-19 is not recommended as a routine procedure for diagnosis (WHO, 2020), this is because it is time consuming, needs expertise and expensive, however rapid diagnostic methods are needed which are cheaper, do not require much expertise and can give results at the shortest possible time,.

Antigen and Antibody Detection

Serology helps in the investigation of the ongoing pandemic especially in cases where NAAT assays are found to be negative and the link between illness (clinical manifestations) and COVID-19 is very strong, so that samples can be collected for both acute and convalescent phases for serology (WHO, 2020).

In response to SARS coronavirus infections, different types of immunoglobulins (antibodies) including IgG and IgM are produced and can change in level in the course of infection. The antibodies can be undetectable at the initial infection stage; IgG can be detected even after the illness has been resolved. The antibody tests for the disease include ELISA to detect both IgG and IgM antibodies which is more reliable especially 21 days of infection, and Immunofluorescence assay to detect only IgM or IgG antibodies after about 10 days of infection. Positive antibody test signifies infection whereas the reverse indicates no infection has taken place (WHO, 2003). Immunoblot is another serologic technique for SARS-CoV antibodies (Maschinen et al., 2005).

Serologic techniques for identification of COVID-19 antibodies including IgA, IgG and IgM from clinical specimen such as ELISA are less reliable than molecular tests and can potentially be utilised for early diagnosis. There is limitation around the onset of the symptoms when incubation and transmission are high, and the body may not likely start producing antibodies. The response of antibodies to the viruses usually takes many days or weeks to be clearly detected, and, negative outcome do not count out infection particularly at early stage (Cheng et al, 2020; USFDA, 2020). Cross reactivity with antibody to non COVID-19 proteins must also be put into consideration, so that positive result may be as a result of recent or past infection with other coronaviruses. Serology is more relevant in a situation whereby patient present with complication of late disease when RT-PCR is likely to produce false negative result as a result of dropping of viral load over time (Cheng et al, 2020).

Detection of SARS-CoV directly using ELISA have not been possible, rather, its nucleocapsid protein from respiratory specimen, faeces and urine; potential nucleocapsid protein has been used as reliable diagnostic tool to detect the virus (Lau et al., 2004). ELISA was known to be highly specific, less expensive and labour intensive than RT-PCR. Western blotting (WB) and IF assay have also been used to detect serum SARS-CoV (Leung et al., 2004). Detection of influenza virus antigen by IF directly from clinical sample have been available and is less complex, providing results at point of care but have suboptimal sensitivity

to rule out disease, this challenge may exist probably in the case of COVID-19, therefore implementation of test of this nature need clear guidance on correct interpretation.

It was already recommended that negative serologic results do not rule out SARS-CoV-2 infection, rather molecular testing should be conducted particularly on samples of highly suspected individuals, furthermore, the results of antibody testing should not be relied on for confirmation or exclusion of COVID-19 infection or its status as positive outcome may be as a result of present or fast encounter with other non COVID-19 coronavirus strains (USFDA, 2020). Monoclonal antibodies against nucleocapsid of 2019-nCoV is currently generated for future antigen detection test (Cheng et al., 2020). Recombinant spikes protein (S-protein) and nucleocapsid protein (N-protein) are currently in use to manufacture diagnostic kit for 2019-nCoV serum antibodies (Xu, 2020).

Polymerase chain reaction

The development of reverse transcriptase polymerase chain reaction (RT-PCR) methods to detect RNA sequences has been a major advance in viral diagnostics (Dembert & Kaiser, 1983). Principally, PCR tests are very specific but less sensitive, therefore, negative test do not rule out infection; positivity does not indicate quantity large enough to be transmitted (WHO, 2003). The highest yield for the detection of SARS-CoVs has been assessed by RT- PCR (Chan et al., 2004); positive RT-PCR results is usually confirmed by repeat testing of the original specimen and through retesting in an independent laboratory using a validated assay (Wadsworth, 2003). This is because PCR is useful at early stage of the disease but can produce many false-negative results, creating dangerous security issues that could aid transmission (Roos, 2003).

Cases of both SARS-CoV and SARS-CoV-2 are routinely confirmed on the basis of detection of RNA sequences that are unique to the viruses by NAAT such as nucleic acid real-time reverse-transcription PCR (rRT-PCR) (Wadsworth, 2003; WHO, 2020). Nucleic acid sequencing can serve as confirmatory test if necessary, especially in areas that is not known with incidence of the disease, hence SARS-like coronaviruses or beta coronaviruses have to be considered before sequencing. In the COVID-19 isolated areas however, a single rRT-PCR can be reliable (WHO, 2020).

Members of the family coronaviridae have between 29,000 to 31,000 nucleotides (CDC, 2003a). The genomic nucleotide length of SARS-CoV is 29,727 and there is similar genomic organisation with that of COVID19. The S and N proteins, small membrane protein (E), membrane protein (M), and several other open-reading frames of unknown function, have been identified with SARS-CoVs (CDC, 2003b). Oligonucleotide primers that are used to detect COVID-19 were selected from the N-region of its genetic region of its nucleocapsid, designed with dual primer/probe sets with additional set for human RNase P (RP) gene as control (CDC, 2020). COVID-19 detector that employ simultaneous use of rRT-PCR and loop-mediated amplification techniques has been produced, which does not require repeated heating and cooling cycles of PCR and the amplified nucleic acid is incubated with a guide RNA (gRNA) molecule that target the viral E and N genes with the help of single strand Deoxyribonucleic acid (ssDNA) that confirm the presence of the target RNA (Sheridan, 2020; Huang et al., 2020).

POINT OF CARE DIAGNOSTICS

Point of care diagnosis in epidemics is very essential and can be achieved by the use of rapid analysis which can be conducted at ease outside laboratory or near patient in order to facilitate prompt diagnosis, monitoring, management and quick medical decision at early stage of disease development (Vashist, 2017). It is highly needed at the time of epidemics and pandemic such as SARS-CoV and SARS-CoV-2.

Molecular detection of both SARS-CoV and COVID-19 was known to be their recommended means of diagnosis (WHO, 2020). The fact that NAAT require expertise, and is expensive, complex and not easily obtainable at environments outside laboratories, other devises to aid diagnosis that are easy to use are currently recommended at point of care to detect antigens or antibodies or both (WHO, 2020; Ying et al., 2020). Rapid diagnostic test (RDT) is used to detect viral antigens present in the clinical sample if they are in sufficient concentration to bind with the specific antibody against the virus that are fixed on paper

and detectable visually within 30 minutes, they are best used in acute stages when the viruses are actively replicating (WHO, 2020). It has been experienced from the antigen based RDTs for respiratory viruses such as influenza and ASRS-CoV that have variable sensitivities and could lead to missed diagnoses (Bruning et al., 2017; Wang et al., 2018), hence further studies are required to understand their accuracy in COVID-19 cases so that if any of the antigen detection strip demonstrate adequate performance they can be used as triage for individuals that have likelihood to harbour COVID-19 (WHO, 2020).

Being that RT-PCR is the gold standard for viral diagnosis and NAAT is globally recommended method for detection of SARS-CoVs, detection of antibodies to COVID-19 become a supplementary option even if a combined IgG/IgM device is to be used. This can be supported with the fact that about 86% of the RT-PCR positive samples have been found to be positive for SARS-CoV-2 IgG/IgM test kit with 91% specificity as demonstrated by Ying et al. (2020).

RDTs that detect the presence of COVID-19 antibodies in the blood are readily available (Cheng et al., 2020). In some infected individuals that are confirmed positive by RT-PCR, there was reported weak or absent antibody responses till after a week or more (Li et al., 2020), which indicate that antibody testing are more reliable at later stage of the disease (Zhao et al., 2020; Okba et al., 2020). For point of care diagnosis, there is limitation for such tests as a result of delayed outcome, but can be useful epidemiological vaccines research and risk assessments (Cheng et al., 2020; WHO, 2020),

At point of care, neither immunoassay nor RT-PCR are ideally reliable, however the later is better in terms of accuracy and speed of development but complex and delays results; the former is less accurate and usually takes long time to develop but easier to use, with results within 20-60 minutes. Immunoassays usually detect antibodies to pathogens from samples and must unavoidably be contended with the variable human polyclonal antibody response that take time to characterize (Sheridan, 2020), efforts are therefore needed to produce more reliable devices.

BIOMARKERS ASSOCIATED WITH COVID-19 PATIENTS

Blood samples from patients suspected of COVID-19 were found to have low albumin, elevated lactate dehydrogenase, elevated reactive proteins, and lymphopenia. Increased erythrocyte sedimentation rate, elevated alanine aminotransferase, aspartate aminotransferase and creatinine kinase levels have also been assessed, there is also leucocytosis, increased creatinine and bilirubin levels and leukopenia (Cheng et al., 2020). Protein profiles and serum clinico-pathological features had also helped in the diagnosis and epidemiological surveys of SARS-CoV (Ren et al., 2004).

CONCLUSION

Presently, the diagnosis of SARS coronaviruses have been exclusively on the basis of rRT-PCR and serology and none of these methods is solely reliable at early stage of the diseases. Specific methods for early diagnosis of SARS-CoVs are very crucial in order to effectively control and manage epidemics. Sensitivity and specificity of IgG-IgM RDT kits were found to be acceptable as they are speedy, easy to use and cheap procedures which contribute to their application in mass testing. However, they cannot be employed instead of rRT-PCR to detect COVID-19 but can be optional and complementary test. Development of rRT-PCR/IgG-IgM combined test kit could be helpful to ease diagnosis and management of epidemics, therefore more efforts are needed to investigate alternative diagnostics for coronaviruses.

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ETHICAL STATEMENT

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CONFLICT OF INTEREST

No conflict of interest to declare.

DATA SHARING

Data sharing is not applicable to this article as no new data were created or analysed in this study

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