RESEARCH ARTICLE

Clinical laboratory role in viral pandemic response: Focus on COVID-19

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Impact Statement

Clinical Laboratory workers are both practitioners and a specific population of at-risk individuals who play a critical role during a viral pandemic threat such as the COVID-19 pandemic. This article describes the activities of laboratory professionals during a pandemic threat including how to mitigate the spread of disease among this specific healthcare population as well as the types of testing that emerge during an early window for an emerging viral pandemic. Knowledge regarding the role and response of the clinical laboratory detection of viral pandemic disease provides insights for professionals examining scientific and research strategies for confronting infectious viral outbreaks.

Abstract

Background: The global focus on COVID-19 provides a spotlight for the critical role of the clinical laboratory scientist in monitoring and managing pandemic disease. Laboratory management and the laboratory team must understand the signs, symptoms and routes of transmission of the pandemic infectious disease, ensure the safety of their employees, and determine the most effective testing methods for implementation in a rapid time frame.

Methods: An examination of the current literature regarding characteristics of airborne viral outbreaks, safety practices, and emergency use testing methods were compiled.

Results: The protection of laboratory employees is mediated by emergency preparedness plans prior to pandemic threats, insurance that personnel are vaccinated when possible and have appropriate PPE available; includes respirators or face masks. The primary method of early detection of novel pandemic viral threats, such as COVID-19, is molecular testing via nucleic acid extraction from patient specimens and rRT-PCR as indicated from the issuance of FDA Emergency Use Authorizations (EUAs) from February to April of 2020.

Conclusions: Prior pandemics and standard laboratory practice have prepared clinical laboratories for mitigating the spread of disease among employees. NAAT (nucleic acid amplification testing) has emerged as the earliest testing modality for monitoring pandemic viral disease presence and other methods include viral culture, serological assays, and biomarker testing will be used to monitor progression and treatment. The clinical laboratory investigations and response to viral pandemics has the benefit of increasing our technical knowledge in handling future pandemic threats.

Keywords: COVID-19, NAAT, PCR, Pandemic, Viral, Infectious Disease, Laboratory Medicine

1. Introduction

The global focus on COVID-19 provides a spotlight for the critical role of the clinical laboratory scientist in monitoring and managing pandemic disease. The fundamental role of the medical or clinical laboratory professional is to engage in the diagnosis of the disease in association with the extended health care team. As such, their role in any response to an epidemic or pandemic response is to investigate specific cases for the occurrence of the purported agent of epidemic or pandemic disease. To perform this role effectively and efficiently, laboratory management and the laboratory team must understand the signs, symptoms and routes of transmission of the pandemic infectious disease to ensure safety of employees and aid clinicians in their interpretation of results, work with other health officials to coordinate their efforts to monitor the spread of disease, ensure the safety of their employees, and determine the most effective testing methods for implementation in a rapid time frame. This review provides an overview of the laboratory response to pandemic infectious disease response with a focus on the COVID-19 pandemic.

The major goal of the health care team, in response to epidemiological spread, is to establish a systematic process for managing and mitigating the spread of disease. With the advent of laboratory diagnosis, the overall mortality rate for infectious diseases has steadily declined from the beginning of the 20th century to today; with influenza and pneumonia accounting for roughly 40% of all infectious disease mortality between the years 1980 to 2014 within economically developed countries like the United States with pneumonia amounting to a significantly larger portion of the total[1,2]. The role of the clinical laboratory in specifically identifying the source of a disease supported both an increase in general hygiene and establishment of the efficacy of antibiotic treatment of infectious disease. By the end of the 20th century infectious disease was only one of the top ten causes of death; forth on the list, amounting to 3.5% of total deaths per year in developed countries[3], declining in the U.S. from 797 deaths in 100,000 people in 1900 to 36 deaths per 100,000 people in 1980 with a surge within the 1980's to around 63 deaths in 100,000 due to the AIDS pandemic[2]. A pandemic is defined as the spread of epidemic disease that goes beyond the boundaries of one or very few geopolitical regions. Pandemic disease may therefore increase the number of deaths related to viral disease during selected time periods if a disease spreads globally.

2. Review the signs, symptoms and transmission

One of the first steps in establishing definitive laboratory procedure for the appropriate tracking and management of disease is a careful review of the signs, symptoms, and routes of transmission implicated in the spread of a given disease based on the existing research and data gathering activities of epidemiologists and public health organizations. In the case of emerging infectious diseases, such as the COVID-19; SARS-CoV-2, only limited evidence may be available as to the primary routes of exposure and disease spread, which confounds efforts to manage and mitigate spread. Prior diseases with similar routes of transmission, signs, and symptoms, can allow for a preliminary set of policies and practices. For example, the prior emergence of

coronaviruses such as the SARS and MERS pandemics can be of significant value[4], as can the policies and practices established for similarly spread viruses including the influenza viruses. The general consensus is that infections can transmit via five significant routes; direct contact, fomite, aerosol, oral, and vector-mediated, and many microorganisms causing disease can be transmitted via more than one route. Influenza virus and coronavirus are both primarily transmitted through aerosol; i.e. influenza virus is spread through droplets that remain airborne and are distributed when expelled through sneezing and coughing[5], and the coronavirus SARS-CoV, the causative agent of SARS, was primarily spread through airborne droplets and droplet exposed fomites (surfaces/objects)[6].

Like influenza; and unlike many other coronaviruses identified in human populations since the 1960's, SARS-CoV-2, SARS-CoV, and the Middle East Respiratory Syndrome coronavirus (MERS) can lead to a variable clinical presentation ranging from flu-like symptoms to acute respiratory distress syndrome[7]. The outbreak of SARS-CoV in 2003, which began in the Guangdong Province of China and spread to 26 countries, areas before being declared contained by the World Health Organization (WHO) in July 2003, provides us with a perspective on the timeline for research into newly discovered epidemic disease[6]. None-the-less, the severity of the viral spread also plays a significant role on the number of researchers turning their attention to the task of understanding pathogenesis, exposure risks, and the development of diagnostics and therapies. These studies also point to the risks to healthcare workers associated with epidemics who manage and mitigate the disease spread. For instance, 21% of affected persons in the 2003 SARS outbreak were heathcare workers[6]. The coronavirus disease 2019 (COVID-19), first detected in Wuhan, China, identified as SARS-CoV-2, while having a lower incidence of mortality to the original SARS-CoV, spread worldwide far more successfully than its precursor. Furthermore, the majority of individuals showed no or only mild symptoms when infected with SARS-CoV-2 (COVID-19), presenting a challenge for healthcare workers looking for signs and symptoms to reduce dissemination. Additionally, its rapid spread meant that critical cases overwhelmed unprepared healthcare organizations around the world. In these critical cases, individuals presented to the hospital with cough and shortness of breath, with the most common ICU admission reason described as hypoxemic respiratory failure requiring the use of mechanical ventilation[8].

The route of transmission is a key indicator for determining a preparedness plan. Unlike airborne viruses, vector-borne viruses such as Zika and chikungunya viruses, present increased risks for regions harboring the vector host; specifically the *Aedes aegypti* and *Aedes albopictus* mosquito, and present a reduced risk for countries outside the endemic zones where the vector may not be found[9]. In a hospital and laboratory setting, dissemination can be mitigated or eliminated by restricting or eliminating the vector-host. Likewise, diseases that are spread via direct contract with an infected individual, such as sexually-transmitted and blood-borne pathogens including HIV, HBV, and HCV require different precautionary steps within the laboratory and healthcare environment to prevent dissemination. The transmission of blood-borne viruses between healthcare workers and patients was found to be negligible except during specific invasive

procedures where accidental blood to blood contact occurred[10]. However, the spread of respiratory airborne viruses such as influenza and coronavirus can be either significantly contagious or responsible for significant morbidity, thus the CDC's recommendation to quarantine or isolate suspected COVID-19 infected individuals; a recommendation that was similarly made for persons suspected of infection with the original SARS virus[11]. Additionally, extensive efforts to vaccinate for influenza in healthcare settings is practiced to reduce infection rates[12]. To underscore the significance of airborne transmission of respiratory disease, a case report describes the admission of a single person with MERS-CoV to a Hospital in Daejeon, South Korea in 2015 which led to an outbreak of 23 infections in patients and healthcare workers in two hospitals leading to 11 additional deaths[13]. Specifically, MERS-CoV is estimated to cause mortality in roughly 34.4% of infection cases, SARS-CoV to cause roughly 11%, and SARS-CoV-2 to cause roughly 2.6% mortality[7]. Dissemination of disease is often inversely proportional to morbidity, i.e. severe acute respiratory syndrome causing coronaviruses like MERS-CoV led to 2494 cases but high mortality, SARS-CoV led to 8422 cases but intermediate mortality[7], and SARS-CoV-2 led to an astounding two million plus confirmed cases as of April 18, 2020 according to the World Health Organization (WHO) with the lowest mortality rate; <1%[14].

Thus the routes of transmission and rate of viral spread are not only important for indicating rates of infection but also the probability of dissemination into a health facilities geographic area and within the healthcare facility. Airborne transmitted viruses present an increased risk to laboratory workers and healthcare workers generally and appropriate measures should be in place to protect these workers; whose job is critical for the monitoring and containment of emerging pandemic disease.

3. Establish internal safety guidelines for laboratory personnel

For the clinical laboratory worker and healthcare workers generally, estimates of both propagation rates and mortality are strong indicators of risk. During an emerging epidemic viral disease, it is the primary responsibility of the laboratory manager to implement safe practices that mitigate or eliminate the spread of disease among their workforce. This may be done in association with a larger effort within the health care institution, including coordination with public health authorities or with guidance from an internal emergency response system, commonly developed in major health care systems[15]. Unlike the general population, who may be guided to avoid locations that increase the spread or contraction of a virus and practice social distancing, clinical laboratory workers are among a specific population that must continue with their work in order to aid in the monitoring and diagnostics of disease. Furthermore, the close proximity of work stations and equipment may preclude consistent social distancing recommendations. The United States Center for Disease Control and prevention (CDC) has established four competency domains for safety in the clinical laboratory which include determining potential hazards, hazard controls, administrative controls, and emergency preparedness and response[4,16]. The reduction in laboratory personnel, resources, strict

regulations, and increase in the usage of reagent-kit-specific high throughput instruments may all negatively impact a laboratory ability to flexibly change operations during a pandemic, while increasing regional laboratory networks, installing mobile labs, stopping the cuts to lab resources, and establishing emergency plans in advance may increase preparedness within the laboratory[17].

An important key to that effort is to ensure that all precautions, including available vaccinations and personal protective equipment (PPE) are on hand and in use by laboratory personnel; such as face masks, shields, gloves, lab coats, and eye protection. While rates of influenza annually account for around 133,900 hospitalizations and 36,000 deaths per year[18], hospital acquired infections among healthcare workers can be significantly reduced through vaccination initiatives as was shown in a University of Virginia Health System retrospective analysis where an increase in vaccination led to a decrease in worker infections and nosocomial-acquired influenza among patients[12].

However, vaccination, while effective, is not always available for newly emerging epidemic viruses; as was the case with COVID-19. Most established biological risk assessments include the following steps; identify hazards, evaluate/prioritize risks, determine necessary controls, implement control measures, and evaluate effectiveness of controls[4,19]. The CDCs guidance for healthcare responses to epidemic and pandemic infectious disease containment include minimizing exposure opportunities, through the use of Standard Precautions, and enhanced protection when performing aerosol-generating procedures[19]. In the lab centrifugation and sample manipulation during procedures, including removing of swabs from sample containers, are potential sources of aerosol. Aerosol precautions include the use of N95 or higher-level respirators, eye protection, gloves, and a gown[20]. Updated CDC guidelines suggest that face masks can be used if respirator availability is limited; a situation encountered during the spread of pandemic COVID-19[21]. The CDC Standard Precautions provide guidance for handling all patient specimens[22] and are appropriate guidelines for safety within the clinical laboratory during an epidemic and pandemic response. Unlike the prior CDC guidelines; i.e. Universal Precautions and Body Substance Isolation, Standard Precautions introduced in 1996 includes precautions for the potential exposure to respiratory secretions[22].

Studies comparing N95 to medical masks showed no statistically significant different in number of laboratory confirmed incidents of influenza between these types of face protectors[23], other reports suggest that N95 respirators provide slightly superior protection for droplet-borne pathogens when compared to surgical masks[24]. Significantly a study using avian influenza showed that N95 masks could block 99.98% of the virus, while medical masks and homemade masks made of 4-layer kitchen paper and 1-layer of cloth could block 97.14% and 95.15% of virus respectively[25]. Coronaviruses are the largest known RNA viruses with a size ranging from 26,000 to 37,000 bases[7], which is perhaps double the size of the influenza A genome at roughly 14,000 bases[26]. However, these numbers do not correlate to the particle size of the virus; with coronaviruses ranging in size from 60 to 140 nm[27] versus influenza virus sizes estimated at 80-120 nm[28], suggesting that similar protection can be

achieved with the above mentioned devices for either virus.

The WHO suggests that alcohol based antiseptics containing either ethanol, isopropanol or n-propanol alone or in combination; at concentrations of 60-80%, are effective against influenza and other enveloped respirator viruses[29]. Other additives such as chlorhexidine and iodophors may also be effective agents for protection against viruses and bacteria[29]. The use of surface disinfection with products containing alcohols, formic acid, sodium hypochlorite, and phenolic compounds; specifically in areas that are frequently touched or assumed contaminated, is critical in reducing the spread of microbial infections and have low contact-sensitivity and toxicity risks[30].

Evidence suggests that an employee's willingness to work during a crisis influenced by infectious disease include perceptions from the healthcare worker regarding the availability of PPE, prior education, age, gender, and confidence in one's employer[31]. Given the shortage of laboratory personnel; specifically a 10-11% vacancy in core laboratories and immunology department labs[32], confidence in well-established safety protocols will bolster institutional efforts to maintain staffing necessary to mitigate and monitor the spread of epidemic and/or pandemic disease. Studies that evaluated gender and age further suggested that a willingness to work during a public health emergency was decreased for women and age greater than 40 years of age[31]. This has a particular impact on clinical laboratory operations where the population of employees is predominantly female; approximately 80%[33], and nearing retirement age; 15 and 30% in core laboratories[32].

4. Laboratory methods for Management of Viral Pandemic

During an epidemiological crisis many regulatory policies may be relaxed in order to facilitate the rapid entry of new diagnostic tests for emerging disease, for instance under the FDA's Emergency Use Authorizations (EUAs). The relaxation of regulation may increase the number of tests that enter the market. Efforts should be made to ensure high quality testing in the clinical setting in order to prevent the spread of misinformation that could impact the course of medical action taken. The questions clinical laboratory managers and their teams ask when adopting a new test include whether the test provides diagnostically useful information, whether a newly implemented test can replace a less specific/sensitive/timely or more costly assay, whether the test can be performed with the current staff, and are the benefits increased for on-site versus referral testing[34].

In the 2 months following the FDA's EUA for SARS-CoV-2 testing on February 4th 2020, several dozen EUA's for in vitro diagnostics were issued (as of April 2th 2020, see **Table 1**). All but one of the EUA approved assays rely on nucleic acid extraction and reverse-Real-Time Polymerase chain reaction (rRT-PCR). While many of these assays use similar methodology, a number of the assays improved timeframes to result and increased reliance on automation. Approximately half of the authorized tests (13/25, 52%) were issued by companies that have an instrument foothold in established clinical laboratories for use on their specific instruments. All assays were qualitative assays, defining a positive or negative value to prior infection with COVID-19. Several assays were

multiplex assays, using a 96-well plate method, that looked at not only COVID-19 markers but also markers for other infectious diseases that may mimic the symptoms of COVID-19, such as the NxTAG CoV Extended Panel Assay from Luminex for use on the MAGPIX instrument assessed the major the major genet targets of SARS-CoV-2 including the ORF1ab (RNAse P gene), N and E genes[35], and another a cartridge-based panel from Qiagen called the QiAstat-Dx-Respiratory SARS-CoV-2 Panel detected not only SARS-CoV-2, but 22 bacteria and viruses leading to respiratory disease including influenza A, RSV, parainfluenza, adenovirus, and other coronaviruses[36]. Only one test relies on a serological approach, providing for detection of COVID-19 specific IgG and IgM[37].

Table 1: FDA Emergency Use Authorization approved assays for COVID-19 from February 2nd to April 2nd 2020

EUA Issued	Manufacturer	Test Name	Method of detection	specimen source	Instrument requirements	target for SARS-CoV-2	time to result
2/4/20	Centers for Disease Control and Prevention's (CDC)	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	rRT-PCR	upper and lower respiratory specimens	any authorized real-time PCR instrument	RP and N gene	~1 to 2.5 hours*
2/29/20	Wadsworth Center, New York State Department of Public Health's (CDC)	New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel	rRT-PCR	nasopharyngeal/oropharyngeal swabsand sputa	any authorized real-time PCR instrument	RP gene	~1 to 2.5 hours*
3/12/20	Roche Molecular Systems, Inc. (RMS)	cobas SARS-CoV-2	rRT-PCR	nasopharyngeal and oropharyngeal swab	cobas 6800/8800 system	RP and E gene	~1 to 2.5 hours*
3/13/20	Thermo Fisher Scientific, Inc.	TaqPath COVID-19 Combo Kit	rRT-PCR	nucleic acid extracted from nasopharyngeal swab, nasopharyngeal aspirate, and BAL specimens	Applied Biosystems 7500 Fast Dx Real-Time PCR instrument	RP, N, and S sequences	~1 to 2.5 hours*
3/16/20	Laboratory Corporation of America (LabCorp)	COVID-19 RT-PCR Test	rRT-PCR	nucleic acid fromSARS-CoV-2in upper and lower respiratory specimens	Applied Biosystems QuantStudio7 Flex (QS7)	N1, N2, and N3 sequences	~1 to 2.5 hours*
3/16/20	Hologic, Inc.	Panther Fusion SARS-CoV-2	rRT-PCR	nucleic acid from SARS-CoV-2 extracted from nasopharyngeal and oropharyngeal swab	Panther Fusion System	RP sequence	~1 to 2.5 hours*

3/17/20	Quest Diagnostics Infectious Disease, Inc.	Quest SARS-CoV-2 rRT-PCR	rRT-PCR	upper and lowerrespiratory specimens	Applied Biosystems 7500 Real Time PCR System	N1 and N3 seqeunce	~1 to 2.5 hours*
3/17/20	Quidel Corporation	Lyra SARS-CoV-2 Assay	rRT-PCR	nasopharyngeal and oropharyngeal swab	Applied Biosystems7500 FastDx Real-Time PCR instrument	Undiscolsed COVID-19 primer and probe mix	~1 to 2.5 hours*
3/18/20	Abbott Molecular	Abbott RealTime SARS-CoV-2 assay	rRT-PCR	nasopharyngeal and oropharyngeal swab	Abbott m2000 System	N and RP sequence	~1 to 2.5 hours*
3/19/20	GenMark Diagnostics, Inc.	ePlex SARS-CoV-2 Test	rRT-PCR	nucleic acid in nasopharyngeal swab specimens	ePlex	RP sequence	~1 to 2.5 hours*
3/19/20	DiaSorin Molecular LLC	Simplexa COVID-19 Direct assay	rRT-PCR	direct extraction from nasopharyngeal swap specimen	LIAISON MDX	RP and S sequence	~1 to 2.5 hours*
3/20/20	Cepheid	Xpert Xpress SARS-CoV-2 test	rRT-PCR	nasopharyngeal swab and nasal wash/aspirate specimens	GeneXpert Dx and GeneXpert Infinity systems	N sequence	~15 minutes
3/20/20	Primerdesign Ltd.	Primerdesign Ltd COVID-19 genesig Real-Time PCR assay	rRT-PCR	oropharyngeal swab specimens	any authorized real-time PCR instrument	Undiscolsed COVID-19 primer and probe mix	~1 to 2.5 hours*
3/23/20	Mesa Biotech Inc.	Accula SARS-Cov-2 Test	PCR and lateral flow	throat swab and nasal swab specimens combined	Accula SARS-CoV-2 test cartridge on AcculaDock and SilarisDock	N sequence	30 minutes
3/23/20	BioFire Defense, LLC	BioFire COVID-19 Test	rRT-PCR	nasopharyngeal swabs	FilmArray2.0 and the FilmArrayTorch Instrument Systems	RP and ORF8 sequence	50 minutes
3/24/20	PerkinElmer, Inc.	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	rRT-PCR	oropharyngeal swab and nasopharyngeal swab specimens	any authorized real-time PCR instrument	RP sequence	~2.5 hours
3/25/20	Avellino Lab USA, Inc.	AvellinoCoV2 test	rRT-PCR	nasopharyngeal and oropharyngeal swab specimen	any authorized real-time PCR instrument	N and Rp sequence	~1 to 2.5 hours*
3/26/20	BGI Genomics Co. Ltd	Real-Time Fluorescent RT-PCR Kit for Detecting	rRT-PCR	throat swabs and bronchoalveolar lavage fluid (BALF)	any authorized real-time PCR instrument	RP sequence	~1 hour

		SARS-2019-nCoV					
3/27/20	Luminex Molecular Diagnostics, Inc.	NxTAG CoV Extended Panel Assay	multiplex rRT-PCR	nasopharyngeal swab specimens	MAGPIX instrument	Rnase P (RP), N, and E sequence	~1 to 2.5 hours*
3/27/20	Abbott Diagnostics Scarborough, Inc.	ID NOW COVID-19	isothermal rRT-PCR	direct or eluted nasal, nasopharyngeal or throat swabs	ID NOW Instrument	RP sequence	13 minutes
3/30/20	NeuMoDx Molecular, Inc.	NeuMoDx SARS-CoV-2 Assay	rRT-PCR	Nasopharyngeal, oropharyngeal, or nasal swab	NeuMoDx™ SARS-CoV-2 Test Strip using NeuMoDx System	N sequence	~1 to 2.5 hours*
3/30/20	QIAGEN GmbH	QIAstat-Dx Respiratory SARS-CoV-2 Panel	mutliplex rRT-PCR	nasopharyngeal swap	QIAstat-Dx Respiratory SARS-CoV-2 Panel Cartridge for e QIAstat-Dx Analyzer 1.0	RP and E sequence	~1 to 2.5 hours*
4/1/2020	Cellex Inc.	Cellex Inc. qSARS-CoV-2 IgG/IgM Rapid Test	lateral flow immunoassay	serum, plasma (EDTA and citrate) and whole blood	Test cartridge	IgM and IgG antibodies to SARS-CoV-2	15-20 minutes
4/1/2020	Ipsum Diagnostics, LLC	COV-19 IDx assay	rRT-PCR	nasopharyngeal and oropharyngeal swab samples	QS12 instrument	N and RP sequence	~1 to 2.5 hours*
4/2/2020	Becton, Dickinson & Company (BD)	BioGX SARS-CoV-2 Reagents for BD MAX System	RT-PCR	nasopharyngeal and oropharyngeal swab samples	BD MAX ExK TNA-3 kit using BD Max System	N1, N2 and RP sequence	~1 to 2.5 hours*

Table 1: FDA Emergency Use Authorization approved assays for COViD-19 from February 2nd to April 2nd 2020. A total of 25 assays received Emergency Use Authorization (EUA) for detection of COVID-19 from the FDA from February 2nd when the FDA announcement was made, up until April 2nd; two months later. All data collated in this table is collected from package inserts and other materials found on the FDA's EUA webpage (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations), unless otherwise indicated. *Estimated based on typical rRT-PCR cycle times allowing for cycling time and estimates of preparation and system variation where specific guidance from manufacturer has not been provided. **Key:** N: Nucleocapsid (N1 and N2) gene, RP: Rnase P (Orf1ab) gene, E: envelope gene, S: Spike protein (S1 and S2) gene, ORF: open reading frame

Fundamentally there are three methods used to detect infectious viral disease in a laboratory setting. The first and oldest method of viral detection is cell/tissue culture, in which growth of a viral organism is expanded within a suitable host cell, the second is detection of either the viral antigen or nucleic acid in a patient specimen, and the finally through detection of antibodies against the virus; serology, and viral antigen detection[34]. The final two methods are useful for adoption in regional and local clinical laboratories for the tracking and diagnosis of newly emerging viral disease.

4.1. Viral Culture

Growth via tissue culture, has been used for over 70 years[34] and is primarily a historical approach to testing in contemporary clinical laboratories, where more rapid molecular methods now predominate and cost effective serological and antigen tests provide shortened times to diagnosis. Viral culture is still considered a standard approach for research into viral pathogenesis and is particularly useful in the initial phase of an epidemic when other assays remain undeveloped, however viral culture for diseases such as MERS-CoV and other deadly coronaviruses require BSL-3 facilities that are not the norm in the traditional clinical laboratory[38]. Viral cultures, when possible, are also a significant source of data regarding the cellular modifications that the virus may make in the host cells. With MERS-CoV for example, growth in multiple immune cell types was shown to mediate aberrant production of inflammatory cytokines and chemokines and upregulate pathways of apoptosis[38]. The CDC provided guidelines for growth in culture of the SARS-CoV-2 virus and provides SARS-CoV-2 generated by cell culture for distribution to external institutions for use in antiviral and pathogenesis research[39].

4.2. Molecular Testing

The most significant expansion in viral detection has occurred using highly sensitive molecular amplification methods including the emergence of rapid nucleic acid amplification testing (NAAT)[40]. These assays, which can detect directly the target pathogen, are considerably more sensitive than rapid antigen detection immunoassays which can be low in volume during acute disease. NAAT within the laboratory environment plays a central and early role in pandemic viral responses, primarily due to the time frame to viral detection via nucleic acid (within 24 hours in many cases) versus the development of antibody responses (potentially weeks to develop)[41]. Thus during the COVID-19 pandemic, significant early development occurred in establishing testing nucleic acid based on rRT-PCR (Table 1). Existing practices to detect genetic material include reverse transcription-polymerase chain reaction (RT-PCR), real time RT-PCR (rRT-PCR), and others such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), including for coronaviruses SARS-CoV and MERS-CoV[42] and others[43]. As well as the isothermal amplification technique which allow for rapid, simpler, and less expensive genetic detection[44], at least one assay developed for COVID-19 utilizes isothermal nucleic acid amplification and can be conducted in 13 minutes[45]. These prior assays, as well as genetic mapping of other coronaviruses such as MERS-CoV[38], led to the first validated rRT-PCR diagnostic assays for SARS-CoV-2 which detected the envelope (E) gene and

RNA dependent RNA polymerase (RdRP) gene (also known as *ORF1ab*), used for screening and confirmation respectively[42]. Subsequent to this a signification number of rRT-PCR assays were developed for an increasing number of COVID-19 genes. The first emergency authorized assay for qualitative determination of COVID-19 in the US, developed by the CDC; CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, contained primers and probes for the detection of SARS-CoV-2 nucleocapsid (N) gene[46]. A list of target genes used in the first emerging rRT-PCR assays for COVID-19 can also be found in Table 1.

While traditionally requiring longer timeframes to results than serological assays, as late as 2018 rapid point of care (POC) NAATs were available, via kits, from commercial vendors for three respiratory viruses; namely influenza A and B and respiratory syncytial virus (RSV)[40]. Increased research and development efforts following a pandemic viral threat will lead to newly developed NAATs for emerging pandemic pathogens. A meta-analysis comparing rapid tests for Influenza A and B by either traditional rapid influenza diagnostic tests (RIDTs), digital immunoassay (DIA) or NAAT suggested that all have a very high specificity (≥98.3%), allowing physician confidence regarding a positive result[47]. One assay, the XPRSARS-COV2-10 assay for use on the GeneXpert Xpress System from Cepheid can be performed in roughly 15 minutes and was authorized for emergency use in patient care settings outside the clinical laboratory[48]. While a number of microfluidic-based molecular systems, that significantly reduce size and cost, have reached the proof of concept stage, requiring less sophisticated equipment and reducing manual preparation steps, they are only now emerging for many infectious diseases[44]. However, during emerging pandemics centralized instrument-based approaches, including RT-PCR for NAAT, often still require laboratories with molecular biology capabilities[18].

As with all diagnostic testing, careful adherence to specific test procedure materials and laboratory standard operating procedures should be followed as assays vary (See **Box 1**). During an emerging viral pandemic, molecular testing may be hampered by the lack of personnel, lack of appropriate primers and probes, and the availability of positive control probes. A survey from January 2020 at the onset of the COVID-19 pandemic suggested that 38 out of 45 laboratories across Europe performed diagnostics without the availability of a positive control and only 11 of 45 had validated the assays against other known coronaviruses[49], for instance.

Box 1: Generalized Nucleic Acid Amplification Techniques

Collection of specimen

The optimal specimen for NAAT-based diagnosis is based on the symptoms encountered in the patient. For example, bronchitis/pneumonia or flu-like symptoms traditionally require a nasopharyngeal swab (NPS) to be collected and in some cases throat swabs are acceptable[34,50]. Lower respiratory tract specimens, such as tracheal aspirates and bronchoalveolar lavage (BAL), often contain the highest viral RNA load, but are invasive, especially in individuals with mild illness, thus NPS and oropharyngeal swabs (OPS) are more obtainable[38]. Follow collection guidelines for the assay kit in use. Face masks are strongly recommended PPE by the CDC[51] and WHO[52] when collecting specimens for

potentially airborne or droplet-borne viruses in order to mitigate transmission to healthcare workers. Samples should be collected within 3 to 5 days of symptom presentation, transported to the lab on ice and refrigerated if testing is to be performed within 48 hours; extended storage can be maintained at -80°C[50].

Performance of RNA extraction

A multitude of good manual and automated methods for the extraction of nucleic acids are commercially available[53]. The fundamental steps involved in nucleic acid preparation are first the lysis of the cell or organism, separation of the material from protein and other material, washing away debris material and inhibitors, and optionally concentrating of the nucleic acid. Lysis can be performed using simple salt solutions, or using detergents and lytic enzymes when dealing with viral capsids[53]. Nucleases and proteases are often used in the later steps for isolating debris and degradation of inhibitory factors. Common separation methods include liquid phase extraction; e.g. phenol solvent mixed with chloroform or isoamyl alcohol, which can separate other components from nucleic acid through centrifugation, and solid phase extraction; which use less hazardous chemicals and use chromatographic techniques such as gel-based size exclusion, ion exchange, and reversible affinity columns[53]. For RNA viruses special efforts must be made to reduce the degradation of RNA by RNases found in specimens and the environment. Kits like the RNeasy kit from Qiagen (Valencia CA) require approximately 500µl of swab material for accurate testing according to manufacturer recommendions[54]. Significant variations in kit designs for RNA extraction when used in conjunction with rRT-PCR should be considered, however, referring to pre-established SOP recommendations is advised for developing a rapid response during pandemic planning. Furthermore, advances in automation have led to robotic nucleic acid extraction methods that reduce labor and contamination concerns[55].

Performance of PCR

RT-PCR is the most sensitive method for the detection of RNA in low volume isolates from specimens when compared to viral culture, immunofluorescence, and existing tests for rapid identification of virus[43,56]. PCR is often cited as the new gold standard for nucleic acid amplification in infectious disease, surpassing viral culture and signal amplification methods[57]. Though methodology varies, in general RT-PCR kits allow for either one or two step processing[54]. A significant number of advances in rRT-PCR diagnostic technologies, for the detection of viral RNA, have come from the investigation of influenza and other respiratory viruses associated with pandemic threats[43,54-57]. Some available modalities include conventional gel-based RCR (cRT-PCR), multiplex PCR (mRT-PCR)[43], and most recently real time-RT-PCR (rRT-PCR)[56]. Both qualitative and quantitative rRT-PCR can be performed used a one-tube method in which labelled target probes and primers are added to extracted nucleic acid and temperature cycled for RNA amplification using a thermocycler; often within an automatic instrument. A general cycling procedure includes 30 minutes at 50°C followed by 2 minutes at 95°C followed by multiple cycles at 95°C for 15 seconds and 60°C for 30 seconds[58], however manufacturer recommendations should be followed for diagnostic assays. While traditional RT-PCR techniques may take several hours to complete[57], newer systems such as the Applied Biosystems 7500 Fast RT-PCR

Instrument boast cycle times as low as 35 minutes[59].

4.3.Antigen and Antibody Detection Methods

Companies worldwide began developing serological methods of detection and gaining approval for use in both the US and European markets for pandemic COVID-19 within months of its first detection in Wuhan, China. Specifically, EUROIMMUN has developed two Anti-SARS-CoV-2 ELISAs for immunoglobulin classes A and G, which gained CE (certification mark) approval, for use in the European Economic Area (EEA), on March 26th, 2020[60]. In the US market, the first EUA approval for a serological antibody was for detection of IgM and IgG antibodies to SARS-CoV-2, posted to the FDA site on April 1st, 2020 to Cellex Inc. for its qSARS-CoV-2 IgG/IgM Rapid Test[46].

While evidence of infection is possible using molecular methods such as the nucleic acid amplification techniques that proliferated at the start of the 2020 COVID-19 pandemic **(Table 1)**, the cost and availability of these assays create significant restrictions to their distribution, specifically in small facilities and financially limited locations. However, prior research into the RNA genome of coronaviruses, such as SARS-CoV, has highlighted the coding for a total of eight accessory viral proteins that may have both a significant role in virus to host response and act as potential targeting for antibody-based diagnostics[61]. For instance, that the coronavirus spike glycoprotein found on the surface of all coronavirus and used for cell fusion with host cells and viral entry, share 96% nucleotide sequence identity between the original SARS-CoV and SARS-CoV-2[62]. Furthermore, the SARS-CoV-2 spike proteins; consisting of two subunits; S1 and S2, share 74% amino acid homology with SARS-CoV[62], suggestive of its evolutionary derivation from the later and the applicability of prior strategies for detection. Additionally, early evidence from 173 SARS-CoV-2 infected patients showed a seroconversion rate for antibody in 93% of cases[63]. Beta versions of the EUROIMMUN ELISA assay, now approved for use in Europe[60], specifically reacted with SARS-CoV-2 S1 antibody containing sera, with limited cross-reactivity with SARS-CoV and no other tested coronaviruses according to prepublished finding by Ou at al.[64].

None-the-less, sensitivity restrictions and time until utility of serological and antigenic assays are often cited as limitation to the use of these testing methods[50]. The inherent sensitivity issue with immunoassay detection is based on the method of discovery in which neither the target nor the signal is amplified as is done with the nucleic acid techniques described above[40]. However, the utility of immunoassays, particularly in the rapid surveillance with POC assays of viral disease has been well established for Influenza A and B and Respiratory Syncytial Virus[40,65]. Two advantages of immunoassay-based diagnostics is cost of testing and time to results; with rapid influenza testing taking 10 to 15 minutes versus approximately 1 hour for nucleic acid testing[65]. Furthermore, assays that reduce time to results may be effectively implemented in public health monitoring of disease, in such cases there is an emphasis on developing immunoassays with minimal false negatives; or negative predictive values, while accepting assays with higher false positive values[66]. The most commonly performed laboratory immunoassay is the ELISA (enzyme-linked immunosorbent assay) and the most common POC

immunoassay is the lateral flow immunochromatography assay (LFIA; also known as the strip test). During the 2002-2003 SARS-CoV outbreak a number of serological assays were developed including ELISA, IFA (Immunofluorescence assays), and Western blot assays, with IFAs being some of the first to emerge[67]. However IFAs, while inexpensive and easy to use, are hard to standardize because they rely on the subjective assessment of visual staining patterns[67]. Furthermore, all assays requiring antibody detection depend on the time to seroconversion. Assessments of SARS-CoV during 2002 suggested a potential two week delay for antibody expression[67]. Similarly a case report from Finland showed that SARS-CoV-2 antibody response; IgG and IgM, was present at day 9 post infection but not before[68]. Antibody testing must be carefully interpreted, as IgM is typically a sign of early infection, IgG of past or recurrent infection, and the results may be impacted by the presence of therapeutic neutralizing antibodies[69,70].

The impact of a serological response to a pandemic infectious disease is more important for determining a survey of public health[71], indicating recovery from illness[72], and assessing the impact of therapeutic interventions[73]. Thus the first attempts to develop assays during the 2020 COVID-19 pandemic focused almost entirely on early detection methods predominantly using molecular methods. However, the later phase of a pandemic response includes the development of serological detection assays to monitor recovery and treatment efforts. Only one serological method was developed for commercial release in the US within the first 60 days after the FDA EUA for COVID-19 and approved for use, on day 59; the Cellex Inc. qSARS-CoV-2 IgG/IgM Rapid test[37] (Table 1). None-the-less, the developments of serological assays in Europe, such as the EUROIMMUN ELISA assay[60], and in the US suggest that the second phase; monitoring recovery and tracking treatments of newly emerging pandemic viral diseases, is still based on the immunological response.

5. Laboratory Role in therapeutic interventions to Pandemic Disease

Beyond detection, the response to viral pandemics should focus efforts around the most affected populations. Of significant importance in this response, the clinical laboratory has a pivotal role in accessing not only the detected incidence of pandemic viral disease, but also in assessing the indications of severity of disease and the efficacy of intervention/therapeutic strategies. One key to this effort is defining and providing appropriate testing algorithms for severe disease cases.

The clinical laboratory, central in all these efforts, will continue to have a role in informing testing algorithms and selection of treatments. To this end, significant efforts have been made in the last few decades to understand the host response to infectious pathogens through single analyte and genomic, transcriptomic, and proteomic panel assays[74]. For example, along with viral load, detected via rRT-PCR, initial studies of COVID-19 patients suggested that clinical scores assessing the severity of acute lung injury, specifically CRP (C-reactive protein) levels, are indicative of negative outcomes and progression to pneumonia and respiratory failure in patients[72]. Similarly ELISA testing of angiotensin II from patient plasma, showed a marked increase, indicating a potential

association of SARS-CoV-2 to binding of the ACE2 receptor and acute lung injury[72,75]. Studies of both the SARS-CoV-2 spike protein and human ACE2 indicate that structural and sequence variations may mediate host-virus interactions[76] and thus be effective at determining individual risk for severe disease. This mechanism of host interaction and association with acute respiratory distress syndrome is potentially similar to patient response to influenza virus infection[77], which means prior research into ARDS interventions for influenza may aid in treating at-risk populations for COVID-19. Additionally, monoclonal antibody based therapeutics, previously studied in SARS-CoV and MERS-CoV, are a perceived immunotherapeutic approach to treating COVID-19[75] and will require additional laboratory testing to determine effectivity. Finally, as the case for COVID-19 suggests, early assays to detect novel infectious diseases may only provide us with qualitative results, whereas clinical triage of patients effected by an emerging viral disease may be better performed using quantitative viral load testing in association with assays of immune response. An early study examining viral load for COVID-19 suggested that viral load increased in early and progressive stages of infection but dropped in recovery stages[78,79], for instance.

Emerging pandemics often require significant efforts on the part of the entire health care team in determining the rate, demographics, and sources of infectious disease. The clinical laboratory, central in all of these efforts, will continue to have a role in determining treatments and algorithms for laboratory testing. As a result of the 'all hands on deck' approach to combating viral pandemics, significant advances will be made regarding the pathogenesis and potential reaction to future viral threats. Past efforts including massive vaccination schedules to eradicate polio and measles[80] as other infectious diseases[81], technological gains in detection seen with the AIDS pandemic[82], and the proliferation of diagnostic testing for influenza[65], are suggestive of the research efforts that will enhance the healthcare response to future pandemics. Initial increase in testing for a novel pandemic viral disease within the laboratory may give way to increased efforts focused on tracking and monitoring disease progression for the most severe cases. Thus the laboratory should be prepared to re-tool their efforts throughout their pandemic response.

6. Conclusions

Like with prior pandemic disease, the true heroes in the effort to curb the spread of viral disease are the clinical laboratory professionals, often hidden from view during business as usual medical coverage, who are tirelessly working to monitor the spread of viral illness. The solutions will not ultimately come from public health efforts at containment or flattening the curve, but from advances in technology and into the pathogenesis of disease.

The clinical laboratory plays a critical and pragmatic role in the initial characterization of viral pandemic threats, and will maintain critical involvement in tracking hospitalization cases for individuals with severe disease. Key to that effort, clinical laboratory organizations must understand the signs, symptoms and transmission routes for viral infections in order to protect workers from succumbing to disease and the resultant reduction in available labor. Provisions must be made in advance of a pandemic to ensure

appropriate resources are available and sensible precautions are taken; including vaccination and emergency response plans. Proper PPE for respiratory viral pandemics include adherence to Standard Precaution such as hand hygiene and use of appropriate face protection.

Laboratories' must exercise judgement in selecting the appropriate testing methods for early stage diagnostics; such as rRT-PCR and other NAAT, and late stage diagnostic goals. Rapid developments in the availability of laboratory tests will occur once public authorities, such as the CDC and FDA in the US, conclude that the virus presents a significant threat to public health and authorizes commercial manufacturers to release emergency use assays for the detection of the viral threat. The case for COVID-19 suggests that such early test methods will predominantly be qualitative molecular assays that require detection within a CLIA approved moderate to high complexity laboratory. Later laboratory responses will include tracking through both molecular and serological assessments of the virus within the patient as well as tracking of biomarkers associated with the severity of the disease.

A road-map to securing the safety and providing efficient diagnostics during a pandemic will be defined by an examination of prior efforts, clear indications are that laboratory studies will ultimately advance our understanding of viral disease and led to new technologies that will govern our more efficient response to future viral threats. Furthermore, laboratory workers and managers should be prepared for a significant increase in specific testing for the pandemic threat, which will give way to later efforts at tracking and patient management assays.

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