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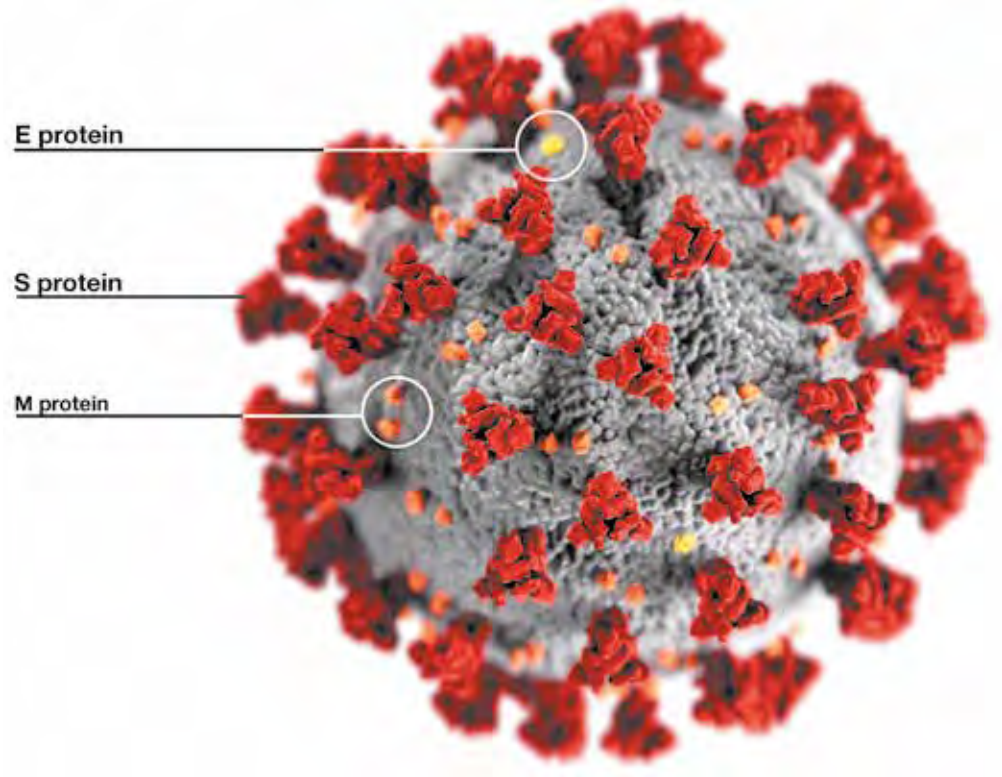
Special Issue on COVID-19



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The Aga Khan University Hospital, Karachi

LABRAD

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From the Editor's Desk

Dear Readers

I hope all our readers are in good health and managing these uncertain times as best they can. The rapid spread of the Coronavirus Disease 2019 (COVID-19) poses unprecedented challenges in Pakistan and across the globe. To serve the health care community at such a critical time, the LABRAD team has created a special issue pertaining to this global pandemic. One of the biggest lengthiest issue of its time, LABRAD offers a great variety of articles all related to COVID-19 to keep you informed on how to address this ever-evolving pandemic using the best available information. This issue also includes an interview of the Chair of the Department of Pathology and Laboratory Medicine and how she led the department in these challenging times. As the current pandemic problem extends to all parts of the country, we anticipate that this issue will be helpful to all our readers from across the country in providing high-quality, safe, effective and optimal care to their patients.

The day we decided to have a special issue of LABRAD on COVID-19 my whole newsletter team sprang into action. Whether, it was sending call for articles, gathering pictures (don't forget to check the Polaroid section of LABRAD), taking appreciative inquiries (Best of the Past) reactions, or writing. Every single person in my team was ready to contribute and give it their best. The newsletter team remain connected throughout these past few weeks via WhatsApp and emails and compiled this issue completely remotely. The information in the current newsletter was conceived and compiled by staff, residents and faculty of the Departments of Pathology and Laboratory Medicine and Radiology.

Headed into the summer, there are umpteen questions we don't have answers to. In this anxiety producing environment of pandemic, I would encourage all of you to stay positive. Keep on looking over the horizon and come up with innovative ideas to fight this COVID-19 pandemic. Stay connected to each other and keep discussing your ideas. The wealth of knowledge that can be gained from the experience of others should never be underestimated. Transmission of ideas should be sticky and as aggressive as COVID-19!

We truly are in this together, and that is precisely how we will get through it to a better future. Please take care, and stay safe.

Dr Lena Jafri
Clinical Chemistry

Pathophysiology of Lung Injury in Context with Novel Coronavirus 2019

Dr Qurratulain Chundriger and Dr Nasir Ud Din
Section of Histopathology

Coronaviruses are large enveloped RNA viruses. The human coronaviruses cause lower respiratory infections apart from being the causative agents of common cold and gastroenteritis in infants. COVID-19 or SARS-Cov-2, a type of beta-COV, is a novel strain of the Human Coronaviruses that was first identified in the latter part of 2019 as a cluster of pneumonia cases. In January 2020, the Chinese authorities identified a novel type of coronavirus, which was later named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Until recently, only two beta coronaviruses — severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus

(MERS-CoV) — have caused significant human morbidity and mortality.

Pathophysiology: The COVID-19 has four major structural proteins: the spike surface glycoprotein, small envelope protein, matrix protein, and nucleocapsid protein. Coronaviruses exhibit a tropism for the epithelial membranes of the respiratory tract in particular. When a person is infected by COVID-19, the petal shaped glycoprotein spikes on the surface of the viral capsid attach to the epithelial cell via receptor binding domains of the Angiotensin Converting Enzyme II (ACEII) which is most abundant in type II pneumocytes. It is also found in other organs like gastrointestinal tract, kidney, lymph nodes, thymus, bone marrow, spleen and brain. The RNA of the virus is then endocytosed into the cytoplasm of the infected cell, where it first creates the viral RNA polymerase and then starts replicating. Multiple newly formed virions are then shed from the cell and infect other nearby cells.

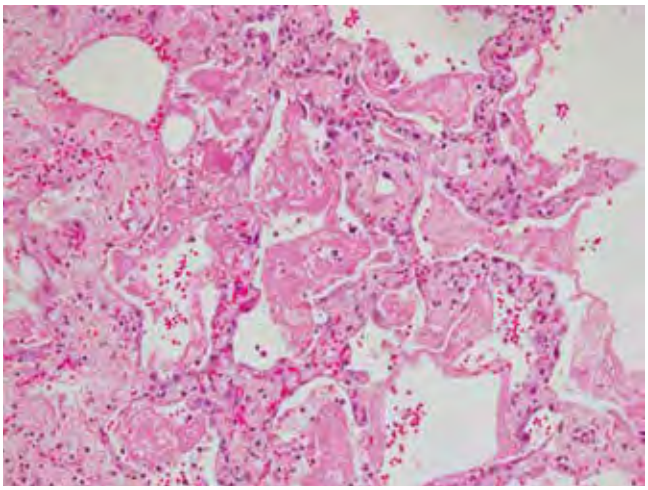
Clinical presentation: In 80 percent of cases, immune system fights back resulting in mild to moderate symptoms of cough, chest pain, fever and some degrees of shortness of breath. Upto 14 percent of COVID-19 infected individuals develop severe disease, most of these patients have a pre-existing condition that makes them highly vulnerable. These may be geriatric patients, immunocompromised individuals, chronic smokers, those with underlying pulmonary or cardiac dysfunction and diabetics to list a few. These patients present with hypoxemia or multi organ dysfunction and may require mechanical ventilation.

ARDS and Radiological findings: About five percent cases develop Acute Respiratory Distress Syndrome or ARDS, which shows characteristic radiological findings on standard X-Rays and CT scans. According to the studies published so far, the imaging features of COVID-19 pneumonia are diverse, ranging from normal appearance to diffuse changes in the lungs. In addition, different radiological patterns are observed at different times throughout the disease course. One study showed slight predilection for the right lower lobe. The most common findings were ground glass opacities, variable degrees of septal thickening, nodules, cystic changes, pleural effusions and thickenings. Of these, patients with severe disease and those who succumbed, exhibited severe ground glass opacities and white lung on CT scans.

Microscopic findings: Only a handful of studies have been published so far which have described the histopathological findings of patients with severe disease and those who died of COVID-19. The clinical picture is that of pneumonia and ARDS. The pathological findings are of Diffuse Alveolar Damage (DAD) which is the most common histopathologic correlate of ARDS. DAD shows an early exudative phase and a late organizing phase. In the exudative phase, there are rounded organizing plugs of exudate in the terminal bronchioles and protein-rich edema in the alveolar space, with fibrin-rich eosinophilic hyaline membranes along the surface of the alveolar septa (Figure 1).This results in inability of the lungs to perform the vital function of gaseous exchange through the damaged and so-called blocked pulmonary alveolar spaces. There can be proliferation of type II pneumocytes, accompanied by reactive nuclear changes in these cells, i.e. enlargement of the nuclei and mild atypia. The interstitium shows edema with scant number of inflammatory cells including lymphocytes, plasma cells and/or neutrophils, depending on the underlying etiology. The term “diffuse” refers to complete involvement of the pulmonary lobule and not necessarily the entire lung tissue. One study has also described multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the intra-alveolar spaces, showing viral cytopathic-like changes. Viral inclusions were not identified. Large areas of intra-alveolar hemorrhages and intra-alveolar fibrin cluster formation were observed in another study. All these changes have been described in autopsy findings of COVID-19 decedents, who had increased weights of lungs due to edema or consolidation and adhesions grossly. The organizing phase may show interstitial thickening and fibrinoid necrosis of small vessels along with alveolar edema and pleural fibrosis with adhesions. Some or may be most of the cases under the microscope show a variable mixture of areas of both exudative as well as organizing phases. Findings representing the fibrotic phase (e.g. dense collagenous fibrosis, architectural remodeling) are not reported so far.

Figure 1. Lung tissue showing formation of hyaline

membranes which line the alveolar spaces with loss of alveolar pneumocytes. In addition, there are changes of pneumonia i.e. interstitial inflammation and widening, along with congested vessels and areas of hemorrhage.



No significant histopathological abnormalities were reported in other tissues like the heart and liver in patients who died of COVID-19. These organs showed changes pertaining to either an underlying condition, like liver cirrhosis and or changes related to prolonged hospitalization and drug related injury, such as microvesicular steatosis in hepatocyte lobules. Only few autopsy studies and a couple of studies of core biopsies from patients who recovered from COVID related illness have been reported in the literature so far, all of these from china and the western world. In Pakistan, we are way behind them as autopsies are not routinely performed for non-medico legal purposes and there is no trend of doing core biopsies from patients with any degree of symptomatic COVID-19 illness. As reports of atypical presentation are continuously being made, this strain of the coronavirus presents an entire horizon of clinical, radiological and pathological features to be explored.

Imaging in COVID-19 Pandemic: Salient Features

Dr Shaista Afzal
Radiology

The entire world is experiencing an outbreak caused by the novel Corona virus that originated from Wuhan, China in December 2019. The disease was officially announced as a pandemic by World Health Organization (WHO) on the 11th of March 2020. At first, the disease appeared in a few clusters affecting individuals with advanced age or co-morbidities; however, it is now widespread. COVID-19 can cause severe pneumonia and fatal respiratory disease especially acute respiratory distress syndrome (ARDS). The predominant clinical features of COVID-19 infection are fever, fatigue, malaise, dry cough and other respiratory symptoms. Early detection and diagnosis of this highly contagious disease is vital to control the outbreak; hence, it is important to separate suspected cases and contacts. In the present times the diagnosis is mainly based on the patient's epidemiological history, clinical features, laboratory tests especially the reverse transcriptase polymerase chain reaction (RT-PCR) for COVID-19 and chest imaging features. The radiologist plays an important role in the early diagnosis and identification of a suspected COVID-19 individual which can be of great help not only to the patient but also for public health surveillance and response authorities. This short communication briefly introduces the chest X-ray and CT imaging features of this novel corona virus infection with focus on the value of imaging in its diagnostic workup.

Chest X Ray

Chest x-ray is not routinely performed in the clinical workup of a suspected COVID-19 patient because it is not sensitive in the early stage of the disease. However, with the progression of disease beyond the early stage, the chest x-ray may show multiple patchy opacities, predominantly at peripheral and bases, and in bilateral lung fields. (Figure 1 & 4a) With further severity of disease, there develops confluence of

opacities that results in “whited out lung”. Pleural effusion has also been reported in severe cases. The findings have been reported to develop over the time course of the disease.

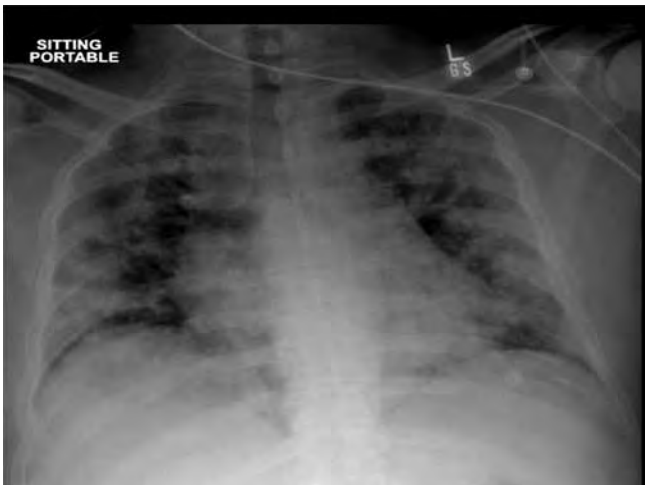


Figure 1: X ray chest, AP sitting projection, shows multiple inhomogeneous opacities in the periphery of bilateral lung fields

CT (Computed Tomography) Chest

For COVID-19 diagnosis, RT-PCR is regarded as the reference standard. However, as reported in recent studies, CT chest has shown a sensitivity of 98%, especially in patients with false negative RT-PCR. CT chest also plays an important role in the evaluation of therapeutic efficacy and monitoring of disease progression. The common imaging features of COVID-19 on CT chest are ground glass opacities which are multiple, patchy, bilaterally distributed mostly along the bronchovascular bundle, with or without consolidation, and predominantly in the posterior and peripheral lungs. (Figure 2 , 3 &4b) Other features include reversed halo sign, crazy paving pattern and airway changes. Pleural changes

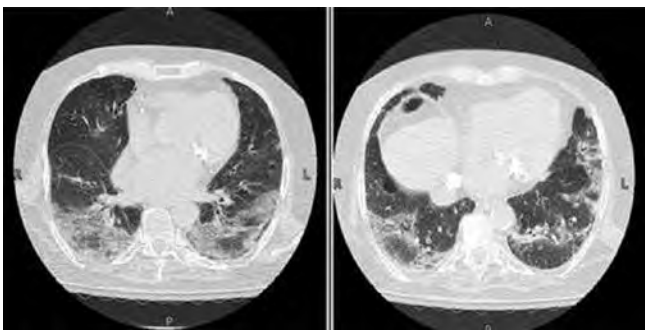


Figure 2: Unenhanced CT chest examination: Bilateral, basal, ground glass opacities with some crazy paving. The disease is predominantly peripheral in distribution with perihilar sparing.
Classic imaging features of COVID-19 infection are seen

comprising of pleural thickening and pleural effusion have also been reported, with the former feature being more prevalent. Poor prognosis is reported in patients who develop pleural effusion. Recent literature further reports the presence of lymphadenopathy and pericardial effusion in critical COVID-19 patients.

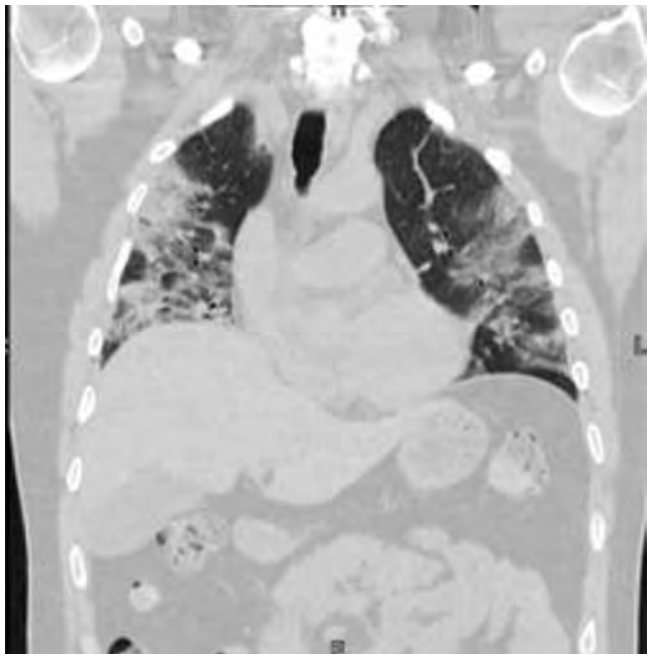


Figure 3: Multiplanar reformatted coronal image, with HRCT protocol. There is evidence of groundglass appearance and patchy areas of consolidation in the periphery of bilateral lung fields, predominantly on the right side in keeping with COVID-19.

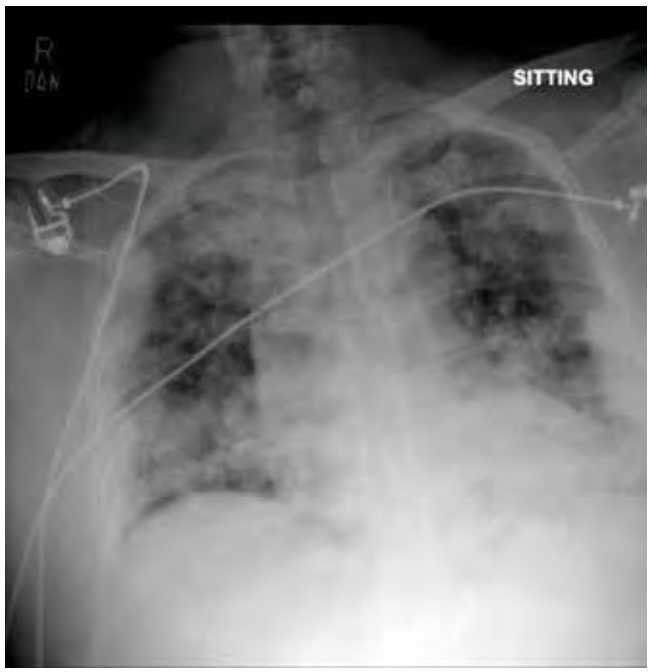


Figure 4a: X-ray chest AP sitting view.
Patchy areas of consolidation in bilateral lung fields predominantly involving subpleural location

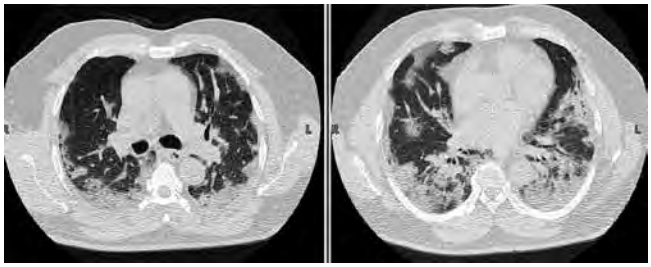


Figure 4b: HRCT examination of the above patient (4a), Multifocal, patchy areas of dense groundglass haze as well as scattered consolidations identified in both lung fields with predominant subpleural involvement, appearances are highly suggestive of COVID pneumonia.

Conclusion

In the present situation, imaging of COVID-19 patients especially with CT Chest is very valuable because it shows characteristic imaging features of COVID-19 infection. This shall enable frontline physicians to diagnose patients even when laboratory results are false negative. An early diagnosis with the help of imaging may thus facilitate containment of the disease and overcoming of the outbreak.

Qualitative Real-Time RT-PCR For Detection of SARS-Cov-2 RNA

Dr Kiran Iqbal and Dr Najia Ghanchi
Molecular Pathology

The global outbreak of novel coronavirus, SARS-CoV-2 (COVID-19), caused over 100,000 deaths and more than 1 500,000 infected sending several countries to lock down. COVID-19 is caused by SARS-CoV-2 which belongs to a family of beta corona viruses. It was first discovered in the Wuhan, a city in the Hubei Province of China in 2019. It belongs to the same subgenus as that of the severe acute respiratory syndrome (SARS) virus but to a different clade. SARS-CoV-2 virus appears spherical in shape. It has spike like proteins protruding from the surface of the virus particle. Recent work shows that SARS-CoV2 attaches to human cell receptor angiotensin-converting enzyme two (ACE2) using its spike like proteins which than undergoes a structural change allowing the viral membrane to fuse with the human cell membrane. The viral genes then enter into the host producing more viruses.

Transmission is through droplets and the incubation period is 14 days following exposure. Patients who are affected with SARS-CoV2 presents with fever and acute respiratory symptoms including cough and dyspnea. In case of severe manifestation, patient presents with pneumonia having bilateral infiltrates on chest X-ray. Patients with compromised immune responses including old age or underlying comorbidities are reported to develop more severe

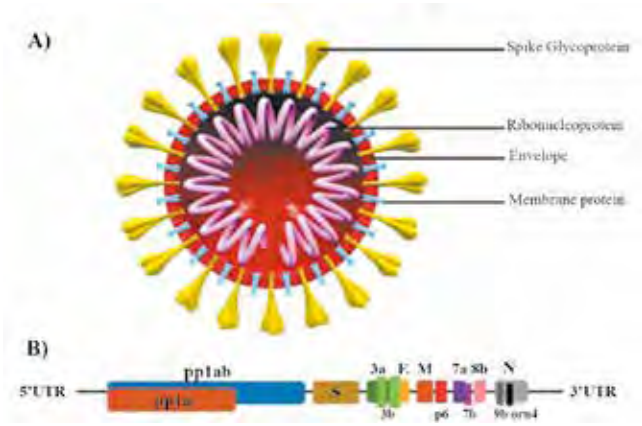


Figure 1: Structure and genome organization of SARS-CoV-2.
A) structure of SARS-CoV 2 viron.
B) Relative positions of amplicon targets on SARS-CoV genome.
N: nucleocapsid, ORF: open reading frame.

disease. The global fatality rate is reported to be around two percent. People who are at risk of getting COVID 19 are the ones who live in or have travelled to the areas where community transmission has been reported or the ones who have had a close contact with confirmed cases. Patients suspected of COVID-19 should undergo testing for SARS-CoV2 along with testing for other possible respiratory pathogens. Appropriate sample including upper respiratory specimen (oropharyngeal and nasopharyngeal swab) or lower respiratory specimen (sputum) is sent to the lab in a viral/ universal transport medium. Nucleic acid extraction

is performed and the sample is subjected to real-time reverse transcription polymerase chain reaction (rRT-PCR) for the detection of SARS-CoV2 RNA. The viral genes targeted for testing include the N, E, S and RdRP genes. E gene is targeted as a first line screening assay for pan-Sarbecovirus detection. For confirmation RdRp and ORF1/a non-structural gene are being tested. PCR detects SARS-CoV-2 RNA that are detectable in nasopharyngeal and Oropharyngeal swab samples during infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions and must be correlated with clinical, patient history, and epidemiological information.

Patients tested positive should be isolated and provided with supportive treatment. Antiviral treatment for COVID-19 is under investigation. Patients with mild infections can be managed at home provided they stay at home in a separate room from other people and animals in the house. They should wear a facemask when with someone or when visiting health care settings. No vaccine or specific treatment for COVID-19 is available and care is supportive

Many aspects of the virus and disease are still not understood. A better understanding will be needed to provide improved guidance.

Coagulopathy and Anticoagulants in COVID-19

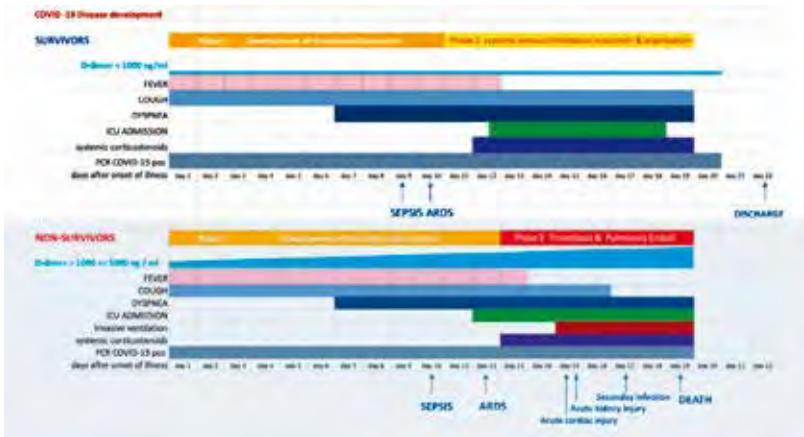
Dr Kanta Devi
Hematology Transfusion Medicine

The 2019 novel coronavirus (COVID-19) presents with a variety of phenotypes that range from asymptomatic to profound, rapid multiple organ dysfunction syndrome (MODS) and death. Proposed mechanisms for MODS in COVID-19 are multifactorial but include a hypercoagulable state with micro and macro-circulatory thrombosis.

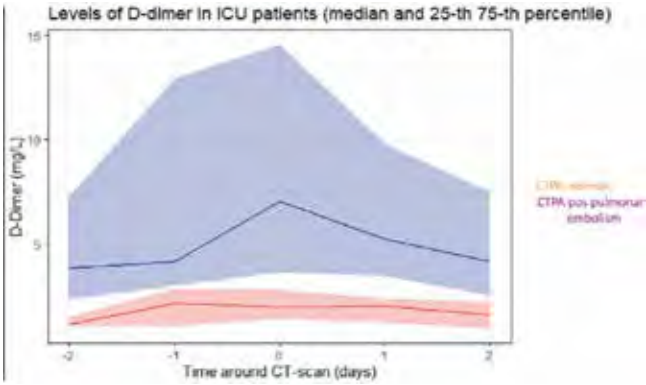
Recent observations and autopsy findings suggest that respiratory failure in COVID-19 is not driven by the development of the acute respiratory distress syndrome (ARDS) alone, but that (microvascular) thrombotic processes may play a role as well. This may have important consequences for the diagnostic and therapeutic management of these patients. There is a strong association between D-dimer levels, disease progression and chest CT features suggesting venous thrombosis.

A strong predictor of mortality is disseminated intravascular coagulation (DIC). A significant increase in D-dimer and prothrombin with a decrease in fibrinogen in non-survivors at days 10-14 is also reported. This highlights the importance of regular and continued monitoring of these levels.

D-Dimer is a degradation product of cross-linked fibrin and reflects blood clot formation and its subsequent fibrinolysis. Testing uses an enzyme-linked immunoabsorbent assay (ELISA) or microlatex agglutination assay . D-dimer has a very high sensitivity for thrombotic disease, but its specificity



is poor. Elevated D-dimer (above 1 µg/mL) was a strong and independent risk factor for death in this population.



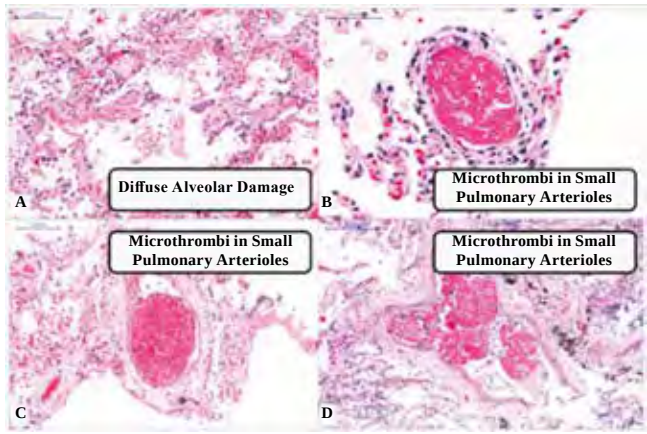
Pathology Findings

Microvascular thrombosis is hypothesized to be involved in hypoxemic respiratory failure in some patients with COVID-19. Autopsy studies to date have been limited, with some suggesting microvascular thrombosis and others showing pulmonary hemorrhage. Whether the coagulation cascade is directly activated by the virus or whether this is the result of local or systemic inflammation is not completely understood. High plasma levels of proinflammatory cytokines (interleukin-2, interleukin-7, granulocyte colony-stimulating factor, IP10, MCP1, MIP1A and tumor necrosis factor-α) have been observed in COVID-19 patients admitted to intensive care units. . While many pro-inflammatory cytokines trigger the coagulation system, showed that the increase in IL-6 was discrepant with the elevations in D-dimer; IL-6 levels appeared to increase only 13 days after disease onset, whereas D-dimer levels were already 10-fold increased by that time. This observation suggests that the very high D-dimer levels observed in COVID-19 patients are not only secondary to systemic inflammation, but also reflect true thrombotic disease, possibly induced by cellular activation that is triggered by the virus.

● **Bottom Line:** this report supports the concept of hypercoagulative status, showing high frequency of pulmonary microthrombosis

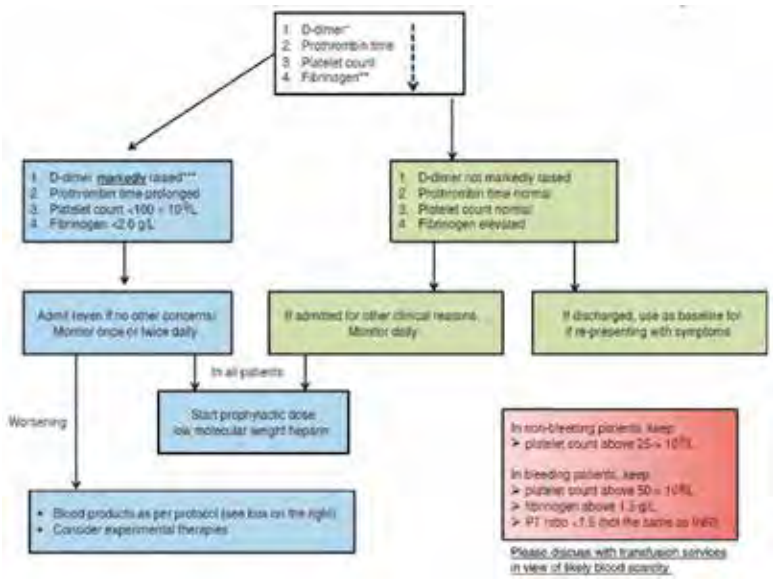
Role of Antithrombotic Therapy

Notably, a Chinese single-center retrospective



cohort study (Tonghi hospital) of 449 consecutive patients classified as having severe COVID-19 indicates that prophylactic doses of heparins might be associated with improved survival (20 percent) in patients with evidence of sepsis induced coagulopathy (SIC)/DIC). Severe COVID-19 was defined as either a respiratory rate ≥30/min, arterial oxygen saturation ≤93 percent at rest, or PaO2/FiO2 ≤300 mmHg. Exclusion criteria included bleeding diathesis, hospital stay <7 days and lack of information of coagulation parameters and medications. Heparin was associated with lower 28-day mortality.

American Society of Hematology recommends all hospitalized patients with COVID-19 should receive thromboprophylaxis with LMWH or fondaparinux (suggested over unfractionated heparin to reduce contact) unless the patient is judged to be at increased risk of bleeding. In patients with a history of heparin-induced thrombocytopenia (HIT) use fondaparinux.



Serum Procalcitonin - A Marker to Differentiate Bacterial from Viral Infection

Dr Zaib-un-Nisa
Clinical Chemistry

Procalcitonin (PCT) is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid and involved in calcium homeostasis. Procalcitonin arises from endopeptidase-cleaved preprocalcitonin. It is also produced by the neuroendocrine cells of the lung and intestine and is released as an acute-phase reactant in response to inflammatory stimuli, especially those of bacterial origin. It is a biomarker that exhibit greater specificity than other pro-inflammatory markers (eg, cytokines) in identifying sepsis and can be used in the diagnosis of bacterial infections.

PCT production is induced in response to microbial toxins and to certain bacterial-induced cytokines, particularly interleukin (IL)-1β, tumor-necrosis factor (TNF)-α and IL-6, and is released in the bloodstream where it can be measured Conversely, PCT production is attenuated by certain cytokines released in response to a viral infection, particularly interferon-γ (IFN-γ). This selective cellular mechanism makes PCT a useful diagnostic biomarker, which is more specific for bacterial infections and helps to distinguish bacterial infections from other inflammatory reactions or viral infections. The raised Procalcitonin level during inflammation is associated with bacterial end toxin and inflammatory cytokines. Increased levels of serum procalcitonin in response to viral infections and noninfectious inflammatory stimuli such as autoimmune disease and chronic inflammatory processes are much less pronounced, rarely exceeding 0.5 ng/mL. The reference cutoffs are shown in Table 1.

PCT levels intensify within two–four hours of infection and the maximum level is reached by six–eight hours and with continued infection or sepsis the elevated level persists and decreases once infection is controlled. Its half-life is about 20–24 hours. Its levels persist as long as the inflammatory process continues and the level correlate with the severity of sepsis. The applications or indications of testing PCT include to:

- Aid in the diagnosis and risk stratification of bacterial sepsis.
- Aid in distinguishing bacterial from viral infections, including meningitis.
- Screen therapeutic response to antibacterial therapy and reduce antibiotic exposure.

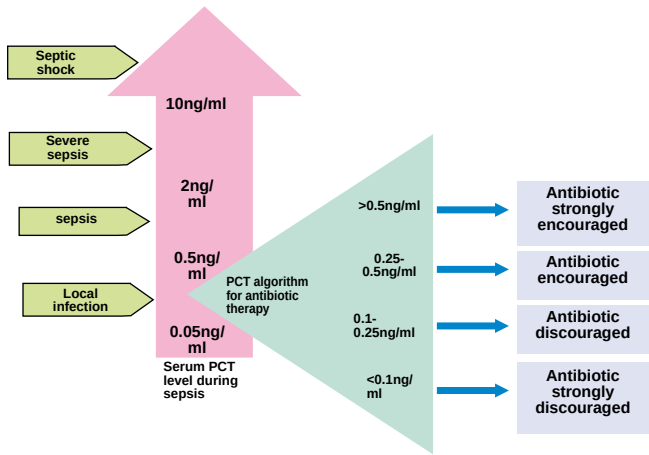


Figure 1: Algorithm for use of PCT for antibiotic Therapy. (Reference:Vijayan et al. Journal of Intensive Care (2017) 5:51; DOI 10.1186/s40560-017-0246-8)

- Aid the preference and timing of the initiation of antibiotic treatment (Procalcitonin Algorithm)

Table 1: Interpretation of Procalcitonin Levels:

Test Name	Reference Cutoffs	Conditions associated
Pro Calcitonin	< 0.5 ng/mL represents a low risk of severe sepsis and/or septic shock	• Localized mild-to-moderate bacterial infection • Noninfectious systemic inflammatory response • Untreated end-stage renal failure
	2.0 ng/mL represent a high risk of severe sepsis and/or septic shock.	• Bacterial sepsis • Severe noninfectious inflammatory stimuli (e.g., major burns, severe trauma, acute multiorgan failure, major abdominal or cardiothoracic surgery). • Medullary thyroid carcinoma (may exceed 10,000 ng/mL).

for improved antibiotic stewardship. The figure 1 shows the algorithm of PCT which can be utilized in antibiotic therapy.

- Aid in the diagnosis of systemic secondary infection after surgery and in severe trauma, burns, and multiorgan failure.

COVID-19 and Procalcitonin

The PCT is now considered an important parameter for earlier identification of COVID-19 patients at risk of developing bacterial co-infection. A report by Guan W. et al. (NEJM Feb 2020) showing data from different medical centers of China, including 1099 COVID-19 patients reported that their PCT levels were low (<0.5 µg/L) in > 96 percent of cases with low disease severity and absence of adverse outcome (combined endpoint of ICU admission, invasive ventilation, death). Another report by Zhou et al. reported that COVID-19 patients may even present with PCT levels <0.25µg/L or even below 0.1 µg/L (Lancet Mar 2020). PCT testing on admission seems to be a valuable additional piece of information to aid in early risk assessment and rule-out of bacterial coinfection in COVID-19 patients.

Role of C-Reactive Protein (CRP) in COVID-19

Ms Iffat Arman
Clinical Chemistry

COVID-19 is a viral disease, for which there is currently no treatment. It is therefore necessary to explore biomarkers to determine the extent of lung lesions and disease severity. New coronavirus pneumonia (COVID-19) is a health emergency due to its high infectiousness and high case fatality in critically ill patients. Clinical monitoring and appropriate treatment strategies were essential to improve case fatality. The pathological and physiological processes and diagnostic methods of COVID-19 are still in the exploratory stage.

The CRP levels are used in the early diagnosis of pneumonia and patients presenting with severe pneumonia have high CRP levels. Correlation between CRP levels, lung lesions, and disease severity to provide reference for clinical treatment. It was first discovered in a patient with lobar pneumonia in 1930 by Tillet & Francis. The CRP is a protein produced in response to infection or inflammation and it is widely used in clinical tests to diagnose and manage patients with sepsis. It is an acute phase reactant whose synthesis in the liver is up- regulated by Interleukin-6. Its response is stronger in acutely ill patients and levels decrease as patients recover. It should be used cautiously in patients with sepsis, as it is also a marker of inflammation that also increases after surgery, burns, myocardial infarctions, and rheumatic diseases. A rapid decrease in CRP levels has been reported to correlate with good response to initial antimicrobial therapy in septic patients. The plasma half-life of CRP is 19 hours. The sensitivity and specificity of CRP as a marker for bacterial infections are 68–92 percent and 40–67 percent, respectively.

Although CRP does not normally elevate significantly in mild viral respiratory infections, levels have shown to increase in severe cases, such as in avian influenza H1N1 and H7N9, and during SARS epidemics in 2003. A similar significant increase of CRP has also been reported in COVID-19 patients. One possible explanation for this phenomenon is the overproduction of inflammatory cytokines that take part in the defense against the pathogen, but also cause more severe symptoms and damage in lung alveoli and stimulate CRP production. Therefore, CRP testing may be useful in the initial evaluation of coronavirus patients. It provides an important clinical evaluation index, and levels can reflect disease changes, especially for patients who are not in critical condition.

A Snapshot of the Biochemical Markers Used in the Monitoring of COVID-19 Cases

Dr Sibtain Ahmed
Clinical Chemistry

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus Two (SARS-CoV-2) has emerged as a life-threatening global pandemic. The role of clinical laboratories begins with the etiological diagnosis based on real-time reverse transcription polymerase chain reaction and extends beyond to disease surveillance, monitoring disease severity, evaluating prognosis and therapeutic drug monitoring via numerous biochemical markers.

The table below summarizes the role of various biochemical markers alongside their interpretation and clinical significance in COVID-19 cases.

Biochemical Test	Specimen Type	Clinical Significance	Abnormality in COVID-19 Cases	Interpretation
Albumin	Serum	Useful for assessment of nutritional status and liver dysfunction	Decreased Increased	Impairment of liver function, malnourished Dehydration
Lactate Dehydrogenase	Serum	Useful for assessment of tissue damage	Increased	Indicate severe shock, and hypoxia. Widespread tissue damage particularly pulmonary injury
Alanine Aminotransferase (ALT)	Serum	Useful for assessment of liver dysfunction associated with hepatic necrosis	Increased	Destruction of hepatocytes
Aspartate aminotransferase (AST)	Serum	Useful for assessment of myocardial infarction, acute liver cell damage	Increased	Tissue damage, particularly liver injury
Creatinine	Serum	Useful for diagnosing and monitoring treatment of acute and chronic renal diseases -Estimation of Glomerular filtration rate -Adjusting dosage of renally excreted medications	Increased	Renal injury, declining renal function, therapeutic dose adjustment
Procalcitonin	Serum	Useful for diagnosing Diagnosis of bacteremia and septicemia	Increased	bacterial coinfection in those developing severe form of disease
C-reactive protein	Serum	Useful for detecting systemic inflammatory processes	Increased	Increase associated with severity of viremia and sepsis, associated with prognosis
Lactate	Plasma	Useful for monitoring of lactic acidosis	Increased	Septic Shock, associated with prognosis

AKUH Blood Bank During SARS-COV-2 Outbreak

Muhammad Hasan
Section of Hematology & Transfusion Medicine

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus recently renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is transmitted primarily via droplets, respiratory secretions and direct contact. The presence of the virus in feaces and blood suggest other probable modes of transmission. Since the start of this epidemic it had been speculated that it has the potential to reduce the supply of blood components and adversely affect blood system activities. Blood services across the world, therefore, took steps to assess, plan, and respond appropriately and proportionately. AKUH blood bank took the blow very early, when on 26th February 2020, the first patient of Pakistan was diagnosed with SARS-CoV-2 infection in Karachi, resulted in the closure of all schools in Sindh by the government. On 27th February, we had a well-planned annual blood donation camp at one of the leading schools in Karachi which got canceled at the eleventh hour because of the school closure. That camp was promising to collect the donation of at least 500 blood units.

To maintain safe and adequate blood supply during this outbreak we took the following measures:

1. Reduce the potential risk of transmission through the transfusion of blood

Transmission of a respiratory virus by transfusion is theoretically possible, but unlikely based on precedent. Any actions taken to reduce risk are therefore precautionary.
On 19th March, in the pre-donation assessment, we added the following questions to be asked from the potential blood donors:

- Within the last 14 days, travel history of the donor (traveled to Karachi from an outside country or from inside country)
- Within the last 14 days, travel history of any person who has been in close contact with the donor (traveled to Karachi from an outside country or from inside country)
- The donor has a fever within last 14 days
- Cough, cold, flu or sore throat in the last 14 days

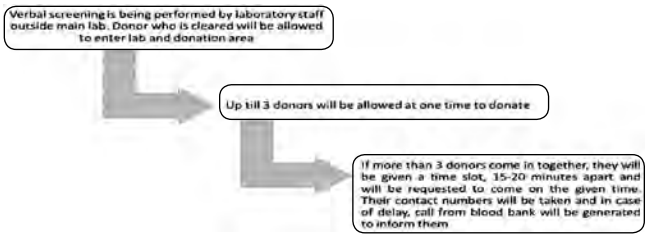
If the answer to any of these questions is YES, he is deferred from donating blood for 28 days.
Since March 2020, we have deferred a total of 335 blood donors out of which 35 donors were deferred due to the above-mentioned points.

2. Alleviate the impact of reduced availability of blood donors

The reduction of donor numbers during the outbreak is a major risk for the blood bank. So, we considered the sufficiency risk early to enable preparedness and response. Blood donation numbers were closely monitored so that measures can be taken quickly to pre-empt any decline in donor attendance. Our aim has been to balance donor deferrals for protecting the blood supply with an estimate of any negative impact on the adequacy of the blood supply. Furthermore, we conveyed our concerns about the shortage of blood supply to clinical areas and ask them to ensure exchange donation by sending blood donors to the blood bank, while arranging blood products for their patients. At the time of writing, we have been able to equilibrate the supply with demand. But as the month of Ramadan has approached we fear a decrease in the number of blood donations and working upon the strategies to overcome it.

3. Mitigate the risk of staff and donor exposure to SARS-CoV-2

Any transmission from a donor is far more likely to occur through the respiratory route than through parenteral routes (including phlebotomy during blood donation). It is possible that an infected donor who is asymptomatic, pre-symptomatic, or has very mild symptoms may infect other donors and staff.
Besides following the standard laboratory biosafety practices advised by laboratory management we adopted the following workflow for blood donation:



4. Collection of Convalescent Plasma

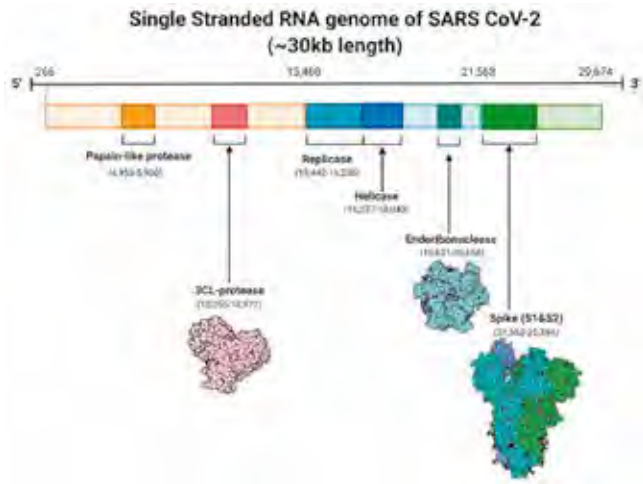
While specific new drugs and vaccines are being researched for COVID 19, certain drugs that are already present in medical arsenal are under trial too. One investigational treatment being explored for COVID-19 is the use of convalescent plasma (CP) collected from recovered COVID-19 patients. Convalescent Plasma is a source of passive immune therapy- the administration of specific antibodies against a given agent for preventing or treating an infectious disease due to that agent. Section of Hematology & transfusion medicine in collaboration with Section of Infectious disease has started an open-label clinical trial, in which we are collecting plasma from the persons (CP donors) who have been recovered from SARS-CoV-2 infection and transfusing it to the patients having active severe COVID-19, to assess the efficacy and safety of this therapy.

Significance of COVID-19 Whole Genome Sequencing

Samina Ghani
Molecular Pathology

Coronaviruses are a large family of single-stranded RNA viruses that can infect humans as well as animals. In humans, coronavirus infection is typically associated with mild to moderate respiratory illnesses such as the common cold Sometimes these viruses can mutate and jump to humans, which results in severe respiratory illness, as in the outbreak of Severe Acute Respiratory Syndrome (SARS-CoV) in 2002 and, more recently, Middle East Respiratory Syndrome (MERS-CoV) in 2012. Coronavirus disease 2019 (COVID-19) was discovered in Hubei Province, China in December 2019. Initially, the new virus was called 2019-nCoV. Subsequently, the task of experts of the International Committee on Taxonomy of Viruses (ICTV) termed it the SARS-CoV-2 virus as it is very similar to the one that caused the SARS outbreak (SARS-CoVs).

Coronavirus is an RNA virus consisting of positive-sense single-stranded RNA of approximately 27–32 kb. The genome encodes 27 proteins including an RNA-dependent RNA polymerase (RdRP) and four structural proteins. The four structural proteins include the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N).



Single-stranded RNA genome of SARS-CoV2

Routine confirmation of COVID-19 cases is based on detection of COVID-19 virus nucleic acid (RNA) by real time RT-PCR assays. A major advantage of real-time RT-PCR assays is that amplification and analysis are done simultaneously in a closed system to minimize false-positive results associated with amplification product contamination.

Sequencing

Sequencing played an important role in identifying

the novel coronavirus SARS-CoV-2. Following next-generation sequencing, where partial and full genomes were obtained, Sanger sequencing was used in the connection of viral contigs, resulting in acquisition of the sequence of full length genomes. Additionally, by analyzing these genomes and comparing to other known coronaviruses, it was determined that the virus originated in an animal reservoir that evolved from bats, with a likely intermediate animal host. Whole genome sequencing will help understand whether multiple strains of Covid-19 exist among population and dispersal patterns of mutations in various geographic areas. Data generated through WGS may predict effectiveness of the response to drugs and variation from one strain to another.

Next-generation Sequencing (NGS)

During viral pandemics, next-generation sequencing (NGS) is proven as a valuable tool for the characterization and detection of viruses in the environment, animals, and humans. The biggest challenge during any outbreak is to predict the pattern or evolution and disease spread, especially with RNA viruses such as the Covid-19, which have a very high genetic variability making it difficult to understand the transmission pattern, number of active strains and their geographical locations, or if a person is affected with multiple strains. By sequencing the entire viral genome, researchers can pinpoint the genetic changes that occur in the virus as it spreads through the population. This approach is useful to

1) Understand the transmission of the virus

Understanding changes in the genetic sequence of the viral genomes collected from different patients allows researchers to build a viral 'family tree' and contribute to efforts to monitor disease spread within and between populations over time. This can help with identification of infection 'hot spots', or of super spreaders – individuals who transmit the infection to a larger than expected number of people. This information is valuable for planning targeted public health interventions to reduce disease spread.

2) Design treatments and vaccines

Understanding the viral DNA sequence will assist researchers designing therapies and vaccines that target specific features of the virus. It will also allow better understanding of how therapy and vaccine effectiveness might change as the virus evolves.

3) Monitor viral evolution

Continually tracking the virus will alert researchers to genetic changes that might give rise to less virulent or more virulent strains. Early warning of a more virulent virus or emergence of treatment resistance will be vital to support measures to minimize disease spread and for designing new treatments and vaccines.

Convalescent Plasma Therapy for COVID-19

Dr Anila Rashid
Haematology & Transfusion Medicine

Convalescent plasma: Convalescent plasma refers to plasma from an individual who has recovered from an infection. It is a means of antibody transfer to provide passive immunity until the individual can develop an active immune response, with the hope that clinical outcomes can be improved in the recipient. Convalescent plasma is not routinely available, nor is it a licensed FDA product; instead, it has been made available for specific agents at times of disease epidemics or pandemics. During the 2019-2020 coronavirus disease (COVID-19) pandemic, community blood centers around the world are establishing programs for recovered individuals to donate convalescent plasma. Once the pandemic subsided, convalescent plasma is likely to become unavailable. Monoclonal antibodies with neutralizing potential are another approach, although this requires additional development and manufacturing steps. Convalescent plasma is generally most appropriate for individuals who are severely ill and do not have access to better alternatives.

Historical Use: Prior to SARS-CoV-2, convalescent plasma was used for treating the following severe acute respiratory syndromes

Coronaviruses and Convalescent Plasma Therapy

SARS-CoV (2003 SARS epidemic) – There was reduced mortality (on the order of 7-23 percent lower mortality). There was a reduced hospital length of stay, especially when convalescent plasma was administered within two weeks of symptom onset. No major complications were reported.

Influenza viruses and Convalescent Plasma Therapy

H1N1-pdm09 (2009 pandemic) – One study reported an 80 percent reduction in mortality, especially with early treatment. There was a reduced length of intensive care stay.

H5N1 (avian influenza; several outbreaks) – Non-significant benefits (one study).

H1N1 (1918-1919 flu epidemic) – There was reduced mortality (approximately 20 percent reduction). Convalescent blood was used rather than plasma.

Plasma Administration – Criteria for optimal preparation of plasma (or hyperimmune globulin) include

- High enough titer of neutralizing antibody to be effective
- Lack of infectious particles (either the target pathogen or others)
- Blood type compatible (ABO; Rh in women of reproductive potential)
- Evidence for efficacy and lack of harm, ideally from randomized trials or prospective studies
- Informed consent with discussion of the risk of disease and risks and benefits of therapy

Risks of exposure to plasma include infection; volume overload and febrile, allergic, anaphylactic transfusion reactions, and transfusion-related acute lung injury (TRALI).

Patient Eligibility: The use of COVID-19 convalescent plasma to treat patients includes the following criteria:

- Laboratory confirmed COVID-19
- Severe or immediately life-threatening COVID-19
  - Severe disease is defined as one or more of the following:
    - shortness of breath (dyspnea),
    - respiratory frequency ≥ 30/min,
    - blood oxygen saturation ≤ 93percent,
    - lung infiltrates > 50 percent within 24 to 48 hours
  - Life-threatening disease is defined as one or more of the following:
    - respiratory failure,
    - septic shock,
    - multiple organ dysfunction or failure

COVID-Donor Eligibility

COVID-19 convalescent plasma must only be collected from individuals who meet all routine donor eligibility requirements. Donation testing for relevant transfusion-transmitted infections must be performed and the donation must be found suitable. COVID-19 convalescent plasma is collected from individuals who meet the following qualifications:

- Evidence of COVID-19 documented by a laboratory test either by:
  1. A diagnostic test (e.g., nasopharyngeal swab) at the time of illness OR
  2. A positive serological test for SARS-CoV-2 antibodies after recovery, if prior diagnostic testing was not performed at the time COVID-19 was suspected.

- Either one of the following
  1. Complete resolution of symptoms at least 28 days prior to donation
  2. Complete resolution of symptoms at least 14 days prior to donation, AND Negative results for COVID-19 either from one or more nasopharyngeal swab specimens or by a molecular diagnostic test from blood.

- SARS-CoV-2 neutralizing antibody titers, if available
  1. Neutralizing antibody titers of at least 1:160. A titer of 1:80 may be considered acceptable if an alternative matched unit is not available.
  2. When measurement of neutralizing antibody titers is not available, consider storing a retention sample from the convalescent plasma donation for determining antibody titers at a later date.

Source: *UpToDate April 11, 2020, https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma.*

Optimizing the Availability and Rational Use of Personal Protective Equipments (PPES) Among Health Care Workers

Dr Moiz Ahmed Khan
Clinical Microbiology

Frontline health care workers involved in the screening process and providing care for COVID-19 patients are playing a pivotal role in mitigating the effects of the disease in the community. Hence, prioritizing the protection of health care workers is of utmost importance by prompt provision of all essential PPEs. Currently overwhelming demand and scarce supply of PPEs has created a serious threat to frontline workers across the globe. It has now become an essential responsibility of any institute to lay down strategies that ensure prompt and regular availability of PPEs as well their rational use. For the effective utilization required Risk assessment. Following are the generic recommendations for the usage of PPE during COVID pandemic among health care workers:

1) The type of PPE used will vary according to the setting, type of personnel, and activity (require risk assessment).
2) Those involved in direct care of COVID-19 patients should use PPE according to indications.
3) For aerosol-generating procedures and support treatments (tracheal intubation, noninvasive ventilation, tracheotomy, cardiopulmonary resuscitation, manual ventilation before intubation, and bronchoscopy) use respirators, eye protection, gloves and gowns; aprons should also be used if gowns are not fluid-resistant.

Recommended PPE during the outbreak of COVID-19 outbreak, according to the setting, personnel, and type of activity:

Setting	Target personnel or patients	Activity	Type of PPE or procedure
Screening Clinical triage for prioritization of care according to severity (e.g. Manchester classification) should be performed in separate area for individuals with symptoms and signs	Health care workers	Preliminary screening not involving direct contact·	• Maintain physical distance of at least 1 meter. • Ideally, build glass/plastic screens to create a barrier between health care workers and patients • No PPE required. • When physical distance is not feasible and yet no patient contact, use mask and eye protection.
	Patients with symptoms suggestive of COVID-19	Any	• Maintain physical distance of at least 1 meter. • Provide medical mask if tolerated by patient. • Immediately move the patient to an isolation room or separate area away from others; if this is not feasible, ensure spatial distance of at least 1 meter from other patients. • Perform hand hygiene and have the patient perform hand hygiene
	Patients without symptoms suggestive of COVID-19	Any	• No PPE required • Perform hand hygiene and have the patient perform hand hygiene
Patient room/ward	Health care workers	Providing direct care to COVID-19 patients, in the absence of aerosol-generating procedures	• Medical mask • Gown • Gloves • Eye protection (goggles or face shield) • Perform hand hygiene

Setting	Target personnel or patients	Activity	Type of PPE or procedure
	Health care workers	Providing direct care to COVID-19 patients in settings where aerosol- generating procedures are frequently in place[ii]	• Respirator N95 or FFP2 or FFP3 standard, or equivalent. • Gown • Gloves • Eye protection • Apron • Perform hand hygiene
	Cleaners	Entering the room of COVID-19 patients	• Medical mask • Gown • Heavy-duty gloves • Eye protection (if risk of splash from organic material or chemicals is anticipated) • Closed work shoes • Perform hand hygiene
	Visitors	Entering the room of a COVID-19 patient	• Maintain physical distance of at least 1 meter • Medical mask • Gown • Gloves • Perform hand hygiene
Laboratory	Lab technician	Manipulation of respiratory samples Specimen handling for molecular testing would require BSL-2 or equivalent facilities. Handling and processing of specimens from cases with suspected or confirmed COVID-19 infection that are intended for additional laboratory tests, such as hematology or blood gas analysis, should apply standard precautions	• Maintain physical distance of at least 1 metre • Medical mask • Eye protection • Gown • Gloves • Perform hand hygiene
Administrative areas	All staff, including health care workers.	Administrative tasks that do not involve contact with COVID-19 patients.	• Maintain physical distance of at least 1 metre • No PPE required • Perform hand hygiene

In the scenario of severe shortage of PPE despite application of the above-mentioned strategies, it is crucial to ensure to protect frontline health care workers. This includes urgent increased production of PPE, including, if needed, through advance market commitments, public-sector mandated scale up of production by the private sector, pursuing donation options, international solidarity through financial support of PPE purchase and distribution for the needs of the most vulnerable countries, and engaging with the general public to prevent irrational use of PPE at community level, among other strategies.

Setting	Target personnel or patients	Activity	Type of PPE or procedure
Screening/triage	Health care workers	Preliminary screening not involving direct contact	• Maintain physical distance of at least 1 metre. • Ideally, build a glass/plastic screen to create a barrier between health care workers and patients • No PPE required • When physical distance is not feasible and yet no patient contact, use mask and eye protection. • Perform hand hygiene
	Patients with symptoms suggestive of COVID-19	Any	• Maintain spatial distance of at least 1 metre. • Provide medical mask if tolerated. • Perform hand hygiene
	Patients without symptoms suggestive of COVID-19	Any	• No PPE required • Perform hand hygiene

Grand Exit of The World's Famous Pandemics

Dr Tamana Asghari and Dr Khurram Minhas
Histopathology

With the human race constantly evolving and improvising through the ages, the microorganisms did not seem to have been left behind at all. Infact, every few decades they make sure to make their presence felt (not that they need to) globally by making a magnanimous entry and a grand exit. Pandemics have been mankind's oldest enemy, the earliest reported pandemic dates back to 165 AD and was known as Antonine Plague. At a glance, all of these pandemics have maintained their individuality in terms of causative organisms, their vector, mode of transmission, signs and symptoms etc. But that one thing that has been unanimously followed by them is the mass death they caused before finally saying Ciao! Here are few of the famous pandemics and their unforgettable exits:

1. **Plague Of Justinian-** No one left to die:
With its arrival in Constantinople in 541 CE after the plague-ridden fleas hitched a ride on the black rats that snacked on the grain, this pandemic which got its name from a land conquered by Emperor Justinian over the Mediterranean Sea spreaded over Europe, Asia, North Africa and Arabia killing an estimated 30 to 50 million people, perhaps half of the world's population.
Causative organism: Yersinia pestis.
Estimated death: 30-50 million.

2. **Black Death-** The invention of Quarantine:
800 years later Yersinia pestis made an impactful comeback and this time with quite a grim name The black death, it hit Europe in 1347 and finally bid adieu in 1351 with a death of 200 million people on its name. Though it is said to have killed more people than WWII, it did brought the idea of Quarantine as a saviour. Imposed by Venetian laws as "terentino", it was first implemented on the arriving sailors for 30 days and was later extended to 40 days forced isolation.
Causative organism: Yersinia pestis.
Estimated death: 200 million.

3. **The great Plague of London-** Sealing up the sick:
The plague didn't seem to leave the British capital the easy way and it experienced around 40 outbreaks in 300 years (1348-1665) killing 20 percent of its population. As the saying goes desperate times call for desperate measures, the Englishmen started separating and isolating the sick, homes stricken by plague were marked with a bale of hay strung to a pole outside. The family members of the infected person had to carry a white pole when out in public, cats and dogs were massacred since they were believed to carry the disease and the dead were buried in mass graves. Red crosses were painted on their

doors along with a plea for forgiveness: “Lord have mercy upon us”.
Causative organism: Yersinia pestis.
Estimated death: 100,000 people in seven months.

4. **Smallpox**- The first vaccine:
Smallpox was endemic to Europe, Asia and Arabia for centuries but its arrival in 15th century with the European exploreres bought devastation to the indigenous population of US and Mexcio. 90 to 95 percent of the indigenous population of Americas were wiped out over a century and the population of Mexico went down from 11 million to one million. 18th century saw the coming of smallpox vaccine in quite a dramatic way, a British doctor Edward Jenner, after learning that the milkmaids who were infected with cowpox virus were immuned to smallpox, inoculated his gardener’s nine year old son with a milder cowpox virus before exposing him to smallpox and voila!! In 1980 WHO finally declared the complete eradication.
Estimated death: 56 million.

5. **Cholera**- A new way into public health research:
Tens of thousands became prey to Cholera in early to mid19th century in England. The then theory of it being spread by foul air prompted Dr John Snow that there was more to this rapidly fatal disease than what meets the eye. And with his integrated investigations of hospital records, morgues and precise location of outbreak, the Broad Street pump for drinking water was found to be the culprit behind the outbreak that claimed 500 lives in just over 10 days. Though cholera is still prevalent in some parts of third world countries lacking proper sanitization but the overall education of urban sanitization has been widely accepted by many.
Causative organism: Vibrio cholera.
Estimated death: Approximately one million.

COVID-19 & Risk assessment for Clinical Chemistry Procedures

Ms. Iffat Arman and Dr Hafsa Majid
Clinical Chemistry

Risk assessment is a systematic process of collecting data and evaluating the likelihood and outcomes of exposure to workplace hazard(s) and so that appropriate risk control measures can be implemented to reduce the risk to an acceptable level. It is recommended to start with performing a local risk assessment for each process step, that is, from sample collection to testing and reporting. Certain hazards will then be considered for each process step, such as aerosol exposure or eye splash during sample processing; infectious biological specimen spill or leakage that can elevate the level of risk. For each identified risk, appropriate risk control measures should be selected and implemented, to mitigate the residual risks to an acceptable level.

In the context of SARS-CoV-2 pandemic, a risk assessment exercise was done for processes and procedures that are performed in Section of Chemical Pathology. The exercise was led by the Sectional safety officer, faculty and manager. The team reviewed the laboratory processes and procedures to identify the potential hazards, followed by evaluation of the risk associated with all procedures and control measures which can be implemented to avoid risk to our staff. A risk control strategy was developed based on international guideline and strategies on biosafety and biosecurity. Once these control measures were implemented another review was carried out by the team to again assess the risk.Different types of specimen received at the Section of Chemical Pathology are shown in table 1. It is recommended that all laboratories should perform risk assessment activities to assess and mitigate the risks associated.

Table 1: Types of Specimen, High Risk Activities and Risk Mitigation Strategies (Taken from WHO-Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. Interim guidance, 19 March 2020)

Specimen Type	High Risk Activities and Concentration of virus	Risk Mitigation strategies adopted
Serum/Plasma/ Whole blood Specimen	Low viral concentration. Centrifugation is a risk for aerosol generation.	■ Within-lab movement and non-essential handling/processing minimized ■ All samples treated as potentially infectious, wear surgical mask, gloves and full sleeves gowns at all times ■ After centrifugation, wait for 10 minutes before opening the centrifuge. In case of Breakage follow the defined protocol ■ While handling breath and fecal specimen, Perform sample processing/analysis in Biosafety cabinets. ■ While collecting/processing/analyzing fecal/breath/sweat specimen: treat all samples as infectious, wear N-95 mask, goggles, double gloves and full sleeves gowns. ■ Liquid waste disposal process: use 5% Hypochlorite, to prevent splash exposure wear face shields, surgical mask, full sleeves gowns and handle carefully.
CSF Specimen	highly infectious[1]	
Urine Specimen	Not established yet[2]	
Fecal Specimen	highly infectious[2]	
Breath Specimen	highly infectious	
Sweat specimen	highly infectious	

1. *Earlier studies on COVID-19 from Wuhan China have reported cases where specific SARS-CoV-2 RNA was not detected in the nasopharyngeal swab but was detected in a CSF (https://doi.org/10.1016/.ijid.2020.03.062)*

2. *Duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is unknown. However warlier reports of COVID-19 have reported that shedding and thereby transmittance of virus is through oral–fecal route (https://www.tandfonline.com/doi/full/10.1080/222 21751.2020.1729071)*

Safety Considerations in the Laboratory Testing of Specimens Suspected or Known to Contain the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Sony Siddiqui
Molecular pathology

Emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), the World Health Organization and the Centers for Disease Control and Prevention (CDC) has released documents to provide interim biosafety guidelines for the collection, handling, and testing of clinical specimens that might contain SARS-CoV-2. WHO guidelines suggests all specimens (swabs, body fluids, blood, feces) must be considered as potentially infectious, and appropriate PPE must be donned. However Prior studies on the coronaviruses showed that a much lower concentration of the virus was noted in stool, urine, and blood so the risk to the laboratorian can be mitigate while using standard blood borne pathogen biosafety level (BSL)–2 precautions. Aerial transmission is considered the major exposure risk to the coronaviruses such as SARS-CoV-2 however currently information is still not available to define specifically the infectious dose for SARS-CoV-2. As a standard for any laboratory biosafety program, it is also important to realize that the first step to consider is to identify hazards by performing a biological risk assessment as described below:

Table 1: Simplified Biological Risk Assessment

Focus	Processes
Risk characterization	Identify hazards: Classify the potential for exposure (mode of transmission, organism concentration, virulence, potential for spill or inhalation) ,determine likelihood and consequence of a specific risk)
Risk mitigation strategies	Identify required safety practices to address the risks (PPE, engineering controls, training, operating procedures)
Workforce	Assess competency and experience of laboratory personnel Identify an appropriate training program Enroll staff in an occupational health program

The CDC suggests standard practices used in the laboratory during the manipulation of potentially infected specimens performed in a certified class II BSC within the BSL-2 laboratory, appropriate physical containment devices should be used such as centrifuge with safety buckets or sealed rotors, eye and face protection, double gloves, and fit-tested N95 respirator or surgical mask if N95 respirator is not available. These Standard precautions should be applied for aliquoting or diluting specimens, inoculating bacterial or mycological culture media, performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo, nucleic acid extraction procedures involving potentially infected specimens, and preparation of chemical or heat fixing of smears for microscopic analysis.

For virus isolation in cell culture and initial characterization of viral agents recovered in cultures of SAR2-CoV specimens, the CDC suggests that work should be performed in a BSL-III laboratory while using BSL-III practices (to include fit-tested N95 respirators or powered air-purifying respirators). In general, while working within the laboratory environment, regulatory standards require that all health care facilities provide the appropriate personal protective equipment (PPE) for the laboratorians, that personnel be instructed in the proper use of this PPE, the equipment being used in the laboratory be maintained and safe, and written procedures for the procurement, transportation, and handling of patient specimens be available. Basic Core Processes to Support Laboratory Biosafety Practices are summarized as follows:

Topic	Processes to Consider
Training	Donning and doffing of PPE, specimens collection, Packaging and shipping , waste management
Equipment	Certified BSC, Centrifuge with sealed rotor or safety cups
Inventory control	Adequate supply of specimen collection devices ,disinfection material, transport materials
Communication	Open lines with the medical care team Collaborations in place with the state/local public health laboratory

From the standpoint of the histopathology laboratory, the processing of frozen sections from a possible case of COVID-19 should be performed only in a cryostat where aerosols can be contained while wearing the appropriate PPE. Any SARS-CoV-2 specimens that have been formalin fixed and paraffin embedded, however, should be inactivated based on the previous information reported for the inactivation of SARS-CoV.

The recommendations are often fluid when responding to a new pathogen, strict adherence to the suggested safety practices may not be the best approach and should be based on the needs of the specific facility. As a part of this process, each laboratory should conduct a facility-specific risk assessment that reviews the procedures performed, identifies the hazards involved in the processes and procedures, determines the competency level of the personnel who perform the procedures, and evaluates the equipment and facility design.

Summarized from

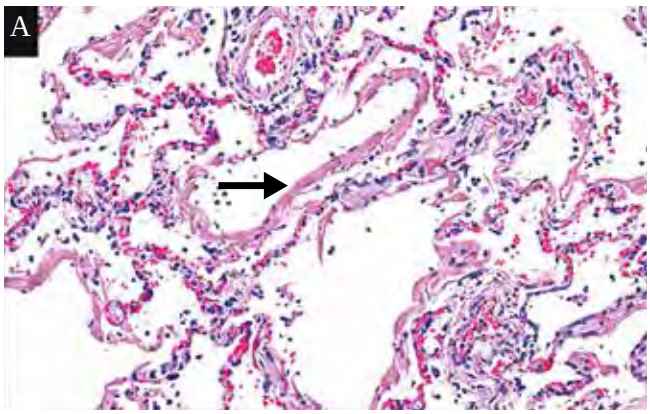
Peter C. Iwen, Karen L. Stiles, SM, Michael A. Pentella. Safety Considerations in the Laboratory Testing of Specimens Suspected or Known to Contain the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).Am J Clin Pathol 2020; XX:1–4 DOI: 10.1093/AJCP/AQAA047

Autopsy Findings in COVID-19 Deceased Patients

Dr Sahar Suleman and Dr Nasir Ud Din
Section of Histopathology

"The key question: did the patient die from COVID-19 or with COVID-19?" pointed out by Dr Sanjay Mukhopadhyay (Director, Pulmonary Pathology at Cleveland Clinic) in this recent research study. It comprised of complete, comprehensive, detailed autopsy reports of two COVID-19 deceased patients in Oklahoma, USA, it is first of its kind report published in an English-language peer-reviewed journal. Earlier Chinese literature review based on postmortem lung core biopsies, from COVID-19 patients have all described similar histopathological changes including Diffuse alveolar damage (DAD) in most , with superimposed bacterial pneumonia in one and several nonspecific changes like edema, patchy inflammation, exudates (fibrinous/proteinaceous), mononuclear inflammatory infiltrates, giant multinucleated cells etc. The objective of this study was to offer insight based on complete autopsy on underlying pathology. After proper biosafety and infection control practice Oklahoma forensic pathologists with adequate PPE conducted the autopsies.

The first decedent was a 77-year-old male known case hypertension, Deep venous thrombosis (DVT), status post total knee replacement (TKR), cholecystectomy and splenectomy, with complaints of fever and chills for 6 days. There was no travel or exposure history. He experienced shortness of breath, suffered sudden cardiac arrest during transfer to urgent care, and died soon after reaching the hospital. Postmortem nasopharyngeal swabs and lung parenchymal swabs were positive for SARS-CoV-2, however basic respiratory pathogen panel was negative. Histological findings in the lungs revealed patent airways, no mucus plugging, DAD with hyaline membrane formation and mild chronic interstitial inflammation comprising of mainly lymphocytes in airway mucosa. T-lymphocytes were positive by immunohistochemistry for CD3, CD4 and CD8. Other significant findings were coronary artery disease with no myocarditis, arterionephrosclerosis, and a right renal mass (oncocytoma).



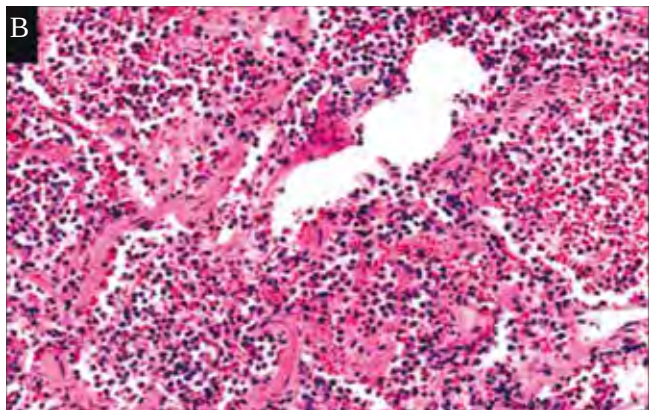


Figure 1A. Diffuse alveolar damage in the acute stage. Note hyaline membranes (arrow). **B).** Patchy interstitial chronic inflammation. This image is taken from one of the few areas where interstitial inflammation was obvious even at low magnification. In most areas, the inflammatory infiltrate was very sparse or absent.

The second decedent was a 42-year-old male known case of myotonic muscular dystrophy, status post cholecystectomy. He had a short history of fever, cough and shortness of breath, no travel or exposure history. Two days before his demise he started having abdominal pain. CT chest preceding death showed bilateral ground-glass opacities and consolidations.

However, he passed away due to cardiac arrest after few hours in hospital. Postmortem nasopharyngeal swabs were positive, and lung parenchymal swabs were negative for SARS-CoV-2. Basic respiratory pathogen panel was negative but bacterial cultures were positive for nontoxigenic Escherichia coli, Proteus mirabilis, and Candida tropicalis. Histological findings in the lungs revealed airspaces filled by mixture of neutrophils and histiocytes (acute bronchopneumonia) with rare aspirated vegetable particles. No DAD or airway mucus plugging. IHC results were same as case1 along with CD68 positive macrophages. Other findings were liver cirrhosis, gynecomastia, testicular atrophy, nephrosclerosis, mild coronary atherosclerosis no myocarditis.

The authors concluded that patients may occasionally die of other disease processes although being positive for COVID-19 as illustrated in case two; who "died of acute bacterial bronchopneumonia likely caused by aspiration, therefore, this patient likely died with COVID-19, not from COVID19". The reports also denied presence of viral myocarditis which is being currently speculated by community of physicians. They believe these autopsies has allowed adequate collection of involved tissue hence increasing accuracy of reporting, also multiple organs can be assessed simultaneously.

Donning, Doffing and Fit Testing of N95 Respirators

Dr Joveria Farooqi
Clinical Microbiology

In the advent of the COVID-19 pandemic, various biosafety precautions have been advised for healthcare workers. One of these precautions is the use of N95 respirators while working in a situation where there is a possibility of exposure to aerosols, i.e. droplets smaller than 1.0 micron in size that do not settle down within three feet of the patient. This may occur while suctioning airways, intubating patients, being in a room with an intubated patient collecting respiratory samples, or centrifuging clinical material which may contain the virus. The N95 respirators are so named because they can the filter or prevent the passage of >95 percent particles 0.3 microns or more in size.

Donning N95 Respirators

The steps of donning the N95 mask are as given in Table 1

Step 1: Check integrity of the mask.	Mask must not be cracked, wet, folded or soiled. The mask must seal well (Step 5)
Step 2: Perform hand hygiene before touching the mask. Hold the mask in the palm of your hand with the straps hanging below.	

Step 3: Position the mask in front of your face, covering nose and mouth, with straps hanging over your hand	
Step 4: Move the top strap over your head and above your ear, move the lower strap below your ear around the neck.	
Step 5: Using index and middle fingers of both hands press down on the metal bit on the bridge and mold it according to the shape of your nose and facial structure. Do not pinch the bridge as it can tent the mask and result in leaking.	
Step 6: Test the mask for leakage or seal by placing your hands around the edge and exhaling. If you feel air leak from the bridge, press down on the metal bit and retest for leakage. If air leaks from the sides, adjust the straps till a proper seal is achieved. The mask edges should be sealed to your face and no air should leak from them if the efficiency of the mask is to be ensured. **Seal test must be performed every single time the mask is worn.**	

Fit-testing

To confirm if the make of N95 is actually appropriate for you and it really is sealing well to your face, fit test may be performed. If fit-test is passed, it remains valid as long as the make of the mask does not change, and the individual does not have any great change in facial structure due to accident, surgery or significant change in weight. A fit-testing kit is required to perform a fit test.

The steps of fit-testing are given in Table 2

Step 1: The person being tested should not have ingested anything in the last 30 minutes. Sensitivity of the taste receptors of the person being tested is checked by administering a puff of the diluted reagent through a hand-held nebulizer (both provided in the kit) while wearing a hood over their head. This reagent may be sweet (saccharin) or bitter (Bittrex) in taste. The individual is asked if they can taste the reagent; if not, another puff is administered every 20 seconds till the person can taste it or 20 puffs are complete. The person is asked to remember the taste, then asked to gargle and rinse out the taste completely from their mouth. If the reagent cannot be tasted after 20 puffs, the fit test cannot be performed for this person using this reagent, it may be attempted with another reagent eliciting an alternate taste.	 *Courtesy: 3M™ FT-10 (sweet) and 3M™ FT-30 (bitter) Qualitative Fit Testing Kits. https://youtu.be/PthSES4O9d8*
Step 2: Ask the person being fit-tested to don the N95 and seal test it. Now place the hood over the person's head and administer five puffs of the undiluted fit-testing reagent and ask them to breathe normally for 30 seconds. Ask them to indicate if they can sense the taste they were sensitized with. If yes, the fit test has failed, ask them to remove mask rinse mouth repeat donning and seal test and restart from step 2. If they cannot taste it, proceed to next step.9	 *Fit testing being performed in Aga Khan University Hospital, Karachi.*
Step 3: Administer another five puffs of reagent and ask the person being tested to take deep breaths for 30 seconds. If they can still not taste the reagent, then proceed to step 3. If they can, then doff mask, rinse out taste, and repeat from step 2.	
Step 4: Administer another five puffs of reagent and ask the person being tested to move their head side to side and up and down, bend at the waist, reach up and to the side in measured movements. Continue these movements for 30 seconds. If they can still not taste the reagent, then proceed to step 5. If they can, then doff mask, rinse out taste, and repeat from step 2.	
Step 5: Administer another five puffs of reagent and ask the person being tested to talk continuously for 30 seconds. You may keep a written passage for them to read out. If they can still not taste the reagent, then fit test is passed. If they can, then doff mask, rinse out taste, and repeat from step 2.	

Doffing N95 respirators

The steps of doffing the N95 mask are as given in Table 3

Step 1: Perform hand hygiene and hold the lower strap from the back and pull it off	
Step 2: Pull the upper strap from the back and pull it over the head	
Step 3: Hold the mask from the straps and store in a paper bag labeled with your name or discard if soiled, cracked or wet. Perform hand hygiene.	

Personal Protective Equipment (PPE) for Coronavirus Disease (Covid-19)-As Recommended by World Health Organization

Dr Shayan Sirat
Radiology

In this era of COVID-19 pandemic, it has been a challenge to fight this novel virus efficaciously without compromising well-being of health professionals. The frontline healthcare providers and staff, who are in direct contact with the patient or deal with Aerosol Generating Procedures (AGP) have high risk of transmission of this infection and therefore need effective protection. Below is the summary of the rational use of personal protective equipment (PPE) for coronavirus disease (COVID-19) as proposed by WHO on 19th, March 2020 for health care and community setting. Though these are interim guidance but have been well formulated to achieve maximum protection. PPE includes gloves, medical masks, goggles or a face shield, and gowns. For specific procedures, respirators (i.e. N95 or FFP2 standard or equivalent) and aprons. (Figure 1)

Preventive Measures for COVID-19 Disease

Based on the available evidence, the COVID-19 virus is transmitted between humans through droplets and close contact. There is no airborne transmission. Personnel in close contact or caring for COVID-19 patient are at most risk of contracting infection. Preventive and attenuating measures are key. The most effective preventive measures in the community include:

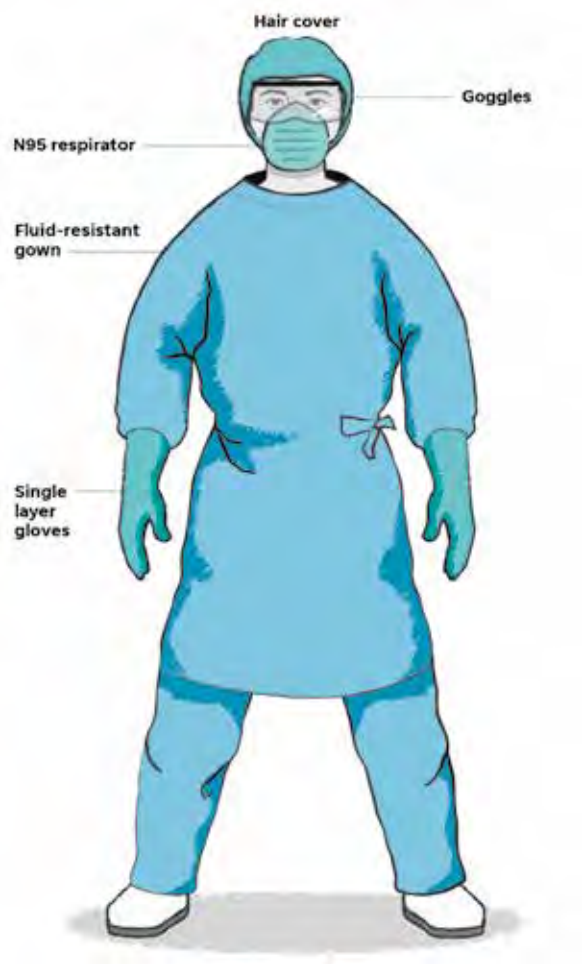


Figure 1: Personal protective equipment, https://www.usatoday.com/in-depth/news/2020/03/31/coronavirus-protection-what-health-care-workers-need-stay-safe/2917179001/

- Performing hand hygiene frequently with an alcohol-based hand rub if your hands are not visibly dirty or with soap and water if hands are dirty;
- Avoiding touching your eyes, nose, and mouth;
- Practicing respiratory hygiene by coughing or sneezing into a bent elbow or tissue and then immediately disposing of the tissue;
- Wearing a medical mask if you have respiratory symptoms and performing hand hygiene after disposing of the mask;
- Maintaining social distance (a minimum of 1 meter = 3 feet) from persons with respiratory symptoms.

Additional precautions to be taken by frontline health care workers includes using PPE appropriately; this involves selecting proper PPE and being trained in how to put on, remove, and dispose of it. Besides PPE, administrative and environmental and engineering controls, has also been described in WHO's Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. These controls are summarized here.

Administrative controls include safeguarding resources for infection prevention and control (IPC) measures, such as appropriate infrastructure, development of clear IPC policies, facilitated access to laboratory testing, appropriate triage and placement of patients, adequate staff-to-patient ratios, and training of staff.

Environmental and engineering controls aim at minimizing the spread of infection and pollution of surfaces and inanimate objects. They include providing adequate space to allow physical distance of at least one meter (three ft) to be maintained between patients and between patients and health care workers and assuring availability of well-ventilated isolation rooms for patients with suspected or confirmed COVID-19.

Recommendations for optimizing the availability of PPE. In view of the global PPE shortage, the following strategies can facilitate optimal PPE availability (Figure 2).

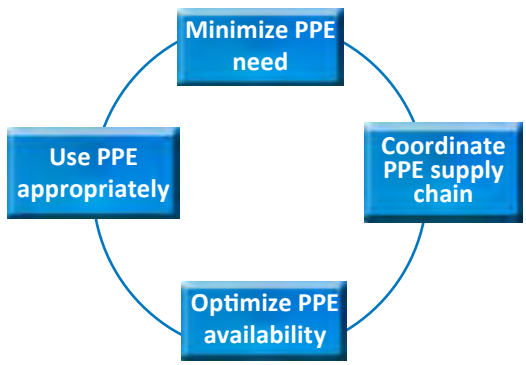


Figure 2: Strategies to optimize the availability of PPE

1. Minimize the need for PPE:
The following interventions can minimize the need for PPE while protecting health care workers and others from exposure to the COVID-19 virus in health care settings.

- Prefer using telemedicine to evaluate suspected cases of COVID-19.
- Use physical barriers such as glass or plastic windows, in areas such as triage, the registration desk and pharmacy window to reduce exposure.
- Health care workers not involved in direct care of COVID-19 patients should be restricted from entering the isolation rooms.
- To minimize the number of times a room is entered, consider bundling activities e.g. check vital signs during medication administration or have food delivered by health care workers while they are performing other care.
- Ideally, visitors shall not be allowed, but if unavoidable, restrict the number of visitors to isolation areas; reduce the amount of time visitors are allowed to spend in the area; and provide clear instructions about how to put on and remove PPE and perform hand hygiene to ensure that visitors avoid self-contamination.

2. Ensure PPE use is rational and appropriate:
PPE should be used based on the risk of exposure (e.g. type of activity) and the transmission dynamics of the pathogen (e.g. contact, droplet or aerosol). The overuse of PPE will have a further impact on supply shortages. Following recommendations, if observed, will ensure rational use of PPE.

- The type of PPE used when caring for COVID-19 patients will vary according to the setting and type of personnel and activity (Table 1).
- Health care workers involved in the direct care of patients should use the following PPE: gowns, gloves, medical mask, and eye protection (goggles or face shield).
- Specifically, for aerosol-generating procedures (e.g. tracheal intubation, non-invasive ventilation, tracheostomy, cardiopulmonary resuscitation, manual ventilation before intubation, bronchoscopy) health care workers should use respirators, eye protection, gloves and gowns; aprons should also be used if gowns are not fluid resistant.

Table 1 Recommended PPE during COVID-19 outbreak

	Health care workers -providing direct care to COVID-19 patients- medical mask, gown, gloves, eye protection (goggles or face shield)
	Health care workers - aerosol-generating procedures performed on COVID-19 patients- respirator N95 or FFP2 standard, or equivalent, gown, gloves, eye protection, apron.
	Cleaners- entering the room of COVID-19 patients- medical mask, gown, heavy duty gloves, eye protection (if risk of splash from organic material or chemicals) boots or closed work shoes.
	Visitors- entering the room of a COVID- 19 patient- medical mask, gown, gloves.)

In addition to using the appropriate PPE, frequent hand hygiene and respiratory hygiene should always be performed. PPE should be discarded in an appropriate waste container after use, and hand hygiene should be performed before putting on and after taking off PPE.

Radiology Department Preparedness for COVID-19

Dr Ismail Alvi and Prof Tanveer ul Haq
Radiology Department.

The Coronavirus Disease 2019 (COVID-19) pandemic began in December 2019 in Wuhan, China. The outbreak is due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In March 2020, the World Heath Organization declared COVID-19 as a pandemic, due to the rapid increase in the number of cases outside China.

Due to the nature of the outburst in China, Chest CT findings (e.g., peripheral ground-glass infiltrates and/or organizing pneumonia) temporarily became part of official diagnostic criteria of COVID-19 as a substitute for viral nucleic acid testing. In addition to COVID-19, Radiology departments have to cater a large number of other patients and make sure that there is no infection spread from the confines of the department, while maintaining high quality of diagnostic and interventional services.

AIMS

Radiology department, according to the Hospital infectious disease department prepared a set of policies and procedures tailored to department's needs.
The aims were

a) to reduce patient morbidity and mortality related to infection through early diagnosis and appropriate treatment
b) to prevent disease dissemination to our employees, patients and the general community.
c) to maintain radiology diagnostic and interventional services for the entirety of the hospital and health system.

Outpatient Services

With the guidelines of the infectious disease department, radiology department setup patient screening desk (Figure 1) at the entrance of department, where screening of all patients and visitors in carried out. A second layer of screening for symptoms is carried out at the radiology front desk.

Figure 1 - Screening desk at Radiology Entrance.

Patient screening is also undertaken at the time of radiology exam scheduling on phone. For elective radiology exams, suspected patients are referred to the designated hospital screening area (initially CHC) for further workup. In addition to patient screening this desk helps in enforcing the 'one patient one attendant' policy.

Furthermore, radiology department has limited the number of elective appointments to ensure that social distance can be maintained during department stay. This is being accomplished via automated patient texting services, direct calls to patients from radiology

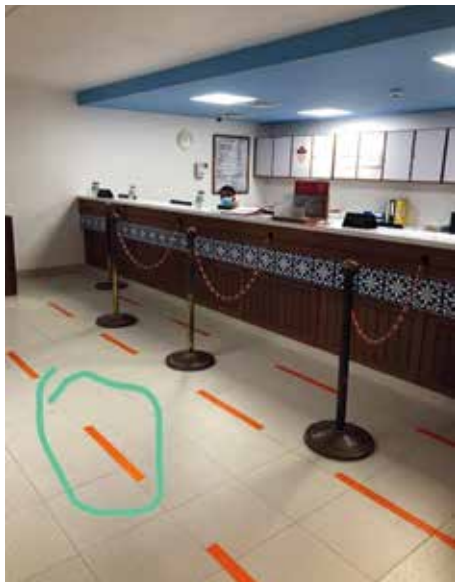


Figure 2 - Safe Distance Floor markers at Radiology Reception

appointment desk and through Radiology coordinator assistance. The seating arrangement (Figure 2) in the waiting areas have also been transformed to ensure social distancing.

Figure 3 - Waiting area with distant seating

Inpatient and ER Services

After coordination with inpatient areas, all radiology requests should mention COVID status of the patient in one of the following categories.

a) Negative for COVID-19.
b) Suspected for COVID-19.
c) Positive for COVID-19.

Separate procedure rooms in all modalities (x-rays, CT-scan, MRI) are allocated for negative and suspected/positive patients for COVID-19. Similarly, separate portable X-Ray and Ultrasound machines are designated for COVID areas and general inpatients.

Imaging of COVID-19 Positive or Suspected Patients

The decision to image patients who are COVID-19 positive or suspected is based on how the imaging will impact patient care. Currently we do not routinely use imaging for COVID-19 screening, but imaging is performed in COVID-19 positive or suspected patients, to rule out other diagnoses that can be treated, including pulmonary embolism. In addition, emergent imaging may be necessary for evaluation of other urgent conditions, including stroke, trauma, infection and other disease conditions.

Clinics and inpatient units are directed to call ahead regarding COVID-19 positive or suspected cases so that precautionary preparations to receive the patient can be made moreover we have assigned the pathway through which we transport the patient to radiology so our elective patients are not exposed with these population and GR,US, CT and MRI system to provide services for these patients.

Figure 4 - Housekeeping staff cleaning with hypochlorite solution.

Implementation of "social distancing" strategies for Staff, Trainees, and Faculty

The radiology reading room has been restructured to promote social distancing (Figure 5). A number of diagnostic workstations have been shifted to other locations (like radiology library, teaching room, etc.) so that social distancing can be practiced easily. The reading room is equipped with hand sanitizers and alcohol wipes (Figure 6) for disinfection of the computer peripherals (microphone, keyboard, mouse).

Figure 5 - Social distancing in Radiology Reading Room.

Figure 6 - Hand sanitizers and Alcohol wipes for Disinfection of Workstation peripherals.

With the aim to decrease foot traffic in radiology reading rooms' remote consultations by telephone rather than in-person is encouraged.
Recurring departmental meetings and multiple clinical conferences have been moved to video conferencing. We are exploring strategies to allow diagnostic radiologists to work from home and developing guidance for when this is appropriate.
In addition to these measures, and taking in consideration of the workload, the staff roster has been changed to allow only the essential workforce presence in the department.
In conclusion, the department's response, policies and protocols to this pandemic have been adapted up to the maximum standards achievable.

COVID-19: An Alarm Bell for Healthcare System in Pakistan!

Lessons to learn from COVID Pandemic to effectively cope with new, more severe contagious diseases in future.

Dr Mohammad Zeeshan
Microbiology

Heraclitus, a Greek philosopher once said, 'the only constant in life is change'. And how so true this statement is! We constantly discover, invent, and improve to survive and grow in this world. Interestingly, for those of us who don't improve, life creates situations and circumstances that force us to bring in the much needed change.

The COVID-19 Pandemic is one such situation for healthcare system in Pakistan. Today, the entire healthcare industry from basic healthcare units to multidisciplinary hospitals, has been given a wake-up call. Medical personnel are grappling to provide services and care to their communities, and desperately putting their lives at stake to save patients. The gaps and holes, inefficiencies and discriminations in our system have been laid bare for all. Fixing this falling system is a tall ask, and would require purity of intent and persistence; however, few aspects need to be address urgently.

Personnel and Institutional Safety

The concept of safety within our healthcare system is almost non-existent and barring few exceptions, a functional Hospital Safety Committee is an abstract notion in most health facilities. Similarly, the wellbeing of healthcare providers is a long neglected case. The attitude of both the government and hospital managements towards medical staff's safety is appallingly indifferent.

In recent COVID-19 scenario, the unprecedented demand but interrupted, inadequate supplies of PPEs (personnel protective equipment) has hit the panic button for health care systems across the entire globe. And in Pakistan, we are struggling even more. We claim our healthcare staff to be frontline troopers, and yet we fail to provide appropriate, ample safety gears for them. The staff is exposed to health threatening environment, putting their lives at jeopardy. This also

has a ripple effect; these staff become source of spread to other patients, attendants, colleagues, and even their families.

Therefore, now is the right time for all small scale healthcare centers and multidisciplinary institutes to enforce a Hospital Safety Committee. For smaller outfits, any senior doctor or head nurse with training in infectious disease and an understanding of safety regulations can be given the responsibility to oversee affairs. For hospitals, a multi-disciplinary committees should be formed that reports to the facility CEO or Administrator. This committee must preempt situations and liaise with health ministry and provincial governments to ensure availability of protective gears, testing and lifesaving equipment and medicines through local and international manufacturers. The committee should also ensure safety regulations are being fully followed vis-à-vis usage and disposal of gears and equipment, and handling and disposal of corpses in case of infectious epidemic.

Training of Healthcare Workforce

The entire healthcare workforce requires regular upgrading of skills and rigorous training in new approaches. This sadly is another neglected aspect of our system. Despite being frontline defense, our nurses and paramedics are not trained at grass root level to handle expected panic situations such as infectious disease outbreaks. Large medical facilities are better off in coping with these situations through mobilizing local resources and international health agencies. However, BHUs and smaller medical centers, which cater to the masses, struggle to serve. The government should engage large hospitals to assist in training these healthcare providers with regular training (at cost) and secondments to keep abreast. There can also be cascade training programs where senior professional from each facility can be trained as Master Trainers to disseminate learning to others.

Data Sharing

During any ongoing outbreak, epidemic or large-scale calamity, it is imperative to share reliable, real time data with national and international public health agencies. This helps in devising strategies to combat the situation, formulate or modify policies and guideline, and avoid duplication of efforts across. Therefore, our healthcare system must have a uniform, structured, user friendly database, compatible and integrated with other national and global databases. There should also be clear SOPs for sharing such data of reportable diseases with national health agencies on regular basis.

Diagnostic Facilities

A rare, inspiring medical moment during this COVID-19 Pandemic was Germany's exemplary effort of investing preemptively in laboratory diagnosis, massive testing-kit production and performing screening at mass level against modern-day Frankenstein which lead to effectively and proactively implemented policies for early controls. The survival ratio of Germans is far more encouraging contrary to the rest of Europe. For Pakistan, it is a pressing need to develop capacity for rapid procurement of reliable, authentic diagnostic kits to perform testing with recommended validation. Facilities with better infrastructure and workforce situation must facilitate resource limited setting in their capacity building.

Infection Prevention and Control Department

An active, efficient Infection Prevention and Control Department is considered a linchpin in any healthcare facility. The core responsibility of this team is to ensure spread control of infections between patients, healthcare personnel and visitors by breaking the chain of transmission through introducing and implementing stringent protocols and SOPs.

In our hospitals and clinics, the position of Infection *Preventionists* is regrettably understated. This professional has never been prioritized to be in the list of essentials by healthcare managers. Therefore, while reflecting on COVID-19 and conceiving post pandemic strategies, the roles of Infection *Preventionists* must considered critical and an empowered infection prevention team must be put in place.

- **Objectives and Responsibilities**
  The objectives and responsibilities of this department must be clearly specified with a

strong underlying principle of well-being and safety of workers, patients and visitors, and ensuring a safe premises.

- **Standard Operation Procedures**
  The department must establish clear SOPs for the Healthcare facility and workforce with additional rules and regulations for any functional teams and sub-committees.
- **Administrative Strategies**
  Implementation of SOPs has to be ensured through a dedicated and professional 'Safety Officer' and 'Infection Control Practitioner'. In case of resource limitation, a single Healthcare professional be designated instead of a team. This individual can work in close contact and under the supervision of a senior doctor.
- **Risk Assessment**
  The infection prevention and control department or individual is responsible for developing a 'risk assessment and mitigation framework' for the Healthcare facility and staff. Based on a multidisciplinary approach, the framework should define leveled scenarios from low risk to high and state procedures for key aspects such as modification in engineering systems, change in hospital traffic flow, use of PPEs, proper waste disposal, etc.

The COVID-19 Pandemic is a testing time for all. There is sickness and death; and the visible future is uncertain. The only hope in this maddening fear is having a better Healthcare system that can fight the pandemic and sustain till help arrives in from of vaccine or other treatment. Therefore, we must latch on the one opportunity this adversity offers – steer our Healthcare system in the right direction. The gaps in this system are most visible today. Let's work together – government, large hospitals and medical fraternity – to bridge the gaps and build on!

Emergency Online Undergraduate Medial Education During COVID 19

Dr Romana Idrees
Histopathology

The Covid 19 pandemic has adversely affected our lives and the scales of its impact are unprecedented in our life time. The deteriorating economic conditions lock down, and safety issues of us and the family, makes the situation quite stressful and uncertain. In view of all this we faced a challenge of continuation of educational activities for undergraduate medial students so that they continue the learning activities without risk of losing an academic year. We were well aware of certain limitations specific for our country such as lack of internet access in remote areas of the country, laptops accessibility, and trained technology savvy man power.

Detailed plans were devised for delivering content, attendance record, assessments, number of sessions per day and faculty student adaptability for the online platform. As most of the faculty was comfortable with delivering face to face sessions and had never used an online platform for teaching learning purpose, this was and added challenge. Beside this students were also thinking it as boring and far-fetched.

After exploration and thorough discussion with the IT team, we decided to use Microsoft teams as an online portal that best fit for conducting our classes. Following this, we planned a series of scheduled training sessions for faculty and administrative staff where they received orientation on how to operate MS team. Finally we conducted an orientation session with the students to inform them regarding the newly established online system for learning. Students were guided on how to use MS team. In addition, we developed video tutorials and easy to follow flow charts to be shared with all faculty members, detailing the basics of platform and delivering online sessions.

To address any issues, separate admin support

WhatsApp groups were developed for faculty and students. These served as command and response centers during the online sessions. Minor hiccups like disruptions of internet connection or login issues for the students were faced in the first orientation session. However, these issues were resolved in real time. With the commencement of online teaching we were able to complete all the essential component of the module in the form of LCF,PBL and tutorials. Few Labs were also converted into LCF.The clinical skill sessions, sign off labs and few embryology lectures will be done one students join back the campus.

Despite some challenges, we noted few advantages of this emergency shift. Faculty members who were earlier reluctant to deliver content online or to pre-record their lectures; were happily recording the lecture sessions and/ or converting them as Flipped classes. This resulted in providing students an opportunity to learn as per their convenience as they can review the lecture anytime. We have received encouraging and positive students' feedback as their classes schedule is almost the same keeping them busy. We conducted online mid module and end of the module formative exams comprising of 20-30 MCQs and two SAQs.The passing percentage was 70 percent. All students took the exam and found it useful in enhancing learning.

In addition there was an increase in student attendance and engagement. During the online lectures, students put forth numerous logical questions on the session chat which were addressed by the teachers. Therefore, the fear of lack of connects or student interaction was rebutted.

During this whole exercise we acknowledged the core values of teamwork, support from the leadership, and consistent effort by the faculty along with the eagerness to learn displayed by our very academically gifted students. These were the major driving forces which made this learning experience a great success.

Challenges and Success Factors of Going Online with the Endocrine and Reproduction Module in Undergraduate Medical Education at Aga Khan University

Dr Lena Jafri
Clinical Chemistry

Online teaching takes a lot of preparation! Once COVID 19 hit Pakistan, as Chair of Endocrine and Reproduction Module in Undergraduate Medical Education (UGME) at Aga Khan University, I was informed to shift the module to an online format just few weeks away from commencement of the module. Like everyone else we were also not prepared for COVID 19, however in order to continuity in teaching-learning a plan was devised. We knew we will be facing hiccups as we had to work with tools we were not familiar with.

Challenges Faced

- Rush to get teaching moved to an online format.
- Poor connectivity was faced by few students and faculty at times during online sessions.
- The myriad complexities involved in moving a face-to-face course into the online format.
- Training of faculty to teach online.
- Students Assessments.
- Conducting 'Flipped Class Room' in an online format. With students feedback we tried to make it case based or with quiz and guided faculty to refrain from didactic online lectures.
- How to teach clinical and communication skills?

Success Factors

- Planning in no time! Find time to plan.
- Creating a Game plan with clear final outcomes.
- Creating a project timeline.
- Clarify for your team the specific set of expectations and responsibilities associated with their roles.
- Recognize and encourage positive energizers in your organization who have interest in your module/ teaching and involve them.
- Experience of Year 2 UGME team. Gave the provision of online training to faculty on how to conduct online lectures and PBLs.
- Available assistance from Office of UGME, Aga Khan University
- Available assistance and experience of institute's IT and Academics and Blended and Digital Learning Network.
- Tech savvy students.
- Energized faculty ready to adapt to change.
- Faculty and staff adapted to cope with the situation.
- Focus on the positive and reframe obstacles into positive challenges.
- Identify strengths of your team members, and use to the benefit of the team.
- Two years' experience of running our module in blended format with handful of faculty and staff trained on virtual learning environment.
- We had Moodle Champs within our Department that we had trained in the past and were engaged.
- Express appreciation to students, faculty and staff (e.g., making appreciative comments, emails and notes a daily practice).
- Staying connected with your module team members, with students, with faculty and staff.
- Due to COVID 19 Pandemic, change was easy!

Recommendations

All involved in teaching should be prepared to teach online once in a while because after this pandemic is over who knows when a similar situation like this may arise again. Sometimes even in harsh weather issues like heavy rainfall in Karachi it might be wise and more desirable for students and faculty to stay home and teach from a distance. It may be wise for educational institutes to encourage faculty to spend some of their time teaching online to gain experience and prepare themselves. As online teaching and learning continues to grow, a growing number of faculty will be requested or even required to develop curricula and teaching in this environment. Beyond the pandemic can online teaching learning ever be as effective as the face to face traditional teaching methodology and is teaching-learning online here to stay?

Adopting Virtual Teaching During the Pandemic- An Overview of the Utility of 'Microsoft Teams' from an Educator's Perspective

Dr Sibtain Ahmed
Clinical Chemistry

Owing to the leading health crisis of the COVID-19 pandemic, most educational institutes including medical universities worldwide have shut their campuses to mitigate the spread. In response, teaching is being transformed from physical class rooms to online, on an unprecedented scale; certainly, with a hand full of trial and error and experience building for the educators, students, curriculum developers and institutional administration.

Why *'Microsoft Teams'*, for Online Teaching?

Though there are numerous online platforms available for virtual learning, Microsoft Teams offers a suite of communication tools that are user friendly and more importantly easily accessible alongside the company's Office 365 subscription for

windows at no additional charges. The application (app) can be accessed from windows, iOS and Android based platforms ranging from PCs to handheld devices with internet connectivity on the go.

How to Learn, to Use *'Microsoft Teams'*?

To begin with, it is advisable to skim through the variety of tutorials, freely available on YouTube and Microsoft 365 online help, although mastering the utility of the app comes with hands-on real-time practice, preferably with a small group initially.

What are the *Features* of the *App* in a Nutshell?

Class rooms which the app defines as 'TEAMS' can be constructed based on specific criteria. The occupancy of the team can range from the entire batch for instance for a lecture or a small group of students for problem-based learning with facilitator. A video chat with up to 250 people at once or a live presentation with up to 10,000 people can be held via the app. A specific URL or invitation is sent by a team owner, allowing the participants to connect. Certain topics for discussion can be set up within the teams by the teacher which the app terms as 'Channels'. Pupil can reply via text as well as images, GIFs. Educators can also use direct messages to pursue individual feedbacks.

Real time online classroom can be scheduled or created ad-hoc which the app terms as 'Meetings'. The participants are able to see that a meeting is currently in progress and the facilitators have the option of sharing their screens. Additionally, the app also offers a plugin for Microsoft Outlook to invite others into a Teams meeting. This feature proves to be handy in cases you want a guest lecturer or invited facilitators to join.

How to Plan a *Flipped Classroom* with the *App*?

For the Flip classrooms, the pre reading material, videos, animations etc. can be circulated within the team using the app. This option is especially helpful for those institutes which do not have prior virtual learning environment in place like MOODLE.

Is there any option for *Assessment?*

As far as assessment is concerned, the app has a 'Assignments tab' which allows teachers to distribute, provide feedback, and grade student assignments. Pre and Post Test activities for e.g. a quiz can also be assigned to students through an integration with Office Forms.

Is there option for *Recording* and online *Cloud-based Storage*?

Another substantial benefit is ease of recording lectures and presentations and sharing with the team. Also comes built in is shared storage in SharePoint Online and participants get personal storage in OneDrive, consisting of 2GB for a free account and 1TB for a fully licensed Office 365 account. This resource pack can be utilized by the students as well as the teachers from anywhere, with merely a phone and internet connectivity for future reference and learning without carrying heaps of books and notes.

Does it offer *Connectivity with other Apps* used by the Institution?

Moreover, if there are other tools that your institutions have already standardized on like ZOOM; Microsoft Teams has an option via "Apps' to connect those apps directly and then switch to that platform as per the requirement.

What are the *noteworthy advantages* of this virtual teaching platform?

Furthermore, other numerous advantages include cost saving in terms of overhead cost for setting up and attending physical classrooms both for the institution as well as the students. Space constraints specially pre-booking of rooms for classes has been overcome by the virtual podium. Disruption of educational activities owing to various unfavorable circumstances like natural calamities and low and order situations in certain locations can no-longer become a hindrance for teaching and learning activities. With this user-friendly app even for the novice users, no hardcore and time-consuming trainings are required.

Any distinct advantage for *Postgraduates*?

From a medical educator's standpoint, the undergrad medical students have dedicated and scheduled learning activities, on the contrary postgraduate trainees owing to their workplace duties suffer from time constraints and are often unable to attend physical class rooms. This is where, this app can do wonders, allowing ease of learning, live as well as remotely for the trainees as well as the 'busy clinical faculty'.

Are there any Limitations?

On the other side of the picture, there are certain limitations associated with this mode of learning. The lead is lack of adaptivity, fluctuating comfort levels and engagement of students with their online instructors or their virtual classmates. **Disruption in internet services is also a issue in certain areas often restricting the use of technology for certain users.**

Way Forward

From a broader perception, changing the mode of delivery of education, is not just a switch from one app to another, rather it's about changing behavior and habits of the learners and the facilitators, that have been ingrained over decades. Building effective online classrooms, will require self-discipline on both ends, time, experience and commitment. However, the present world situation has given us all the reasons to move online and reform our educational system for better productivity.

Reframing rare in Pakistan: An Experience of Conducting a Successful Multidisciplinary Conference in the face of COVID-19 Uncertainty

Dr Hafsa Majid
Clinical Chemistry.

A Multidisciplinary Conference on 'Newborn Screening for Rare Disorders in Pakistan' was held from March 4th to 7th 2020 at the Aga Khan University (AKU), Karachi, Pakistan. Conference was arranged by Department of Pathology and Laboratory Medicine and the Division of Women and Child Health, AKU in collaboration with Departments of Biological & biomedical Sciences and Obstetrics and Gynecology, AKU and national societies including Pakistan Society of Chemical Pathologists, Pakistan Medical Association, Pakistan Pediatric Association, Pakistan Society of Hematology and Pak IMD-Net. Rare diseases affects many aspects of an individual's life including their social, educational and employment opportunities and is an emerging global public health priority. When consider together, "rare" diseases are not so rare after all. According to an estimate around 300 million people, i.e. four percent of the world's population suffers from these conditions. Therefore public health policies at global and national level are needed to address this issue. Such a policy is becoming reality in many countries around the globe. However, non-availability of diagnostic facilities, delayed diagnosis, misdiagnosis, difficulty accessing appropriate information, difficulty accessing care, availability of experts help and poorly coordinated care are still the norm for rare disease patients and their families in Pakistan.

In this scenario, it is important for us as a nation to think that how can we form collaborations to facilitate resource development. Health care providers, and experts from the different institutes of the country participated in it. The primary

Picture 1: Picture of Conference Organizers and Speakers.

aim was to break the silos and bringing synergies between healthcare personnel's working in the field of rare diseases in Pakistan, building bridges between then, bringing them on one platform where they can come together, understand the issues of rare diseases and come up with concrete steps that can be implemented within tight timeframes after the conference. On February 1st, the programme looked fine with some 21 oral presentations, 70 posters, four pre-conference workshops, four international guests and nearly 15 national experts coming from different cities of Pakistan. We also planned four pre-conference workshops including *'Establishing Registries for Tracking Patient Outcomes', 'Genetic Counseling: How to do it right?', 'Bioinformatics hands-on on IMD genomics' and 'Essentials of establishing a newborn screening program'.* Things were looking good. Then Corona hit furiously. In Pakistan the first corona patients were reported on February 26th, 2020, before the meeting even began. We were all on tenterhooks, whether our participants and speakers, especially international speakers will be able to come because of travel restrictions. We kept in close contact with them, made back-up plans in case they were not able to come. Our administrative team played an important role in it they made sure that all the participants/speakers face no hiccups in registrations. Fortunately, the most if not all of all of the International and National speakers were able to attend the conference. And very good discussions were generated in all the workshops and conference talks, participants were enthusiastic. The workshop participants were from all over Pakistan, and overwhelmingly engaged into talks, and interactive hands-on learning activities. The participants gave a good evaluation for each workshop both formally and informally indicating that these activities helped them brainstorm ideas of common interest. It was a huge learning experience for us in terms of conducting a conference in these times of uncertainty and many of the lessons we learned can be applied to future conferences.

The Best of the Recent Past

#COVID19 # #Pathologists #Followtheirlead #changeagents

Interview of the Chair of Pathology and Laboratory Medicine, Professor Afia Zafar

Recorded by Dr Lena Jafri

1. As we navigate through the uncertain times of 'COVID19' do you feel the same level of motivation every morning while coming to AKU?

Professor Afia: Since the beginning of the lockdown in Karachi in March I have been coming to AKU everyday with the same zeal and commitment. I feel responsible and I am happy to serve my department, my institute and my community. As an infection control expert, I have also been occupied and engaged in various activities related to Covid-19 within the institute.

2. What challenges are you facing in a time like this and what were the success factors that helped you respond to these challenges?

Professor Afia: These are challenging times and we expect our staff to work in such difficult circumstances. For me trying to provide safe environment for all was one of the major concerns. The leadership my peers and colleagues have remained connected, supportive and helped me find solutions to any kind of problem during these times. Despite, the financial constraints, and uncertain future both in terms of the disease and their own future everyone in my department is still contributing for the love of humanity. Everyone at every level has been supportive and has been working for humanity and I really appreciate it.

3. Was there any 'AHA moment' at workplace during these difficult COVID times?

Professor Afia: The AHA moment for me was when we started the diagnostic testing for COVID-19 patients. With this service we were the first ones to serve the community. Another big moment was the 'Fit Test Service' by my department through which till to date more than thousand personnel have been trained which was all possible with mutual teamwork.

4. Any advice to prestige readers on how to stay positive in this dire time?

Professor Afia: Stay connected with junior staff and keep supporting each other. Do not limit your efforts to your section, department or hospital in short have a holistic approach. We as medical practitioners are trained to cope with challenges and to serve mankind in any circumstances. Our seniors in this profession have set the bar high for us to follow and we will continue to endeavor.

The Best of the Recent Past

#COVID19 # #Pathologists #Followtheirlead #changeagents

Interview of the Professor of Pathology and Laboratory Medicine, Professor Zahra Hasan

Recorded by Dr Najia Ghanchi

1. How is the typical day at work now different from the one before the COVID-19 outbreak?

Professor Zahra: For the past two months the work days have been long and engaging – it's a marathon rather than a sprint. There is an urgency in the laboratory to work through the COVID-19 testing at different levels. It took us some time to set up workflows that could be easily followed as the teams have grown. The operations of testing are running smoothly with the cooperation of all sectional faculty and technical staff who have been fantastic. Further, we are constantly trying to bring in new innovations into the testing, pooling of PCR samples in batches, validating new kits, trying out alternate specimens, each day brings new queries and due to the nature of the disease there is an urgency to deal with things as rapidly as possible.

2. What challenges did you face in establishing and leading COVID-19 testing at AKUH clinical lab and what were the success factors that helped you respond to these challenges?

Professor Zahra: The first challenge was procurement of necessary reagents when we decided to go ahead and establish a laboratory developed test (LDT) when we saw the virus spreading in and outside of Wuhan, China in January 2020. Waiting three weeks for reagents seemed like eternity. However, the reagents did come in and with the help our fantastic faculty and staff team at Molecular Pathology we were able to set up the assay. Dr. Kiran Iqbal and Dr. Najia Ghanchi have been relentlessly working on improving the testing methods and have done validations and verifications of many commercial assays for SARS-CoV-2 as we now run CE-IVD marked assays. The whole team is involved in this effort. Dr. Zeeshan, Section Head has facilitated all the necessary ordering, all of us have a rapid reporting rota. Nazneen Islam, the lab manager has done a fantastic job of coordinating staff rotas, training, coordination with purchase. A special thanks to Sohail Baloch for keeping up with all of our demands. Due to the biosafety requirements of working with SARS-Cov-2 testing collaboration with Section of Microbiology, Dr Rumina and Dr. Erum who have helped use the Biosafety Level 3 laboratory as effectively as possible. The lab management has been very supportive and it has been a multi-dimensional and multi-sectional effort to keep up the requirements for COVID 19 testing.

3. How do you feel when you see your laboratory team work tirelessly day and night responding to patients need in this time of crisis?

Professor Zahra: I feel very proud that we have been able to sustain the workflow, work ethics and do our best each day in this demanding time. I do worry about keeping them safe and feel that the PPE needs to be taken care of at all time.

4. How rewarding it is to be working behind the scenes and to tackle this pandemic head-on supporting patient who are battling with COVID-19?

Professor Zahra: I believe we are already seeing the impact that we at AKUH have had on diagnostics of COVID-19 testing at the institutional, provincial and

national levels. We are involved in guidance to other institutions for SARS-CoV-2 testing. Initially for RT-PCR and now regarding the serology based assays as well. The innovations and improvements we bring in can benefit a maximum number of people when we work cooperatively.

5. Times of crisis have historically also been opportunities for change. Are you optimistic that as we emerge from this, it could be a chance to be a health care facility a lot more equipped to face the challenges like this better than before?

Professor Zahra: Yes, indeed. The Clinical Laboratory management, Hospital management and the Dean have all been involved in making this a concerted and rapid effort. We have learned a lot the past two months and hope that it will leave us in a better place to work quickly if faced with other challenges.

6. As per daily updates, the incidence of COVID-19 is on rise. How is your team responding to meet the needs of huge number of patients coming in requiring rapid testing?

Professor Zahra: We are staying on track with our plans for testing through the day with regular cut-offs. We have involved additional staff from other sections to organize the set-up of testing. It has helped to streamline the organization of samples, preparation of batches and set up of large batches for the high-throughput testing by sending people to BSL3 in pairs. We have learned that as long as every step of the process is organized properly it is possible to scale up effectively.

7. Do you see a process map in near future that will support the extensive patient testing throughout the country?

Professor Zahra: I believe we have the organization capacity to support expanded testing throughout the country provided that bottlenecks such as space for sample organization, storage and additional equipment can be provided. We have an opportunity to impact COVID-19 diagnostics throughout Pakistan

The Best of the Recent Past

#COVID19 # #Pathologists #Followtheirlead #changeagents

Interview of the Chief Resident of Pathology and Laboratory Medicine, Dr Ruhul Quddus

Recorded by Dr Anila Rashid

1. As we navigate through the uncertain times of 'COVID19' do you feel the same level of motivation every morning while coming to AKU?

Dr. Ruhul Quddus: Yes, I do feel the same level of motivation each day. Although in the start there were uncertainties but just like any other medical professional I relied on my clinical instincts regarding the situation and continued to practice the preventive and precautionary standard practices accordingly

2. What challenges are you facing in a time like this and what were the success factors that helped you respond to these challenges?

Dr. Ruhul Quddus: The challenges that I personally and in general most of us faced in the start were: the risk of exposure to or contracting the COVID. Also, I had to come up with a plan in order to manage the situation, according to the hospital guidelines, i.e.

reducing the exposure without compromising the work and patient care. Additionally, to look after, to morally support and to counsel my colleagues/ residents/medical officers that were on frontline or those who went to isolation or quarantine were at times overwhelming.

As in every case, it wasn't a one-man effort at all. We achieved success through coordinated teamwork which in part was also contributed by the ability of everyone to listen to us and to understand us. The other half of success, obviously, is everyone staying positive dissociating all negative news/information, and sticking to the standard precautions and preventive measures.

3. Was there any 'AHA moment' at workplace during these difficult COVID times?

Dr. Ruhul Quddus: One of the big moment for me, despite all the negativity, was seeing the commitment of faculty, residents, medical officers who continued their duties and the facilitation by others staff. Some of our colleagues actually going on the frontline was a real inspiration. While others volunteered for and conducted awareness sessions and facilitated training sessions.

4. Any advice to prestige readers on how to stay positive in this dire time?

Dr. Ruhul Quddus: Always stay positive and be hopeful. Avoid anxiety-provoking news and articles on social media. Do something that brings you joy and which keeps your time and mind occupied. You can utilize your time to study, read or complete your other unfinished work and researches. Share your anxiety and fears with those colleagues who can give you positive and optimal response.

5. As an expert what changes do you propose to the institute to gear up for another such eventuality in the future?

Dr. Ruhul Quddus: In any emergent or disastrous situation, time is of the critical essence. There should be contingency plan in place all the times which can be used should the situation or need arise. It will help us saving the time and utilizing this time directly on the implementation of the plan. We should also have backup plans in case if we cannot cope with the situation or if the primary contingency plan fails. In order to contribute to general public, we should also have good social media coverage in order to reach to them. It will also help us to conduct public awareness sessions.

The Best of the Recent Past

#COVID19 # #Pathologists #Followtheirlead #changeagents

Interview of the Medical Officer of Pathology and Laboratory Medicine, Dr Aniqa Arshad Raza

Recorded by Dr Siraj Muneer

1. **As we navigate through the uncertain times of 'COVID19' do you feel the same level of motivation every morning while coming to AKU?**

Dr Aniqa: Yes, it's true that the prevailing situation due to spread of COVID-19 is unprecedented yet realizing the hopefulness and trust of the patients on us to keep our doors open even during times of pandemic, makes me feel motivated. I believe the AKU plays an important role in keeping us motivated as well, everyone here follows the safe practice recommendations, we are provided with appropriate PPEs and the trust that here at AKU staff safety comes first and foremost. I feel blessed to be working at AKU and be able to serve the humanity through this noble profession.

2. What challenges are you facing in a time like this and what were the success factors that helped

you respond to these challenges?

Dr Aniqa Raza: Direct interaction with the patients who are suffering from this lethal virus can expose even the doctors to critical hazards, especially patients who are asymptomatic. However our repeated trainings have ingrained in us to follow the safe practice guidelines and use appropriate PPEs. With the support of our head, chief resident and cooperation of my colleagues we were able to respond to all challenges with the determination to be successful in this turbulent time.

3. Was there any 'AHA moment' at workplace during these difficult COVID times?

Dr Aniqa: Yes, I'm amazed at the team spirit with which we are all enthusiastically working together to achieve a common goal of countering COVID-19 and I'm proud of being an active member. It was also an 'AHA moment' for me to be chosen to provide training of propharangeal swabs and nasopharangaeal swabs techniques to fellow doctors and technical staff to defeat this pandemic. Also safe COVID-19 testing zone for sampling of patients for screening Corona was an experience that amplified my own expertise and further enhanced my understanding about the pandemic.

4. Any advice to our prestigious readers on how to stay positive in this dire time?

Dr Aniqa: I would like to encourage my counterparts to keep serving the humanity indefatigably for the triumph of health over disease. To all those heroes I dedicate this quote, "Not all angels have wings some have stethoscopes". They are truly angels. For the public I would advise to STAY HOME STAY SAFE unless it's extremely important to go out. In that case don't forget to adopt all the precautionary measures.

5. As an expert what changes do you propose to the institute to gear up for another such eventuality in the future?

Dr Aniqa: A pandemic situation demands a rapid response and huge capacity building. To treat and accommodate maximum number of patients while all departments of the hospital should remain operational too is a mammoth task. Training of the paramedics should be multiplied further to respond to any emergency situation in a better way. The proper gear and equipment should be in functional position and in sufficient quantity to deal unforeseen circumstances of high magnitude.

Polaroid

Picture 1: Some of the Histopathologists reporting during COVID 19 pandemic days

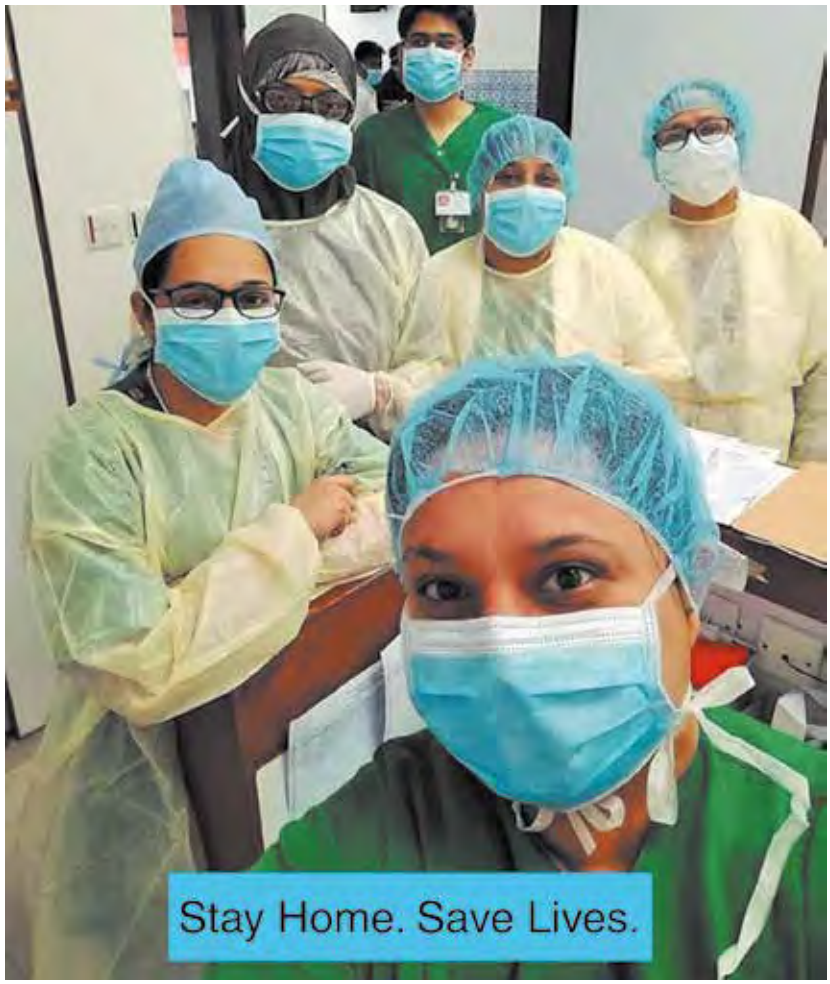
Polaroid

Picture 2: CME Webinar "Covid-19 Imaging, An Essential Tool to Diagnose & Treat" held on 19th April, 2020 Presentations, Course design and Moderation by AKU Radiology faculty (Dr Nadeem Ahmad, Dr. Kiran Hilal and Dr Muhammad Azeemuddin

Picture 3: Staff at the Section of Chemical Pathology has been working day and night to deliver accurate and timely reports. Picture shown is of COVID-19 warriors from the Section of Chemical Pathology taken in April 2020.

Polaroid

Picture 4: Multiple COVID-19 Awareness and training sessions were conducted for the staff at the Section of Chemical Pathology. Picture shown is of one such awareness session conducted on 27th February 2020 at the Section of Chemical Pathology.



Picture 5: Health care team, Ultrasound Suite-Department of Radiology during COVID times.

Polaroid

Picture 6, 7 & 8: N95 Fit testing performed by microbiology faculty and residents for health care workers of Aga Khan Hospital

Polaroid

Picture 9: Meet the Molecular Pathology Faculty who developed the COVID-19 test at Aga Khan University. Reporting first case of SARS-CoV2 from Pakistan.

Picture 10: COVID 19 warriors at section of molecular pathology, AKUH clinical laboratories working day and night performing complex critical testing and delivering quality results on time

Polaroid

Picture 11: COVID warriors working in Biosaftey level 3 (BSL-3) laboratory taking appropriate precautionary measures performing SARS CoV 2 testing according to international standards.

Polaroid

Picture 12 & 13: In service teaching and training session for health care workers regarding Infection prevention in health care setting during COVID 19 pandemic at Abbasi Shaheed hospital by Microbiology faculty and resident

Picture 14: COVID-19 Sample Collection Booth at Aga Khan University Karachi. These single modular booths provide a physical barrier separating patients and the healthcare staff.

Polaroid

Picture 15: Dr Bushra Moiz delivered talk on Inherited thrombophilia testing in a CME "Reframe Rare in Pakistan", held at AKU in March 2020

Picture 16: COVINAR, special course series with over 3500 registrations, organized jointly by DCPE and DED.

THE AGA KHAN UNIVERSITY HOSPITAL CLINICAL LABORATORIES

UPDATE
SARS-COV-2 ANTIBODY ASSAY

VOL. XXVI No. 9, 2020

Introduction:
SARS-CoV-2 is the causative agent of coronavirus disease 2019 (COVID-19) in humans. Given the acute and rapid onset of COVID-19, molecular testing of respiratory tract sample(s) to detect SARS-CoV-2 RNA remains the preferred diagnostic test for symptomatic patients. With disease progression the viral shedding decreases as the circulating antibody titers rise to detectable level. Thus, there is increasing interest for use of serologic assays to detect antibodies against SARS-CoV-2. Unlike molecular testing, **detection of an immune response to the virus is an indirect marker of infection.** Antibody tests may aid diagnosis and management of COVID-19 and provide insights into the kinetics of the immune response.

AKU clinical Microbiology section is pleased to inform the availability of new test COVID-19 antibody test which detects antibody against SARS-CoV-2.

Intended Use:
1. Clinical Diagnosis and Screening for infection:
Reports from China suggest combining PCR and serological testing significantly increased the sensitivity of COVID-19 diagnosis. The combination of antibodies plus PCR detected 98.6% of cases versus 51.9% with a single PCR. Antibody based test is particularly valuable in patients presenting in late phase of disease (day 7 onwards) and exhibit 90% sensitive after day 12 post-onset.

2. SARS-CoV-2 Seroprevalence Studies:
Antibody test is also valuable in assessing the disease prevalence in the community for seroprevalence studies at local and national level, for epidemiology and government-based surveillance agencies. Serologic testing to detect IgG-class antibodies against SARS-CoV-2 will play an essential role in determining the true prevalence of this virus in the community.

3. Plasma Therapy Donor Selection:
The reference method for detection of neutralizing antibodies, as a correlate of protective immunity, remains plaque reduction neutralization tests (PRNTs). These tests are not routinely performed in clinical laboratories as they involve live viral culture. SARS-CoV-2 serologic testing for the presence of anti-SARS-CoV-2 antibodies is currently recommended for selection of COVID-19 recovered donors1. If present, convalescent plasma from these donors may be used to treat acutely ill patients with COVID-19.

Principle:
In-vitro detection of anti-SARS-CoV-2 antibodies by:
1. Lateral flow immunoassay to detect IgM / IgG
2. Electrochemiluminiscence immunoassay to detect total antibodies (will be available soon)

Specimen collection, storage and transport:
3-5 mL blood in gel tube is required for testing. Specimens should be stored and transported at room temperature.

Rejection criteria:
Haemolysed, lipemic and icteric specimens should not be accepted because free hemoglobin, high levels of lipids and bilirubin may interfere with the test.

Schedule:
The assay will be performed daily (Monday to Sunday) and reported same day by 7:00 p.m. (cut-off time: 11 a.m.).

References:
1. Theel et al. The Role of Antibody Testing for SARS-CoV-2: Is There One? J Clin Micro Apr 2020, JCM.00797-20; DOI: 10.1128/JCM.00797-20

THE AGA KHAN UNIVERSITY HOSPITAL

CLINICAL LABORATORIES

UPDATE

Detection of Novel Coronavirus-2019 (COVID-19)

by Real Time PCR

VOL. XXVI No. 3, 2020

Introduction

Coronaviruses, named for the crown-like spikes on their surface. Around the world, most common mild to moderate respiratory coronavirus infections in humans are by 229E, NL63, OC43, and HKU1. Sometimes coronaviruses that infect animals (zoonotic viruses) can evolve and transmit to humans and become a new human strain. Two zoonotic coronaviruses, SARS-CoV (2002-2003) and MERS-CoV (since 2015), are known to cause severe illness in humans. A recent example of such emergence from Wuhan, China is the novel coronavirus, COVID-19.

Person-to-person spread of COVID-19 is via respiratory droplets produced when an infected person coughs or sneezes. The incubation period is 2-14 days. Symptoms include fever, cough and shortness of breath; suspicion increases when patient has history of travel to affected areas (especially China and other Far Eastern countries) in the last 2 weeks. Suspected cases with respiratory infection and recent travel to affected countries should be tested for COVID-19. All such patients who need hospitalization for management, should be tested at the earliest and contact isolation precautions instituted till COVID-19 infection has been ruled out.

Principle of the Assay:

Real-time (RT- PCR) assays for the *in vitro* qualitative detection of Novel Coronavirus-2019 (COVID-19) is used. Results interpretation is based on detection of fluorescent signals.

Specimen Collection:

Note: For initial diagnostics testing of COVID 19, Centers for Disease control and Prevention(CDC) and World Health Organization(WHO) recommend to collect nasopharynx and oropharynx swab in a same tube to increase the viral load.
Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts as they may inhibit PCR testing or inactivate some viruses.

Collecting the Oropharynx swab.

Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.

Collecting the Nasopharyngeal swab.

Insert swab through the nares parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient indicating contact with the nasopharynx. Gently, rub and roll the swab. Leave the swab in place for several seconds to absorb secretions before removing.

Sputum

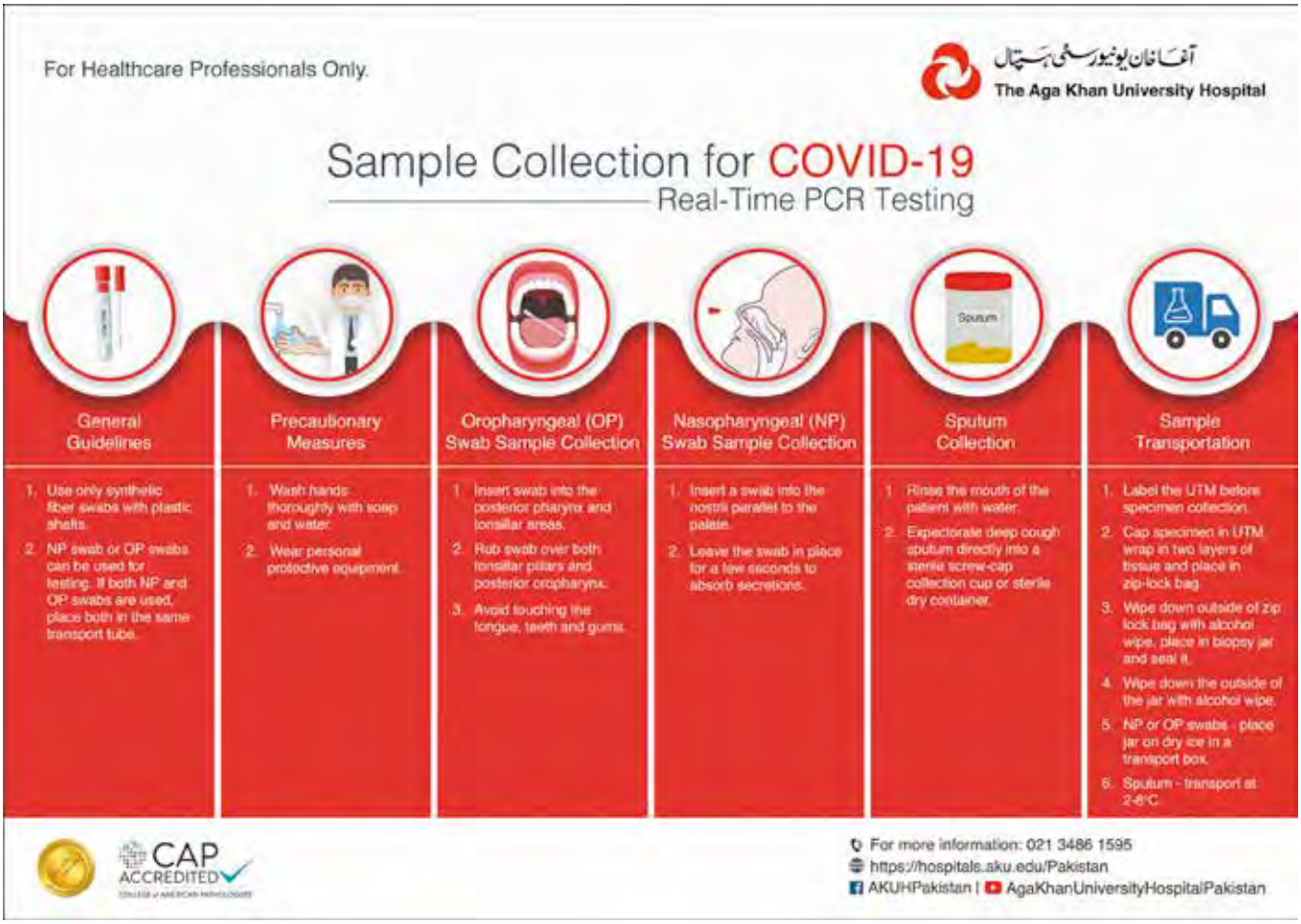
Educate the patient about the difference between sputum and oral secretions. Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap collection cup or sterile dry container

Upper respiratory specimens including nasopharyngeal or oropharyngeal swabs in a special tube containing Universal Transport Medium (available from the clinical laboratory)

Lower respiratory specimens including Broncho-alveolar Lavage (BAL), tracheal aspirates and sputum in sterile container.

Reporting Schedule:

COVID-19 virus RT-PCR is performed and reported daily if specimen is received before cut off 11:00 am (excluding Sunday)



hospitals.aku.edu/Karachi/clinical-laboratories