Script for lab diagnosis of covid-19

Slide 1

Welcome all!
Ever since being declared a pandemic on 11th March 2020, COVID-19 has spread across the globe affecting 213 countries and claiming over 600,000 lives. Knowledge of
diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are still evolving, and it is important that their understanding and interpretation is clear.

Slide 2

Here is a brief outline of the presentation, we will be touching upon the case definitions, latest guidelines for testing, different samples used and various testing modalities available

Slide 3

The novel SARS-CoV2 is a RNA virus around 200 nm in size.

[Animation 1] it binds to the ACE2 receptors through the “spike” glycoprotein, which is central to its pathogenesis.
[Animation 2] Neutrallizing antibodies are seen as early as 1 week after symptom onset, they are directed against the S (spike) and the N (nucleoprotein)

Slide 4

WHO has listed case definitions for the purpose of risk stratification of individual patients and optimal utilization and prioritization of testing resources in the setting of a pandemic.
A **suspect case** is defined as any person with fever and respiratory symptoms like cough, breathlessness, sore throat, (this symptom complex is also referred to as “Influenza like illness”) So any person with ILI AND history of travel/residence in a area where there is ongoing local transmission of COVID-19, for example any containment or hotspot areas within an incubation period i.e, 14 days of symptom onset.
Similarly if the person has had a contact with probable/confirmed case within the last 14 days or in absence of an alternate diagnosis the fully explains the clinical presentation.
Individuals fitting into these definitions are highly likely to be infected with the virus and have to be prioritised for testing and isolated in a timely manner to prevent further spread.

Slide 5

A **probable case** is one where a suspect case could not undergo testing for some reason, or the testing was inconclusive.
A **confirmed case** is one with confirmation of infection through nuclic acid detection.

Slide 6

ICMR has issued guidelines on testing patients with ILI who have in the last 14 days from symptoms onset travelled overseas, or have had contact with a confirmed case. Migrants or returnees within 7 days of symptom onset and all hospitallised patients with ILI are to be tested with a Nucleic acid amplification based test.

Assypmtomatic individuals between 5-10 days of exposure should also be tested. This period is keeping in mind the average incubation period (5 days) and maximum viral load in respiratory specimens being present in the initial days of symptom onset, hence testing during this period is likely to have a better yeild.
Severe Acute Respiraory illness, i.e, patients with fever and respiratory symptoms requiring hospitallisation should be tested regardless of history of prior exposure.

Slide 7

Upper respiratory samples like nasopharyngeal and throat swabs are the most widely used for COVID-19. Sample collection is an aerosol generating procedure and pose a potential risk to health care workers and warrants adequate PPE. Saliva is currently emerging as a substitute specimen for virus detection. Data is still emerging but it is said to be 90-95 % sensitive. Salivary glands are said to be rich in ACE2 receptors and could potentially harbor the virus during acute symptomatic phase. Sample collection is safe and cost effective as it does not require trained personnel and PPE.
Broncho-alveolar lavage is primarily used in intubated patients and have a better yield than upper respiratory tract spercimens.
Antibody testing is done in the serum,its utility and role in COVID-19 will be discussed in subsequent slides

Slide 8

Coming to the Gold standard for lab diagnosis of COVID-19, RT-PCR, which basically detects certain special sequences of RNA within the virus, by using primer targets and taqman probes. Primer sequence binds to the particular nucleotide sequence like the N gene/S gene, which is amplified and further detected by taqman probes. Since it is a novel virus with high rate of mutation more than one sequence are targeted by the primer to ensure high specificity.

Slide 9

Pooled RT-PCR tests a maximum of 5 specimens in a single session of RT-PCR, a positive result thereby warrants repeat individual testing of the samples. This can be applied in settings while testing asymptomatic individuals/ in low prevalence regions where the pre test probability is low, thereby decreasing the burden on limited testing resources.

Slide 10

CBNAAT works on similar principles of RT-PCR and is already well established for the diagnosis of tuberculosis. They are an “automated form of RT-PCR” and have a shorter turnover time of about 45 minutes. Cepheid Xpert Xpress SARS-Cov2 has been validated by a multicentric trial and was found to have comparable sensitivity and specificity as RT-PCR. ICMR considers a CBNAAT test to be as valid as RT-PCR. It be used when when results are required fast and patients are not symptomatic or mildly symptomatic: going for urgent procedures like surgery, endoscopy.

Slide 11

TruNAT is a “mini” version of the CBNAAT/GenXpert, it is portable and battery operated, with a turnover time of approximately 1-1.5 hours. These features make it ideal for testing in peripheral health care centers. It has found to have similar sensitivity, specificity to CBNAAT. A negative trunat result does require confirmation with a RT-PCR.

Slide 12

Rapid antigen testing kits have quenched the thirst for a much needed “point of care” test that is reasonably reliable. It gives a result within 15-30 minutes of sample collection. It has been validated by both ICMR and AIIMS and has been found to have a sensitivity of 60-80 % and very high specificity. Current recommendation is to test all suspect cases with rapid antigen test, and positive results are interpreted as “**true positives”** ; negative results have to be confirmed by a RT-PCR.
After sample collection,
[Animation 1]
The swab is drenched into the buffer solution, where the viral particles if present, are extracted.
{Animation 2]
The solution is then dripped onto the specimen well and [animation 3] card is read within 15-30 minutes.
This assay detects the “S protein” of the virus, which if present in the specimen will give out a [animation 4] visible readout on the card as seen here.

Slide 13

Repeat testing prior to discharge is recommended only in “severe cases” that is patients with respiratory rate >30 and saturation less than 90% at room air or ARDS or SHOCK.
Immunocompromised patients are have been shown shed viruses for a prolonged period of time, testing them negative prior to discharge is also being practriced.

Slide 14

Next, we come to the serological tests, which are ELISA based tests detecting IgM/IgG antibodies against the Nucleocapsid/Spike antigen of COVID. These tests indirectly measure the host immune response and are particularly useful in children with mild to moderate illness who usually present late. {animation 1] IgM and IgG titres have been shown to appear at similar times points, IgG lasting for a longer duration.
Serological tests seem to address the pitfalls of RT-PCR, such as dependence of the accuracy of RT-PCR on the sampling technique, lower viral load getting missed by RT-PCR and logistical issues of performing a RT-PCR in remote areas.
Recent tests have shown to have high specificity with sensitivity of 90% at the end of 1st week and 96% at two weeks.
Positive antibody tests could give a fasle belief that a person is immune to COVID-19. There is NO EVIDENCE so far of these antibodies of being protective.

Slide 15
Other non specific lab parameters seen include lymphopenia, rauised CRP,D-Dimer, fertin, fibrinogen and IL-6 levels.
Additinally MIS is characterised by elevated inflammatory markers along with raised CPK, Trop I and dilated coronary artteries with reduced ejection fraction seen on ECHO.

Slide 16

Studies have shown that the most common CT finding in children is the presence of ground glass opacities, subpleural in location and mainly involving the peripheral and posterior lungs. These GGOs are distinct from the adults, as they tend to be more localised.

Image ‘a’ shows a distinct GGO involving the posterior aspect of the right lung, which is well localised. Image ‘b’ shows a consolidation in the inferior lobe of the left lung surrounded by GGO.

Image ‘c’ is of a child with severe disease showing diffuse consolidations and GGO in both the lungs.

Slide 17

Summary

Suspect COVID in the appropriate setting

RT PCR in an appropriately collected nasopharyngeal swab is the gold standard for diagnosis

CBNAAT and TRUNAT have been validated and do not need a confirmatory RT-PCR

Antigen test is a good point of care test if available though it has limited sensitivity

A negative RT PCR does not rule out COVID-19

Antibody tests have limited role in diagnosis at this time.