COVID-19 Virology

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1. SARS-CoV-2 detection, viral load and infectivity over the course of an infection[1] Walsh et al.

This paper summarises evidence on the detection pattern and viral load of SARS-CoV-2 over the course of an infection. It is a systematic review of 113 studies conducted in 17 countries. It highlights three key points.
Firstly, the viral load of COVID peaks around symptom onset and becomes undetectable about two weeks after symptom onset. In symptomatic patients, there is reduction in infectivity 7–10 days after onset of symptoms, hence WHOs recommendation to stop discontinue transmission-based precautions 10 days after symptom onset and when symptom-free for at least three days (or 10 days after first testing positive if asymptomatic).
Second, no study definitively measured the duration of infectivity; however, patients may not be infectious for the entire duration of virus detection, as the presence of viral RNA may not represent transmissible live virus. However, the early peak of viral load in COVID-19 patients, and the detection of virus in asymptomatic and pre-symptomatic patients underlines how important ongoing widespread public health and social measures are. In particular, evidence suggests that due to the potentially high viral load in the early stages of the infection, often prior to symptom onset, contact tracing should include a period of at least 48 hours prior to symptom onset in the index case.
And third, although viral load is used as a proxy for infectivity, it doesn't necessarily mean it translates into transmissibility.

2. Airborne or droplet precautions for health workers treating COVID-19?[2] Bahl et al.

Does the 1-2m rule of separation protect health care workers? The 1-2m rule of spatial separation is based on the idea that larger droplets are unlikely to travel more than 2m, and therefore this separation reduces transmission risk. SARS-COV2 is likely spread through contact, airborne or droplet routes. This systematic review looked at the evidence supporting the 1m rule of separation suggested by the WHO for health care workers caring for suspected COVID-19 patients (alongside other precautions). They included ten papers in their review. Methodology differed widely between papers as did the definition of particle size. Eight of the papers showed more than 2m trajectory of small droplets (<60μm). Seven relying on modelling

showing this horizontal spread to be 2-8m. The approaches used for modelling have downsides and make assumptions affecting interpretation; however, most still showed transmission greater than 2m. They also discuss disagreement about the definition of droplet transmissions and the lower limit of particle size to be considered droplets (the WHO uses 5>µm as droplets and less than this is aerosol), but larger droplets can remain airborne for long enough to be aerosols and droplet sizes are dynamic, which make this particularly hard to look at. It is also affected by temperature and humidity and ventilation and therefore, location-specific. They also discuss studies that have shown aerosol particles 3 hours after procedures and hence the importance of airborne precautions. They demonstrate the difficulty in interpreting the evidence and the lack of it with regards to droplet transmission of SARS-COV2 and spatial distancing as part of public health guidance for health care workers. They highlight a need for a combination of measures to protect health care workers but that more work is needed to understand transmission and that current spatial distancing guidance for health care workers may not be appropriate and will need updating as evidence develops.

3. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis.[3] Bastos et al.

This systematic review determines the diagnostic accuracy of serological tests for COVID-19, given they could improve diagnosis and be useful tools for epidemiological surveillance. 40 studies were included. There was no current evidence base supporting the diagnostic accuracy of these tests.

It adds four keys points: Firstly, the available evidence on the accuracy of serological tests for COVID-19 is characterised by risks of bias and heterogeneity, and as such, estimates of sensitivity and specificity are unreliable and have limited generalisability. Second, the evidence is particularly weak for point-of-care serological tests. Third, caution is warranted if using serological tests for COVID-19 for clinical decision making or epidemiological surveillance. And finally, current evidence does not support the continued use of existing point-of-care tests. It highlights the importance of not wasting limited resources on serological tests when there is no evidence base for them.

4. Suboptimal Biological Sampling as a Probable Cause of False-Negative COVID-19 Diagnostic Test Results[4] Kinloch et al.

RT-PCR of nasopharyngeal swabs has been a common testing method for SARS-CoV2. It has been pretty sensitive, but varying proportions of false negatives have been reported. Although several things such as test timing and storage affect these results, Kinloch et al. looked at suboptimal sampling as a cause of these false negatives. They used ddPCR to evaluate human DNA levels on the swabs as a marker of swab quality (however this does not account for anatomical location of where the swab is taken from, but it is a good marker for the amount of material collected on the swab). They looked at 40 suspected false-negative samples and found they had significantly less human DNA levels against a convenience sample of consecutive tests submitted in the same time frame. This highlights the importance of proper sampling for

tests, and a need to ensure this is being done to an appropriate standard and suggests that a certain proportion of false negatives are due to poor sampling, and not just PCR sensitivity.

5. Nasopharyngeal Swabs Are More Sensitive Than Oropharyngeal Swabs for COVID-19 Diagnosis and Monitoring the SARS-CoV-2 Load[5] Wang et al.

This study compared Nasopharyngeal Swabs and Oropharyngeal Swabs. they found Nasopharyngeal Swabs to be far superior. Nasopharyngeal Swabs had a better detection rate than Oropharyngeal Swabs (46.7% vs 10.0%). Nasopharyngeal Swab were more sensitive than Oropharyngeal Swabs (98.3% vs 21.2%), Nasopharyngeal Swab had a longer median duration of detectable SARS-CoV-2 than Oropharyngeal Swabs (25.0 vs 20.5 days), Nasopharyngeal Swab had a lower mean cycle threshold value than Oropharyngeal Swabs (37.8 vs 39.4) indicating that the SARS-CoV-2 load was significantly higher in Nasopharyngeal Swab specimens than Oropharyngeal Swabs, and Nasopharyngeal Swab samples were less likely to make patients nauseous or vomit. If testing capacity is limited, this suggests using Nasopharyngeal Swab. The study has limitations however, most patients in the study were in recovery, the median duration since symptom onset was 27 days, so patients may have no longer been shedding virus, therefore detection rates may not accurately reflect diagnostic sensitivity.

6. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of SARS-CoV-2[6] Lau et al.

Reverse transcription polymerase chain reaction (RT-qPCR) is the recommended method for detecting SARS-CoV-2. However, it is too expensive to be widely used in many developing countries. It also requires experienced personnel, maintenance of reagents in cold storage facility, and use of a high-precision thermal cycler. Loop-mediated isothermal amplification (LAMP) is an alternative amplification method for the detection of nucleic acid. It can take less than 1 hour to perform at a constant temperature. It does not require any major equipment and is simple to perform, therefore an ideal diagnostic tool for use in areas with limited resources. In this study, to further reduce costs and enable detection by the naked eye, they used hydroxynaphthol blue dye for the colourimetric detection of the amplification reaction. Testing on 47 RNA samples, they found LAMP detected one copy/reaction of SARS-CoV-2 RNA in 30 min. Both the clinical sensitivity and specificity of this assay were 100%. The RT-LAMP showed comparable performance with RT-qPCR. Because of its simplicity and cost-effectiveness, this assay is therefore recommended for use in resource resource-limited settings. It is worth noting however that the authors were unable to determine limits of detection due to limited resources, and samples were from only one hospital in Malaysia using nasopharyngeal swabs alone.

7. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people[7] Lohse et al.

There have been a number of asymptomatic infected individuals, and therefore testing is important to help confirm infection and reduce spread. Could pooling of samples help lessen the

burden on and expand diagnostic capacity? Lohse et al. propose a testing strategy where pools of asymptomatic individual samples are tested using RT-PCR, then following the pool being positive, further testing could be initiated. They looked at a range of pool sizes and assays comparing the cycle threshold (the number of cycles needed for the fluorescent signal – from a positive PCR reaction -to accumulate and exceed the background level.) There was little difference between the assays for the envelope protein gene and spike protein gene. Pooling of up to 30 samples could accurately detect positive infections. They found using pools of 30 samples and retesting in smaller sub-pools of 10 if positive infections were identified, allowed them to test 1191 samples, using 267 tests to detect 23 infections. This method could help increase testing infrastructure as it is built and developed, or be useful if tests are in short supply and could be readily adapted to various contexts.

8. Rapid identification of SARS-CoV-2-infected patients at the emergency department using routine testing(preprint)[8] Kurstjens et al.

Can we rapidly evaluate an individual′s risk of SARS-CoV-2 infection in the Emergency Department (ED)? This study designed a score for patients entering the ED with respiratory symptoms. Scores were compared between those who tested positive and negative. 967 patients were included and the following were used to calculate the score: C-reactive protein, lactate dehydrogenase, ferritin, absolute neutrophil and lymphocyte counts), demographic data and the chest X-ray/CT result. The study found patients testing negative for SARS-CoV-2 showed a median corona-score of 3 versus 11 (scale 0-14) in patients testing positive for SARS-CoV-2 ($p<0.001$). Using cut-off values of 4 and 11 the model has a sensitivity and specificity of 96% and 95%, respectively. Given that RT-PCR testing can be time-consuming and many hospitals deal with a shortage of testing materials, this score can be used as an adjunct. It must be noted however, this algorithm performed poorly in patients with a gastro-intestinal presentation of COVID-19, but without respiratory symptoms. Therefore, it should only be used for patients at the ED with respiratory symptoms. Also, it cannot differentiate between other respiratory viruses.

9. A predictive tool for identification of SARS-CoV-2 PCR-negative emergency department patients using routine test results[9] Joshi et al.

With limited available testing, Joshi et al. looked at components of full blood counts as predictors for positive SARS-COV2 tests. They used neutrophil, lymphocyte counts and haematocrit as well as sex in their model. They validated this in their own population (33 confirmed COVID-19 and 357 negative patients) with a c-statistic of 0.78. It was able to rule out SARS-CoV-2 in 40% of patients. It had a NPV of 99 % and sensitivity of 93 %. It was also validated in cohorts across the US and South Korea and performed similarly. Whilst not perfect this decision support tools could help rule out SARS-COV2 infection and potentially allow better resource allocation of SARS-COV2 tests, using results from a more widely and less resource-constrained test, reducing the burden on testing facilities and supplies and potentially helping to better risk-stratify patients in hospital.

10. Temperature screening has negligible value for control of COVID-19[10] Mitra et al.

This Australian retrospective study looked at the incidence of fever in patients that had tested positive for SARS-COV2. Fever has been reported as a common symptom of COVID-19, and fever screening has been used in many settings to try to limit the spread. They looked at patients' temperatures at the time of their tests and at repeat testing 24 hours later. They found 16 of their 75 SARS-COV2 patients (19%) had a fever on testing and 18 (24%) 24 hours later. In this case of hospitalised patients fever itself was not very sensitive amongst their patients positive for SARS-COV2. There has been a wide range of previously reported prevalence of fever in COVID-19 patients. This population of Australian hospitalised patients may differ from the wider population in presenting symptoms as they have needed to seek medical attention and one should bear in mind Australia was an area that has had a fair amount of testing and a relatively low infection rate at the time. However, nonetheless, this suggests that temperature measurements do not provide the thorough screening method some have thought and that temperature screening may provide a false sense of security and on its own is not a sufficient screening method.

11. Augmented curation of clinical notes from a massive EHR system reveals symptoms of impending COVID-19 diagnosis[11] Wagner et al.

By analysing the clinical notes of 77,167 patients subjected to COVID-19 PCR testing, this study compared the symptoms of COVID-19-positive and negative patients for the week preceding the PCR testing date. They found COVID positive patients had the following symptoms more frequently than COVID negative patients: anosmia/dysgeusia (27.1-fold), fever/chills (2.6-fold), respiratory difficulty (2.2-fold), cough (2.2-fold), myalgia/arthralgia (2-fold), and diarrhoea(1.4-fold). The combination of cough and fever/chills has 4.2-fold more likely in COVID positive patients during the week prior to PCR testing, and along with anosmia/dysgeusia, is the earliest symptom of COVID-19. Identifying the risk of a positive diagnosis earlier is essential to mitigate the spread of the virus. Patients with these symptom risk factors could be tested earlier, undergo closer monitoring, and be adequately quarantined to not only ensure better treatment for the patient but to prevent the infection of others. Additionally, as businesses begin to reopen, understanding these risk factors will be critical in areas where comprehensive PCR testing is not possible. There is however, a bias for those who sought testing (more likely those who had severe symptoms). Also, there was limited testing availability early on in pandemic, so these patients are less likely to be included.

12. Predictors of progression from moderate to severe coronavirus disease 2019: a retrospective cohort.[12]Cheng et al.

This retrospective study from the Central Hospital of Wuhan, China looks at blood markers and how well they predict progression from moderate to severe/critical condition or death disease. They used all adults with COVID-19 of moderate severity diagnosed using quantitative RT-PCR from 1 January to 20 March 2020. Two particular findings were associated with increased OR of poor prognosis: 1. higher neutrophil count: lymphocyte count ratio on admission (OR 1.032) and

2. higher C-reactive protein on admission (OR 3.017). In limited-resource settings, simple blood tests could determine outcomes and therefore better allocation of resources. This is, however, from a single centre, and most individuals with moderate COVID-19 enrolled in this study were older and had multiple co-morbidities, so were more likely to have adverse outcomes. So the rate of disease progression in the study may not reflect the true rate.

13. Potential fecal transmission of SARS-CoV-2: Current evidence and implications for public health[13] Amirian.

Amirian looked at the evidence surrounding the faecal transmission of SARS-CoV2 and its potential implications. Evidence has suggested other coronaviruses can be shed faecally and reports have suggested SARS-CoV2 viral RNA in stool and anal swabs of COVID-19 patients. There have been reports of viable virus in patient stool samples; however, these have been from small patient samples. There is no clear evidence about the association of virus detection in stool and disease severity, symptoms or duration of illness. There have been reports of the virus in the stool of exposed asymptomatic infants and convalescent adults. However, no studies have yet been able to provide strong evidence for viable virus transmission from stool. Still, there are small reports of high viral RNA numbers and live virions in stool from a very small number of samples. Amirian suggests that in view of this public health precautions for sewage and sanitation need to be considered– also applicable to laboratory transmission or nosocomial transmission. This is of particular relevance in areas with limited access to uncontaminated drinking water. There is no data surrounding the viral load that would be needed to propagate infection in such drinking water. The CDC has suggested that chlorination is likely sufficient to inactivate the virus. Care needs to be taken with the disposal of faecal material and appropriate PPE use and hand hygiene essential when also dealing with faecal material.

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