Preanalytical issues affecting the diagnosis of COVID-19

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INTRODUCTION
Significant challenges exist to develop sensitive and specific tests to diagnose COVID-19 caused by infection with the RNA virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 1

Diagnosis of COVID-19 in the acute stage relies on detecting SARS-CoV-2 genomic RNA in naso/oropharyngeal (NOP) swabs. The swab is transferred to the laboratory in either viral or universal transport medium (VTM/UTM) and reverse transcriptase PCR (RT-PCR) performed to detect SARS-CoV-2 RNA. 1

This same algorithm is used in the diagnosis of seasonal flu caused by the RNA virus, influenza with few false-positive and false-negative results leading to specificity and sensitivity greater than 95%, respectively. 2

ISSUES WITH SARS-COV-2 RT-PCR TESTING
The false-negative rate for RT-PCR in COVID-19 has been reported to be as high as 41% 3 and there are several reports of swab negative patients, who are subsequently positive on repeat testing. 4 It is possible that the amount of virion present in NOP in COVID-19 is much less than that in influenza as sore throat is a major symptom of COVID-19. Accurate data to define a false-positive rate are unknown as a defined normal (uninfected) population has not been tested. However, in the absence of sample contamination, PCR design is such that the probability of a false-negative result is low.

The false-negative rate has important ramifications for gaining accurate clinical and epidemiological data. 3 False-negative results may lead to misdiagnoses in both patients and healthcare workers, with increased risk of infection transmission. Current guidance suggests that a single negative result is insufficient to exclude infection. 4

EXPLANATIONS FOR FALSE-NEGATIVE SARS-COV-2 RT-PCR RESULTS
Over 60% of errors occur in the preanalytical phase of any diagnostic process, with relatively few analytical and postanalytical errors. 5 RT-PCR is robust for detection of other RNA viruses making analytical errors the unlikely reason for diagnostic inaccuracies in COVID-19. We hypothesise that the high false-negative rate with SARS-CoV-2 testing may be associated with preanalytical factors.

Timing of swab
The swab should be taken at the time of symptom onset when highest viral load occurs in COVID-19. This will be logistically challenging for both patients and healthcare systems, but swabs taken at later time points may be falsely negative. 6

Swabbing practices
NOP swabs are established practice in the diagnosis of influenza infection and are performed by trained healthcare professionals, who in the UK are offered vaccination against the current strain of influenza.

UK health agencies advocate both NP and OP sampling on a single swab to test for COVID-19. Swabbing is a complex task requiring training and competency assessment. This will be associated with anxiety due to the uncertain immune status of the healthcare professional in the context of a potentially lethal infection. These factors coupled with a lack of a standard swabbing practice may contribute to the high false-negative rate. 4


RNA instability and laboratory practices
RNA integrity may be adversely affected by both ubiquitous RNases and hydrolysis (water is a constituent of VTM and UTM). Preanalytical inconsistencies have been demonstrated to reduce the stability of extracted viral RNA. 7

RNA instability may be an important cause of false-negative results in COVID-19 compounded by low viral load. Delays from time of sampling to extraction due to transport or processing issues may exacerbate false negativity. 8

Incorporation of RNA stabilisation medium to the swab tube should allow for samples to be batch tested, while preserving RNA integrity: addition of RNA stabilisation reagent has been validated in a multicentre study and been shown to improve yield, integrity and reduced assay failure rates of RT-PCR for external quality assurance schemes. 9

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The approach could be particularly important when countries move to the next phase of pandemic mitigation with the start of community testing to identify COVID-19.

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