Impact of false-positives and false-negatives in the UK's COVID-19 RT-PCR testing programme

Carl Mayers & Kate Baker, 3rd June 2020

Background

The UK's COVID-19 testing programme uses a network of laboratories to detect SARS-CoV-2 in nasopharyngeal swabs. Pillar 1 (those with a clinical need, critical essential workers in the NHS) pillar 2 (essential workers, wider public through NHS portal, care home staff and residents regardless of symptoms) and pillar 4 (national surveillance, such as ONS surveys) of the UK testing programme use reverse-transcription polymerase chain reaction (RT-PCR) tests to detect viral RNA. These RT-PCR tests are carried out across a network of government, commercial and academic labs across the UK to meet the high demand and fast turnaround required.

RT-PCR tests are highly sensitive, but can show false negatives (giving a negative result for a person infected with COVID-19) and false positives (giving a positive result for a person not infected with COVID-19). The RT-PCR assays used for the UK's COVID-19 testing programme have been verified by PHE, and show over 95% sensitivity and specificity. This means that under laboratory conditions, these RT-PCR tests should never show more than 5% false positives or 5% false negatives.

It is important to remember that laboratory testing verifies the analytical sensitivity and analytical specificity of the RT-PCR tests. They represent idealised testing. In a clinical or community setting there may be inefficient sampling, lab contamination, sample degradation or other sources of error that will lead to increased numbers of false positives or false negatives. The diagnostic sensitivity and diagnostic specificity of a test can only be measured in operational conditions.

Operational false-positives and false-negatives will have significant impact in the way we respond to the COVID-19 pandemic. They will affect national surveillance, and the functioning of the UK track-and-trace programme. We have been unable to find any data on the operational false positive and false negative rates in the UK COVID-19 RT-PCR testing programme. This short paper suggests this must be measured as a priority, and makes recommendations on managing operational false positive and false negative rates.

What causes false positives?

- **Cross reactions with other genetic material.** Other sources of DNA or RNA may have cross reactive genetic material that can be amplified by the RT-PCR test. False positives were observed unexpectedly in norovirus assays in patients with enterocolitis, due to unusually high levels of human DNA in samples [1]
- **Contamination during sampling**. This may happen if the swab head accidently contacts, or is placed on a contaminated surface (e.g. latex gloves, hospital surface).
- **Contamination during swab extraction.** Viral RNA is extracted from swabs in solution; accidental aerosolization of liquid can cause cross contamination between samples.
- **Contamination with PCR amplicon.** The PCR amplification process generates millions of copies of the DNA target (amplicon) that can cause false positives in subsequent PCR reactions. If a testing lab is accidently contaminated with amplicon it can lead to sporadic false positives.
- **Contamination of PCR laboratory consumables.** Contamination can spread from a post-PCR lab into a pre-PCR lab by transfer of equipment, chemicals, people or aerosol. Even experienced national labs can be affected. In early-March 2020, COVID-19 RT-PCR assays produced by the CDC were withdrawn after many showed false positives due to contaminated reagents. [2]

What is the UK operational false positive rate?

The UK operational false positive rate is unknown. There are no published studies on the operational false positive rate of any national COVID-19 testing programme.

An attempt has been made to estimate the likely false-positive rate of national COVID-19 testing programmes by examining data from published external quality assessments (EQAs) for RT-PCR assays for other RNA viruses carried out between 2004-2019 [7]. Results of 43 EQAs were examined, giving a median false positive rate of 2.3% (interquartile range 0.8-4.0%).

Why are false positives a problem?

DHSC figures [3] show that 100,664 tests were carried out on 31 May 2020 (Pillar 1 and 2 RT-PCR tests). 1,570 of those tests were positive for SARS-CoV-2 (1.6%). The majority of people tested on that day did not have SARS-CoV-2 (98.4% of tests are negative). When only a small proportion of people being tested have the virus, the operational false positive rate becomes very important. Clearly the false positive rate cannot exceed 1.6% on that day, and is likely to be much lower. If the operational false positive rate was 0.4%, 400 of the 1,570 positive tests would be false positives. That would represent 400 people being isolated when they are well, and much wasted effort in contact tracing. It is possible that a proportion of infections that we currently view as asymptomatic may in fact be due to these false positives.

Unless we understand the operational false positive rate of the UK's RT-PCR testing system we risk overestimating the COVID-19 incidence, the demand on track and trace, and the extent of asymptomatic infection.

What causes false negatives?

- **Poor sampling technique.** Nasopharyngeal sampling is invasive and can feel unpleasant. It may be less effective when carried out unsupervised, so the false negative rate may increase as sampling at home becomes more common.
- **Sample degradation.** Samples may degrade when stored or while being transported.
- **Sampling too early.** Viral shedding from individuals peaks just before, or at the onset of symptoms [4,5]. If samples are taken early in infection (1-4 days after infection) they have an increased false negative rate.
- **Sampling too late.** Viral shedding declines after symptoms have peaked [6]. Samples taken at this stage of infection will show an increased false negative rate.

What is the UK operational false negative rate?

The UK operational false negative rate is unknown.

A recent study [6] combined results from seven studies (>1300 swab test results associated with time of disease onset) to create a model of the false negative rate for SARS-CoV-2 RT-PCR assays against time since infection. Their model suggested that in the first four days of infection (pre-symptomatic phase) the probability of a false negative in an infected person decreased from 100% on day 1 (i.e. a false negative was certain) to 67% on day 4. It then decreased to 38% on day 5 (day of symptom onset) to a minimum of 20% on day 8 of infection (i.e. one in five people still give a false negative result despite having experienced three days of COVID-19 symptoms). The false negative rate then increased from day 9 (21%) to day 21 (66%). Point estimates and confidence intervals are shown in Figure 1 [taken from 6].

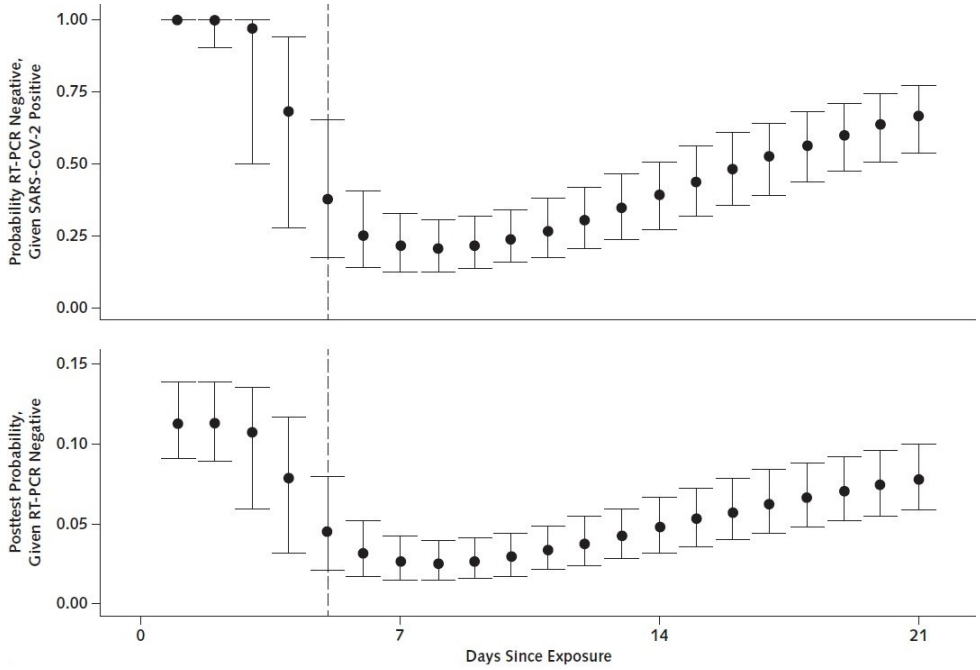

Figure 1. RT-PCR false negatives by days since exposure [From 6]. The upper chart shows the probability (out of 1, vertical axis) against days since infection/exposure (horizontal axis). The dashed vertical line at day 5 indicates the typical time of symptom onset. For each day, an estimate (solid dot) and 95% confidence interval (error bars) are shown. The upper chart shows the probability that a COVID19-infected individual would test negative by RT-PCR when sampled on that day. The lower chart is an extra analysis showing the probability that an individual who had an absolute chance of 11% of being infected (e.g. lives in the household of a COVID19 patient) would be infected, given that they have tested negative by RT-PCR. This indicates that the chance of confirming a lack of infection is maximised at days 5 – 7 post contact, and that there is less value in testing before then.

Why are false negatives a problem?

The false negative rate changes over the course of infection, and this will be further reduced by poor sampling technique and sample degradation. This will lead to an underestimate of incidence. False negatives will also allow an asymptomatic or paucisymptomatic patient to be released from quarantine to infect other people and propagate the epidemic. They represent a missed opportunity

for control in the test and trace programme, and would remain as a source of infection in a care home or hospital. The impact of false negatives is greatest when the absolute risk of infection is high (e.g. close contact with a known case) and where identifying and isolating infectious individuals is critical, especially where negative tests will be used as a 'release' mechanism (e.g. returning to work, entering social care from hospital, release from quarantine).

Recommendations

1) An External Quality Assessment (EQA) must be carried out for the UK National COVID-19 RT-PCR testing programme. This would introduce known positive and known negative samples into the testing programme. Samples would be submitted blindly, to ensure they follow an identical process as operational samples. This would provide a national estimate for the operational false positive and false negative rates. This could be carried out quickly, and at relatively low cost.

2) A continual rolling EQA (a low volume of blind samples submitted every day) should be used to monitor performance of labs across the UK COVID-19 RT-PCR testing network. Labs with higher false positive and false negative rates would be alerted, and could improve their performance.

3) Negative testing as a release mechanism (e.g. return to work, discharge from healthcare or isolation) should be used with extreme caution. Current ECDC guidance for high risk contacts is to quarantine for 14 days where possible, even when a contact has tested negative [9]

4) Where negative RT-PCR testing is used as a release mechanism, two negative tests, 24 hours apart, should be evaluated to reduce the risk from false negative tests. This approach is taken in China, Italy, and Singapore, and is recommended by the CDC [10] and ECDC [11].

5) COG-UK should continue to monitor the genome of SARS-CoV-2 lineages to monitor mutations that occur around PCR primer binding sites that could lead to false negative tests in future.

6) Studies evaluating the benefits of RT-PCR testing of case contacts within the track and trace programme should consider false negative and false positive rates. There will be much less value in testing before day 5 after contact, due to the high false negative rates early in infection.

7) Methods of sampling should be clearly communicated to home samplers. Sampling recommendations were recently (22 May 2020) updated by the CDC [12] and are reviewed by NERVTAG.

8) Additional diagnostic methods could be considered to compensate for the limited window of reliable performance of RT-PCR for SARS-CoV-2. Early antibody testing has the potential to add benefit. PHE recently validated a commercial antibody assay that appears to work in the early stages of infection [8]

References

1. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0109876
2. https://www.washingtonpost.com/investigations/contamination-at-cdc-lab-delayed-rollout-of-coronavirus-tests/2020/04/18/fd7d3824-7139-11ea-aa80-c2470c6b2034_story.html
3. https://www.gov.uk/guidance/coronavirus-covid-19-information-for-the-public
4. Temporal transmission dynamics in viral shedding and transmissibility of COVID-19 , now published in Nature Medicine
5. Virological assessment of hospitalised patients with COVID-19 Nature
6. Variation in False Negative Rate of RT-PCR Based SARS-CoV-2 Tests by Time Since Exposure Annals in Internal Medicine
7. https://www.medrxiv.org/content/10.1101/2020.04.26.20080911v2
8. https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays
9. https://www.ecdc.europa.eu/sites/default/files/documents/Public-health-management-persons-contact-novel-coronavirus-cases-2020-03-31.pdf
10. https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html
11. http://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-Discharge-criteria.pdf
12. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html