Confirmatory testing with a second lateral flow test may mitigate false positives at low levels of SARS-CoV-2 prevalence in English schools

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Summary

- There is currently concern over the possibility of false-positive lateral flow test (LFT) results in the mass asymptomatic testing programme in English schools, with calls for positive LFTs to be confirmed by Polymerase Chain Reaction (PCR) tests.
- However, delays in isolating cases and their contacts due to PCR test delays may lead to increased transmission risk and should be avoided unless strictly necessary.
- Here we show that, at current levels of prevalence in schoolchildren (~0.43%), the chance of a positive test being a true positive (Positive Predictive Value, PPV) is high (88%) and prevalence would need to decrease to below 0.05% in order for the number of false-positive test results to outnumber true positives.
- Were prevalence to decrease below 0.05%, a confirmatory LFT would increase PPV to >99.97%, similar to that of a confirmatory PCR (>99.99%).
- Following up an initial positive LFT with a second LFT provides a high PPV, minimises disruption, and enables faster case isolation and contact tracing than a confirmatory PCR test.
- This analysis makes the assumption that LFT test results are independent of each other, which may overestimate the joint specificity and underestimate the joint sensitivity.

The current policy in English schools is to perform two lateral flow tests (LFTs) per week on each student and member of staff. LFTs return results rapidly (<30 minutes) and have high sensitivity to detect infectious virus (2), allowing for the rapid isolation of cases and their contacts before they cause additional onwards transmission. However, despite the high reported specificity of the test (false positive rate: 3 in 10,000 (3,4); approximately 1 false-positive per class of 30 per year), the proportion of all positive tests which are true (the positive predictive value) may be exceeded by the number of false positives if the prevalence drops to low enough levels. This may cause unnecessary disruption, as a false positive still entails the isolation of a suspected case, their household, and the school bubble.

This disruption can be mitigated somewhat by confirming positive LFT results with a more sensitive and specific polymerase chain reaction (PCR) test. However, acquiring a confirmatory PCR test result may take several days, and if the isolation of a case's

contacts is delayed by the PCR result, this could negate the primary benefit of rapid testing - speed - in reducing onwards transmission (1,5). Here, we investigate the utility of confirmatory testing with either PCR, or alternatively, another LFT taken shortly afterwards, for a range of prevalences. The small delay in getting LFT results means that the two LFTs could be taken minutes or hours apart and be used as part of a testing process that maintains high positive and negative (the probability that you are really uninfected given that you have returned a negative test) predictive value.

Using reported test sensitivities and specificities (Table 1), and varying the prevalence, we estimated the positive and negative predictive values of the following testing strategies: a single (Innova) LFT, LFT + confirmatory LFT, and LFT + confirmatory PCR.

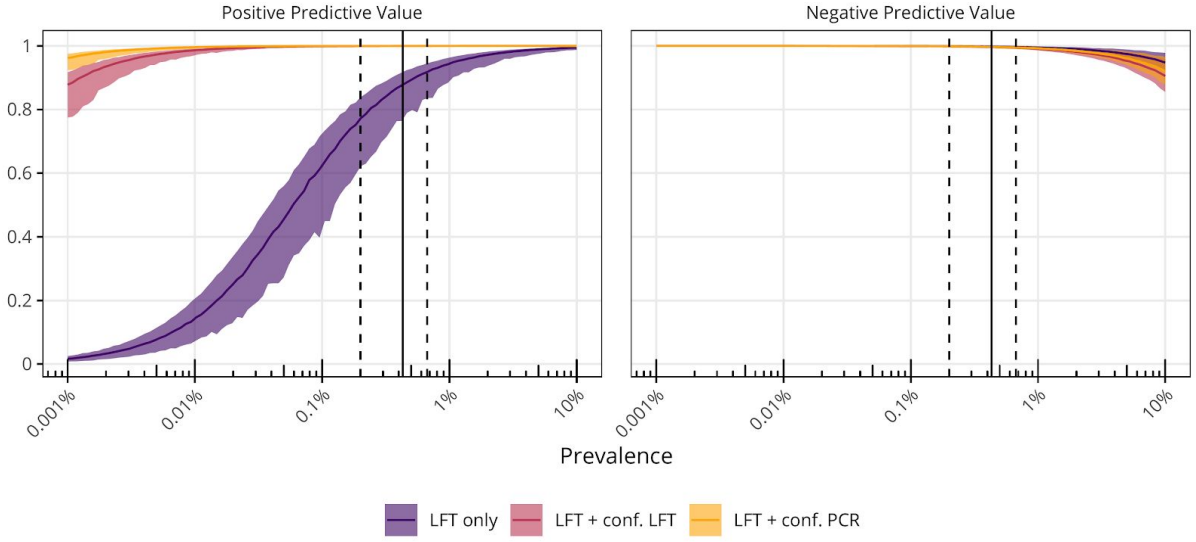

Figure 1: Positive and Negative Predictive Value for different testing strategies at varying levels of prevalence. Coloured lines and bands indicate the median and 95% CI for PPV and NPV for sampled values of prevalence, assuming the same coefficient of variation as current prevalence estimates. Solid and dashed vertical lines are approximate median and 95% CIs of current SARS-CoV-2 prevalence in school children (6). A version with a logit scale on the vertical axis may be found in the Appendix.

We found that the PPV of a single LFT test will decline as the true prevalence of SARS-CoV-2 infection in school-aged children decreases (Figure 1, left). However, current estimates of prevalence (6) put it between 1 in 500 and 1 in 150, which results in a PPV of 88% (95% CI: 78%, 92%) (Figure 1, left).

Confirming positive LFT results with a PCR test increases the PPV to nearly 100% for all but the very lowest prevalence levels (Figure 1, left). However, we estimate that much of this benefit could also be obtained by confirming a positive LFT test result with another LFT. Therefore, the confirmatory LFT strategy has the potential to keep the proportion

of false-positive tests low while providing a faster turnaround than the confirmatory PCR strategy.

This analysis makes a number of assumptions. We assume that false positives in LFTs are the result of random, not systematic error, as there is no evidence currently to suggest that false positives are influenced by any specific factor. We use the value of sensitivity of LFTs vs. PCR positives reported in the Liverpool Community Testing pilot (7), where PCR positives may be considered "infected" individuals. However, individuals testing positive by PCR with viral loads >25 CT are unlikely to yield infectious virus (2). This, consequently, underestimates the ability of LFTs to detect currently infectious individuals, and recent studies have shown that LFTs have an estimated mean sensitivity of 97.7% against culturable virus (2). We also conservatively assume that the sensitivity of follow-up tests is independent of the first; as the primary factor influencing the sensitivity of LFTs is an individual's viral load (2), this likely underestimates the sensitivity.

The R code for this analysis can be found on cmmid.github.io.

Table 1: Parameter values. * vs infection. Estimates against viral culture (i.e, infectiousness) are substantially higher (97.7%) (2).

Assay	**Sensitivity***	**Specificity**
LFT	53.4% (7) × 94.4% = 50.4%	99.97% (3)
PCR	94.4% (3)	99.99% (assumed)

References

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Appendix

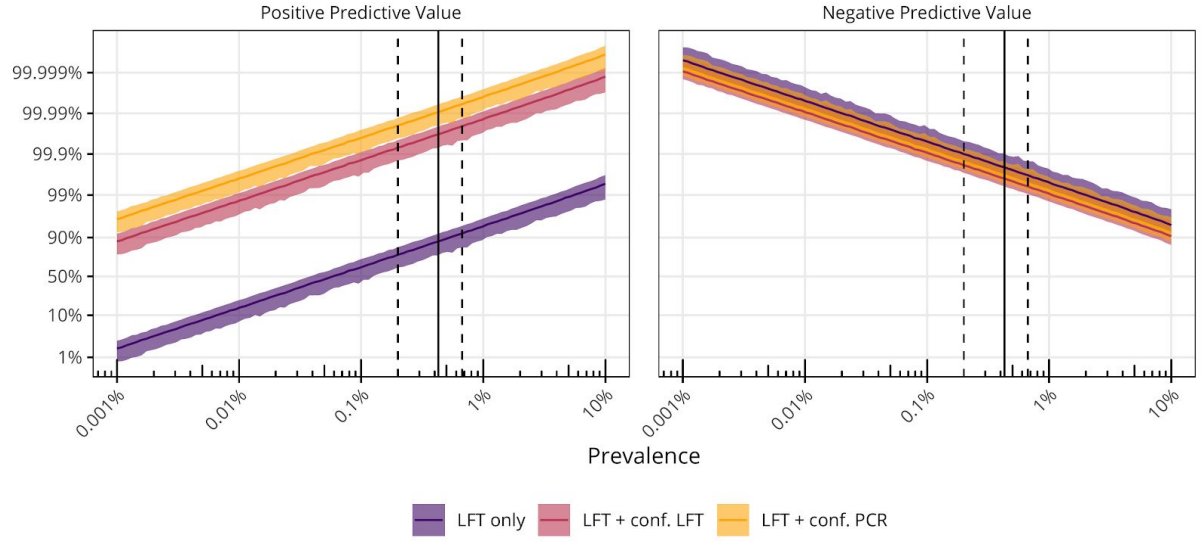

Figure S1: As in Figure 1 but with a logit scale on the vertical axis.