Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Improved detection of Group A *streptococcus* during thermal contrast amplification vs. visual reading of clinical rapid diagnostic tests

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The file includes the following sections:

A. Examples of GAS RDTs (LFA) detection cases in the study

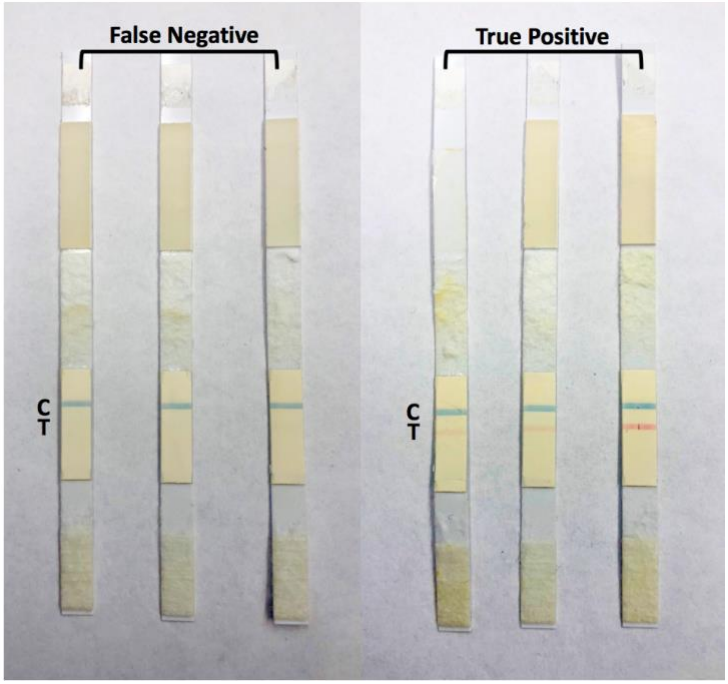
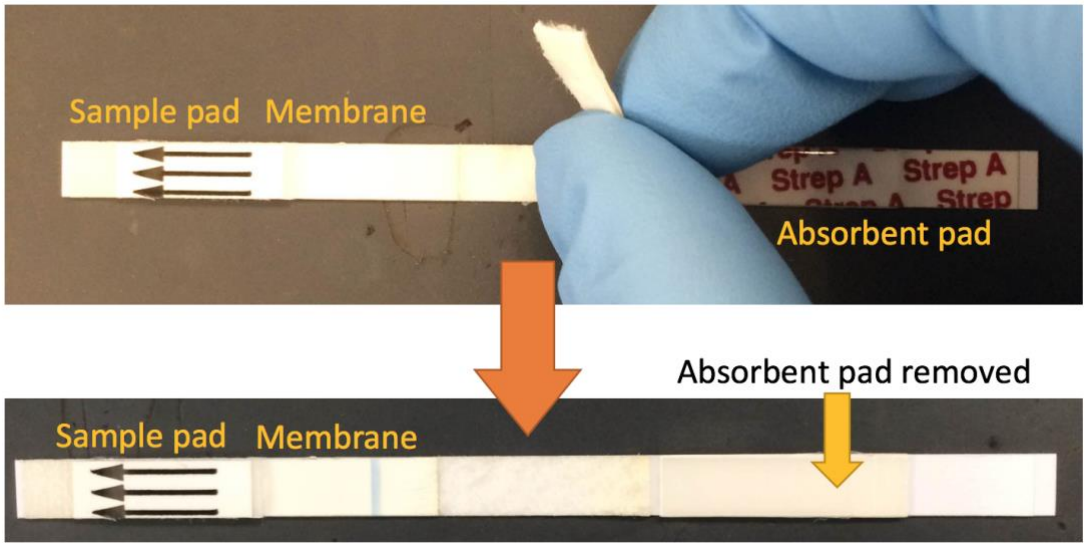


Figure S1. Randomly picked false negative and true positive clinical Quidel QuickVue Strep A Dipstick Test LFAs from the sample stock. The sample pads, conjugate pads and absorbent pads of the LFAs were removed while leaving the membranes intact. Clinically, very faint color test lines are considered as positive. Only blank test lines are considered as negative.

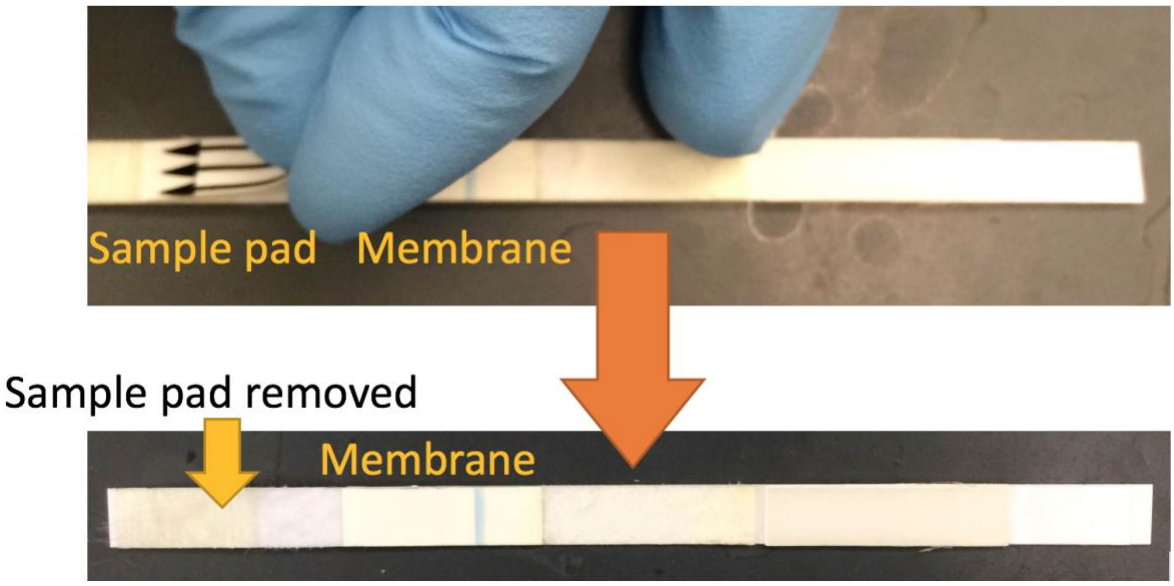
B. Removal of sample and absorbent pads from LFA

1. Run the RDT (LFA) test, incubate, evaluate as usual

2. After visual evaluation, peel of absorbent pad



3. Then remove sample pad, including the top layer and conjugate pad (not visible from top)



4. Store in a plastic bag and document

C. TCA data without removing all the RDT pads

We examined the effects of LFA pads on the TCA detection result by 20 GAS negative fresh swabs. QuickVue package insert protocols were applied. After the visual evaluation, all the components were kept attached unlike the clinical samples in our study. Back flow from the pads was expected due to faster evaporation on the membrane and ensuing capillary force. The wet TCA test was performed immediately after visual evaluation and the dry TCA test was done 3 days later like the calibration curve study. We can see from Fig. S2 that some of the thermal signals increased significantly, which was not observed in the calibration curve study where the pads were removed. Two samples showed false positive TCA results. It can be concluded that backflow should be eliminated by removing sample pads when running TCA reader and that wet (i.e. fresh) study is preferred.

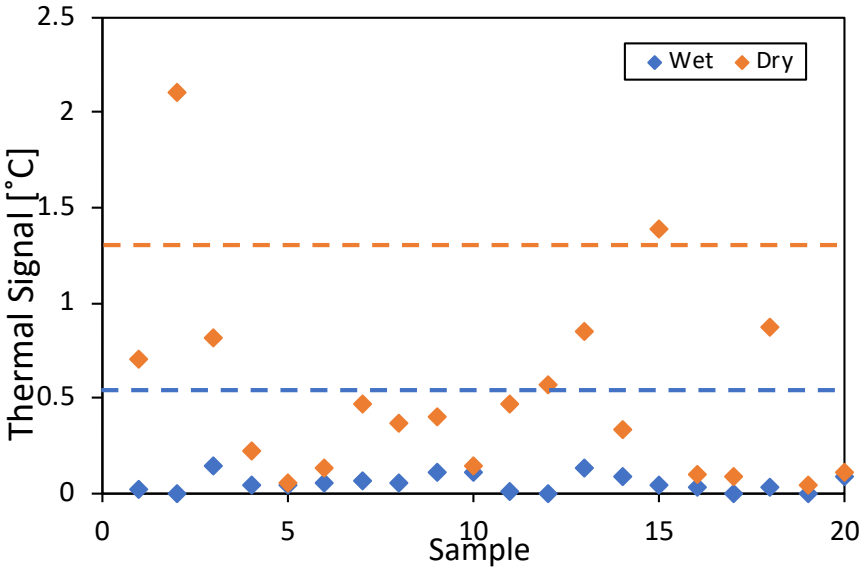


Figure S2. Wet and dry GAS RDTs with backflow shows higher false positives in TCA results. Dashed lines are detection thresholds in both wet and dry conditions.

D. TCA reader repeatability test results

The repeatability of the TCA reader has been shown in our previous work and is demonstrated again here for GAS RDTs. In our original work we showed TCA improvement for detection of *cryptococcus* in CRAG LFAs in the dry state (Qin *et al. Ange.Chem.* 2012). Later, when we developed the TCA reader, we tested the same sample set again and found the results to be repeatable (paired t test p = 0.098 in Figure 2. Wang *et al. Anal.Chem.* 2016). Here for this study, we verify the device repeatability for GAS RDTs by randomly picking 1 false negative and 1 true negative LFA from our clinical sample stock and testing them twice each. The results are shown in Table S1 and suggest they statistically similar. The paired t-test result of these two samples show no statistical difference (p=0.1525).

Table S1 TCA reader repeatability spot check

Sample number*	TCA test 1 (˚C)	TCA test 2 (˚C)	Difference
False negative 21	1.0436	1.0699	2.5%
True negative 16	0.7775	0.8208	5.6%

*Order as plotted in Figure 2 of the paper.