

End-point detection of RNA viruses by recombinase-aided amplification with a nucleic acid lateral flow read-out: the case of SARS-CoV-2

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Introduction

The detection of RNA viruses has been mostly performed by Reverse Transcription real-time Polymerase Chain Reaction (RT-qPCR), which demands specific and costly equipment, and requires specialized technicians¹. Isothermal amplification methods like Reverse Transcription Recombinase-Aided Amplification (RT-RAA) can be an alternative, but there's a lack of solutions for rapid end-point detection of amplicons following amplification. Lateral flow assays (LFA) are simple, fast and cost-effective systems that allow the visual detection of target amplicons using reporter and capture oligonucleotide probes². As such, LFA systems might be combined with RT-RAA to provide simple and accurate detection of RNA viruses, like SARS-CoV-2, without resorting to complex equipment³.

Objectives

- Development of a diagnostic platform for RNA viruses based on nucleic acid detection, by joining nucleic acid amplification (RT-RAA) and a lateral flow assay readout. SARS-CoV-2 was used as a case study and RdRp was chosen as the specific target;
- Evaluation of analytical parameters, including Limit of Detection (LOD) and cross-reactivity;
- Assessment of clinical specimens, in order to draw conclusions about the clinical sensitivity and specificity.

Methods

RT-RAA was performed according with the following conditions:

- Mixing after 4 minutes;
- Temperature of reaction of 42°C;
- In total it takes 20 min.

	Designation	Direction	Sequence (5'-3')	
RdRp	Tail 2 - RdRp - Fw	Fw	CGAAGCTCAATGAA - C3 - TATGCCATTAGTGCAAGAAATAGAGCTCGCAC	480
	Tail 2 - RdRp - Rv	Rv	GCCTGAATTCCTAG - C3 - CAACCACTAGAAATTTGCTTGTTCCAATTAC	630

RT-RAA

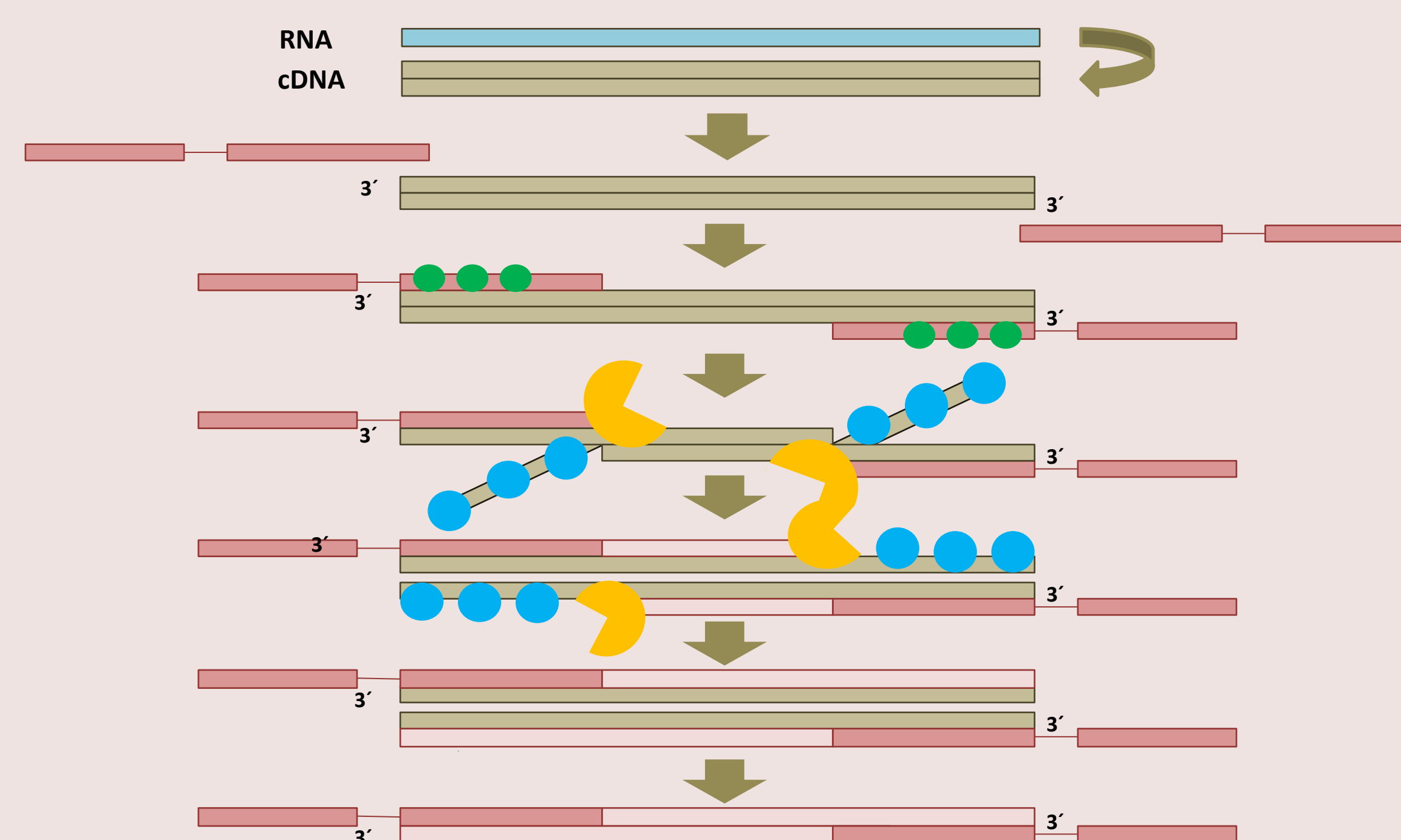


Figure 1 – Scheme of the reaction that leads to the amplification of nucleic acids, departing from RNA. RT and RAA occur at the same time with the cDNA products being exponentially amplified by RT-RAA. The primers were modified with a ssDNA tails at 5' extremity to enable specific recognition through LFA read-out.

LFA Design and Assembly

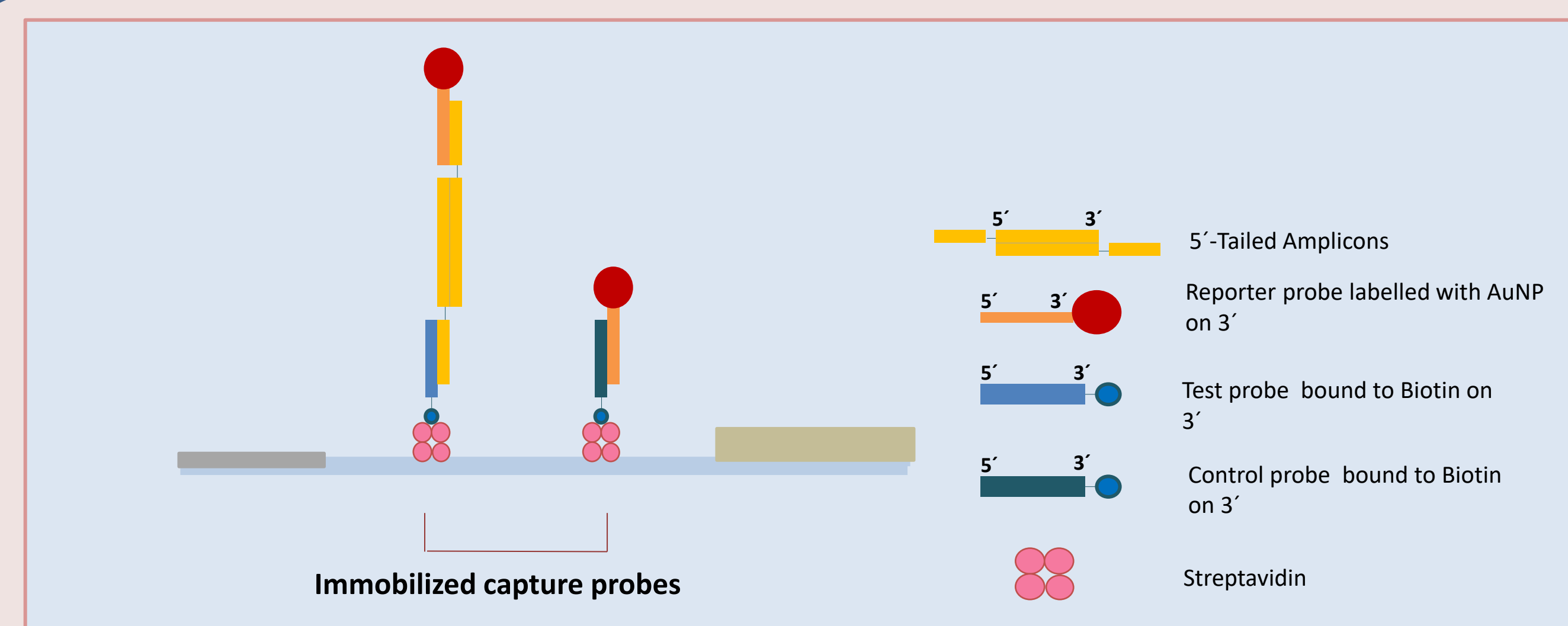


Figure 2– Scheme of the molecular recognition strategy used in the lateral flow assay. In the test line, the tailed amplicons produced by RT-RAA are simultaneously recognized by biotinylated single-strand DNA (ssDNA) test probes anchored on the test strip and ssDNA reporter probes attached to gold nanoparticles (AuNPs) (Table3). In the control line, biotinylated ssDNA control probes hybridize with ssDNA reporter probes. **LFA:** test pad - nitrocellulose membrane (0.5 x 2.5 cm); sample pad – glass fiber (1.5 cm); absorbent pad – absorbent paper (2 cm); **Functionalization:** streptavidin solution (5 mg/mL) and biotinylated ssDNA capture probes (1.5 mg/mL) were conjugated in Phosphate Buffered Saline (PBS), ensuring a 1:4 streptavidin:biotin ratio. Capture probes at 1.5 mg/mL were added to the strip, by pipetting 0.5 µL and air-drying for one hour; **Running protocol:** 10µL of amplicons and 5µL of gold nanoparticles (AuNPs) were mixed in 20µL/RT-RAA of Saline-Sodium Citrate (SSC) buffer. The results were registered after 10 minutes.

Results

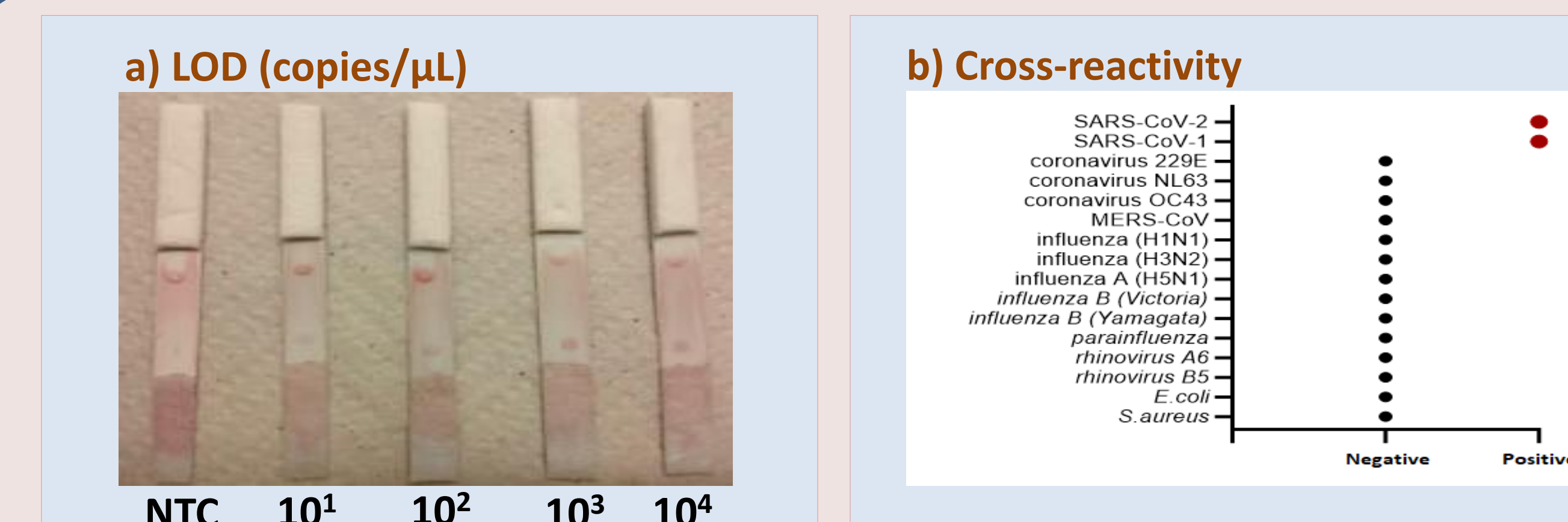


Figure 2 – Results of the evaluation of the analytical performance; a) depicts the results of the analysis of the limit of detection (LOD); b) depicts the results of the analysis of the cross-reactivity with a panel of relevant microorganisms.

Table 4 – Results of the test of the clinical performance of the diagnostic system, resorting to a panel of clinical specimens (30). Positive (P); Negative (N); True Positive (TP); True Negative (TN); False Positive (FP); False Negative (FN); Positive Predictive Value (PPV)= (TP/(TP+FP)x100) and Negative Predictive Value (NPV) = (TN/(TN+FN)x100).

	P	TP	FP	PPV (%)	N	TN	FP	NPV (%)
RT-RAA	14	13	3	81.25	16	13	1	92.86

Conclusions

- The diagnostic platform here purposed for the detection of RNA viruses was successfully implemented, promising a suitable detection of SARS-CoV-2, as evidenced by the LOD (10copies/uL) and cross-reactivity tests (only with SARS-CoV).
- Despite RT-RAA being successfully implemented, it yielded 3 false positives (out of 16 total positives detected), which compromised the PPV value.
- Taking into account that the number of clinical specimens screened was relatively small, further tests are already in progress.

References

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