Addressing variability in rapid antigen detection tests with viral protein reference materials

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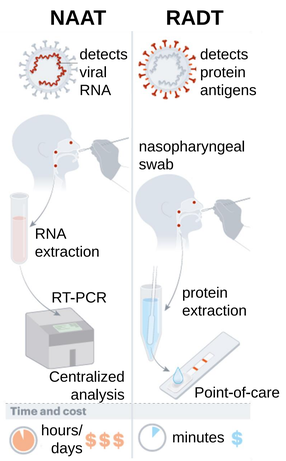
INTRODUCTION

The Coronavirus disease 2019 (COVID-19) pandemic was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As COVID-19 transitions to an endemic disease in much of the world, clinical testing continues to be critical to viral identification and containment. Nucleic acid amplification tests (NAATs) remain the definitive method to diagnose infection by detecting viral RNA, however the associated logistics render their use in widespread testing unrealistic. Alternatively, lateral flow-based rapid antigen detection tests (RADTs) detect the SARS-CoV-2 nucleocapsid protein (NP), can be performed by non-experts at the point-of-care, and offer a quick visual diagnostic result. While RADTs do not offer the same sensitivity as NAATs, they are marketed to detect the viral loads commonly associated with infectiousness.

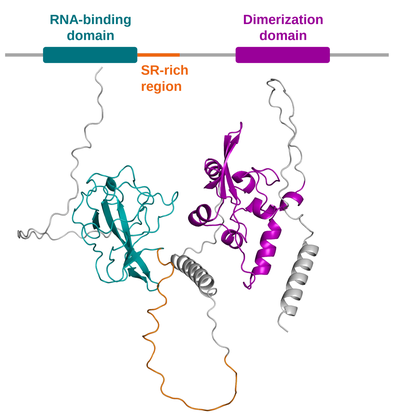
To fulfill the public need for widespread at-home testing, COVID-19 RADTs have been manufactured at an unprecedented pace, yet a shortage of relevant comparative analyses on sensitivity and performance has hindered end-user ability to accurately compare brands for decision making. To address this lack of standardization, we prepared and characterized two independent NP materials and utilized them to evaluate the analytical sensitivity, or limit-of-detection (LOD), of twenty-six RADTs available in Canada and/or Australia.



Comparison of nucleic acid amplification tests (NAATs) and rapid antigen detection tests (RADTs). Adapted from *Nature* (2021) 590, 202-205.

SARS-CoV-2 NUCLEOCAPSID PROTEIN ANTIGENS

The SARS-CoV-2 NP consists of a relatively structured N-terminal RNA-binding domain and C-terminal dimerization domain flanked by largely disordered regions. NPs can self-associate to form higher-order oligomers such as dimers, tetramers, and hexamers. COVID-19 RADTs detect the presence of NP through binding to immobilized anti-NP antibodies on the test strip. Antibody-antigen interactions can be highly selective, yet the exact NP epitopes and structures recognized by the antibodies in each RADT are not easily determined, thus distinct sources of NP may induce variable results within individual RADTs. To address the robustness of commercially-available RADTs, in addition to the analytical sensitivity, we employed two distinct NP materials differing in host-cell expression, fusion tags, buffer, molar mass, etc.



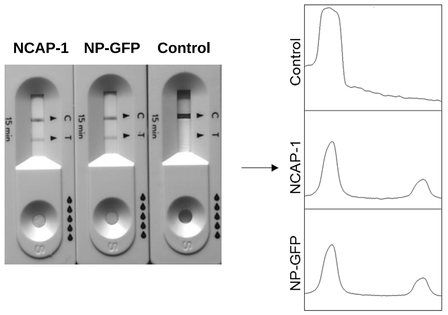
SARS-CoV-2 nucleocapsid protein domain layout and AlphaFold structure prediction of NCAP-1 construct

	NCAP-1	NP-GFP
Expression	CHO	*E. coli*
Fusion tags	C-term: FLAG-2xStrep-His	N-term: His C-term: GFP
Buffer	50 mM Tris (pH 8) 150 mM NaCl	50 mM PBS (pH 7.8) 10 % sucrose
State	Frozen liquid	Lyophilized
Monomer MW	~50 kDa	~75 kDa
Oligomeric state	Hexamer/ tetramer	Tetramer
Autolysis products	Yes	No
Relative host-cell protein amount	0.5 %	38 %

Comparison of NP materials employed in this study
NCAP-1: reference material produced at NRC Canada
NP-GFP: quality control material produced at JCU Australia

ASSESSING RADT ANALYTICAL SENSITIVITY

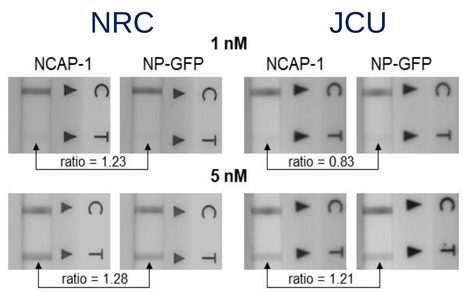
Manufacturers often report sensitivity in terms of the median tissue culture infectious dose ($TCID_{50}$) despite most RADTs actually detecting the viral NP. Thus for each test we spiked NCAP-1 or NP-GFP, at concentrations ranging from 0.5 – 5 µM, into the manufacturers' provided buffers. The spiked buffers were mixed and used according to the specific instructions included with each RADT. Cartridges were after the prescribed duration alongside a control RADT for intensity normalization to facilitate comparison between different RADTs. Band intensities were quantified with ImageJ software.



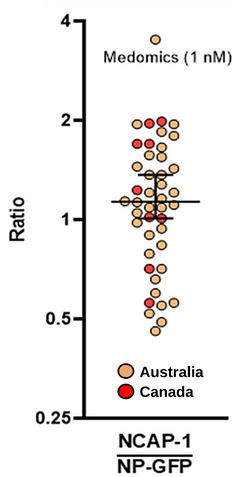
Left: Image of RADTs tested with equal concentrations of NCAP-1 and NP-GFP, and data visualization with ImageJ for band intensity determination.

Right: Results for all RADTs at each NP concentration tested. Similar responses are generated with each NP material, yet a 20-fold difference in LOD is observed between different tests.

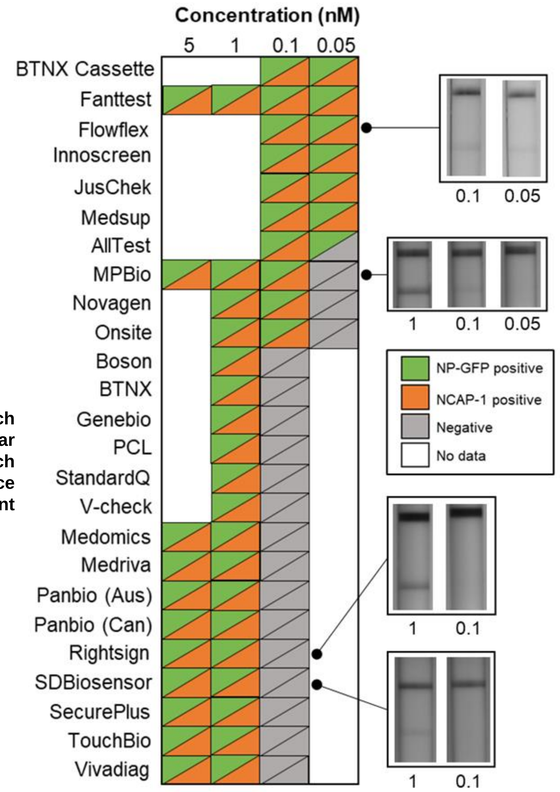
Our study evaluated 26 RADTs, however only one was available in both Canada and Australia – the Abbott Panbio test. We therefore used this RADT to evaluate the response of both NP materials within each lab. Accounting for the fact that the tests used in each lab were likely to have come from different manufacturing lots, we set a 2-fold difference in band intensity between NCAP-1 and NP-GFP as an acceptable threshold to indicate antigenic equivalence.



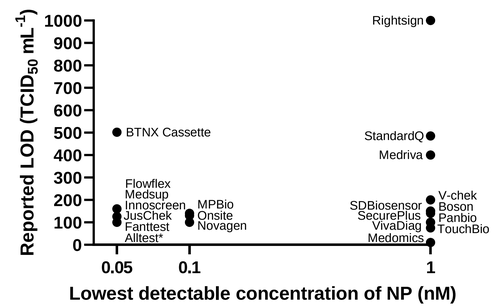
Above: Imaged Panbio RADTs run at NRC (Canada) and JCU (Australia) comparing band intensities for NCAP-1 and NP-GFP at 5 nM and close to the LOD of 1 nM.



Right: NCAP-1:NP-GFP band intensity ratios for all RADTs evaluated in the study. A median value close to 1 and nearly all ratios within a 2-fold difference strongly indicate antigenic equivalence of the two NP materials.



The lowest NP concentration to give a visually-detectable positive response demonstrated little correlation with the $TCID_{50}$ LOD values reported by the RADT manufacturers.



CONCLUSIONS

We demonstrate the utility of assessing RADT LODs with the protein antigen being detected rather than viral cultures. Our data highlight the need for readily available protein reference standards to allow government regulators, as well as end-users, to reliably evaluate and benchmark RADTs during a rapid response to future viral outbreaks.

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