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Analytical sensitivity of COVID-19 rapid antigen tests: A case for a robust reference standard

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ABSTRACT

Aggressive diagnostic testing remains an indispensable strategy for health and aged care facilities to prevent the transmission of SARS-CoV-2 in vulnerable populations. The preferred diagnostic platform has shifted towards COVID-19 rapid antigen tests (RATs) to identify the most infectious individuals. As such, RATs are being manufactured faster than at any other time in our history yet lack the relevant quantitative analytics required to inform on absolute analytical sensitivity enabling manufacturers to maintain high batch-to-batch reproducibility, and end-users to accurately compare brands for decision making. Here, we describe a novel reference standard to measure and compare the analytical sensitivity of RATs using a recombinant GFP-tagged nucleocapsid protein (NP-GFP). Importantly, we show that the GFP tag does not interfere with NP detection and provides several advantages affording streamlined protein expression and purification in high yields as well as faster, cheaper and more sensitive quality control measures for post-production assessment of protein solubility and stability. Ten commercial COVID-19 RATs were evaluated and ranked using NP-GFP as a reference standard. Analytical sensitivity data of the selected devices as determined with NP-GFP did not correlate with those reported by the manufacturers using the median tissue culture infectious dose ($TCID_{50}$) assay. Of note, $TCID_{50}$ discordance has been previously reported. Taken together, our results highlight an urgent need for a reliable reference standard for evaluation and benchmarking of the analytical sensitivity of RAT devices. NP-GFP is a promising candidate as a reference standard that will ensure that RAT performance is accurately communicated to healthcare providers and the public.

1. Introduction

The COVID-19 pandemic has demonstrated the importance of screening and isolating infected people to mitigate the transmission of respiratory viruses. Initially, reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) was the cornerstone of testing [1–3] with its inherent practical limitations becoming rapidly apparent with growing numbers of patients. In addition, the extreme detection sensitivity of RT-qPCR is likely to produce a positive result for an individual that is infected, but no longer able to transmit the virus [4,5]. The increased transmission rate of the later Delta and Omicron variants unequivocally highlighted how valuable point-of-care rapid antigen testing would become [6–8]. Within the span of a year, a multitude of COVID-19 rapid antigen tests (RATs) had been approved for emergency use to curb the pandemic. Development of these RATs underwent an accelerated evaluation framework to hasten their development, scaling and deployment [9]. In the wake of this, several practical concerns about these RATs have arisen along with a more esoteric problem: i.e. which is the most sensitive and on what basis?

COVID-19 RATs predominantly use an antibody-based system to detect the presence of the viral nucleocapsid protein (NP), making them inherently less sensitive than RT-qPCR assays [10] involving a target amplification step. Nevertheless, they are fast, portable and cheaper than RT-qPCR, and shift the focus to identification of infectious individuals [1]. The most commonly referred to and compared metrics of RATs include the clinical specificity and sensitivity, measured by the probability of negative samples testing negative and positive samples testing positive in agreement with RT-qPCR-tested samples respectively.

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The specificity of a COVID-19 RAT is generally very high (up to 100%), due to the specific nature of the antibody-NP interaction. On the other hand, the clinical sensitivity of COVID-19 RATs varies considerably and can dramatically decrease for individuals that are asymptomatic, early or late in disease progression or have a low viral load [11–13]. Of note, the extreme sensitivity of RT-qPCR can produce persistent positives without necessarily indicating the presence of viable or infectious viruses [14,15]. As such, comparison of the performance of COVID-19 RATs using clinical sensitivity data can be misleading as there is no reference standard available. The number of participants in clinical evaluations and their disease states also differ significantly. For instance, as disclosed by the manufacturers, the clinical sensitivity of the 'EcoTest' was evaluated with 184 symptomatic patients who were suspected of COVID-19 and professionally sampled and tested, in contrast to 'Care-Start', which was evaluated using 39 RT-qPCR positive specimens from individuals of an undisclosed disease state that were self-sampled and self-tested. Indeed, when samples from recovering or convalescent patients are used, they are more likely to yield a false positive result by RT-qPCR [15] thereby artificially lowering the apparent RAT sensitivity. Moreover, the Alpha, Delta and then Omicron waves have exposed an ongoing need to re-evaluate and compare RAT performance against emerging SARS-CoV-2 variants [6], which is difficult to achieve using clinical specificity and sensitivity analyses.

The analytical limit of detection (LOD) of COVID-19 RATs varies greatly between manufacturers as does the way the LOD is determined, compounding their cross comparison. Primarily, the LOD is defined as the lowest concentration of cultured virus for which a positive test line is reliably observed. The WHO has defined that an LOD of 1000 $TCID_{50}$/ml, 500 pfu/ml or 10^6 genome copies/ml in viral culture is acceptable [16]. However, the use of viral culture data to determine the analytical sensitivity of an antigen detection device is inherently flawed for two main reasons: (a) the number of infectious virions or viral RNA does not correlate with the number of antigenic NP present in a culture, i.e. viral protein expression can vary greatly depending on promoter strength and gene sequence as well as stage of infection, and (b) the laboratory specific conditions used to culture a virus (e.g. cell type, culture conditions and time) also affect the relative abundance of the NP, RNA and infectious virions making direct comparison of data between laboratories difficult.

Thus, estimating the number of infectious virions can lead to either over or underestimation of the LOD of a RAT due to the presence of varying levels of NP in cultures. As such, culture conditions that increase the relative abundance of NP over the number of infectious virions (e.g. increased cell lysis, production of non-infectious virions, and duration of culture) will yield an improved LOD. This same issue makes it impossible to accurately compare different brands of RATs with respect to $TCID_{50}$-based LOD [17]. Indeed, substantial discrepancies have been reported between the $TCID_{50}$ values disclosed by COVID-19 RAT manufacturers and those from an independent study [18]. This is especially worrying in the current competitive landslide of RATs where a superior LOD is essential and key for appropriate management of infection transmission. Although reported, LOD data based on SARS-CoV-2 NP as a reference protein are scarce [19] and a well-characterized reference material has only recently been produced [20]. The new NP reference standard is yet to be developed and commercialized for evaluation of RATs. In fact, very few manufacturers have disclosed analytical sensitivity data using poorly-characterized NP, most likely due to the WHO recommendation to use $TCID_{50}$ data. In addition, NP oligomerization [21], autolysis and stability issues have also been reported, further delaying the production of an appropriate protein reference standard [20]. In our hands, production of a recombinant GFP-tagged NP (NP-GFP) circumvented these issues using the GFP as a reporter for protein concentration, folding and stability [22–26] which allowed us to readily identify buffer conditions in which NP was stable and homogenous.

Here we describe a novel and robust approach to evaluate the feasibility of using NP-GFP as a diagnostic reference standard and compare the LOD of current RATs. The GFP tag affords additional quality control measures for accurate NP concentration and integrity determination in spiked protein samples (Table S1). Ten commercial COVID-19 RATs were evaluated and ranked using NP-GFP, and our data were compared to their reported analytical sensitivity. The NP-GFP-determined LOD data were highly reproducible within RAT batches. The respective NP-GFP LOD of RATs did not correlate with their reported $TCID_{50}$ LOD. Overall, our results indicate a need to re-evaluate practices and methods by which RATs are benchmarked to ensure that their performances are representative and accurately communicated to healthcare providers and the public. NP-GFP provides an easy and fast solution to this end.

2. Materials and methods

2.1. Expression and purification of NP and NP-GFP proteins

The SARS-CoV-2/NP (P0DTC9) and MERS-CoV/NP (K9N4V7) coding sequences were synthesised in an *E. coli* codon optimized form (Bioneer) and ligated into the pIM013 vector [24] to create pRB305 and pCT309 for expression of hexahistidine tagged SARS-CoV-2 and MERS-CoV NP, respectively. pRB305 and pCT309 were cut with AflII to remove a stop codon between the NP and GFP coding sequences, and re-ligated with T4 DNA ligase to create pRB306 and pCT310 expressing hexahistidine tagged SARS-CoV-2 and MERS-CoV NP-GFP respectively. The H5N1 Influenza A virus (IAV) NP-GFP encoding vector was described previously [25]. All NP and NP-GFP were produced in *E. coli* BL21(DE3)RIPL using terrific broth supplemented with 4 mM glucose and 0.4 mM galactose, containing 100 μg/mL ampicillin and 50 μg/mL chloramphenicol ($TB_{Glu\text{-}Gal\text{-}AC}$). A 1 L flask containing 100 mL of $TB_{Glu\text{-}Gal\text{-}AC}$ was inoculated with a bacterial loop sourced from a fresh overnight culture plate and incubated at 37 °C and 200 RPM until the optical density reached 0.7. Proteins were expressed over 48 h at 16 °C. Lysis and purification procedures were performed as previously described for influenza A virus NP-GFP [25,26] except for the lysis and wash buffers which were supplemented with 1 M and 300 mM NaCl, respectively, and anion-exchange chromatography was not required. Protein concentrations were determined by Bradford Assay and purity assessed by SDS-PAGE. Purified NP and NP-GFP suspensions were stored in buffer A (50 mM sodium phosphate (pH 7.8), 10% glycerol (v/v) and 2 mM β-mercaptoethanol) at - 80°C.

2.2. Evaluation of rapid antigen tests

The LOD of ten commercially available COVID-19 RATs (Table 1) was assessed in triplicate with NP or NP-GFP spiked into the provided buffer and run as per manufacturers' instructions. For each RAT, 10 μL of SARS-CoV-2 NP-GFP suspensions (at 10, 1, 0.55 and 0.1 nM in buffer A) were used to spike the manufacturers' provided buffers. The spiked buffer samples were mixed by inverting five times and used according to the manufacturers' specific instructions. Results were captured using a G:BOX Chemi XRQ (white light, no filter, 50 ms exposure) after the time indicated (Table 1). Band intensities were then quantified using ImageJ. For each RAT, MERS-CoV NP-GFP at 1 μM was used as a negative control. The GFP fluorescence of stock NP-GFP suspensions at 1 μM (SARS-CoV-2 NP-GFP or MERS-CoV NP-GFP) was measured as a quality control measure prior to production of each dilution series and repeats.

Rapid test reading is primarily based on a visual assessment and agreement by three independent readers which leads to different outcome in borderline test line cases. All tests were photographed using identical lighting and photographic settings, avoiding human error due to environmental factors as well as reader fatigue and bias. Then, borderline test lines, i.e. lowest concentration producing a test line that could be seen by at least two readers, were used to arbitrarily set the lower threshold value using ImageJ quantification. As such any ImageJ

Table 1
RATs evaluated in this study.

RAT	Type	Buffer volume (μL)*	Drops added	Time (min)	Reported $TCID_{50}$/mL	Detection limit# of NP-GFP (nM)	Positive tests at detection limit (n=3)
EcoTest	S	700	NA	15	32	0.55	3
Cellife	N	350	3	15	50	0.55	3
Testsea Labs	N	300	3	10	50	1	3
JusChek	N	300	3	15	100	0.1	3
Lyher	N	350	3	15	135	0.1	2
Panbio	N	400	5	15	150	10	3
Clungene	N	ND	3	15	570	0.1	1
VivaDiag	N	300	3	15	675	0.55	3
Maccura	N	300	4	15	750	0.55	3
CareStart	N	450	3	10	800	0.1	1

*10 μL of SARS-CoV-2 NP-GFP sample was used to spike the buffer. # Lowest detectable concentration of NP-GFP in the sample dilution series. N: Nasopharyngeal, S: Saliva, ND: not disclosed, NA: not applicable

value below the threshold value of 1100 AU was considered a negative test line to the average human eye.

2.3. Denaturation of SARS-CoV-2 NP-GFP

SARS-CoV-2 NP-GFP at 5 μM in buffer B (buffer A without glycerol) was treated with RNase A (0.1 mg/ml final concentration) for 30 min at RT. Thermal denaturation of RNase-treated NP–GFP was performed in triplicate with 15 μl samples at temperatures ranging from 39 to 63°C for 10 min (Biorad CFX96/C1000 Touch Thermal Cycler). Samples were cooled on ice for 10 min and centrifuged for 20 min at 18,000 g. Supernatants (5 μl) were transferred into a 96-well black plate (Nunc) containing 50 μl of buffer B in each well, and the fluorescence recorded (485 nm excitation and 520 nm emission) with a FLUOstar OMEGA plate reader (BMG LABTECH). The fraction of folded NP (F_{fold}) is obtained by dividing the residual fluorescence in the supernatant of a heat-treated sample by the fluorescence value of the control (i.e. 25°C).

2.4. Proteolysis profile of SARS-CoV-2 NP-GFP

SARS-CoV-2 NP-GFP (10 μl of 5 μM in buffer B) was mixed with 0.5 μl of trypsin (Sigma) at a concentration of 40 μM in $_{dd}H_2O$ and incubated at 25°C for 30 min. Digested samples (10 μl) were subjected to separation by SDS–PAGE and run at 150 V for 60 min. Protein fluorescence was recorded using a G:BOX Chemi XRQ (Blue LED module and 525 nm filter).

2.5. Statistical analysis

Statistics and the number of repeats are indicated in the relevant figure legends and methods. Statistical analyses were performed using GraphPad Prism 9. Data are expressed as mean values ± SD. The Kolmogorov–Smirnov test was used to evaluate the NP-GFP over NP and interassay agreements. To determine if there was a correlation between the reported $TCID_{50}$ and NP-GFP LOD values, a nonparametric Spearman rank-order correlation was performed. The NP-GFP LOD was defined as the interpolated concentration of the spiked buffer at 1100 band intensity units (the cut-off value for line detection by the human eye as assessed by three independent observers) and was determined by fitting the data with a four-parameter logistic curve.

3. Results and discussion

3.1. Evaluation of NP-GFP as a reference protein for accurate comparison of RAT LOD

In this study, all selected COVID-19 RATs are approved by the Therapeutic Goods Administration in Australia and are designed to detect the presence of SARS-CoV-2 NP in nasopharyngeal or saliva samples (Table 1). As such, the analytical sensitivity reported by manufacturers should include a metric that most accurately mirrors the concentration of NP in a sample. We rationalized that SARS-CoV-2 NP-GFP would be an optimal probe due to its intrinsic characteristics affording valuable rapid quality control measures for protein expression, solubility and stability. Indeed, the fluorescent tag provided a rapid and accurate means to quantify protein concentration, solubility, aggregation [24,26,27], and proteolysis [27,28] that greatly facilitated protein production and quality assessments. To ensure the GFP tag was not perturbating the complex formation between the SARS-CoV- 2 NP and the RAT antibodies, the test line band intensities obtained using a dilution series of NP and NP-GFP were compared for two RATs (Panbio and JusChek) (Fig. 1A–C). No significant difference in band intensities could be observed in a pairwise comparison of NP and NP-GFP demonstrating that the GFP tag did not interfere with NP-antibody complex formation (Fig. 1C).

To highlight the advantages of the protease-resistant and highly-stable GFP tag (e.g. heat tolerance in excess of 70°C and a pH range of 5.5–12 [23,24,27]) in protein quality control, we performed a series of partial denaturation and proteolysis experiments with NP-GFP (Fig. 1D–F and S1). The data clearly demonstrate the advantages of the GFP tag to rapidly evaluate protein denaturation and proteolysis, and facilitate pre- and post-production quality control analyses. These measurements are difficult to perform with untagged NP [20] yet extremely important when evaluating and comparing the LOD of RATs to reduce sources of non-replicability. Here, the fluorescence readout provides an easier, faster, and more sensitive means to assess the integrity and concentration of the reference NP-GFP sample in the dilution series. These essential protein quality control data would be problematic to obtain using classic analytical methods due to the low concentrations used in diagnostic reference samples.

3.2. Systematic comparison of RAT LOD with NP-GFP

The LOD of ten COVID-19 RATs (Table 1) was examined using SARS-CoV-2 NP-GFP spiked into the provided buffers and performed as per the manufacturers' instructions (Table S2). To avoid visual bias due to differences in the eyesight of observers, RAT results were quantitatively assessed using ImageJ integration of band intensities (Fig. 2). Analytical specificity was systematically evaluated using spiked MERS-CoV NP-GFP samples as negative controls.

All ten RATs developed a strong positive test line with their respective buffers spiked with 10 μL of a 10 nM SARS-CoV-2 NP-GFP sample (Fig. 2). Six out of ten RATs yielded a visible test line with a 0.55 nM NP-GFP sample (Fig. 2). The Panbio RAT was the only device unable to consistently yield a visible test line with a 1 nM NP-GFP sample (corresponding to a final NP concentration of 1.1 ng/mL). This is in agreement with recent Panbio RAT studies that reported three negative results for recombinant NP at 2.5 ng/mL in non-standard conditions (i.e. 50 μL

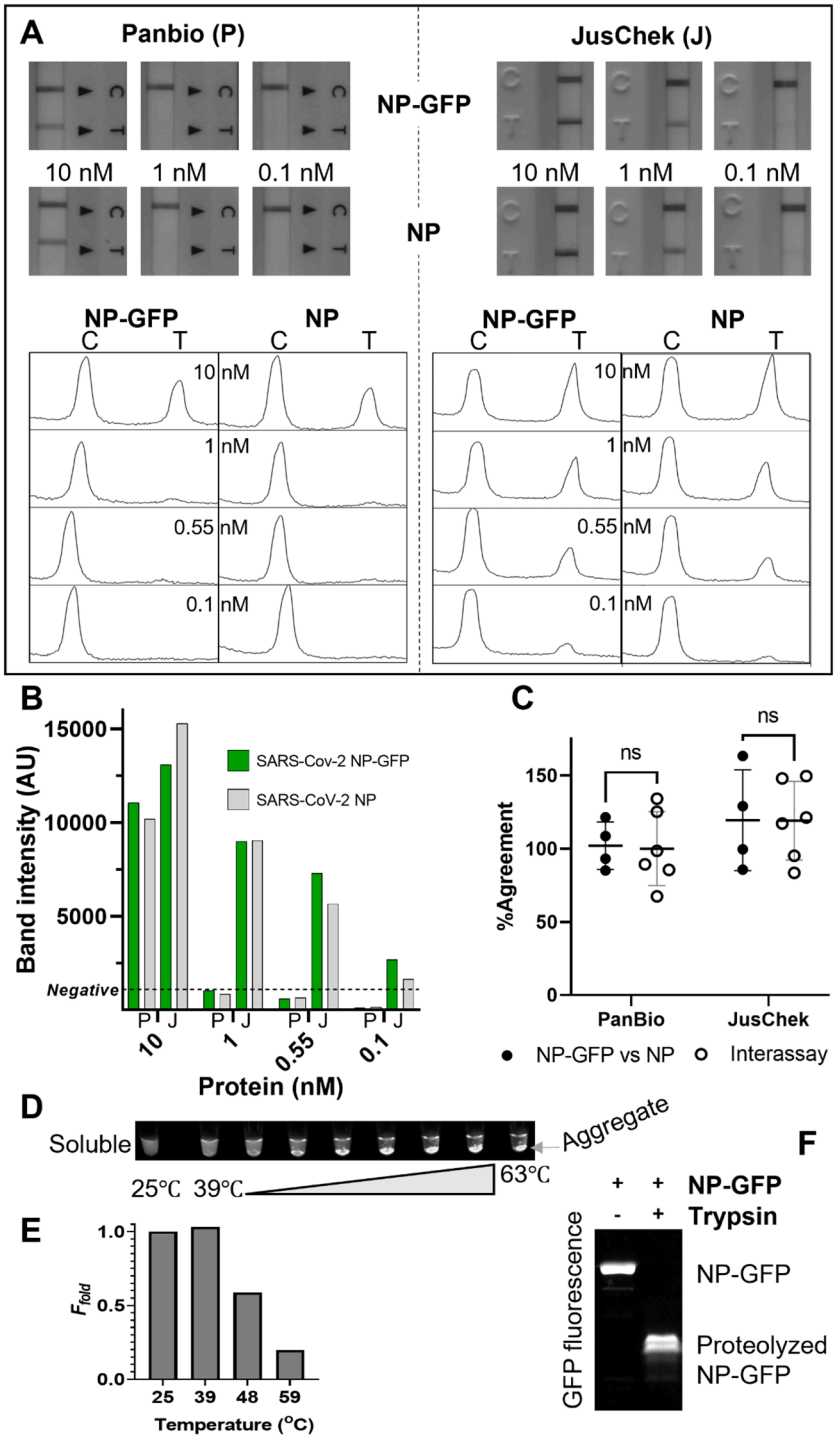

Fig. 1. Comparison of band intensities between SARS-CoV-2 NP-GFP and NP for the Panbio and JusChek RATs. (A) Protein concentrations are indicated and correspond to the concentrations in the samples used to spike the RAT buffers. Band intensities were determined using ImageJ and plotted (B). Visual 'negative' threshold value is 1100 AU. (C) NP-GFP, NP, and interassay agreement. Mean values of replicates were used for normalisation. Transformed data (%) and SD indicate no significant differences between interassay and NP-GFP over NP variability. (D) Rapid assessment of NP-GFP aggregation from (E) the folded fraction (F_{fold}) of NP. F) Rapid assessment of proteolysis. See Fig. S1 for a detailed description of (D–F).

of NP dilution in PBS) [19] and a low clinical sensitivity [29]. The best performing RATs (Lyher and JustChek) consistently detected NP-GFP at the lowest sample concentration (0.1 nM). Of note, only one RAT (Lyher) yielded a detectable test line with the MERS-CoV NP-GFP control sample (1000 nM, Fig. 2). However, no test line was detectable with IAV (H5N1) NP-GFP (1000 nM) [25,26,29] as a negative control sample, suggesting some degree of cross-reactivity of the Lyher RAT antibodies with the related MERS-CoV NP (Supplementary Fig. S2).

Importantly, several RATs included in this study (Panbio, Lyher, TestSea and VivaDiag) have previously been evaluated with SARS-CoV-2 Delta and Omicron variants using dilution series of viral cultures quantified by RT-qPCR (i.e. RNA copy number) [30]. In this previous study, there was no significant difference between these four devices, with consistent detection only at 6.39 and 6.50 $\log_{10}$ copies/mL for Omicron and Delta variants respectively. In contrast, there was clear ranking of RAT performance shown in the current study (Lhyer > VivaDiag > Testsea > Panbio). RNA copy number has not yet been formally correlated with the number of NP produced making direct comparison of data difficult [31]. Indeed, the presence of both canonical and noncanonical subgenomic mRNAs (sgRNAs) complicates quantification, as the vast majority of sgRNAs contain NP sequence [32]. As canonical sgRNAs have been shown to be both nuclease resistant and persistent, they are likely not indicative of active transcription [33]. Additionally, some non-canonical sgRNAs may have sufficient NP

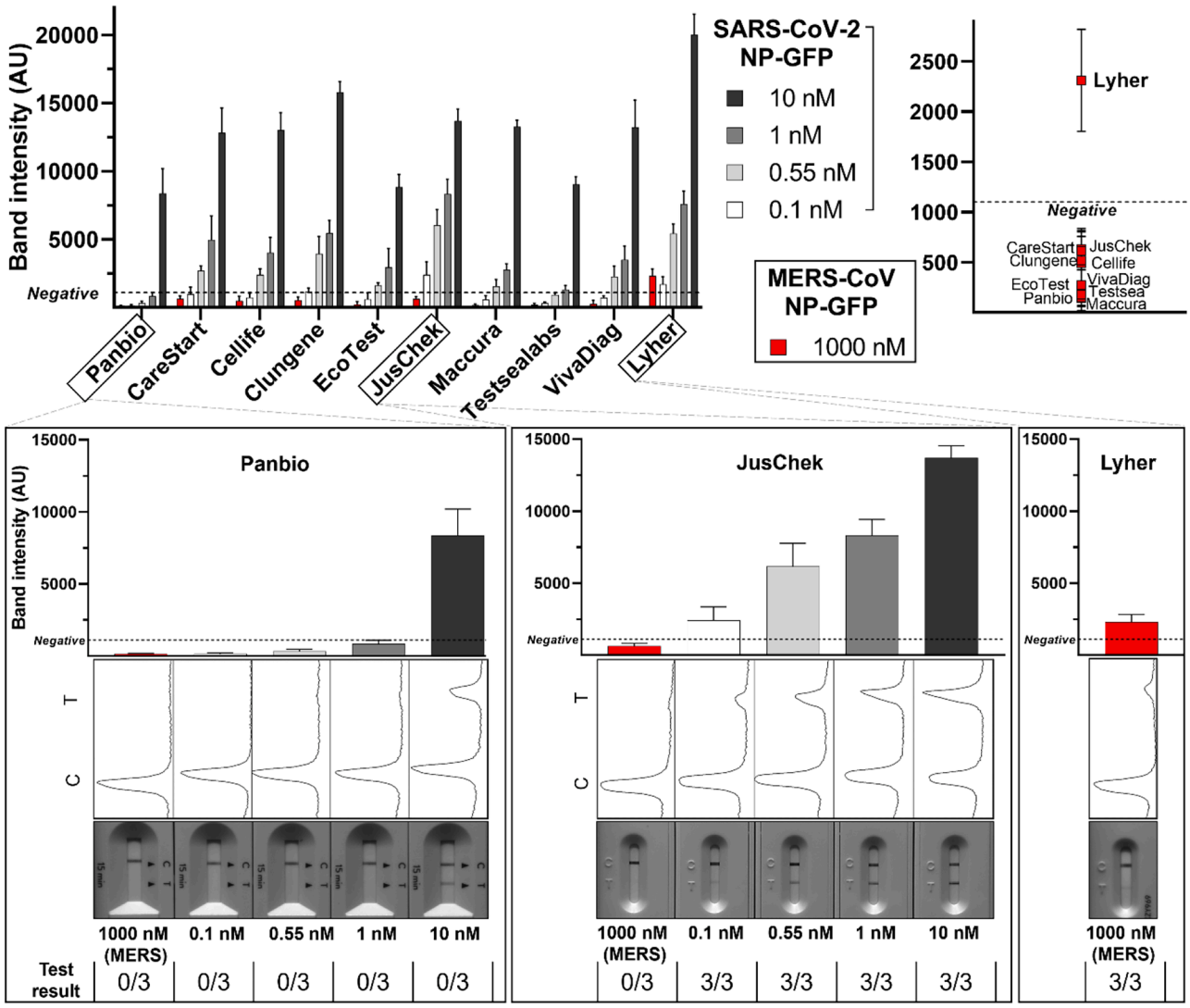

Fig. 2. Comparison of the analytical performance of ten approved rapid antigen tests using SARS-CoV-2 NP-GFP spiked into the provided buffers and run as per manufactures instructions. A 1000 nM MERS-CoV NP-GFP 'spike sample' (red) was used to evaluate test specificity and serve as a negative control. Band intensities (triplicate) were analysed using ImageJ. The visual threshold for a negative test is 1100 AU as determined by three independent observers.

sequence to be detectable via RT-PCR, while not resulting in NP expression [32]. The complications in attempting to correlate RNA copy number and antigen expression highlight the value in using NP antigen as a means of benchmarking device performance.

3.3. *There is no correlation between $TCID_{50}$ and NP-GFP LOD*

The RATs included in this study were selected to provide exemplars with different $TCID_{50}$ LODs ranging from 32 to 800 (Table 1). Our aim was to examine if there was a monotonic relationship between the $TCID_{50}$-based LOD and the NP-based LOD (NP-GFP). Our data demonstrate unequivocally that there is no statistical correlation between the reported $TCID_{50}$ values and the lowest visually detectable concentration of NP-GFP ($r=-0.28$, $P=0.43$, see Fig. 3A). The band intensity data were then fitted for each RAT to calculate NP-GFP LODs and here again these values did not correlate with $TCID_{50}$ values ($r=-0.13$, $P=0.71$, Fig. 3B). Of note, independent studies have shown discordance between $TCID_{50}$-based LOD [18] as well as a lack of correlation between NP-based and PFU-based LOD [19] for several COVID-19 RAT devices.

4. Conclusions and perspective

The $TCID_{50}$-based LOD is the most-commonly reported measure to describe the analytical sensitivity of COVID-19 RATs. While the method is useful for comparative assessment of RATs in single laboratory evaluation studies, it cannot be used to measure the analytical sensitivity of RATs in absolute terms or compare these when using different viral cultures. This study and other comparative studies [19,30] clearly show that $TCID_{50}$-based LOD values are intrinsically flawed and unreliable for cross-comparison of RATs, most likely due to challenges in the production of standardized and well-characterized viral cultures (Table S1). Other viral culture-based reference standards (e.g. pfu/mL and RNA copies/mL) [34] have similar drawbacks. While more RATs are being approved for NP detection, their cross-comparison with respect to analytical sensitivity will become virtually impossible. Although scarce, NP-based LOD values have been reported by some manufacturers (e.g. LOD of Lyher device is 0.5 ng/mL) and an independent study [19]. Of note, the NP LOD of 0.5 ng/mL reported for the Lyher device by the manufacturer is in agreement with the NP-GFP LOD in this study (i.e. 1/3 positive tests at a corresponding NP concentration of 0.13 ng/mL and 3/3 at 0.7 ng/mL).

It is evident that well-characterized NP reference standards [20] are

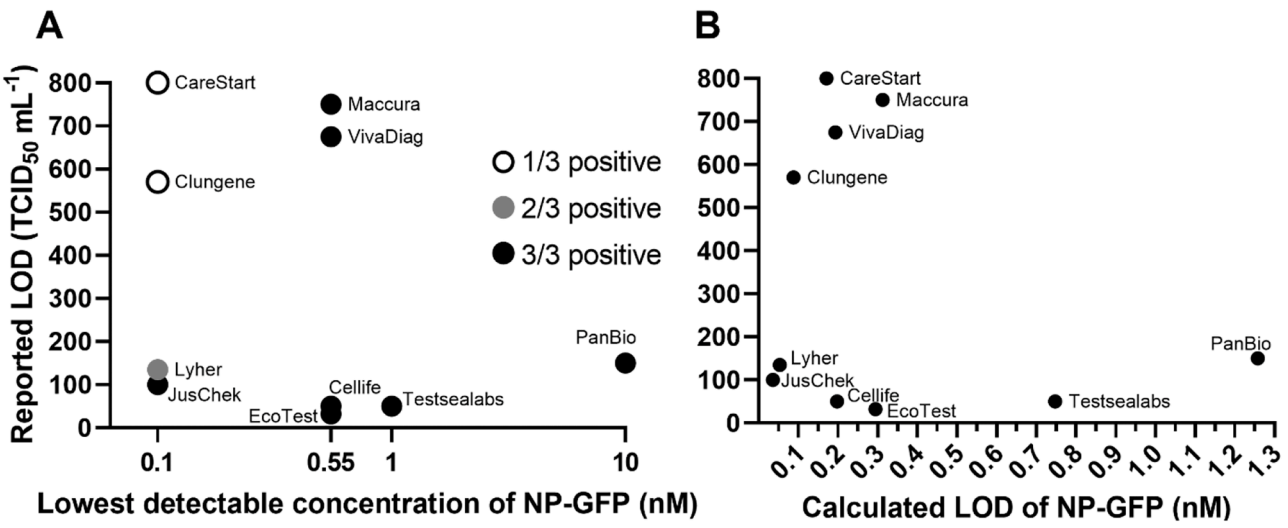

Fig. 3. Correlation between $TCID_{50}$ values reported by RAT manufacturers and their LOD as measured by NP-GFP. (A) Lowest detectable concentrations and number of positive test results ≥1100 AU in the NP-GFP dilution series. (B) Calculated LOD values determined at the negative threshold value of 1100 AU from a fitted four-parameter logistic curve (see Supplementary Fig. S3).

optimal to measure and compare the LOD of COVID-19 RATs and eliminate the need to produce and manipulate infectious virus cultures. NP-GFP yields identical LOD values to NP and it also provides several advantages to this end: (a) NP-GFP can be expressed and purified in high yields and its fluorescence streamlines the entire process, (b) the GFP-tag does not interfere with antibody biorecognition, (c) the GFP-tag affords faster, cheaper and more sensitive quality control measures for protein production, solubility and stability assessments, (d) the GFP-tag offers a more sensitive and accurate measurement of protein concentration in spiked protein samples, altogether improving replicability when evaluating the LOD of a given RAT, (e) the NP sequence within the NP-GFP expression vector can easily be replaced with sequences from new variants to evaluate RAT target failure and (f) NP-GFP is safe and stable permitting cheap and reliable conveyance to end-users. With respect to the limitations of our study, ten RATs were evaluated out of 64 approved for COVID-19 self-test in Australia. The study could be extended to examine more RATs in a multi-centre approach, aiming at comparing the robustness of NP-GFP as a reference standard against NP and different viral cultures. Ultimately, a commercially-viable large scale purification and extended protein characterization studies will be needed to fully develop NP-GFP into a protein reference material.

The diagnostic industry has been operating at lightning speed. Indeed, RATs are being developed and manufactured faster than at any other time in our history. This acceleration is shining light on the need for reliable and comparable analytical technologies that will help developers and producers maintain the batch-to-batch quality and accuracy of RATs. NP-GFP affords a simple, yet relevant and robust reference standard that will help developers and producers to this end. It will also aid public health and government agencies, as well as health and aged care facilities to reliably benchmark different RAT brands to select the best possible device to curb the transmission of current and future SARS-CoV-2 outbreaks. As society attempts to return to pre-pandemic normalcy, the central focus has become the rapid identification of infectious individuals to avoid the spread of SARS-CoV-2 in vulnerable populations such as immunocompromised patients and aged-care residents. Thus, international public health and government agencies should encourage the adoption of relevant quantitative analytics that will inform on absolute RAT sensitivity and help facilitate brand comparison, decision making and approval processes. This will ensure both the quality and reliability of RATs, and that the huge demand for these can be met with minimal batch out-of-specification and target failure events [35].

CRediT authorship contribution statement

Casey J. Toft: Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Data curation. **Rebecca A. Bourquin:** Investigation. **Alanna E. Sorenson:** Supervision, Writing – review & editing. **Paul F. Horwood:** Writing – review & editing. **Julian D. Druce:** Writing – review & editing. **Patrick M. Schaeffer:** Conceptualization, Methodology, Supervision, Writing – review & editing, Visualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2023.100187.

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