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Short communication

# Optimization of magnetic bead-based nucleic acid extraction for SARS-CoV-2 testing using readily available reagents

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ABSTRACT

The COVID-19 pandemic has highlighted the need for generic reagents and flexible systems in diagnostic testing. Magnetic bead-based nucleic acid extraction protocols using 96-well plates on open liquid handlers are readily amenable to meet this need. Here, one such approach is rigorously optimized to minimize cross-well contamination while maintaining sensitivity.

## 1. Introduction

The COVID-19 pandemic has placed unprecedented strain on instrument and consumable supply chains for SARS-CoV-2 nucleic acid (NA) testing (The Scientist, 2021). NA protocols involve lysis and purification of NAs on columns or magnetic beads. Bead-based protocols are amenable to automated workflows and are widely available, rendering them attractive alternatives to proprietary commercial offerings (He et al., 2017). Recent reports have suggested that generic bead-based protocols can be successfully deployed on generic open-deck liquid handling instruments (Esbin et al., 2020; Anon, 2021; Klein et al., 2020); yet lack rigorous measures of specificity and sensitivity that are required for clinical deployment.

## 2. Methods and materials

Step-by-step protocols are described in the supplementary file.

## 3. Results and discussion

We sought to establish an automated protocol to support extraction-based SARS-CoV-2 NA testing using generic reagents, automated on an

*Abbreviations:* NA, nucleic acid; qPCR, quantitative polymerase chain reaction; Ct, PCR cycle threshold.

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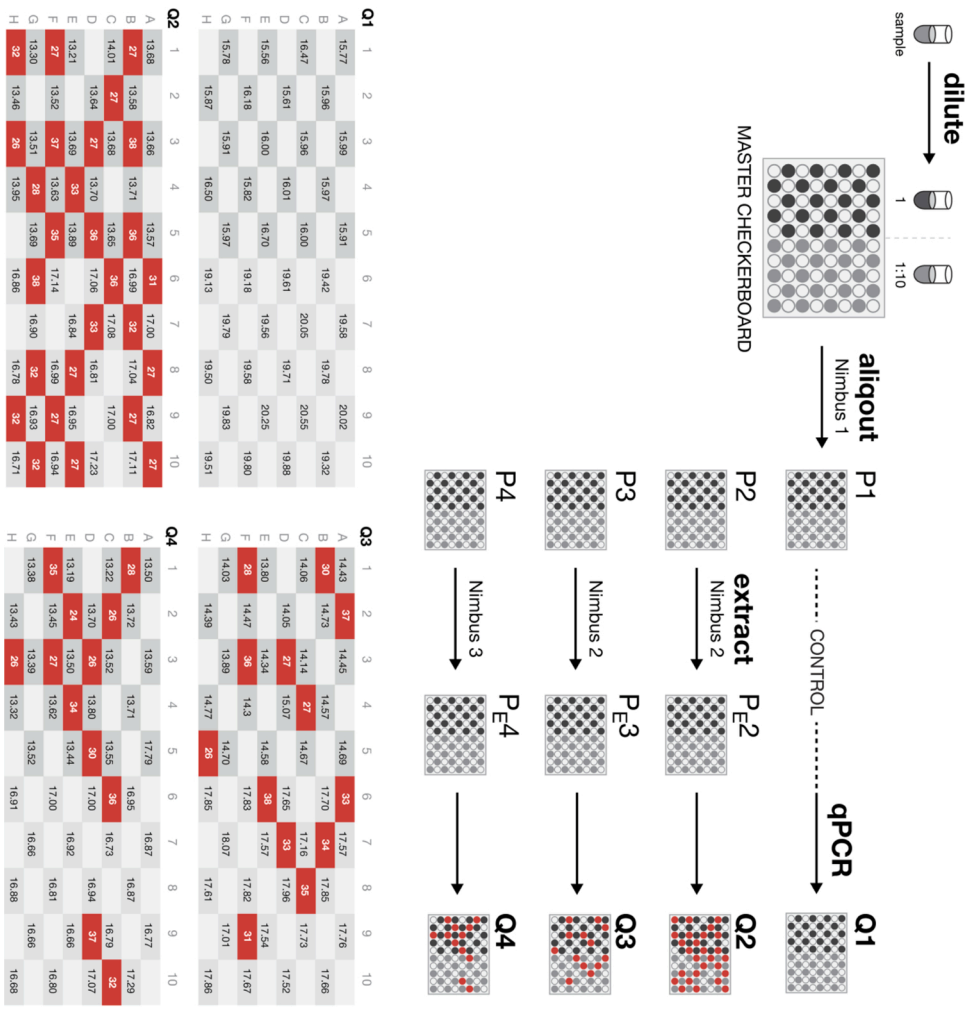


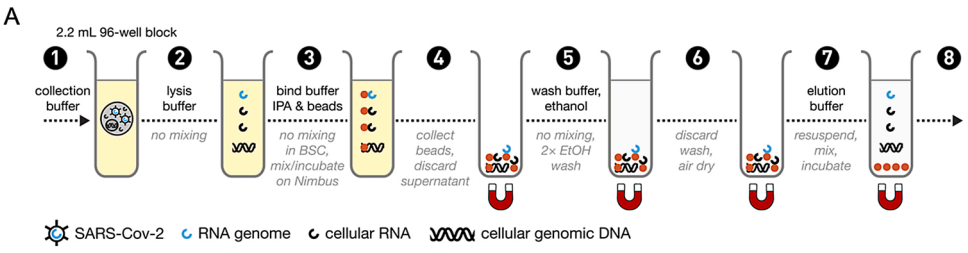

**Fig. 1. Assessment of cross-well contamination associated with a liquid handler.** The contamination assessment Workflow is shown in the upper panel. This assay is designed to decouple the manual upstream BSC steps from the steps on the liquid handler (in this case a NIMBUS). A synthetic DNA fragment (g-block) is used as a starting material and a master g-block checkerboard plate is manually generated. The g-block DNA is aliquoted into a plate that contains elution buffer (control plate) and a deep-well plate that was pre-loaded with a mixture of Copan UTM, RLT Plus, beads, and isopropanol (extraction plates), respectively, using a devoted liquid handler. The samples from the extraction plates are then purified on separate liquid handlers. The g-block DNA eluates from the extraction plates and diluted g-block DNA from the control plates are subsequently used as templates in the same run of qPCR (lower panel).

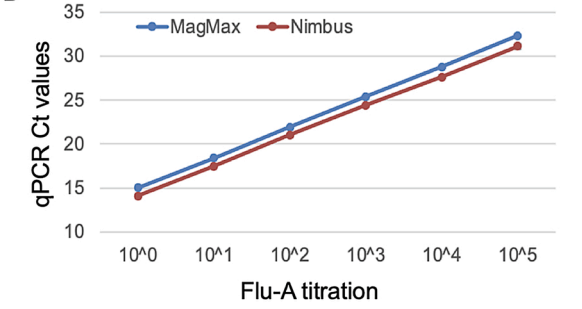
open deck Hamilton NIMBUS96 liquid handler. We benchmarked this against an existing clinical NA testing workflow in place at the BC Centre for Disease Control Public Health Laboratory (BCCDC PHL) (Vancouver, Canada) that relies on the MagMax™ -96 Viral RNA Isolation kit (Thermo Fisher Scientific) deployed on the Applied BioSystems MagMax Express™ 96 platform, hereafter referred to as the "MagMax". MagMax features bead-bound NAs that are transferred serially to five plates containing wash solutions and elution buffer via 96 magnetized rods with disposable sheathes. Widespread adoption of this commercial workflow during the pandemic has driven ongoing shortages in reagents and motivated this study. This reagent shortage extended to many other manufacturers of automated NA extraction systems.

Magnetic bead-based NA purification workflows deployed on open liquid handling platforms support various chemistries and plastic-ware configurations, providing critical flexibility in the face of global supply chain instabilities. Here, we provide a benchmarked standard operating procedure (SOP; **Supplementary File**) that employs a guanidine-thiocyanate containing lysis buffer followed by NA purification using magnetic beads and deployed on a Hamilton NIMBUS liquid handler (**Supplementary** Fig. 1A). The SOP is designed to accept specimens aliquoted into a plate from a variety of commercial transport mediums (Copan UTM, Hologic STM, Roche cobas® PCR Media, YOCON UTM) or common laboratory buffers.

Cultured Influenza A virus (Flu-A) spiked into transport medium was used for initial comparisons between the NIMBUS and MagMax protocols. Following extraction, Flu-A RNA recovery was measured using a TaqMan qRT-PCR assay, developed by BCCDC, that detects Flu-A, Flu-B and RSV. The PCR cycle threshold (Ct) values obtained from the NIMBUS protocol were lower than those obtained from the MagMax protocol across dilutions when Copan UTM was used as virus diluent by an average of 1.13 Ct ($p = 0.0039$; **Supplementary** Fig. 1B). When Hologic STM was used, the opposite was observed, with higher values from the NIMBUS protocol compared to those from the MagMax protocol by an average of 0.22 Ct ($p = 0.0301$; **Supplementary** Fig. 1C). These results suggest that the NIMBUS protocol provides comparable sensitivity to the MagMax protocol.

To measure specificity of our protocol we deployed a "checkerboard" input plate where Copan UTM containing Flu-A was alternated with Copan UTM alone. This test revealed a ~85 % specificity; carryover into blank wells was judged to be an aggregate effect of the manual processes performed in the biosafety cabinet (BSC) and automated liquid handling. To decouple these sources of contamination, we utilized synthetic DNA (g-block) controls and matched primers and probe sets. A master checkerboard plate was first generated by aliquoting an amount of g-block DNA sufficient for an extraction-free control plate and three extraction test plates. Using a dedicated NIMBUS, the master plate was aliquoted into a control plate containing elution buffer only and into a deep-well plate that was pre-loaded with a mixture of Copan UTM, RLT Plus, beads, and isopropanol to mimic the extraction chemical milieu (extraction plates), respectively. The samples from two of the extraction plates were then purified using a second NIMBUS and those from the third extraction plate were purified on a third NIMBUS. As shown in Fig. 1, all the 40 blank wells in the control plate were determined to be negative via the qPCR assay (i.e. undetermined Ct values) (100 %




C

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 14.30 | UNT | 13.59 | UNT | 13.81 | UNT | 14.23 | UTM | 14.22 | UNT |
| UNT | 13.81 | UNT | 14.19 | UNT | 13.73 | UNT | 13.67 | UNT | 13.96 |
| 13.86 | UNT | 13.79 | UNT | 13.90 | UNT | 13.62 | UNT | 13.84 | UNT |
| UNT | 13.76 | UNT | 13.82 | UNT | 13.78 | UNT | 14.00 | UNT | 13.76 |
| 13.73 | UNT | 13.93 | UNT | 13.80 | UNT | 13.86 | UNT | 14.10 | UNT |
| UTM | 13.94 | UNT | 13.85 | UNT | 13.83 | UNT | 13.82 | UNT | 13.83 |
| 13.72 | UNT | 14.25 | UNT | 13.71 | UNT | 13.70 | UNT | 13.71 | UNT |
| UNT | 13.59 | UNT | 14.33 | UNT | 13.62 | UNT | 13.81 | UNT | 13.90 |

D

| *E-gene* | | *RDRP* | |
|---|---|---|---|
| MagMax | Nimbus | MagMax | Nimbus |
| 13.6 | 13.9 | 13.3 | 13.7 |
| 14.1 | 14.8 | 14.5 | 14.6 |
| 14.4 | 14.8 | 14.3 | 14.5 |
| 14.5 | 14.6 | 13.9 | 14.0 |
| 14.7 | 14.9 | 14.7 | 14.7 |
| 14.8 | 15.2 | 14.5 | 15.9 |
| 15.8 | 16.3 | 15.6 | 16.5 |
| 16.3 | 16.5 | 15.9 | 16.5 |
| 16.6 | 16.5 | 16.5 | 16.3 |
| 16.7 | 17.3 | 16.7 | 17.2 |
| 16.8 | 17.0 | 16.6 | 16.8 |
| 17.3 | 17.6 | 17.0 | 17.5 |
| 17.4 | 17.7 | 16.8 | 17.5 |
| 17.9 | 19.3 | 17.8 | 19.0 |
| 17.9 | 19.0 | 18.0 | 19.0 |
| 18.4 | 19.0 | 18.4 | 18.8 |
| 19.6 | 19.4 | 19.3 | 19.6 |
| 20.2 | 21.0 | 20.0 | 20.7 |
| 20.8 | 21.7 | 21.1 | 22.0 |
| 24.2 | 24.5 | 24.0 | 25.3 |
| 24.5 | 25.4 | 24.5 | 24.8 |
| 24.9 | 25.2 | 24.6 | 24.8 |
| 27.0 | 27.6 | 26.6 | 27.3 |
| 27.6 | 28.0 | 26.8 | 27.7 |
| 28.0 | 29.0 | 27.8 | 29.0 |
| 29.6 | 29.7 | 29.2 | 29.5 |
| 30.7 | 31.3 | 30.2 | 30.7 |
| 31.5 | 32.0 | 30.7 | 31.4 |
| 32.6 | 32.5 | 32.0 | 32.3 |
| 34.0 | 35.1 | 33.1 | 34.5 |
| 35.6 | 35.9 | 34.8 | 36.3 |

**Fig. 2. Specificity and sensitivity of the optimized NIMBUS-based protocol.** (**A**) Workflow of the optimized NIMBUS-based protocol. Modifications included: removing the manual mixing steps in the BSC (He et al., 2017), performing all NIMBUS steps in 2.2 mL plates instead of 1.2 mL (1-8); removing the mixing step following addition of the wash buffer on NIMBUS (Klein et al., 2020), reducing the number of ethanol washes (Klein et al., 2020), and reducing the number of mixing in the elution step (7). The other changes that were implemented are summarized in Supplementary Figs. 2-3. The protocol is expected to capture host/cellular RNA and gDNA as well as viral RNA which constitute the viral genome for RNA viruses such as SARS-CoV-2. (**B**) Comparison of sensitivity of the NIMBUS protocol with that of the MagMax. One to five log dilutions of Flu-A virus stocks were spiked into Copan UTM and yield was measured via qRT-PCR. $p$ = 0.000015 (paired, two-tailed t-test; n=4 for each of the dilutions except 10^5 NIMBUS; n=3) error bars=standard deviations. (**C**) Assessment of cross-well contamination levels associated with the NIMBUS protocol. Checkerboard pattern was set-up with alternating wells of Flu-A virus that was spiked into Copan UTM and Copan UTM without Flu-A virus. UNT = undetected. (**D**) Comparison of the improved NIMBUS-based protocol with the MagMax protocol using Covid-19 samples. A heatmap on Ct values obtained from qRT-PCR measurements for both the *RDRP* and *E-gene* targets is shown. These data are from two independent experiments.

specificity). In contrast, the three extraction plates displayed 17, 13 and 12 false positive wells, representing 65 % specificity.

To improve specificity, we switched from 1.2 mL to 2.2 mL deep-well plates, reduced tip mixing steps and number of washes and optimized pipetting techniques to eliminate residual droplets. We wrote new code to eliminate extraneous vertical movement of the robot head between aspirate and dispense steps and to reduce the robot gantry's lateral speed to prevent dislodging of any residual droplets adhering to tips. The details of all the changes are described in **Supplementary Figs. 2 and 3.**

We tested the aggregate effects of all the changes in the optimized NIMBUS protocol (Fig. 2A) against the MagMax protocol using a Flu-A dilution series in Copan UTM. The optimized NIMBUS protocol demonstrated increased sensitivity compared to MagMax by an average of 0.98 Ct ($p$ = 0.000015; Fig. 2B).

Having established comparable sensitivity of our optimized NIMBUS protocol to that of the initial version of the NIMBUS and the MagMax protocols, we next tested its specificity. We performed three independent experiments using g-block checkerboards, each including two extraction-free control plates and two extraction test plates (total of 240 positive wells and negative wells). None of the blank wells had detectable NA and all wells with g-block DNA yielded expected Ct values indicating 100 % sensitivity and specificity. A Flu-A checkerboard was performed and again achieved 100 % sensitivity and specificity (Fig. 2C).

Final benchmarking with nasopharyngeal swabs samples for SARS-CoV-2 testing was performed to compare the NIMBUS to the MagMax protocol. Two independent experiments were run with 34 negative and 31 positive SARS-CoV-2 samples. Results show concordance between the protocols, each detecting 34 negatives and 31 positives. For the positive samples with Ct values that ranged from 13.8–36.8 (Fig. 2D), there was a strong correlation between the two protocols (R>0.996) (**Supplementary Fig. 4**). There were slightly higher Ct values (by a median of 0.43 Ct for *RDRP* and 0.50 Ct for *E-gene*) from the NIMBUS protocol ($p$=1.89E-08 and $p$ = 9.94-E08, respectively). Taken together, our analysis indicates that the optimized NIMBUS protocol yields 100 % specificity and sensitivity and comparable RNA yield, compared to the MagMax protocol used routinely at BCCDC PHL.

## 4. Conclusion

Generic nucleic-acid purification protocols provide an alternative reagent stream for SARS-CoV-2 testing but require optimization and customization to meet clinical sensitivity and specificity requirements. Here we provide a benchmarked SOP for one such protocol deployed on a Hamilton NIMBUS platform.

## Author_statement

Conceptualization: MH, MM, NP, MK; Formal analysis: SH, AMN, DDWT, SP, ES; Funding acquisition: MH, MM, NP, MK; Methodology: SH, PKP, AMN, DDWT, SP, RJC, AW, MW, DE, YZ, MM, QC, DES, JN; Project administration: LMP, JN, FT; Resources: ARM; Supervision: MH, MM, NP, MK, YZ, RJC, SJMJ, AJ; Visualization: MaK; Writing - original draft: SH, PKP, RJC; Writing - review & editing: MH, SH, MM, NP, MK,

AJM.

## Declaration of Competing Interest

MK has received grants/contracts from Roche, Hologic and Siemens paid to British Columbia Centre for Disease Control Public Health Laboratory unrelated to this work.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114339.

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