Detection of multiple respiratory pathogens in air samples collected from K-12 schools using Standard Biotools™ Respiratory Pathogen Panel Assay

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Abstract

Environmental surveillance is a more efficient approach compared to individual testing to determine pathogens present in congregate settings.

Challenges to detection of pathogens in environmental samples include:
- Limited amount of sample
- Less abundant genetic material
- Inhibitors present in samples that affect PCR efficiency
- Sequence diversity of target material may limit detection

Multi-pathogen detection assays can surveil for a large number of targets from a single sample, allowing for effective use of environmental samples.

We previously used a CRISPR/Cas13-based multi-pathogen assay (CARMEN) with air samples collected from K-12 schools.
- Frequency of SARS-CoV-2+ samples with CARMEN highly correlated with frequency of SARS-CoV-2+ samples in qPCR assays for each sampling period.
- High specificity of CRISPR target sequences limited the ability of CARMEN to detect influenza A compared to qPCR.

Hypothesis: A primer/probe-based multi-pathogen RT-PCR assay may improve detection of target genetic material, particularly targets with greater sequence diversity, compared to CRISPR-based assays.

Figure 1: Study Design, Sample processing, and Assay Workflows

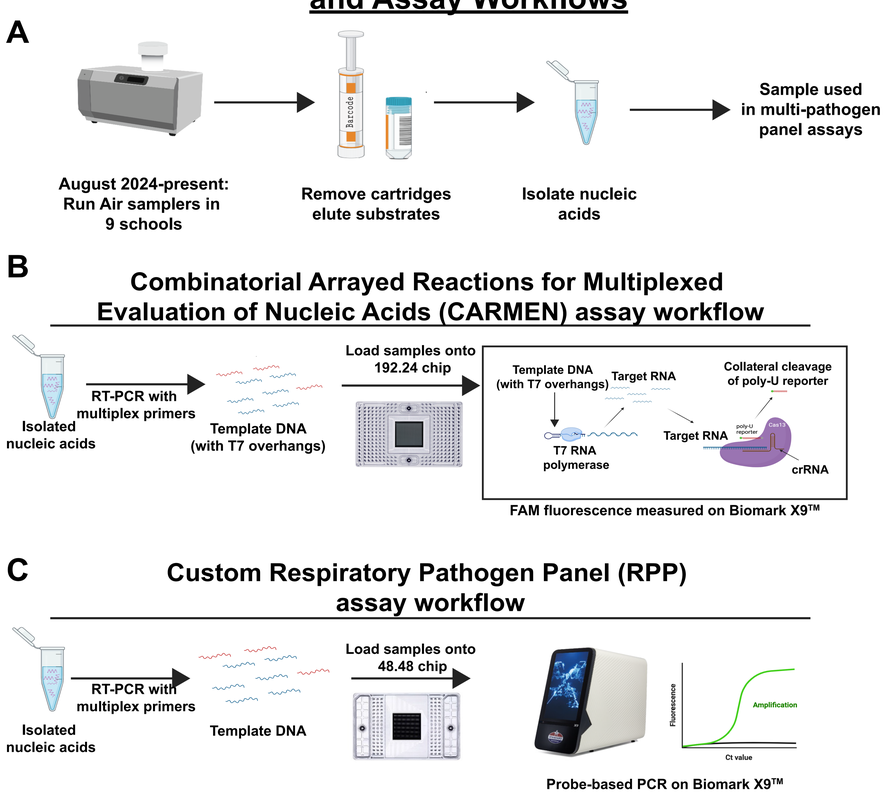


Figure 1. A, Air samplers were run continuously in 9 schools from August 2024 to present day. Every 3-5 days, cartridges were removed/exchanged and substrates were eluted in PBS. Nucleic acids were isolated and utilized in assays described in (B) and (C). B, Workflow for CARMEN assay and C, Workflow for RPP assay. RNA isolated from samples collected in Fig. 1A were run on both CARMEN (B), and RPP (C) assays. Both assays include an RT-PCR step where DNA is generated and loaded into either a 192.24 (CARMEN) or 48.48 (RPP) chip. Target detection is then performed using CRISPR/Cas13 (CARMEN) or primer/probe-based PCR assays. In both assays, FAM fluorescence is measured on the Biomark X9™ platform. For CARMEN, endpoint FAM fluorescence from cleavage of the poly-U reporter is the primary readout, which occurs in individual reaction chambers for each sample/CRISPR target. For RPP, target-specific primer/probe-based PCR assays in individual reaction chambers are performed and FAM fluorescence is measured during each PCR cycle to generate a Ct value for each target for each sample.

Targets for CARMEN and RPP assays

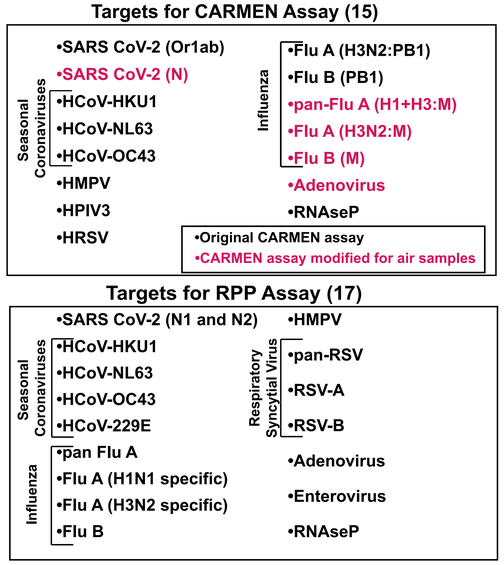


Figure 2: RPP and qPCR air sample results agree more frequently for SARS-CoV-2 than CARMEN and qPCR

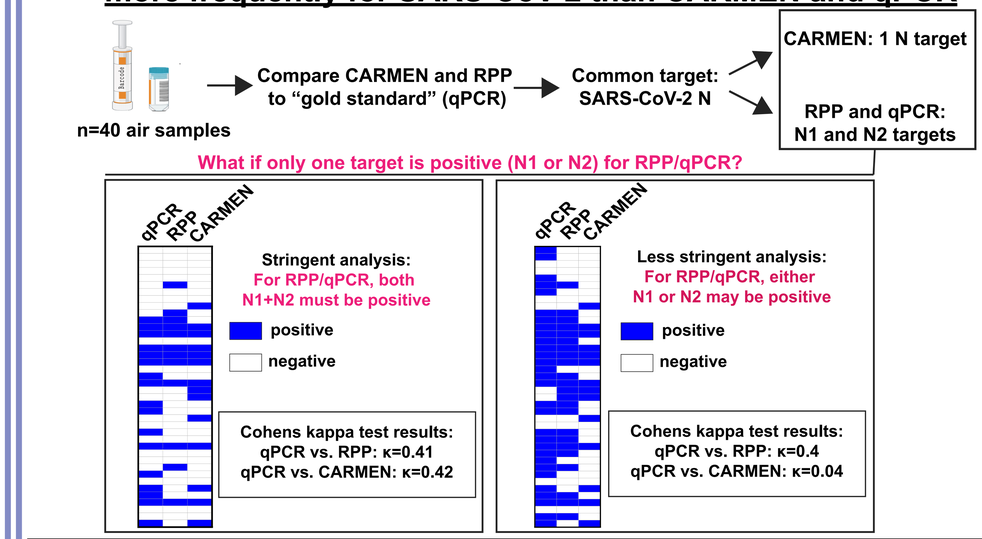
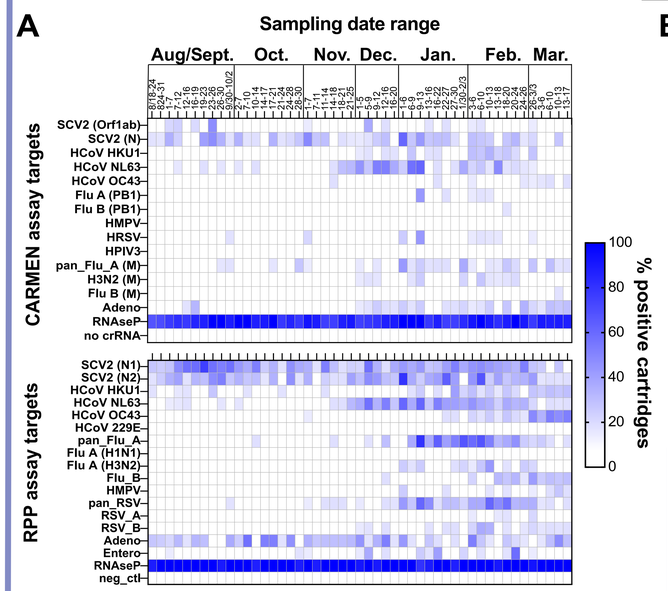


Figure 2. RNA was isolated from 40 air samples collected during the 2023/2024 school year and CARMEN, RPP, and quantitative RT-PCR (qPCR) were performed on all samples. Results obtained for SARS-CoV-2 N was compared for each sample for each assay. For the RPP and qPCR assays, sometimes only N1 or N2 targets were positive. When performing the cohens kappa analysis, we performed two separate analyses. In a stringent analysis, both N1 and N2 had to be positive for the RPP and qPCR assays to be called "true positive" (left). In a less stringent analysis, either N1 or N2 had to be positive for the RPP and qPCR assays to be called positive.

Figure 3: RPP assay detects similar pathogens to CARMEN but at greater frequencies in 2024/2025 air samples



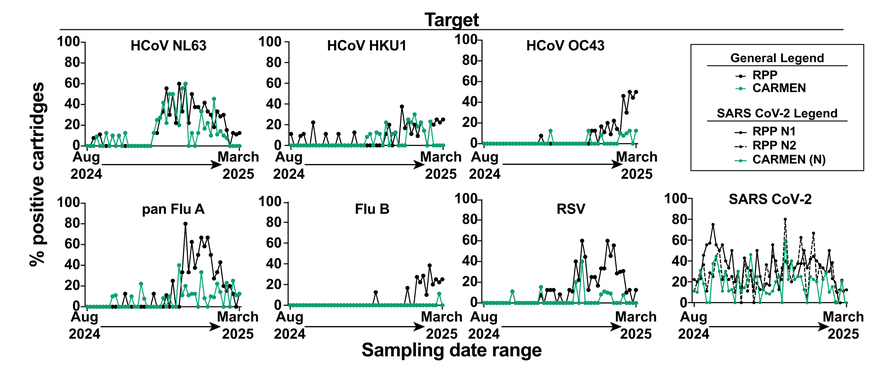


Figure 3. A, CARMEN and RPP assays were performed on RNA isolated from air samples from 9 schools during the indicated sampling date ranges. The percent of cartridges positive during each sampling date range for each indicated pathogen were calculated. The data is displayed as a heatmap where darker blue indicates a higher frequency of cartridges positive for the indicated target, and lighter blue indicates a lower frequency of cartridges positive for the indicated target. B, Line graphs showing the frequency of samples positive for the targets shared by both CARMEN (green lines) and RPP (black lines) over the sampling date range indicated in figure (A).

Conclusions:

- RPP assay detects SARS-CoV-2 similarly to single-plex qPCR
- RPP assay detects similar pathogens in air samples as CARMEN but at greater frequencies

Future Directions:

- Add and test H5N1 target to RPP for air samples
- Add other seasonal pathogens (Ex. HPIV1-4, Rhinovirus)
- Improve sensitivity for some targets by including multiple primers/probes per assay for a single target

Acknowledgements:

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