

# Analysis of Viral RNA Concentration from Dormitory Air Sampling

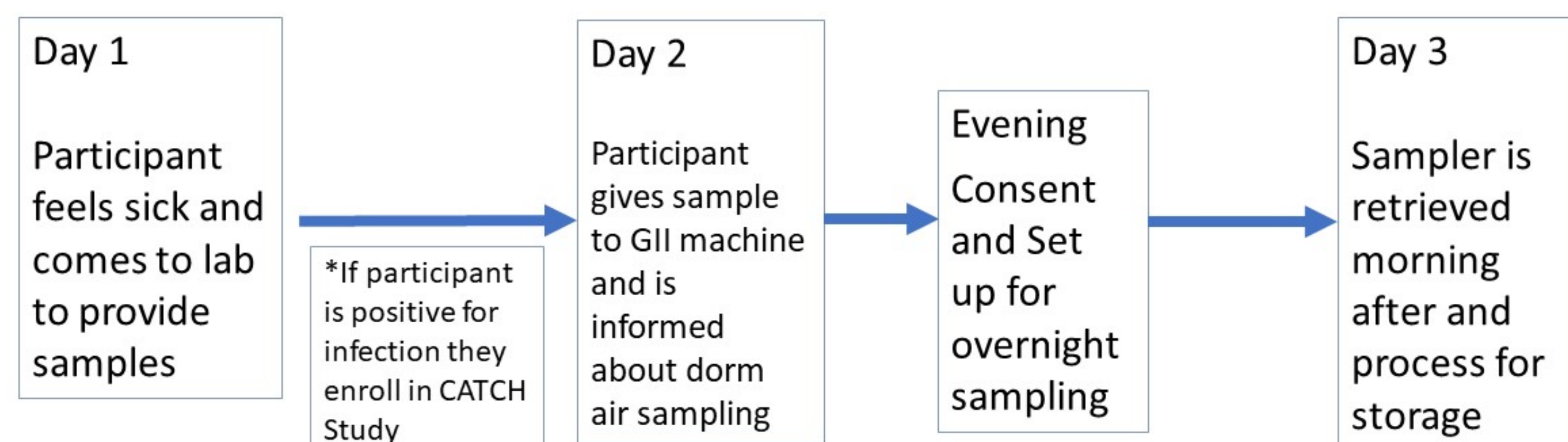
Razeen Khan, P. Jacob Bueno de Mesquita, Donald Milton, and the Prometheus-UMD Consortium

## Overview

- Quantifying airborne risk of respiratory infection transmission is important for quelling pandemics and seasonal outbreaks.
- Measurements of virus shed into exhaled breath from infected people provide helpful estimates of exposure in indoor spaces.
- We asked: do predicted dormitory room exposures based on exhaled breath viral shedding and a rebreathed-air equation correspond with viral quantities directly measured dormitory room sampling?
- Asking such a question helps validate indoor air viral exposure assessment given knowledge of exhaled breath viral shedding and the rebreathed-air equation.

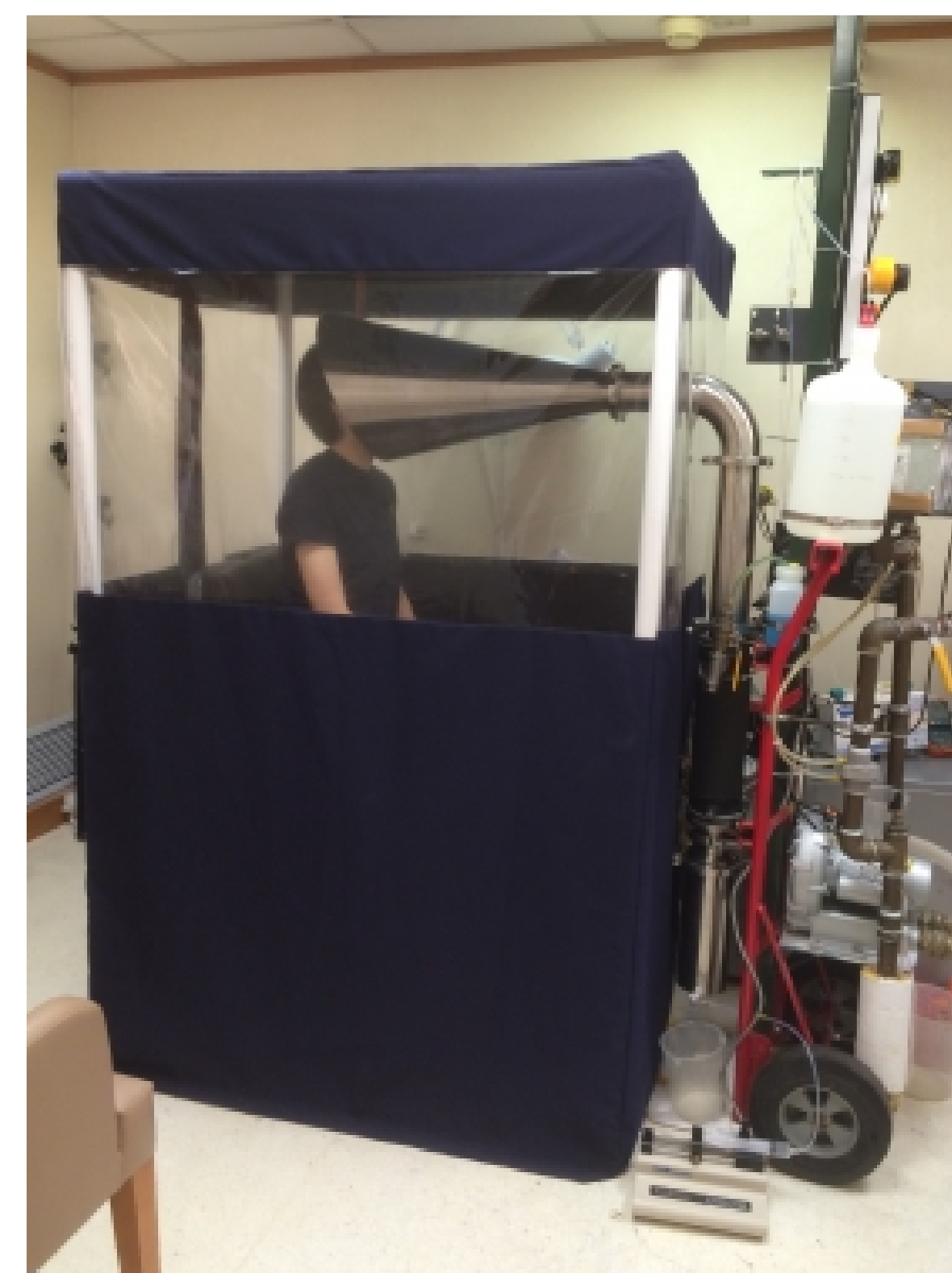
## Methods

### Dorm Sampling Process

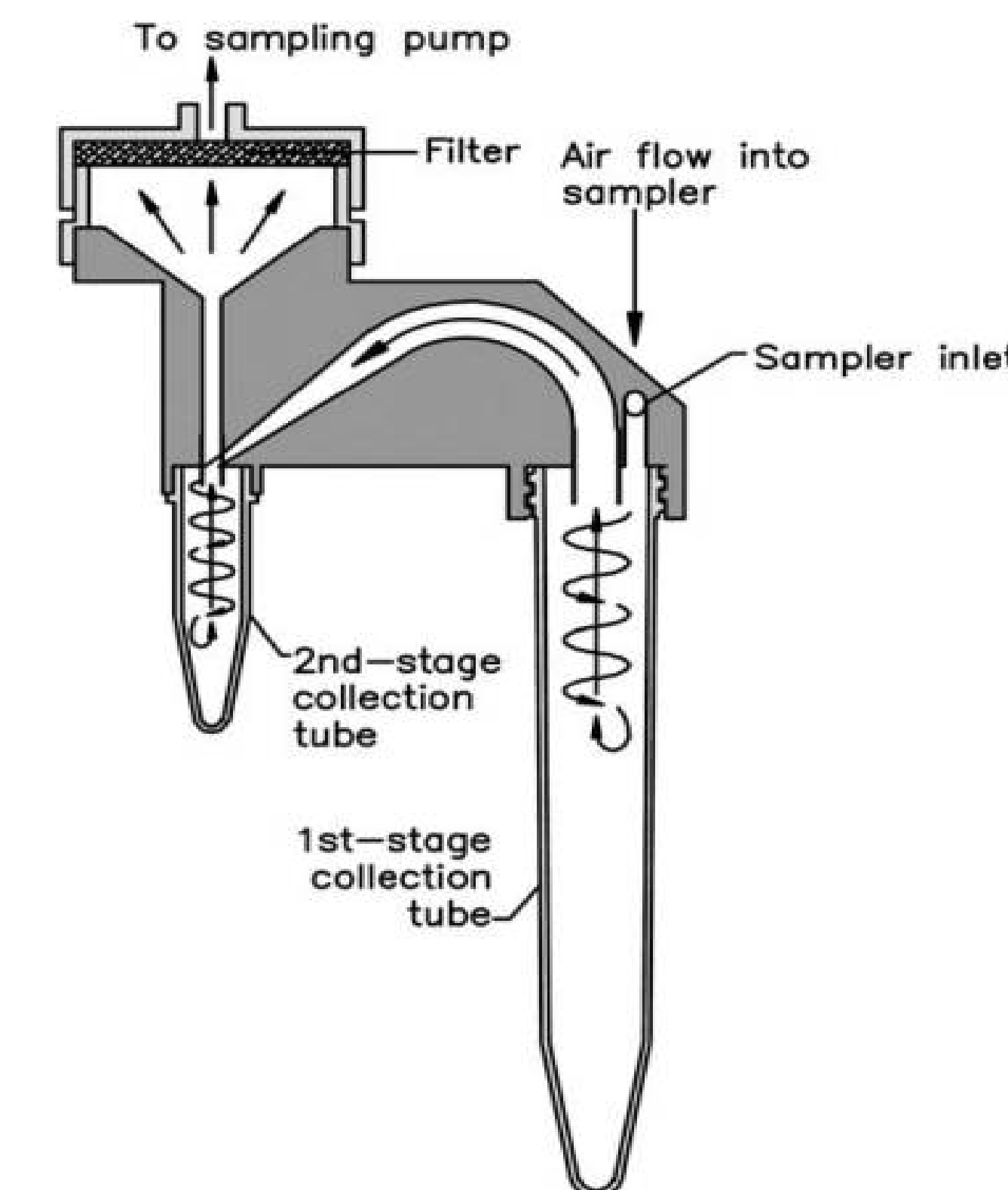


- NIOSH bioaerosol samplers collected air for 6 hours overnight in dorm rooms of those with lab-confirmed respiratory infection.
- Dorm air of those with confirmed Influenza A infection extracted and evaluated by qRT-PCR:
  - Nucleic acid extraction performed with Qiagen© MinElute Virus miniprep using automated Qiacubes.
  - Single well qRT-PCR performed on 96-well plate with primers, probes, and polymerase from ThermoFisher©.
  - Standard curve used influenza A reference strain quantified under electron microscope and extracted with samples.

## Gesundheit-II (G-II) Sampling (quantifies exhaled breath viral shedding)



## NIOSH Bioaerosol Sampler (quantifies room air viral concentrations)



## Are dorm air viral concentrations consistent with what might be expected based on viral aerosol source generation?

$$r = \frac{\bar{f} t V}{n} * (\% \text{ human rebreathed fraction entering NIOSH sampler})$$

Variable	Meaning	Value used in equation
f_bar	(CO2_indoors – CO2_outdoors)/ concentration of CO2 in exhaled breath  (1492ppm – 450ppm)/ 38,000ppm  (Values taken from dormitory surveillance reported in Zhu et al., 2020)	0.0274215
t	Time of sample collection	6 hr
V	Viral shedding detected in G2 machine from infected study participant (note that G-II samples were undetectable for influenza A virus). • If assume shedding at LOD, then we would have 500 virus particles/sample/hr	500 vp/hr
% human rebreathed fraction entering NIOSH sampler	NIOSH sampler intakes air at 3.5 L/s Pulmonary ventilation rate at rest for human averages 8 L/s. We assume that the sampler is exposed to a fraction of the rebreathed fraction that a human inhaling room air = 3.5/8	44%
n	Number of people in the dorm room sharing air	2

Original rebreathed-air equation described in Rudnick and Milton, 2003 and modified to use G-II aerosol shedding data in Bueno de Mesquita et al., 2020.

Predicted NIOSH Sampler Detection = 18 viral particles assuming LOD for G-II fine aerosol shedding

## Results

N=19 confirmed respiratory infection cases whose dorm room air was sampled for virus

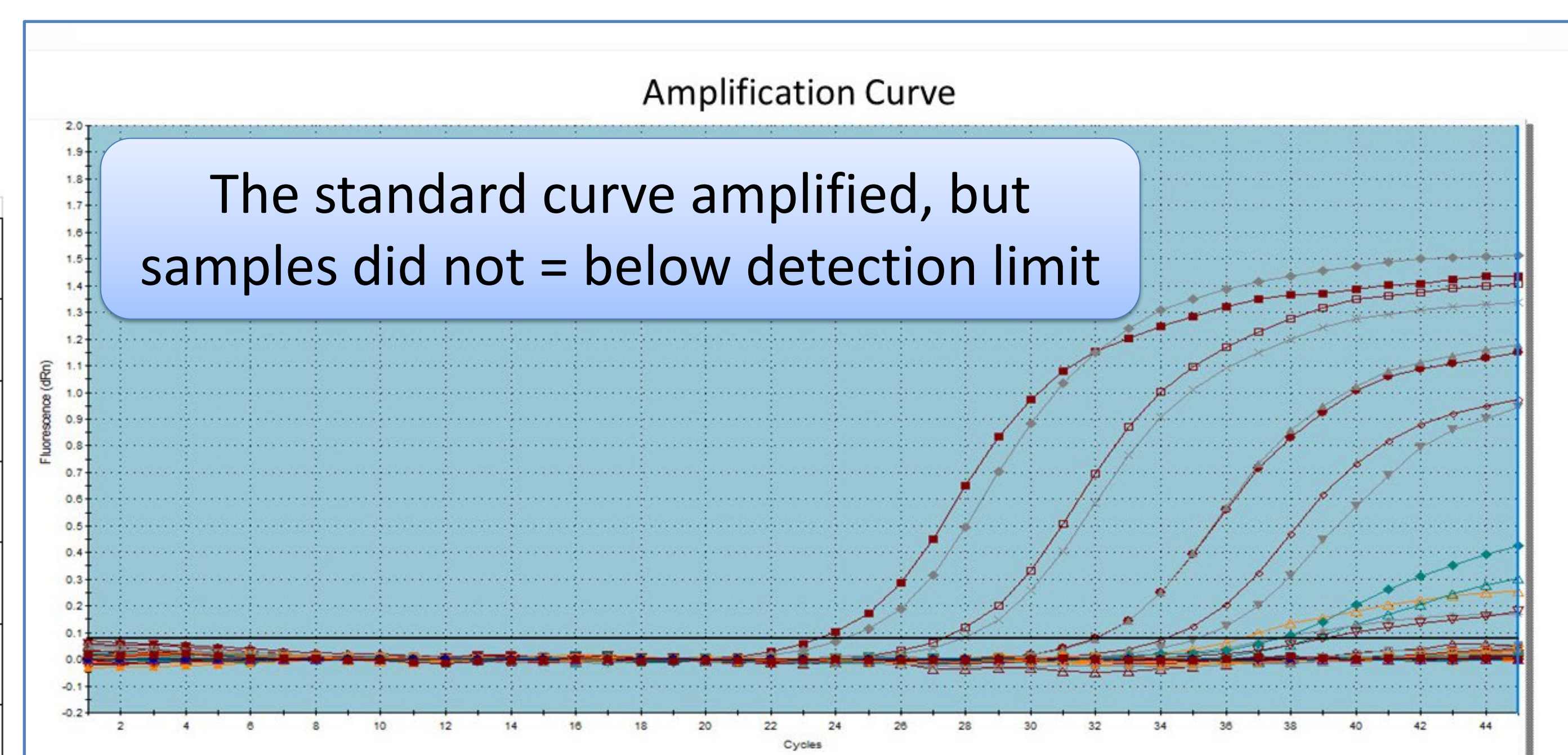
## Study Participation

Dorms and Apartments	Number of Cases	Types of Infections	Number of Samples
Baltimore	1	RSV Variants	2
Cambridge	3	COV Variants	7
Centreville	4	Flu A	3
Cumberland	4	H-Influenzae	1
View 1	1	Rhinovirus	1
View 2	1	hMPV	2
Chestertown	2	Unk.	3
Talbot	1		
Somerset	1		
Worcester	1		

### PCR Plate Set-Up

- Extractions from stage 1, 2 and filter samples from each study ID
  - Three replicates of each
- Flu A control dilutions
  - Two replicates
- NTC-EC
  - Water to control for contamination during:
    - Extraction of flu control
    - Extraction of samples
    - PCR plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	4E+6_PR8vp	4E+6_PR8vp				215_4um_234032	215_4um_234032	215_4um_234032		702_4um_237372	702_4um_237372	702_4um_237372
B	4E+5_PR8vp	4E+5_PR8vp				215_1um_234029	215_1um_234029	215_1um_234029		702_1um_237369	702_1um_237369	702_1um_237369
C	4E+4_PR8vp	4E+4_PR8vp				215_<1um_234027	215_<1um_234027	215_<1um_234027		702_<1um_237367	702_<1um_237367	702_<1um_237367
D	4E+3_PR8vp	4E+3_PR8vp										
E	4E+2_PR8vp	4E+2_PR8vp				NTC-EC	NTC-EC			2090_4um_237364	2090_4um_237364	2090_4um_237364
F	4E+1_PR8vp	4E+1_PR8vp								2090_1um_237361	2090_1um_237361	2090_1um_237361
G	NTC-EC	NTC-EC								2090_<1um_237359	2090_<1um_237359	2090_<1um_237359
H	NTC-Molec_grade_H2O	NTC-Molec_grade_H2O										



Aerosol samples could have no virus in them or viral quantities up to the LOD

## Discussion

- The viral particle quantities expected to be collected in the NIOSH samplers were consistent with the predictions based on source aerosol quantities and the rebreathed-air equation.
  - Efforts to evaluate the correspondence between predicted and sampled air viral levels are limited by uncertainty due to lack of detection in both exhaled breath and room air samples.
  - Extending this project to collect more dorm room air samples and specifically testing the samples from participants known to be shedding high quantities of detectable virus could provide additional insights into indoor air viral aerosol exposure estimates given source strength data.

### References:

Bueno de Mesquita, P.J., Noakes, C.J., Milton, D.(unpublished). Influenza transmission trial aerobiology.  
Rudnick, S. N., & Milton, D. K. (2003). Risk of indoor airborne infection transmission estimated from carbon dioxide concentration. *Indoor Air*, 13(3), 237–245.  
Zhu, S., Jenkins, S., Addo, K., Heidarinejad, M., Romo, S. A., Layne, A., Ehizibolo, J., Dalgo, D., Mattise, N. W., Hong, F., Adeniyi, O. O., Bueno de Mesquita, J. P., Albert, B. J., Washington-Lewis, R., German, J., Tai, S., Youssefi, S., Milton, D. K., & Srebric, J. (2020). Ventilation and laboratory confirmed acute respiratory infection (ARI) rates in college residence halls in College Park, Maryland. *Environment International*, 137, 105537. <https://doi.org/10.1016/j.envint.2020.105537>

### Funding:

Prometheus-UMD was sponsored by the Defense Advanced Research Projects Agency (DARPA) BTO under the auspices of Col. Matthew Hepburn through agreement N66001-17-2-4023 and N66001-18-2-4015. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position or policy of the funding agency and no official endorsement should be inferred.

**Conflicts of Interest:** The authors have no conflicts of interest to disclose.