COVID Screening Platform
#WindsorStrong

Phase 1 Final Report

Date of Study: March 8, 2021 – April 30, 2021

GOALS OF PHASE 1:

- ★ Set up the regulatory processes and initial protocols to permit saliva screening on campus
- ★ Begin the optimization of protocols to reduce the time and cost for testing a large population
- ★ Gather data to improve compliance in moving forward with a larger population

Regulatory processes put in place:

- Research ethics board (REB) approvals are in place, and contacts are made to provide rapid analysis of next steps as the study evolves.
- Research safety (RSC) protocols are in place and contacts made to provide evolution of these protocols as we expand to other sites.

Onboarding participants and data reporting mechanisms established:

- Mobile app (MyCap) that logs data for de-identified individual participant records within a REDCap study and communicates with individual participants has been developed, implemented, and put into place for phase 1 study.
- Swab site was set up, site requirements written out for transfer to other sites and participant satisfaction measured.
- 8 weeks of a study ran with a small participant study. Compliance and overall attitudes measured over the study period.

Training of personnel:

- A study manager trained on REDCap, necessary regulatory components, swab methods and laboratory methods. Study manager developed reporting metrics.
- 1 part-time lab technician (paid for out of Porter Lab grant) trained to aid in running of samples.
- 2 undergraduate volunteers trained to man testing site.
- 2 MSc level students onboard to begin a dashboard for reporting to broader community and trained to handle data coming from study

COVID Screening Platform
#WindsorStrong

OPTIMIZATIONS OF PHASE 1 PROTOCOLS

Sample collection protocol:

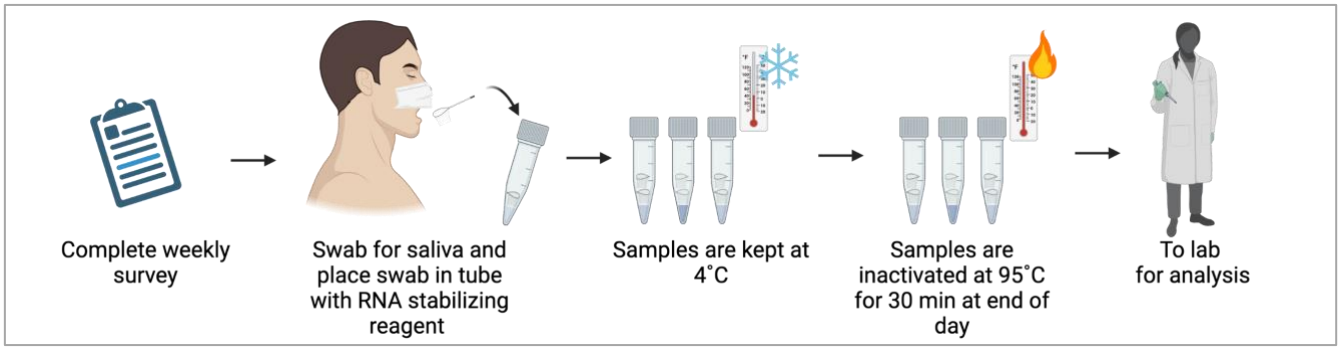


Figure 1 Overview of sample collection protocol for phase 1

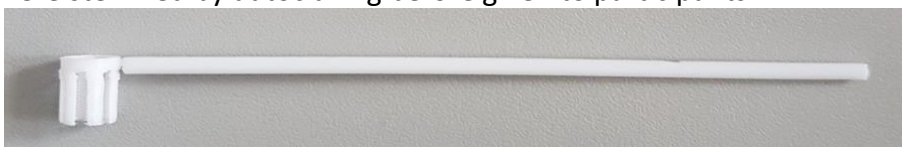
- SMR developed and provided 3D printed poly-lactic acid swabs for phase 1 of the study. The swabs were sterilized by autoclaving before given to participants
  - 

  Figure 2 SMR-designed 3D printed saliva swabs for sample collection

- The volume of saliva sample obtained from the swabs was the very minimum needed to test the samples one time and required us to recall individuals for repeat testing
  - Heat inactivation of the sample for 30 minutes contributed to the small volume of sample
  - There was no consistency in the volume of saliva between participants
- SMR designed another swab and manufactured using injection molding made with polystyrene.
  - There was continued difficulty with saliva collection and logistics of polystyrene sterilization, we will not be moving forward with these swabs.
- The protocol above will be the same for phase 2, but transfer pipets will be used to collect saliva from the participants' mouths to the tube.
  - This will allow us to control the volume of sample from each participant

COVID Screening
#WindsorStrong Platform

Laboratory protocol progress:

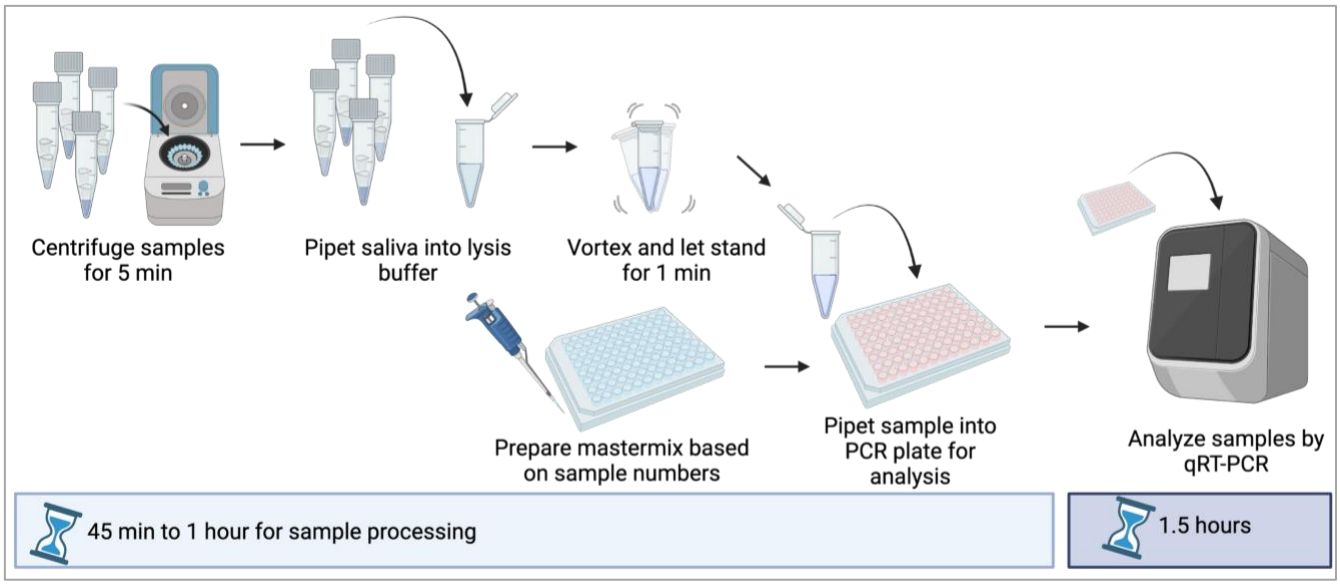


Figure 3 Direct lysis protocol overview used for phase 1

Small sample size has enabled us to test both individual and pooling of samples for participants, as well as running several optimizations within the lab. **Direct lysis protocol** developed by SMR was used throughout the 8 weeks to analyze participant samples. We used the CDC primers for detecting the N gene (N1) which is conserved for variants of concerns (VOC) and will be able to detect VOCs.

The individual testing protocol with the most reliable RNase inhibitor (Lucigen RiboGuard™ RNase Inhibitor) currently costs **$6.16** per sample and takes ~3 hours total to collect samples, run and process data. The new RNase inhibitor that we have tested (Invitrogen™ RNA*secure*™ RNase Inactivation Reagent), our costs for Phase 2 will be **$4.86** per sample for individual testing.

Reproducibility of signal tested with controls (viral like particles: VLPs) in buffer and in saliva and stored at 4˚C for 6 hours or processed immediately.

- In buffer: Method is sensitive and reliable for all controls
- In saliva: variability in sensitivity – likely due to the different composition of individual saliva samples and the ability to stabilize the viral RNA.
- Storage time of the controls in buffer and in saliva did not significantly impact signal

Methods to stabilize viral RNA while samples are heat inactivated at swab sites were tested.

- Heating to 95 degrees for 30 minutes without RNase inhibitors in the sample results in degradation of viral RNA during heating
- The following is a table of reagents tested to stabilize the viral RNA.
    - Different heating times were also tested with the various reagents

COVID Screening Platform
#WindsorStrong

Reagents Tested For Sample Stability	Cost per 50 µL of saliva sample	Overall Success for Controls (5 star)
Lucigen RiboGuard™ RNase Inhibitor ** ($341 for 250 µL)	Use 1 µL per sample $1.36	★★★★ Heat for 30 min
Lucigen NxGen® RNase Inhibitor ($152 for 250 µL)	Use 1 µL per sample $0.61	★★
Zymo DNA/RNA Shield™ ($371 for 250 mL) - Needs RNA extraction step before qRT-PCR - Well proven to maintain sample stability at room temp and 37˚C	Use 50 µL per sample $0.0742	Very well proven to maintain sample stability but cannot go direct to PCR
Zymo DNA/RNA Shield™ Direct (New Product, will be priced similar to the original)	Use 50 µL per sample $0.0742	Did not work at all
Invitrogen™ RNA*secure* ™ RNase Inactivation Reagent ($259 for 10 mL)	Use 2.5 µL per sample $0.0648	★★★★ Heat for 20 min

** Lucigen RiboGuard™ RNase inhibitor was used for all the participant samples in the study.

Reproducibility of signal between participants saliva samples using a process control. Samples were analyzed with a gene common in saliva and the N1 gene of SARS-CoV-2

- Signal detection of the process control (for direct lysis)

COVID Screening #WindsorStrong Platform

- Pooling of up to 4 samples was tested for sensitivity by direct lysis protocol
  - Sensitivity decreases dramatically with pooling.
  - SMR has demonstrated sensitivity in pooling up to 8 samples; to date we have not replicated this. Could be due to RNA stabilization and the buffers being used to break up saliva samples.
- Pooling for up to 4 samples has been tested using TRIzol RNA extraction
  - Works well but will not be compatible for automation
- Methods to incorporate RNA extraction are being tested to increase number of samples being pooled.

Data from phase 1 participants:

Metrics for the Study	Study Population	Total # of Samples Collected	Average # of Samples Collected Per Week	Average PCR Reactions Per Week *	Cost of Analysis per Sample **
	30	159	19.9	98.5	$6.16

* Two reactions per sample, sample pools, and controls
** Cost of Analysis = $1.36 for RNase inhibitor + ($2.40 x 2) for PCR reaction

Suspected Positives in the Study	Suspected Positive Pools from Screening	Re-test of Suspected Individuals	Positives from Suspected individuals	Clinically Diagnosed Positives
	1	2	0	0

Details on suspected positive case:

One participant sample would sporadically report low level signal (high Ct values – 37-40), this was noted for one other participant sample as well but not as consistently. We investigated the sample repeatedly reporting with low level signaling by recalling the individual for repeat testing after a noted potential positive result.

The individual was already on campus when they read the notification. They contacted the research study manager, Jackie Fong, to provide a saliva sample for individual testing and then left the campus to self-isolate, notified their supervisor and obtained a diagnostic test. Another individual who was a close contact also provided a sample as they felt unwell the night before and wanted to confirm their status. The samples from these individuals were analyzed with the saliva study in the afternoon and both tests came up negative. The clinical diagnostic test was also negative.

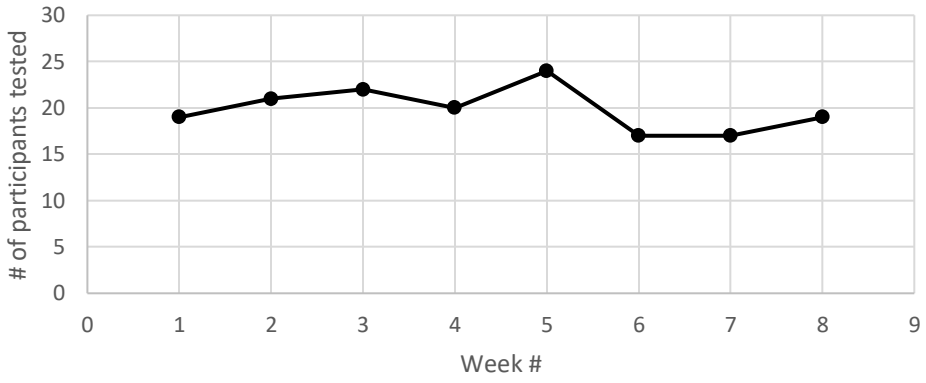
It is notable that this individual wanted to report to the study that they had previously had a COVID diagnosis back in March 2020 and was cleared to return to work by Public Health. The other participant with sporadic positives also self-identified themselves and wanted the study to know that they too had a previous COVID positive result and has been a confirmed ‘recovered’ for several

months. Public health sets a Ct baseline of 35, likely due to this low level of viral shedding that remains in previous COVID positive individuals.

We propose to include whether individuals have had a COVID diagnosis as a portion of the testing questions to be incorporated into Phase 2. We propose that we set our Ct cut off to reflect that of public health, but would like discussions with the University on this.

Participant attendance per week:

Attendance was consistent, ranging from 17 to a maximum of 24 participants. The stay-at-home order was enacted on April 8, with far stricter measures in effect on April 17. The drop in attendance for Week 6 (April 12 – 16) can be attributed to the stay-at-home order.

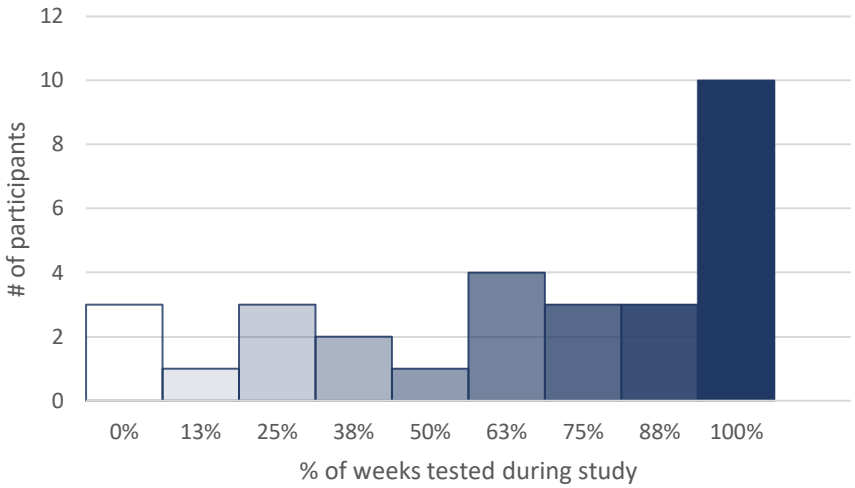


Distribution of percentage of weeks tested during study:

We began the study with 24 participants, but participants reached out during the study and we accrued 'on the fly' recording this data as we went along. There were two participants enrolled, consented, but did not arrive at the testing site for testing. One other participant enrolled, signed the consent form but withdrew from the study before they were tested.

Overall, two-thirds of the participants were tested for 4 weeks or more (50% of the study period). 10 participants provided samples for all 8 weeks of the study.

COVID Screening Platform
#WindsorStrong



Given that this group was researchers who do not have to be on campus every week, and the changing provincial restrictions mid-study 2/3 reporting each week is an encouraging metric.

PSYCHOLOGICAL ANALYSIS OF SURVEY DATA:

During the study, participants were asked to complete a 40-question survey on the MyCap app before providing their saliva sample for testing. The main categories the survey focused on was:

- Personal Safety Habits
- Risk Perception
- Beliefs about Testing
- Testing Site Specifics and Perceptions
- Barriers at the Testing Site
- Specifics of the Procedure
- Whether they felt their participation was voluntary.

At the end of the study, respondents were given an end survey to summarize their final thoughts about Phase 1 and provide feedback. Fifteen participants responded. **Below is an overall summary of the results of both surveys.**

COVID Screening Platform

#WindsorStrong

Thoughts about weekly COVID-19 testing

Do you feel your participation was voluntary?

100% said YES

Are you using the SAFE LANCER App?

100% said YES

Are you following safety requirements on campus?

100% said YES

Feelings about COVID-19

% of participants who agree or strongly agree ...

"I think I am vulnerable to COVID-19." 30%

"I think the university is vulnerable to COVID-19." 78%

"I worry about the number of people infected with COVID-19 on campus". 67%

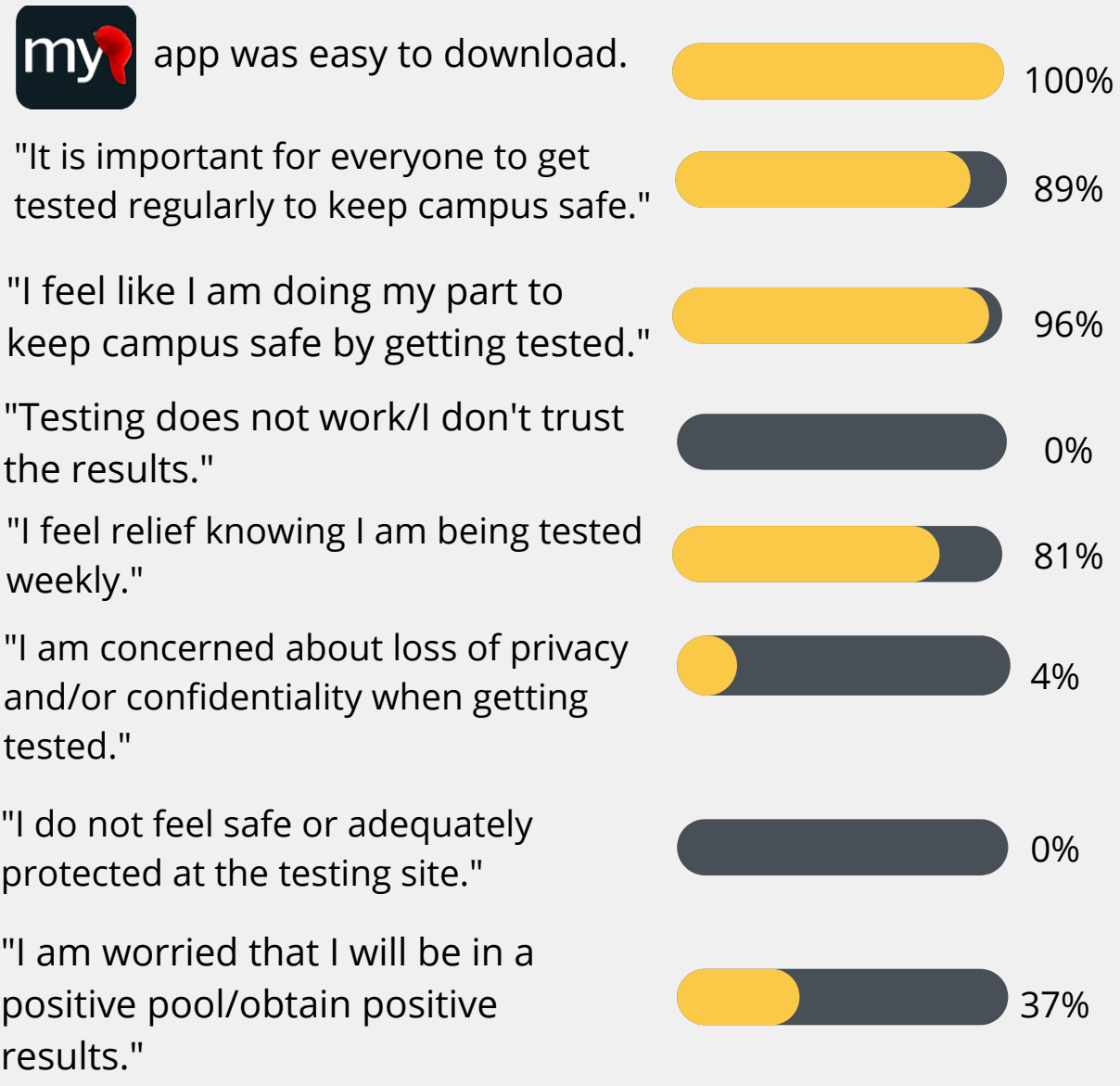
"COVID-19 is a serious disease." 96%

COVID Screening Platform

#WindsorStrong

Thoughts about Phase 1 of Screening Testing

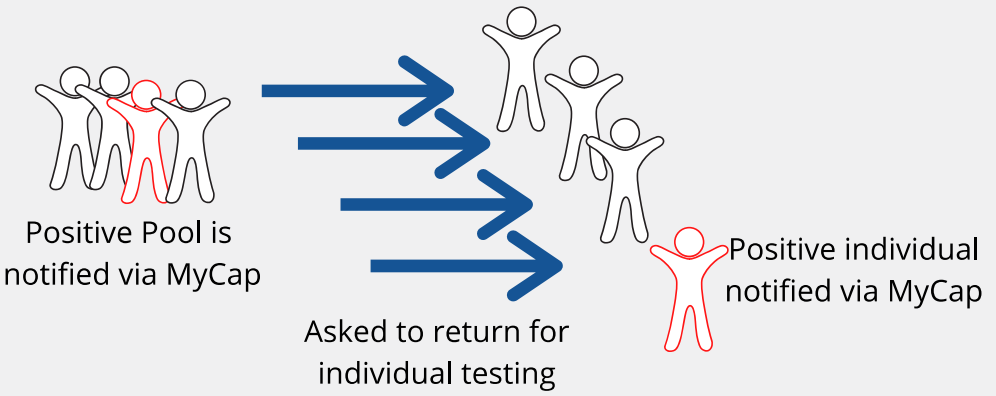
% of participants who agree or strongly agree ...



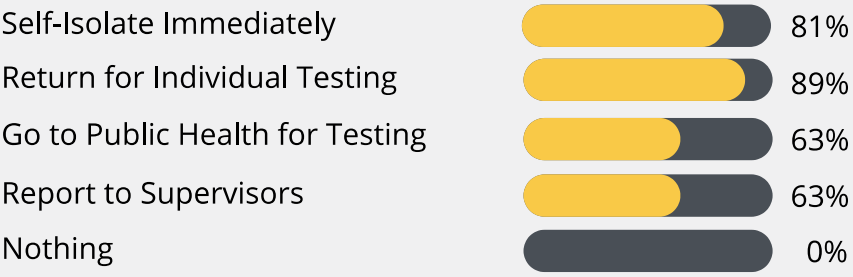
COVID Screening Platform

#WindsorStrong

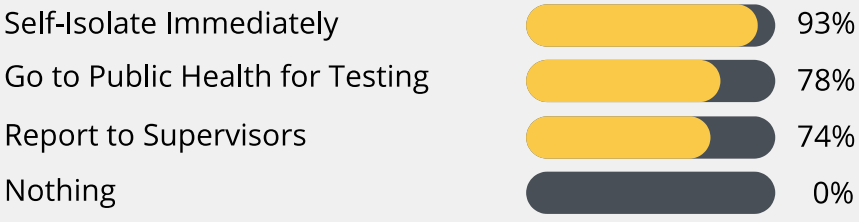
What will they do if they test positive?



What will you do if you are in a positive pool?



What will you do if you test positive individually?



COVID Screening Platform

#WindsorStrong

General Feedback

"Emphasize why it's important to get tested so that people understand the reasons behind why they are doing it weekly"

"Screeners were helpful in directing us throughout Phase 1, I liked how the time slots were open for testing"

"Will need to motivate students who see the case counts and hospitalization are low"

"Receiving notifications from MyCap were stressful. I know that my results will come back within 24 hours, so I don't see the value in letting people know the samples were analyzed "

"I liked the notifications that the samples had been processed"

"Fitting the limited time slots [for sample collection] into my schedule was difficult some weeks"

"I thought it went very well from a logistical standpoint! People could see the [swab site opening] times restrictive depending on the period of time needed to come in each day!"

COVID Screening Platform
#WindsorStrong

CONCLUSIONS:

We have set up the processes necessary to move forward to phase 2 of testing.

Laboratory methods are established for individual testing, sensitivity of pooling needs to be further optimized.

> Current cost and time for individual testing is $6.16 and 3 hours. Replacing the stabilizing reagent with another alternative brings the cost down to $4.86 ($4.80 for mastermix, $0.06 for stabilizing reagent). This is a labour-intensive process and would require significantly more lab technicians to continue running individually. It is our conclusion that we need to continue to optimize pooling.

Participants expressed relief being tested weekly. Participants appreciate confirmation that they do not have COVID-19. Participants know if they test positive they will prevent asymptomatic spread.

> Participants did note “notification anxiety” when receiving a MyCap message, “you have a secure message waiting”. Expressing worry that they were being notified that they were in a positive pool.
>
> Participants felt that the weekly survey was too long and repetitive. Repeating the questions each week was not beneficial to the study as the participants felt that their answers would not change.

NEXT STEPS:

To increase capacity the testing lab needs to move to a new site being prepared by faculty of Science. We also need to onboard more individuals to man screening stations as well as a full-time PhD level person to aid in the optimization of pooling and running of the samples.

Due to current lockdowns, and delays in the new screening lab, onboarding phase 2 will occur weeks of May 17 with anticipated start date of May 31.

We will expand to include our Lancer sports teams and more researchers within Faculty of Science. This will involve educating a larger group about the study, providing access to Essex-CORe building and the set-up of a testing site at the St Denis Centre.

We will automate our MyCap sign up process to eliminate the need for us to send individual emails for recruitment to the study.

We will continue the development and launch of the dashboard and reporting metrics.

Using participants feedback, we will provide an onboarding survey, a very short weekly survey, and an end survey. We will incorporate questions about previous COVID-19 status, vaccination and some general participant demographics that were not collected in the first phase. We will also be looking to create incentives for participants for weekly testing, as a decrease in voluntary participation is a significant issue experienced by US universities with similar programs.