

AWARD NUMBER: W81XWH-19-1-0288

TITLE: Deployable Interstitial Cystitis Urine Diagnostic Technology Development

PRINCIPAL INVESTIGATOR: Laura Lamb, PhD

CONTRACTING ORGANIZATION: William Beaumont Hospital Research Institute

REPORT DATE: August 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT This proposal directly focuses on the PRMRP Topic Area of Interstitial Cystitis (IC), including the Area of Encouragement for the identification of biological markers for making a definitive diagnosis of IC. This is not a clinical trial as there is no intervention. The <i>objective</i> of this grant is to develop a new diagnostic tool, the Interstitial Cystitis Risk Score (IC-RS), to identify and classify IC patients. In year 2, we started both online and clinical collection with all sites; we currently have 1705 samples collected. Although we encountered delays in our research due to the COVID-19 pandemic, we do not expect any further delays and are on track to complete all the aims of this grant. Indeed, we are very proud of all that was accomplished given this unprecedented pandemic including Dr. Lamb was the recipient of the 2021 COVID Pandemic-related Outstanding Research Accomplishment (Individual) to be awarded at the Military Health System Research Symposium in August, 2021.					
15. SUBJECT TERMS Interstitial Cystitis; Bladder Pain Syndrome; Diagnostic Test; Machine Learning; Cystitis, Interstitial; Pelvic pain; Lower Urinary Tract Symptoms; Pelvic Floor Disorders; Biomarkers					
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1. INTRODUCTION:

The subject of this research is to improve diagnosis of Interstitial Cystitis (IC), a devastating disease of pain and altered urinary function that is challenging to diagnose. The purpose of this research is to develop a simple test for diagnosing IC based on urine and symptom scores called the Interstitial Cystitis Risk Score (IC-RS). Our scope of this research includes: 1) Refining and evaluating IC-RS in a large, crowdsourced collection of urine samples from IC patients and normal controls; 2) Comparing patient collected IC-RS from similar collected urine samples and demographic from three academic urology practices that will test civilian and military patients with and without IC and also cohorts of patients with UTI, bladder cancer, and overactive bladder with incontinence to assess specificity of the IC-RS; and 3) Urine kit optimization.

2. KEYWORDS:

Interstitial Cystitis; Bladder Pain Syndrome; Diagnostic Test; Machine Learning; Cystitis, Interstitial; Pelvic pain; Lower Urinary Tract Symptoms; Pelvic Floor Disorders; Biomarkers

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Refine and evaluate IC-RS in a large, crowdsourced collection of urine samples from IC patients and normal controls.

Major Task 1: Study Start Up

- Target Date: 6 months
- Milestones Achieved: Local IRB and HRPO Approval; Trained research team; Study logistics determined; Online tools and urine collection kits ready
- Completed, all milestones achieved by 11 months

Major Task 2: Strategy for Participant recruitment, shipping & returning of collection kits

- Target Date: 28 months
- Milestones: Study begins; 1000 IC, 1000 non-IC human urine samples recruited and processed; Evaluation of shipping kits and recruitment strategies
- Year 2 actual: We have shipped 1000 IC and 1000 non-IC human urine sample collection kits 564 IC and 699 non-IC human urine samples have been collected and processed.
- 85% completed

Aim 1b. Refine and evaluate IC-RS in urine samples from men and women with IC and from normal controls within the military.

Major Task 3: Participant recruitment, shipping & returning of collection kits targeting military personnel.

- Target Date: 28 months
- Milestones: Study begins; 100 urine samples recruited and evaluated; Evaluation and adjustment of shipping kits and recruitment strategies for military personnel (including veterans).
- Year 2 actual: 99 urine samples collected and processed
- 99% completed

Major Task 4: Data Acquisition and Analysis

- Target Date: 34 months
- Milestone: Report and review results from data analyses
- Year 2 Actual: Data acquisition is completed on 90% of collected samples; data analysis is under way
- 75% completed

Specific Aim 2: Compare patient collected IC-RS from similar collected urine samples and demographic from three academic urology practices that will test civilian and military patients with and without IC to assess specificity of the IC-RS.

Major Task 1: Study start-up

- Target Date: 6 months
- Milestones: Local IRBs and HRPO Approvals; Trained research teams; Study logistics determined.
- Completed, all milestones achieved by 10 months

Major Task 2: Recruitment and Collection

- Target Date: 28 months
- Milestones: Study begins; Completed urine sample and corresponding survey collection; 75 IC and 150 non-IC human urine samples recruited and evaluated per site (total: 225 IC and 450 non-IC)
- Year 2 actual: 79 IC and 136 non-IC urine samples collected at Baylor. 32 IC and 96 non-IC urine samples have been collected at Einstein. 15 non-IC urine samples have been collected at Beaumont. Total 111 IC and 232 non-IC samples are collected.
- 51% completed. This is expected due to COVID-19.

Major Task 3: Data Acquisition and Analysis

- Target Date: 36 months
- Milestones: Report and review results from data analyses; manuscript and abstracts submissions
- Year 2 actual: Data analysis under way. Reports on completed parts of the analysis were submitted as a manuscript and several accepted meeting abstracts (2021 annual meeting for American Urological Association and 2021 Military Health System Research Symposium (MHSRS) meeting) are included in appendix.
- 40% completed

Specific Aim 3: Urine kit optimization

Major Task 1: Evaluation of kits and online support tools (e.g. videos, documents, etc.)

- Target Date: 28 months
- Milestones: Optimized urine collection kit ready-for market
- Year 2 actual: Reviewed all comments for optimization of kit and online support materials. Suggested improvements noted and documented.
- Completed, all milestones achieved at 24 months

Major Task 2: As needed, redesign kits to reflect suggested improvements from Task 1 above

- Target Date: 28 months
- Milestones: Optimized urine collection kit ready-for market
- Year 2 actual: Redesign of kit completed; kit manufacturing delays due to COVID-19; need to finish additional analysis for survey design
- 50% completed

Aim 3b: Advance the technology to perform IC-RS in a small table-top system that can be run in any basic clinical laboratories regardless of location and without specialized expertise.

Major Task 1: Develop and optimize assay on new platform

- Target Date: 28 months
- Milestones: IC-RS work-flow includes a small table-top system; verified to work at three different sites; manuscript and abstract submission
- Year 2 actual: We have obtained and setup the ELLA system (Protein Simple) and begin validation testing for initial Quality Controls, a critical step to ensure robust and meaningful results. We have determined that there was cross-reactivity for one of the analytes, GRO/CXCL1, in human urine samples and we are working to resolving this issue.
- 30% completed

Major Task 2: Verify results across platforms at two other geographical locations

- Target Date: 36 months
- Milestones: IC-RS work-flow includes a small table-top system; verified to work at three different sites; manuscript and abstract submission
- 0% completed

Major Task 3: Translation of final product for commercialization with Beaumont Technology Transfer Mi-KickStart

- Target Date: 36 months
- Milestones: Patent issued.
- 20% completed

What was accomplished under these goals?

We are happy to report that, despite significant research limitations due to the COVID-19 pandemic, we were able to complete most of our participant recruitment both online and in the clinic, sample processing, and data acquisition using the Luminex system. We maintained regular research study communication through virtual, online meetings in addition to email and phone calls. Data analysis is underway. Feedback on the urine at-home collection kit and online support tools was completed and optimization is almost done. Despite significant work, we have so far been unsuccessful in transferring the IC-RS to work on a smaller platform due to issues around quality controls and specificity which we will work on addressing these next 6 months. We have submitted a paper describing our online recruitment to PLOS Digital Health. We had a patent issues for the methodology behind our test. We have also had several oral presentations and abstracts presented on our work.

In addition to our work on interstitial cystitis, we are pleased to share how this work on diagnostic test development allowed us to rapidly develop and publish an easy-to-use, inexpensive test for SARS-CoV-2/COVID-19 early in the pandemic. Furthermore, Dr. Lamb is receiving the 2021

COVID Pandemic-related Outstanding Research Accomplishment (Individual) to be awarded at the Military Health System Research Symposium this August.

What opportunities for training and professional development has the project provided?

Study training for all study investigators was done at a meeting in person in Washington DC in September 2019. Due to COVID-19 and travel restrictions, additional study training for study staff has occurred by video conferencing (Microsoft Teams). Webinars that covered laboratory techniques related to this grant were shared with laboratory staff and attended by Sarah Bartolone and Elijah Ward. Dr. Lamb attended several Rapid Acceleration of Diagnostics (RADx) seminars and met with staff regarding the development and commercialization of innovative technologies for diagnostic testing. This project was not intended to provide training and professional development opportunities outside of what was necessary for the research study, as such we have nothing additional to report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Our main focus will be finalizing data analysis, optimization of the technology to perform IC-RS in a small table-top system, and translation of final product for commercialization. Data analysis is already underway and we do not anticipate any issues. We are currently trouble-shooting the ELLA system to be able to run the IC-RS as a small table-top system. We are doing this in partnership with the vendor. Lastly, we will work with Beaumont's Commercialization Center to develop a final product package ready for commercialization in partnership with a vendor who has extensive expertise in this area. We plan to apply for Mi-KickStart program early 2022.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Anything we learn with respect to interstitial cystitis is a step forward as this is an understudied disease. As such, knowledge gained from this study helped inform our other interstitial cystitis projects, including one looking at proteomic analysis of bladder biopsies to understand biological differences, which resulted in a publication and abstract.

What was the impact on other disciplines?

Because of our work on this DoD project, we have an intimate knowledge of how to design good, clinically meaningful diagnostic tests including how to ensure they are sensitive, specific, reliable, and validated. We are also knowledgeable as to the regulatory requirements for FDA approval. Because of this, we were well positioned to lead the development of a rapid test for COVID-19. We had also previously used a similar molecular technology to develop a rapid test to detect Zika virus, another infectious disease, so our team had all the expertise and resources available to rapidly respond to this global unmet need.

This resulted in an open access, peer-reviewed article in PLoS One, a high impact journal (see Appendix). Since then, the global impact has been significant. This test is further being developed and utilized by academia, industry, and even small resource poor clinics on every inhabited continent. This test has been validated by many other research groups, including laboratories from Harvard, MIT, Washington University in St. Louis, and other top medical universities. Importantly, this test

still detects the new, emerging viral strains of SARS-CoV-2 despite their genetic mutations (i.e. alpha, delta, etc.). Furthermore, it was purposely decided not to patent this test, so that it could be freely available to the entire world during this challenging time. Although other rapid tests have since been developed, this work served as the critical foundation that this technology could be utilized for rapid SARS-COV-2 detection in a range of sample types. In fact, the FDA under Emergency Use Approval has approved at least seven COVID-19 tests that utilize this type of technology. Importantly, this technology can be rapidly applied to develop diagnostic testing for new, emerging pathogens in the future.

As a result of this work, Dr. Lamb is being awarded the 2021 COVID Pandemic-related Outstanding Research Accomplishment (Individual) to be awarded at the Military Health System Research Symposium this August.

Furthermore, because of our work on this DoD, we were also well positioned to be the first globally to observe that some COVID-19 patients had severe bladder dysfunction, including inflammation in their urine. This has resulted in several publications, all included in the Appendix.

What was the impact on technology transfer?

We were issued the following patent.

Chancellor MB, Lamb LE, Janicki JJ. Methods for Detecting, Diagnosing, and Treating Ulcerative Interstitial Cystitis. United States Patent US 10,859,579 (filed March 31, 2016, and issued December 18, 2020).

What was the impact on society beyond science and technology?

Given the extensive disruption of COVID-19 on society, and the letters we get from patients and doctors from across the globe, we know there has been an incredible impact on global public health.

5. CHANGES/PROBLEMS:

Due to an initial delay to obtain HRPO approval and then COVID-19, we experienced delays to study start in year 1. However, we have overcome this and are now on track to accomplish our aims without changes required to our approach or SOW.

Actual or anticipated problems or delays and actions or plans to resolve them

1. COVID-19 pandemic completely closed some clinics for a while and has reduced some clinical staff to only 1 day per week. Like any current research study, it is difficult to fully anticipate how the COVID-19 pandemic will continue to impact this study. *If anything, this validates that there is a strong need for research studies that can be completed outside the clinic and instead use alternative strategies like online crowdsourcing and at-home sample collection, as will be done in Aim 1 of this grant.* As such, we have been very successful so far with our crowdsourcing and online recruitment of patients. We will ensure that as our crowdsourcing collection progresses, that we will pay special attention to both effective and ineffective online recruitment strategies, and make sure to disseminate these findings with the larger scientific and medical research communities. We have already shared with the larger urology community our plans for effective crowdsourcing strategies at the annual American

Urological Association meeting and a prepared manuscript submitted to PLoS Digital Health (see section 6).

2. We have obtained and setup the ELLA system (Protein Simple) and begin validation testing for initial Quality Controls, a critical step to ensure robust and meaningful results. We have determined that there was cross-reactivity for one of the analytes, GRO/CXCL1, in human urine samples and we are working to resolving this issue in cooperation with the vendor's tech support team.

Changes that had a significant impact on expenditures

As stated previously, enrollment was initially limited by the COVID-19 pandemic. Subsequently, the rate of enrollment has been slower than anticipated. Therefore, cumulative expenses are less than expected. There is a cost saving in salary, travel, subcontract and other miscellaneous costs. We anticipate that expenditures will increase, as study enrollment increases. Budgeted funds will be needed to achieve the aims of the study.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations

Lamb LE. 2020. Translational Research in Benign Bladder Disorders. 17th Annual Inside Urology, Great Lakes SUNA Chapter 2020 Conference. Livonia, MI, March 13, 2020. *canceled due to COVID-19

Lamb LE, Bartolone SN, Janicki JJ, Peters KM, ICA, Abraham N, Laudano M, Smith CP, Chancellor MB. 2020. National Collaborative Crowdsourcing Method of Diagnostic Biomarker Development for Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS). American Urological Association's 2020 Annual Meeting, Washington DC, May 15. *virtual due to COVID-19; published in journal

Lamb LE. 2020. Developing a Point-of-Risk Molecular Test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). European Society for Translational Medicine (EUSTM), Vienna, Austria, September 25, 2020. *virtual due to COVID-19

Lamb LE. 2020. Developing a Point-of-Risk Test for novel Coronavirus 2019. *Frontiers in Bioengineering Research*, Rice University. Houston, TX, April 30, 2020. *virtual due to COVID-19

Lamb LE. 2021. Improving Diagnostic Testing for Interstitial Cystitis, Radiation Cystitis, and COVID-19. Oakland University William Beaumont School of Medicine Medical Education Week. Rochester, MI, May 20, 2021. *virtual due to COVID-19

Lamb LE. 2021. Interstitial Cystitis Diagnostic Score Development using Crowdsourcing and Machine Learning Algorithm. American Urological Association Annual Meeting/Society of Basic Urological Science Breakout Session. Las Vegas, NV, September 11, 2021. *published in AUA journal

Lamb LE and Chancellor MB. 2021. Improving Patient Outcomes through Research in Benign Urology. Neurourology & Pelvic Reconstructive Surgery (NPR) Joint Urogyn Conference. University of Michigan, Ann Arbor, MI. February 15, 2021. *virtual due to COVID-19

Lamb LE. 2020. Developing a Point-of-Risk Test for COVID-19 and Interstitial Cystitis. University College Cork's Translational Medicine Conference, University College Cork. Cork, Ireland, January 28, 2020. *virtual due to COVID-19

Journal publications

Lamb LE, Dhar N, Timar R, Wills M, Dhar S, Chancellor MB. COVID-19 inflammation results in urine cytokine elevation and causes COVID-19 associated cystitis (CAC). *Med Hypotheses*. 2020 Nov 5:110375. doi: 10.1016/j.mehy.2020.110375. Epub ahead of print. PMID: 33213997; PMCID: PMC7644255.

Dhar N, Dhar S, Timar R, Lucas S, Lamb LE, Chancellor MB. De Novo Urinary Symptoms Associated With COVID-19: COVID-19-Associated Cystitis. *J Clin Med Res*. 2020 Oct;12(10):681-682. doi: 10.14740/jocmr4294. Epub 2020 Sep 21. PMID: 33029276; PMCID: PMC7524562.

Ward EP, Bartolone SN, Chancellor MB, Peters KM, Lamb LE. Proteomic analysis of bladder biopsies from interstitial cystitis/bladder pain syndrome patients with and without Hunner's lesions reveals differences in expression of inflammatory and structural proteins. *BMC Urol*. 2020 Nov 7;20(1):180. doi: 10.1186/s12894-020-00751-x. PMID: 33160333; PMCID: PMC7648270.

Lamb LE, Bartolone SN, Ward E, Chancellor MB. Rapid detection of novel coronavirus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PLoS One*. 2020;15(6):e0234682. doi: 10.1371/journal.pone.0234682. eCollection 2020. PubMed PMID: 32530929; PubMed Central PMCID: PMC7292379.

Preprints on medRxiv:2020.2002.2019.20025155. doi:10.1101/2020.02.19.20025155 and on SSRN: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3539654

Appeared in Top Ten of All Time Lancet Papers on SSRN
Appeared in Top Ten of All Time MedRN: Respiratory Tract Infections on SSRN
Top 10% SSRN Authors by total new downloads for 2020 and 2021
Top Article Downloaded from Institution according to ResearchGate in 2020

Ward EP, Bartolone SN, Carabulea AL, Sharma P, Chancellor MB, Lamb LE. Using Social Media to Crowdfund Collection of Urine Samples during a National Pandemic. PLoS Digital Health. 2021. *Submitted and under review.*

Books or other non-periodical, one-time publications

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

Website(s) or other Internet site(s)

<https://www.icstudy.org/>

This website is dedicated to this study and when active will have all study information available on it, study questions and answers, videos on the study, sample collection, and shipping, contact information, and additional study material. There is an English (default) and Spanish version of the website that triggers back and forth with a clickable button.

<https://www.happybladder.org/>

Website focused on our translational research lab at Beaumont. This includes a description of our research studies including a link to the above study website, publications, funding sources including DoD, study personnel, and contact information.

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Chancellor MB, Lamb LE, Janicki JJ. Methods for Detecting, Diagnosing, and Treating Ulcerative Interstitial Cystitis. United States Patent US 10,859,579 (filed March 31, 2016, and issued December 18, 2020).

Other Products

Dr. Lamb was awarded the 2021 COVID Pandemic-related Outstanding Research Accomplishment (Individual) at the Military Health System Research Symposium. This award “recognizes outstanding research contributions by an individual research scientist with the focus on significant accomplishment(s) of high impact related to the COVID-19 pandemic. This individual has demonstrated outstanding leadership in pursuit of excellence for their country and service.”

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Laura Lamb
Project Role: PI
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0003-0386-4165
Nearest person month worked: 6
Contribution to Project: No Change
Funding Support: No Change

Name: Michael Chancellor
Project Role: co-I
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0001-9480-8972
Nearest person month worked: 1
Contribution to Project: No Change
Funding Support: No Change

Name: Sarah Bartolone
Project Role: Research Coordinator
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0001-9040-7055
Nearest person month worked: 9
Contribution to Project: No Change
Funding Support: No Change

Name: Elijah Ward
Project Role: Research Assistant II (promoted from Research Assistant I)
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0002-9405-6763
Nearest person month worked: 10
Contribution to Project: No Change
Funding Support: No Change

Name: Nitya Abraham, MD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: No Change
Funding Support: No Change

Name: Melissa Laudano, MD
Project Role: Sub-I
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: No Change
Funding Support: No Change

Name:	Andrea Asencio
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Coordinated regulatory and study processes
Funding Support:	No Change

Name:	Christopher Smith MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change

Name:	Haarika Gudlavalleti
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to project:	Sample collection and shipping
Funding Support:	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report for: Laura Lamb, Michael Chancellor, Nitya Abraham, Melissa Laudano, Christopher Smith

What other organizations were involved as partners?

Baylor College of Medicine and Michael E. DeBakey VA Medical Center
Houston, Texas

Contribution: Dr. Smith actively participates in all online meetings and email/phone correspondence along with other key study personnel. He assists in protocol review and the contributes to the development of the patient recruitment plan. Dr. Smith is actively recruiting study participants. Dr. Smith also helps to design strategies to troubleshoot anticipated and unanticipated issues as the study progresses. Finally, he remains engaged with military and Veteran's Affair communities in anticipation of eventual transition of study findings to their IC/BPS patient populations.

Einstein College of Medicine
Bronx, New York

Contribution: Drs. Abraham and Laudano continue to actively participate in all online meetings and email/phone correspondence along with other key study personnel. They assist in protocol review and the contributes to the development of the patient recruitment plan. They are actively recruiting

study participants. They also helped to design strategies to troubleshoot anticipated and unanticipated issues as the study progresses.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not Applicable

QUAD CHARTS:

Not Applicable

9. APPENDICES:

MHSRS Award notice
Patent (first page only)
Crowdsource PLoS Digital Health Paper submission
SARS-CoV-2 Test PLoS Paper
IC Proteomics Paper
CAC Paper
CAC Inflammation Paper
AUA2020 Abstract
AUA2021 Abstract
AUA/SBUR 2021 Invited Presentation – letter and agenda
Rice Invited Presentation - agenda
EUSTM Invited Presentation - agenda
OUWB Invited Presentation- agenda
UCC Translational Medicine Invited Presentation - letter

From: [USARMY Ft Detrick MEDCOM USAMRMC Mailbox MHSRS-INFO](#)
To: [Lamb, Laura, PhD](#)
Cc: [Reilly, Patricia A CIV USARMY HQ USAMRDC \(USA\)](#); [Sparks, Monique R CTR USARMY HQ USAMRDC \(USA\)](#)
Subject: MHSRS Award Notification
Date: Friday, July 2, 2021 8:42:57 AM

WARNING: This email originated from outside of Beaumont Health.
Do not click on any links or open any attachments unless you recognize the sender and are expecting the message.

Good day, Dr. Lamb,

It gives me great pleasure to notify you that you have been selected as the winner of a 2021 Military Health System Research Symposium award in the category of COVID Pandemic-related Outstanding Research Accomplishment (Individual). Congratulations on your award winning write-up!

The award ceremony will be held on Monday, 23 August during the morning plenary session. The award will be presented by Dr. Terry Adirim, Acting Assistant Secretary of Defense for Health Affairs.

At this time, I need several pieces of information:

1. Will you be present to accept this award? If not, will someone be available to accept on your behalf?
2. Can you provide a photo? We project a photo of the winner when the award winner is named.
3. Can you verify the name and credentials for the plaque? (e.g., Name, credentials)
4. We provide winners names and write-ups to the Defense Health Agency Public Affairs Office for a potential article. Can I provide your name as the point of contact?

Congratulations again and thank you for submitting! This package was really exceptional! Please email me through this mailbox if you have any questions, I will be monitoring it as well as Ms. Monique Sparks, who is helping to organize the awards.

Respectfully,

Patricia A. Reilly, Col (RET), USAF, PhD
2021 MHSRS Planning Committee



US010859579B2

(12) **United States Patent**
Chancellor et al.

(10) **Patent No.:** **US 10,859,579 B2**

(45) **Date of Patent:** **Dec. 8, 2020**

(54) **METHODS FOR DETECTING, DIAGNOSING
 AND TREATING ULCERATIVE
 INTERSTITIAL CYSTITIS**

(71) Applicant: **WILLIAM BEAUMONT HOSPITAL,**
 Royal Oak, MI (US)

(72) Inventors: **Michael B. Chancellor,** Pittsburg, PA
 (US); **Laura Lamb,** Beverly Hills, MI
 (US); **Joseph J. Janicki,** McKees
 Rocks, PA (US)

(73) Assignee: **WILLIAM BEAUMONT HOSPITAL,**
 Royal Oak, MI (US)

(*) Notice: Subject to any disclaimer, the term of this
 patent is extended or adjusted under 35
 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **16/089,227**

(22) PCT Filed: **Mar. 31, 2017**

(86) PCT No.: **PCT/US2017/025501**

§ 371 (c)(1),

(2) Date: **Sep. 27, 2018**

(87) PCT Pub. No.: **WO2017/173342**

PCT Pub. Date: **Oct. 5, 2017**

(65) **Prior Publication Data**

US 2019/0107545 A1 Apr. 11, 2019

Related U.S. Application Data

(60) Provisional application No. 62/315,766, filed on Mar.
 31, 2016, provisional application No. 62/421,521,
 filed on Nov. 14, 2016.

(51) **Int. Cl.**

G01N 33/50 (2006.01)

A61K 38/00 (2006.01)

G06F 15/00 (2006.01)

A61B 5/00 (2006.01)

C12Q 1/00 (2006.01)

G01N 33/68 (2006.01)

(52) **U.S. Cl.**

CPC **G01N 33/6869** (2013.01); **G01N 33/6863**
 (2013.01); **G01N 33/6893** (2013.01); **A61B**
5/00 (2013.01); **A61K 38/00** (2013.01); **C12Q**
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None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

8,961,954 B2 2/2015 Chancellor et al.
 2006/0194738 A1* 8/2006 Fong A61K 38/08
 514/8.4
 2010/0166739 A1* 7/2010 Chancellor G01N 33/6869
 424/130.1
 2012/0021407 A1 1/2012 Haj-Ahmad
 2012/0177665 A1 7/2012 Chacko

FOREIGN PATENT DOCUMENTS

W● 2000023082 A1 11/1999
 W● 2014078232 A1 5/2014

OTHER PUBLICATIONS

Tyagi et al. Urinary Chemokines as Noninvasive Predictors of
 Ulcerative Interstitial Cystitis. The Journal of Urology vol. 187,
 2243-2248 (Jun. 2012). (Year: 2012).*

Katsouli et al. The role of the chemokine CXCL1 in epithelializa-
 tion, inflammation and ageing. Experimental Dermatology, vol. 20,
 No. 2, pp. 166-167. Abstract No. P026, Feb. 2011). (Year: 2011).*

Price et al. (Evaluation of a urine biomarker and pain in healthy,
 recurrent urinary tract infections, and painful bladder syndrome
 patients. Female Pelvic Medicine and Reconstructive Surgery, vol.
 18, No. 5, Suppl. Suppl. 2, pp. S126. Abstract No. 62 (Sep.-Oct.
 2012). (Year: 2012).*

Peters et al. (Are Ulcerative and Nonulcerative Interstitial Cystitis/
 Painful Bladder Syndrome 2 Distinct Diseases? A Study of Coex-
 isting Conditions Urology 78: 301-308 (2011). (Year: 2011).*

Arcade et al. Biomarkers for interstitial cystitis/painful bladder
 syndrome. Womens Health (2016) 12(1), 87-90 (published online
 Dec. 23, 2015). (Year: 2016).*

Ercan et al. Stability of urine specimens stored with and without
 preservatives at room temperature and on ice prior to urinalysis.
 Clinical Biochemistry 48:919-922 (2015). (Year: 2015).*

Kouri et al. Preservation of Urine for Flow Cytometric and Visual
 Microscopic Testing. Clinical Chemistry 48:6:900-905 (2002). (Year:
 2002).*

(Continued)

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(57) **ABSTRACT**

The present invention relates to methods for detecting,
 diagnosing and/or treating ulcerative interstitial cystitis
 (UIC) by detecting in a urine sample from a patient the
 levels of each of the proteins IL-6, IL-8 and GRO [also
 known as CXCL 1 (chemokine C-X-C motif ligand 1)]. In
 some embodiments, the method also includes diagnosing the
 patient with UIC when each of the proteins IL-6, IL-8 and
 GRO in the urine sample is at a different level than a
 statistically validated threshold for the respective proteins.
 In some embodiments a companion diagnostic, e.g., a cysto-
 scopy, is used in conjunction with the protein biomarker
 diagnostic. In some embodiments, once UIC is diagnosed,
 the patient is treated for the UIC.

27 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

Only first page shown

Using Social Media to Crowdsourcing Collection of Urine Samples during a National Pandemic --Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Full Title:	Using Social Media to Crowdsourcing Collection of Urine Samples during a National Pandemic
Short Title:	Crowdsourcing urine samples during a national pandemic
Corresponding Author:	Laura E Lamb Beaumont Health Royal Oak, MI UNITED STATES
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Keywords:	sample collection; pandemic; crowdsourcing; online collection; interstitial cystitis
Abstract:	The COVID-19 pandemic and subsequent lockdown had a substantial impact on normal research operations. Researchers needed to adapt their methods to engage at-home participants. One alternative method is crowdsourcing, in which researchers use social media to recruit participants, gather data, collect samples, and publish results. We utilized this method to develop a diagnostic test for interstitial cystitis/bladder pain syndrome. Participants were recruited via posts on popular social media platforms, and enrolled via a study website. Participants received and returned a mail kit containing bladder symptom surveys and a urine sample cup containing room-temperature preservative. Using this method, we collected 1254 Interstitial Cystitis/Bladder Pain Syndrome and control samples in 3 months from all 50 United States. Social media posts containing videos, as well as those posted to multiple websites, drew the largest number of study website visitors. Participants accessed the website primarily using mobile devices, indicating a need for a mobile user-friendly website. Paid advertisements with Google and social media posts by a patient advocacy group attracted the largest number of study website visitors. Our data demonstrates that crowdsourcing is a viable alternative to traditional research, with the ability to reach a broad patient population rapidly. Crowdsourcing is a powerful tool for at-home participation in clinical research, particularly during the national lockdown caused by the COVID-19 pandemic.
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	expert in sample collection during pandemic
Opposed Reviewers:	
Additional Information:	
Question	Response
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the work included in this submission and the role the funder(s) played. This includes grants and any commercial funding of the work or authors. This statement will be typeset if the manuscript is accepted for publication. Please review the submission guidelines and the instructions link below for detailed requirements and guidance.	This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Technology/Therapeutic Development Research Program under Award No. W81XWH-19-1-0288. The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense. We also acknowledge generous philanthropy support from the Taubman foundation.
Competing Interests On behalf of all authors, disclose any competing interests that could be perceived to bias this work. This statement will be typeset if the manuscript is accepted for publication. Please review the instructions link below and PLOS Digital Health's competing interests policy to determine what information must be disclosed at submission.	LEL, JJJ, and MBC have intellectual property associated with methods for diagnosing interstitial cystitis. All other authors have declared no conflicts of interest exist.
Data Availability Before publication, Authors are required to make fully available and without restriction all data underlying their findings. Please see our PLOS Data Policy page for detailed information on this policy.	All relevant data is available within the manuscript.

A **Data Availability Statement**, detailing where the data can be accessed, is required at first submission. Insert your Data Availability Statement in the box below.

Please see the [data reporting](#) section of our submission guidelines for instructions on what you need to include in your Data Availability Statement.

This statement will be typeset if the manuscript is accepted for publication.

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Beaumont

Beaumont Health

3811 West 13 Mile Road, Suite 504
Royal Oak, MI 48073

July 22, 2021

Dear Editorial Board,

We would like to submit our paper “**COVID-19 Inflammation Results in Urine Cytokine Elevation and Causes COVID-19 Associated Cystitis (CAC)**”, for publication consideration. The COVID-19 pandemic has caused unanticipated challenges and difficulties with clinical and translational research due to many issues including lockdowns, patient anxiety, work and clinical restrictions, travel restrictions, loss of workers due to illness, etc. Here we describe a successful effort that collected 1254 patient samples in under 3 months from all 50 US states during the pandemic in the Fall of 2020. We were able to do this utilizing active online and crowdsourcing recruitment and at-home sample collection kits. This crowdsourcing approach utilized social media (patient advocacy websites, Facebook, YouTube, TikTok, and Twitter) to engage and recruit participants to participate in sample collection for Interstitial Cystitis, a chronic and debilitating bladder disorder with no laboratory test. We believe this to be the first manuscript to describe successful large-scale sample collection during significant research restrictions during the pandemic and that the strategy described in the manuscript would be useful to other diseases and conditions outside of urology as well.

Part of our success was due to interest and partnership with a national patient advocacy organization, the Interstitial Cystitis Association. They represent over 50,000 IC patients and are excited about the publication of this research project and would be keen to put out multimedia press releases upon publication to disseminate this news to the medical community.

This is an original manuscript that is not submitted to any other journals. The study has full IRB approval. All the authors have significantly contributed and approve of the submission. This is a highly relevant topic as we anticipate more groups may move to crowdsourcing samples and engaging study participants with social media in the near future. The methods in this paper could easily be extrapolated to developing objective tests for other complicated diseases in other fields. Thank you and I am happy to address any questions.

Sincerely,



Laura E. Lamb, Ph.D.

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Using Social Media to Crowdsourcing Collection of Urine Samples during a National Pandemic

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Short title: **Crowdsourcing urine samples during a national pandemic**

Abstract

The COVID-19 pandemic and subsequent lockdown had a substantial impact on normal research operations. Researchers needed to adapt their methods to engage at-home participants. One alternative method is crowdsourcing, in which researchers use social media to recruit participants, gather data, collect samples, and publish results. We utilized this method to develop a diagnostic test for interstitial cystitis/bladder pain syndrome. Participants were recruited via posts on popular social media platforms, and enrolled via a study website. Participants received and returned a mail kit containing bladder symptom surveys and a urine sample cup containing room-temperature preservative. Using this method, we collected 1254 Interstitial Cystitis/Bladder Pain Syndrome and control samples in 3 months from all 50 United States. Social media posts containing videos, as well as those posted to multiple websites, drew the largest number of study website visitors. Participants accessed the website primarily using mobile devices, indicating a need for a mobile user-friendly website. Paid advertisements with Google and social media posts by a patient advocacy group attracted the largest number of study website visitors. Our data demonstrates that crowdsourcing is a viable alternative to traditional research, with the ability to reach a broad patient population rapidly. Crowdsourcing is a powerful tool for at-home participation in clinical research, particularly during the national lockdown caused by the COVID-19 pandemic.

Author Summary

The COVID-19 pandemic significantly disrupted traditional clinical research, requiring researchers to use new methods. Crowdsourcing is an underutilized method with which researchers can recruit participants, gather data, and publish results. With the rise of social media, it has never been easier for researchers to advertise their crowdsourcing studies. This method is also useful for studying lesser-known diseases, such as interstitial cystitis. We used this method to gather urine samples and develop a urine-based diagnostic test for Interstitial Cystitis/Bladder Pain Syndrome. We completed recruitment in 3 months, gathering 1254 samples from all 50 United States. Our study shows that crowdsourcing is a viable research method with which you can recruit a large number of participants from a wide geographical range, in a short period of time. This is useful in a pandemic, where people may be unable to do clinical site visits, thereby being unable to participate in standard clinical research.

Introduction

The COVID-19 pandemic has massively impacted everyday life, shutting down nonessential services and forcing people to shelter in place for weeks at a time. One of the many effects of the global lockdown was the decrease in clinical research with the closing of research labs. With non-COVID-19 research activities suspended through a significant portion of 2020, nonessential researchers and scientists had no laboratory access [1]. A survey of 881 life scientists revealed that 57% reported some loss of work, stemming from decreased laboratory access [2]. This represents a financial problem, as costly experiments, such as those involving animal models, may need to be repeated. In a survey of postdoctoral fellows, 80% reported difficulty in performing experiments and

discussing ideas with colleagues [3]. The National Institute of Health's survey of principal investigators and research leaders revealed extreme concerns with institutional financial stability and productivity of research [4]. Recruiting for a clinical trial during the pandemic also represents an ethical dilemma, as researchers and study participants could be potentially exposed to SARS-CoV-2 during study visits. Because traditional research recruitment and participation methods were hampered by the COVID-19 pandemic, different approaches were necessary. Crowdsourcing, where researchers employ modern communication methods to recruit participants and gather data, is one such approach.

Crowdsourcing as a research method has gained traction in the medical community. Increasing ease of communication and data collection via the internet and social media has resulted in innovations in public health, education, communication, and ethics [5, 6]. By utilizing social media, researchers are able to advertise their studies, recruit and engage with participants, and gather data. Mail-based collection of samples allows for participation from the safety of home. It also has the potential to significantly lower costs while increasing sample size, compared to traditional research [7]. Crowdsourcing also has the benefit of improved study completion. We believe that these benefits make crowdsourcing an ideal tool for reaching groups suffering from rare diseases that are poorly understood or restrict travel, particularly during the COVID-19 pandemic, when participants were not able to leave their homes. Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is one such disease that benefits greatly from an at-home, social media-based crowdsourcing approach.

IC/BPS is a debilitating chronic disease that affects roughly 3-8 million women and 1-4 million men in the United States [8, 9]. Approximately 10% of IC cases present with Hunner's lesions, which are visible via hydrodistension and cystoscopy; however, the majority of those suffering with IC do not present with lesions [10]. Diagnosis of IC is based on patient reported symptoms and exclusion of other diseases with overlapping clinical presentation. Therefore, a non-invasive, rapid, diagnostic test is required. We sought to develop a urine-based biomarker test by crowdsourcing urine from both IC patients and asymptomatic control participants, utilizing social media and other web-based recruitment tools to reach a large population. We then sought to determine the usefulness and efficacy of these recruitment methods during a nationwide lockdown and pandemic.

Results

Study Enrollment Goal was Met Three Months after Launch

The study was launched September 14, 2020 and closed December 26, 2020. The study recruited 1,254 IC/BPS and control samples from all 50 United States (Figure 1). The first week of study enrollment had the highest percentage of enrolled participants versus unique website visitors (for the purposes of this study, a "unique website visitor" is only counted once for website visitation, regardless of return visits); this percentage slowly decreased through the duration of the study (Figure 2A). Sample return rate was highest at week 3 of the study. There was a 2 week average turnaround time from initial enrollment to sample return. Rate-of-return fell slowly over time but rose slightly at

weeks 8 and 10, when the patient advocacy group sent reminder emails to participants to return their kits (Figure 2B).

Table 1. Crowdsourced IC/BPS and control demographics.

	IC/BPS	Control
No. of participants	559	695
Age (<i>mean ± SD, range</i>)	49.8 ± 16.4 (19-91)	48.0 ± 16.8 (19-95)
No. of males (%)	30 (5%)	401 (58%)
No. of females (%)	529 (95%)	294 (42%)
ICSI/PI (<i>mean ± SD</i>)	23.2 ± 8.1	5.1 ± 5.3
OABq (<i>mean ± SD</i>)	68.9 ± 24.6	25.4 ± 12.1
PUF (<i>mean ± SD</i>)	19.4 ± 6.5	3.3 ± 4.0
VAS (<i>mean ± SD</i>)	5.1 ± 2.3	1.0 ± 0.7

Social media posts using video attracted the most website

Visitors

Seven major social media posts were made during the enrollment period (Figure 3). The posts that were the most effective were posted on multiple social media sites. The social media post with the largest impact on unique website visitors was an 11-second TikTok video advertising the study; this video was also re-posted to Facebook and Instagram.

Majority of participants used mobile devices to enroll and visit website

80% of unique website visitors used their mobile devices to visit the study website (Figure 4A). Mobile devices are responsible for 61% of US website visits, though social networks are predominantly accessed via personal computers [11].

Google and Facebook are Effective Tools for Study

Recruitment

Visitors discovered the study website in multiple ways (Figure 4B). Of these methods, the most common was a direct link, found in recruitment emails. Other methods included Google links (through organic searches or Google Advertisements) and Facebook posts by the ICA and other interested individuals. Finally, the ICA's website and Beaumont Health's website resulted in moderate traffic over the study period.

Discussion

This study confirms the versatility and usefulness of social media-based recruitment and crowdsourcing for clinical sample collection. Social media-based recruitment and crowdsourcing have significant benefits over traditional recruitment methods. This is especially evident as this study was completed during the fall of 2020, in the middle of the COVID-19 pandemic. Social media-based recruitment gathers participants at a significantly faster rate than traditional clinical sample collection. In this study, both clinical sites recruited ten times fewer participants than the at-home, social media-based recruitment in the same three-month period. This may be due to the increased number of people at home and the ease of at-home recruitment and participation during the COVID-19 pandemic lockdown. Online recruitment and crowdsourcing of samples also

bypass the geographical limitations of traditional studies; where a normal clinical study may recruit a population in the immediate geographic area around the site(s), a crowdsourcing study with online recruitment methods can recruit participants from all over the country. Another benefit of social-media/web-based recruitment is the ability to tailor to specific target populations. By tailoring recruitment advertisements and partnering with specialized patient advocacy groups, it is possible to target a subset of the normal population, enhancing recruitment goals. Finally, online recruitment and at-home participation is safer than traditional recruitment methods during a pandemic. As SARS-CoV-2 is not highly transmitted on surfaces, it is advantageous to receive a sample kit containing survey responses and biological samples by mail, rather than in-person, where the risk of viral transmission is significantly higher [12].

Online recruitment and crowdsourcing are not without their disadvantages. The biggest disadvantage of crowdsourcing studies is the absence of a patient medical record. For studies such as this that gather both diseased and control samples, researchers are trusting that the participant answers questions honestly, rather than relying on a clinician-confirmed diagnosis. Studies utilizing these methods also frequently lack a personal component. For example, this study had no face-to-face or video-based interaction with participants; while there were e-mail and phone hotline for participant questions and concerns, the lack of personal interaction may have dissuaded some people from participating. In this study, this could not be avoided, as we sought to eliminate participant-physician interaction due to the COVID-19 pandemic. Lack of control participation was also challenging. Control participants who were not recruited by an IC/BPS participant rarely returned their surveys and urine sample. We have

observed this in a previous study [13], and tried to address it by including paper surveys for control participants, rather than directing them to a website to complete surveys.

One of the challenges in completing a successful social media-based research study is preparing user-friendly study materials, such as advertisements and websites. In this study, 80% of unique website visitors accessed the study website from their mobile devices. This may be due to users accessing social media more frequently from their phones versus personal computers, as these are app-based platforms. It is also important to have multiple advertisement methods in order to reach the largest number of potential participants. In this study, we partnered with a patient advocacy group for primary recruitment, created a Google Ad, and optimized the search engine results for the study website. The patient advocacy group advertised the study via direct e-mails and social media posts on Facebook, Instagram, and TikTok. The posts that were linked to multiple social media platforms resulted in the largest spike in unique website visitors. Social media-based recruitment studies can also grow on their own once they are large enough. In this study, a post advertising the study was made on an IC/BPS forum by a user that was not affiliated with our research group nor the patient advocacy group. In another instance, a clinic in South Dakota discovered the study via a social media post and began to advertise locally for recruitment.

The final consideration for an online recruitment/crowdsourcing study is the timing of study launch, especially during a national lockdown due to a spreading pandemic. This study was initially prepared for launch in the first quarter of 2020; however, there were multiple problems that we attempted to address, resulting in delay of initial study launch. Firstly, we wanted to ensure that the mail kits and urine samples could not transmit

SARS-CoV-2. We waited until publication of research studies that declared surface transmission risk via mail was minimal. We also wanted to confirm that the room-temperature urine preservative prevented transmission of the SARS-CoV-2 virus. The state of the US Postal Service was also considered at the planned time of launch in early 2020. The mail system became backlogged due to the nation-wide lockdown. Finally, full laboratory staff was not allowed in the research facility initially during the pandemic; we were required to stagger our on-site presence, which would have made processing the rapid influx of samples much more difficult. Therefore, the study was postponed until sufficient research staff was available to process incoming samples.

Conclusion

Social media is a powerful untapped resource for researchers. By advertising via social media, it is possible to rapidly gather data from a larger population and geographical region. These new recruitment and participation methods are even more useful during the COVID-19 pandemic, which hampered and sometimes halted normal research operations. Using these methods, we were successful in gathering over 1,254 control and IC/BPS urine samples in 3 months from across the country, aiding us in our goal of developing a urine-based diagnostic test for IC/BPS.

Methods

Study Recruitment and Enrollment

All study materials were approved by the Beaumont Health Institutional Review Board (IRB# 2019-266). A study website was created using Wix, a site development tool. This

website contained study background videos, a link to the enrollment survey, shipping information videos, and frequently asked questions. A form for contacting the research team via email was also included. The enrollment survey was created using SurveyMonkey, a Health Insurance Portability and Accountability Act (HIPAA)-compliant survey website. The enrollment survey included demographic information, health history, and IC/BPS diagnosis information. Participants were asked to provide a name and address to which we sent a collection kit.

We partnered with the Interstitial Cystitis Association (ICA), an IC/BPS patient advocacy group, to reach a large audience of people suffering from IC/BPS. The patient advocacy group sent out emails informing members of the study, with links to the recruitment website. The study was also advertised via Google Ads, appearing under the search terms “IC”, “IC/BPS”, “interstitial cystitis”, and “urology study”. Social media posts on websites and phone software applications Instagram, Facebook, and TikTok were used to advertise the study and recruit participants. Once a potential IC/BPS participant had completed the enrollment survey and met enrollment criteria, and consented to participate, an at-home collection kit was prepared and shipped to them. IC/BPS participants were asked to find a male and female of similar age with no bladder health history willing to participate as control samples; inability to find control participants did not exclude the person with IC from participating in the study. Control participants who discovered the study independently were allowed to enroll via the study website.

At-Home Collection Kit and Mailing

At-home collection kits were sent to the address provided by the study participant via the US Postal Service (USPS). The mailing kits contained 3 boxes for the IC/BPS participant, male control, and female control. Each box was a self-contained independent sample return mailing kit. All three boxes contained study information sheets, bladder symptom surveys, a urine collection cup containing a room-temperature urine preservative (Norgen Biotek, Thorold, Ontario, Canada), and a pre-paid USPS return mailing envelope. The urine preservative stabilizes proteins and nucleic acids in samples and allows them to be shipped at ambient temperatures, while also inactivating bacteria and viruses in the sample. The male and female control boxes also included demographic survey for the control participants, mimicking the information collected from IC participants during their enrollment survey. All return boxes contained a non-reversible temperature recording label, which indicated how long the package was exposed to temperatures exceeding 30° C. At this temperature, the preservative no longer protects the biomarker proteins of interest from degradation. Bladder symptom surveys included the Interstitial Cystitis Symptom/Problem Index (ICSI/PI), the Overactive Bladder questionnaire (OABq), the Pelvic Pain, Urgency, and Frequency questionnaire (PUF), and the Visual Analogue Scale (VAS).

Urine Sample Collection & Return Shipping

Participants were asked to provide a urine sample via midstream catch and place it in the biohazard bag containing absorbent material. They were then asked to fill out the included surveys, place the sample and survey in a pre-labeled USPS envelope, and place the envelope in their mailbox.

Instructions for collecting and returning the urine samples were provided in the kit. The paper instructions included directions to the study website, where videos showed how to collect and ship the samples. The study website also contained a frequently-asked-questions section, which provided answers to common questions, and a “Contact Us” form for questions or concerns about the study.

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Author Contributions

LEL and MC conceived and designed this study. EPW and SNB curated study data. EPW, SNB, ALC, LEL, and PS analyzed data and prepared figures. LEL and MC provided project administration and supervision. EPW wrote the manuscript. SNB, ALC, PS, LEL, and MC reviewed and edited the manuscript.

References

1. Akacha M, Branson J, Bretz F, Dharan B, Gallo P, Gathamnn I, et al. *Challenges in Assessing the Impact of the COVID-19 Pandemic on the Integrity and Interpretability of Clinical Trials*. SBR, 2020. **12**(4): p. 419-426.
2. Korbel JO, Stegl O. *Effects of the COVID-19 pandemic on life scientists*. Genome Biol, 2020. **21**(1): p. 113.
3. Woolston C. *Pandemic darkens postdocs' work and career hopes*. 2020 (cited Jun 24 2021) [internet]. Available from: <https://www.nature.com/articles/d41586-020-02548-2>.

4. Bernard MA, Lauer M.. *The Impact of the COVID-19 Pandemic on the Extramural Scientific Workforce – Outcomes from an NIH-Led Survey*. 2021 Mar 25 2021 (cited Jun 30 2021) [Internet]. Available from: <https://nexus.od.nih.gov/all/2021/03/25/the-impact-of-the-covid-19-pandemic-on-the-extramural-scientific-workforce-outcomes-from-an-nih-led-survey/>.
5. Tucker JD, Day S, Tang W, Bayus B. *Crowdsourcing in medical research: concepts and applications*. PeerJ, 2019. **7**: p. e6762.
6. Swan M. *Crowdsourced health research studies: an important emerging complement to clinical trials in the public health research ecosystem*. J Med Internet Res, 2012. **14**(2): p. e46.
7. Khatri C, Chapman SJ, Glasbey J, Kelly M, Nepogodiev D, Bhangu A, et al. *Social media and internet driven study recruitment: evaluating a new model for promoting collaborator engagement and participation*. PLoS One, 2015. **10**(3): p. e0118899.
8. Berry SH, Elliot MN, Suttorp M, Bogart L, Stoto MA, Eggers P, et al. *Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States*. J Urol, 2011. **186**(2): p. 540-4.
9. Suskind AM, Berry SH, Ewing BA, Elliot MN, Suttorp MJ, Chelemns JQ. *The prevalence and overlap of interstitial cystitis/bladder pain syndrome and chronic prostatitis/chronic pelvic pain syndrome in men: results of the RAND Interstitial Cystitis Epidemiology male study*. J Urol, 2013. **189**(1): p. 141-5.
10. Teichman JM. *Hunner's lesions*. Can Urol Assoc J, 2009. **3**(6): p. 478.
11. Enge E. *Mobile vs. Desktop Usage in 2020*. Mar 23 2021 (cited Jun 27 2021) [internet]. Available from: <https://www.perficient.com/insights/research-hub/mobile-vs-desktop-usage>.
12. NCIRD. *Science Brief: SARS-CoV-2 and Surface (Fomite) Transmission for Indoor Community Environments*. Apr 5 2021 (cited Jun Jun 27 2021) [Internet]. available from: <https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/surface-transmission.html>
13. Lamb LE, Janicki JJ, Bartolone SN, Peters KM, Chancellor MB. *Development of an interstitial cystitis risk score for bladder permeability*. PLoS One, 2017. **12**(10): p. e0185686.

Fig 1. Study workflow for recruitment and sample collection.

Fig 2. Sample return rate depends heavily on reminder posts and advertisements.

- A.** Percentage of enrollments versus number of unique website visitors from study launch to close of enrollment. Rate of enrollment fell steadily as the study progressed.
- B.** Number of samples returned per week was highest approximately 3 weeks after

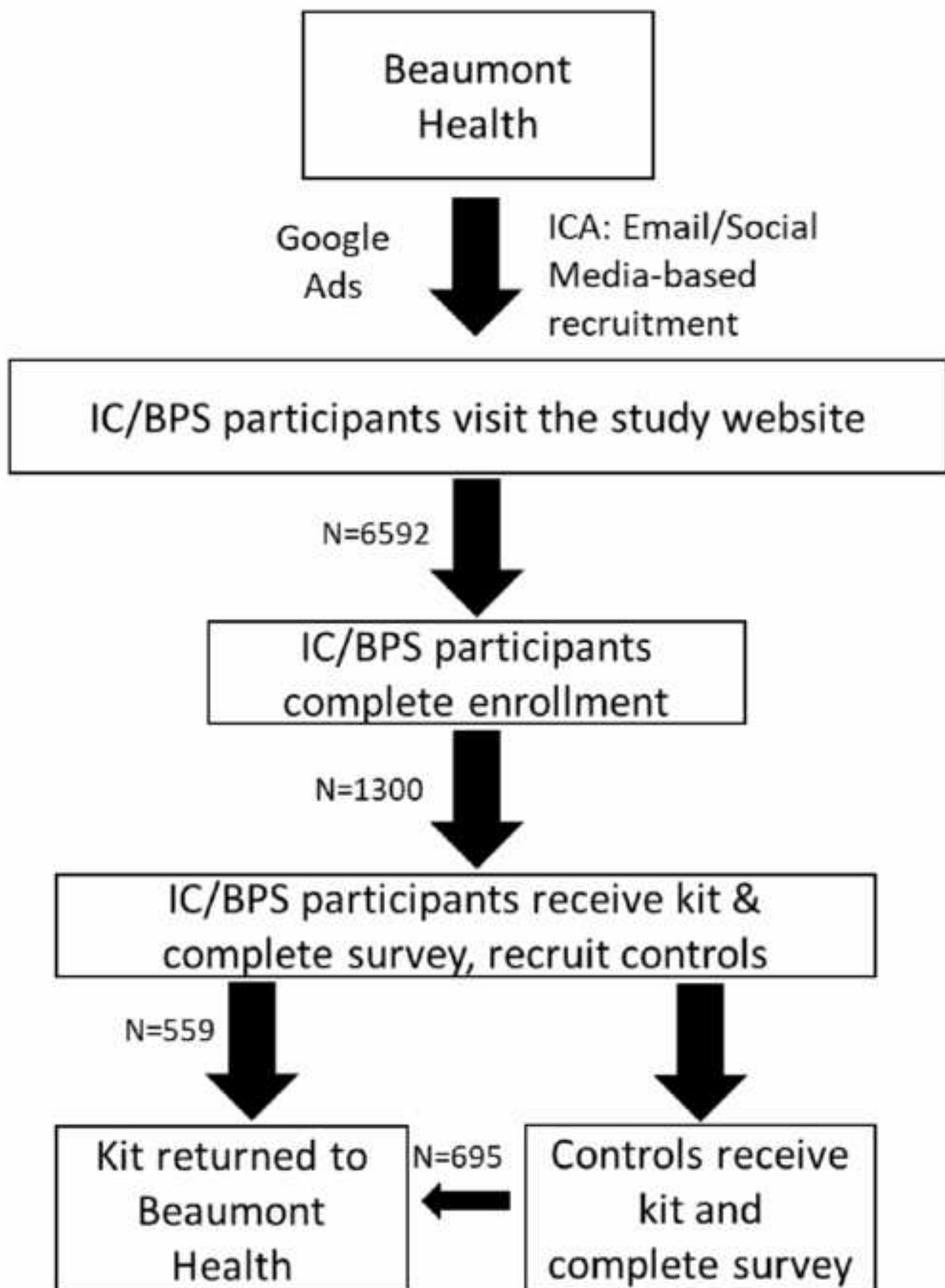
study launch. Subsequent spikes in return rate correspond with study advertisements and reminder emails sent to participants.

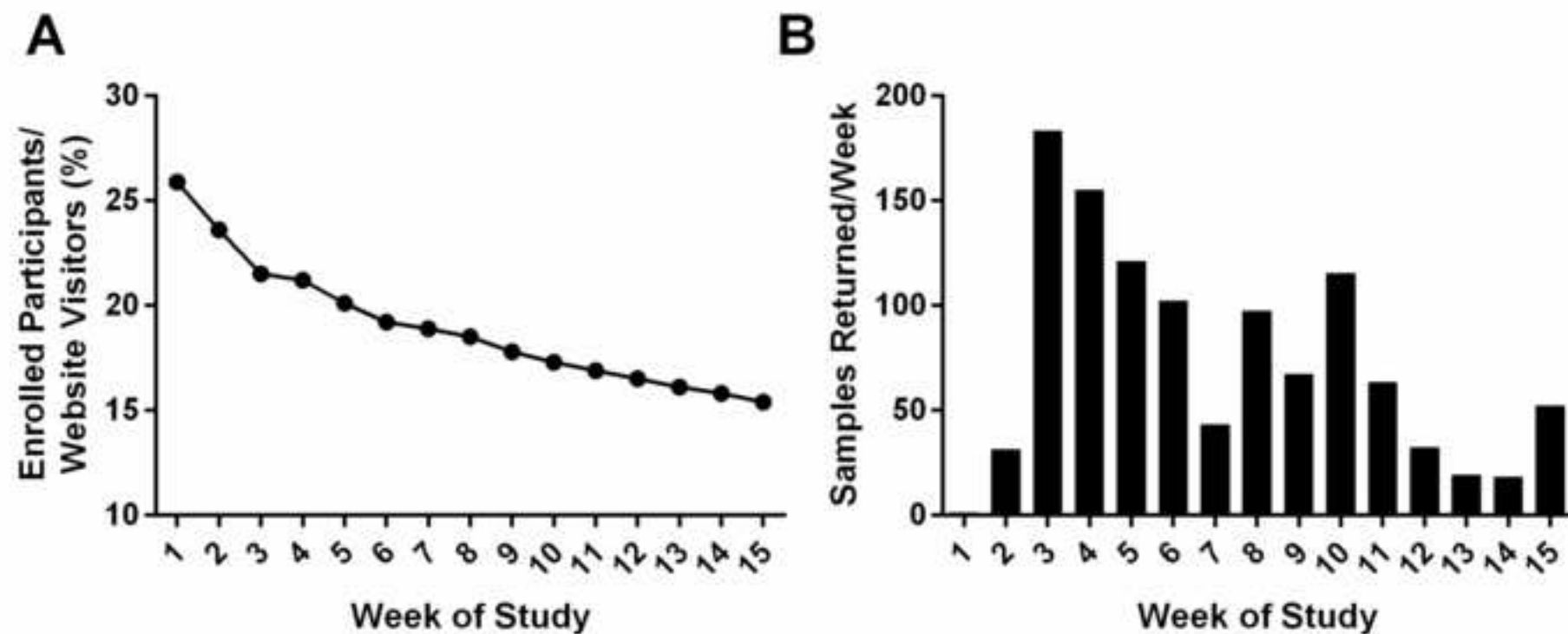
Fig 3. Sharing recruitment posts on multiple platforms increased website visits.

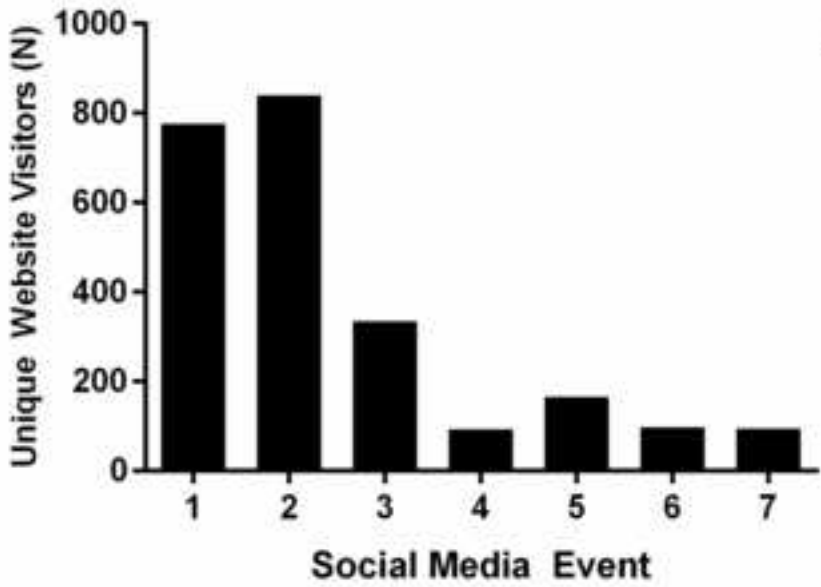
The most effective social media posts were linked to multiple websites and included a short video. Social media posts exhibited decreasing efficacy in attracting unique website visitors as the study progressed.

Fig 4. Participants prefer mobile devices and direct links to access the study

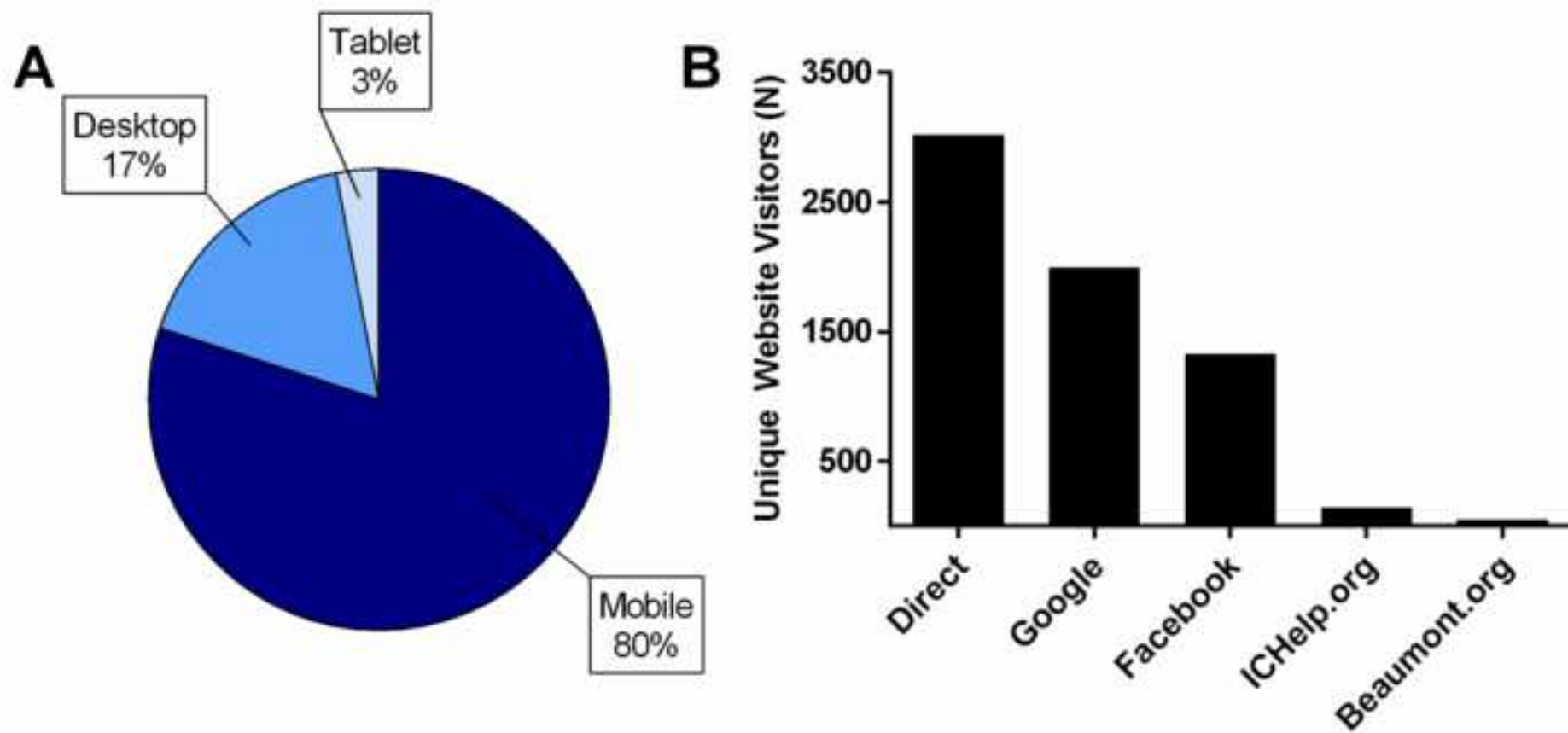
website. A. 80% of unique website visitors used mobile devices to access the study website. **B.** Participants were referred to the website from 5 primary sources, with the most prominent being direct links from emails and non-Facebook social media posts, followed by Google Advertisements and search results, links from Facebook, the ICA's website, and Beaumont's website.







Event	Social Media Post	Methods Used
1	Study Launch	Facebook, Instagram
2	Recruitment Video- Dance	TikTok, Facebook, Instagram
3	Reminder of Study Launch	Email, Facebook
4	Interview with Researchers (ICA Walk for a Cure)	YouTube
5	IC Participant- Kit Content Video	Facebook, Instagram
6	Bladder Health Month Challenge	Facebook, Instagram
7	Shipping Reminder Video	Facebook



RESEARCH ARTICLE

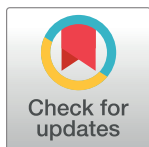
Rapid detection of novel coronavirus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification

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Abstract

Novel Corona virus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or 2019-nCoV), and the subsequent disease caused by the virus (coronavirus disease 2019 or COVID-19), is an emerging global health concern that requires a rapid diagnostic test. Quantitative reverse transcription PCR (qRT-PCR) is currently the standard for SARS-CoV-2 detection; however, Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) may allow for faster and cheaper field based testing at point-of-risk. The objective of this study was to develop a rapid screening diagnostic test that could be completed in 30–45 minutes. Simulated patient samples were generated by spiking serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs with a portion of the SARS-CoV-2 nucleic sequence. RNA isolated from nasopharyngeal swabs collected from actual COVID-19 patients was also tested. The samples were tested using RT-LAMP as well as by conventional qRT-PCR. Specificity of the RT-LAMP was evaluated by also testing against other related coronaviruses. RT-LAMP specifically detected SARS-CoV-2 in both simulated patient samples and clinical specimens. This test was performed in 30–45 minutes. This approach could be used for monitoring of exposed individuals or potentially aid with screening efforts in the field and potential ports of entry.

Introduction

The recent Novel Corona virus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or 2019-nCoV) pandemic, and the subsequent disease caused by the virus (coronavirus disease 2019 or COVID-19), has generated global concern given its rapid spread in multiple countries and possible fatal progression of the infection. Initially, many patients reported exposure at a large seafood and animal market in Wuhan, China, suggesting animal-to-person transmission of the virus. However, since then many patients have reported no exposure to

animal markets, indicating that person-to-person transmission is occurring. There is currently no vaccine or targeted therapeutic for SARS-CoV-2.

SARS-CoV-2 infection is difficult to diagnose early in infection as patients can remain asymptomatic or present with non-specific clinical symptoms including fever, cough, or shortness of breath. Symptoms may appear in as few as 2 days or up to 2 weeks after exposure [1]. Quantitative reverse transcription PCR (qRT-PCR) for COVID-19 in respiratory samples is currently the standard for diagnostic molecular testing. However, this requires expensive equipment and trained personnel.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a one-step nucleic acid amplification method based on PCR technology that has been used to diagnose infectious diseases [2]. RT-LAMP has several advantages making it attractive for clinical utility. This includes high specificity and sensitivity, and ability to be completed in less than an hour. Furthermore, it can work at various pH and temperature ranges which is advantageous for clinical samples [3]. Lastly, the reagents are relatively inexpensive and can be stable at room temperature. We have previously used RT-LAMP to detect zika virus in clinical serum and urine samples, as well as in crude lysates of single mosquitos [4,5].

This study describes an RT-LAMP methodology that can detect SARS-CoV-2 in simulated patient samples as well as clinical samples in 30–45 minutes. The test could be used at the point-of-care by field and local personnel for the rapid diagnosis of individuals for optimal treatment, isolation, and rapid contact tracing. This methodology could be rapidly developed for the investigation of outbreaks of unknown respiratory diseases where the genetic sequence of the pathogen is known.

Methods

RT-LAMP primer design

The consensus sequences of 23 different SARS-CoV-2 strains (EPI_ISL_403929, EPI_ISL_403930, EPI_ISL_403931, EPI_ISL_402129, EPI_ISL_402130, EPI_ISL_402132, EPI_ISL_402128, EPI_ISL_402124, EPI_ISL_402127, EPI_ISL_402121, EPI_ISL_402119, EPI_ISL_402123, EPI_ISL_403962, EPI_ISL_403963, EPI_ISL_404227, EPI_ISL_404228, EPI_ISL_403932, EPI_ISL_403933, EPI_ISL_403934, EPI_ISL_403935, EPI_ISL_403937, EPI_ISL_403936, EPI_ISL_406596, EPI_ISL_406844, EPI_ISL_407079, EPI_ISL_407214) were established when aligned with Lasergene MegAlign (DNASTAR) to identify areas of sequence conservation. To improve specificity, areas of divergence of SARS-CoV-2 (GenBank MN908947) with the sequence-related coronavirus, Bat Severe Acute Respiratory Syndrome (SARS)-like coronavirus (GenBank KY417152.1), were identified using BLAST 2 (Basic Local Alignment Search Tool; NCBI). An area of GenBank MN908947 sequence that had high homology with other SARS-CoV-2 strains and was divergent for Bat SARS-like coronavirus was then targeted for RT-LAMP primer design. The genome equivalent of the SARS-CoV S coding region and Orf8 were purposely avoided. The S coding region was demonstrated to have the most mutations among SARS-CoV isolated strains, suggesting this region may be prone to positive selective pressure, and there were major deletions observed in the Orf8 region of the SARS-CoV genome variants[6]. RT-LAMP primers were designed using LAMP Designer 1.15 (Premier Biosoft), and blasted using Primer-BLAST (NCBI) against genomes of interest. Primer selection was prioritized as described in “A Guide to LAMP primer designing” (https://primerexplorer.jp/e/v4_manual/). In addition, primers were selected to not have four guanines in a row to prevent the formation of tetraplex structures which could disrupt the RT-LAMP reaction. RT-LAMP primers are listed in Table 1 and correspond to the nonstructural protein 3 (NSP3) coding region of open reading frame (ORF) 1Ab. Primer sets include

Table 1. RT-LAMP primers for SARS-COV-2 detection.

Primer	Sequence (5' to 3')
F3	TCCAGATGAGGATGAAGAAGA
B3	AGTCTGAACAACTGGTGTAAAG
FIP(F1c+F2)	AGAGCAGCAGAAGTGGCACAGGTGATTGTGAAGAAGAAGAG
BIP(B1c+B2)	TCAACCTGAAGAAGAGCAAGAAGCTGATTGTCTCACTGCC
LoopF	CTCATATTGAGTTGATGGCTCA
LoopB	ACAAACTGTTGGTCAACAAGAC

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an outer forward primer (F3), outer backward primer (B3), forward inner primer (FIP), backward inner primer (BIP), loop forward primer (LF), and loop backward primer (LB). Primers were ordered from Integrated DNA Technologies and the FIPs and BIPs are HPLC purified.

Control strain details

SARS-CoV-2 PCR-standard was designed from nucleotide 2941–3420 of the SARS-CoV-2 Wuhan-Hu-1 complete genome (MN908947). The SARS-CoV-2 ssDNA control fragment was synthesized through Invitrogen GeneArt. An oligo of GenBank MN908947.3 was used as there were no commercial sources for a SARS-CoV-2 control plasmid at the time this paper was prepared. The fragment was reconstituted to 10ng/μL and used for LAMP and qPCR experiments. Approximate copy numbers were calculated using a Copy Number calculator [7]. RT-LAMP and PCR controls for Middle East Respiratory Syndrome (MERS), Betacoronavirus England-1 (BtCoV), and Murine hepatitis virus (MHV) were similarity made. We were unable to make an RT-LAMP and a PCR control for any SARS sequences as this is controlled under European Union export regulations.

Ethics statement

All studies are in compliance with ethical practices and were approved by Beaumont Health's Institutional Review Board (IRB approval #2020–040 and 2016–044). For studies using samples from healthy participants, the study participants were provided with an information sheet and gave their informed verbal consent to participate, as approved by the IRB. Since samples were deidentified and the study presented no more than minimal risk or harm to the subjects, written consent was not obtained as the principal risk would be potential harm resulting from a breach of confidentiality. The IRB determined that a waiver of consent documentation was appropriate given the study involves no more than minimal risk to human participants per the code of federal regulations. Submission of the sample was interpreted as the participants' informed consent to participate. For studies using samples from with COVID-19 symptoms, full written consent was obtained and documented in the patients' medical record. All experiments were performed in accordance with the ethical standards noted in the 1964 Declaration of Helsinki and its later amendments.

Patient samples

Samples from multiple, consenting, adult volunteers were tested from either fresh or frozen state for each assay. Fresh samples were kept on ice until analysis. For whole mouth saliva and oropharyngeal swabs, participants refrained from eating or drinking anything other than water for 5 minutes prior to collection and rinsed their mouths with water prior to collection. Oropharyngeal swab and nasopharyngeal swabs samples were collected using a Flocked sterile plastic swab applicator which was placed in 3mL of a Universal media for Viruses, Chlamydiae,

Mycoplasmas, and Ureaplasmas vial (Becton, Dickinson and Company). To demonstrate our point-of-care RT-LAMP assay and to test possible interference with agents in serum, urine, saliva, oropharyngeal swab, and nasopharyngeal swab collection, various concentrations of SARS-CoV-2 oligo were spiked in samples for test validation. Water was used as a no template control. For direct testing of clinical samples, of RNA (in 100 μ L of eluate) was isolated from 210 μ L viral transport media from nasopharyngeal swabs using Biomerieux easyMAG and Promega Maxwell (Viral Total Nucleic Acid; AS1330) automated extraction instruments by Beaumont's Clinical Laboratory Improvement Amendments (CLIA)-licensed Clinical Reference Lab. Patients were confirmed to be SARS-CoV-2 positive or negative by one of the following SARS-CoV-2 qRT-PCR tests: fNxTAG CoV Extended Panel Assay on the Luminex MAGPIX instrument; or the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel on a Maxwell Instrument. The CDC assay targets two different regions of the N gene (N1 and N2). We tested 30 SARS-CoV-2 positive samples and 30 SARS-CoV-2 negative samples.

SARS-CoV-2 RT-LAMP primer specificity

Percent mismatch for RT-LAMP primers was determined by aligning the SARS-CoV-2 genome (Wuhan City, Hubei, 2019-12-26, GenBank ID MN908947) to 22 other sequenced SARS-CoV-2 strains from various locations around the world using the Global Initiative on Sharing All Influenza data (GISAID), EpiFlu database and the BLAST Global Alignment tool [8,9]. RT-LAMP primers were also compared to other coronaviruses using the BLAST Global Alignment tool to determine specificity and percent mismatch using the same method. Percent mismatch was calculated using the following equation:

$$\%Mismatch = \frac{\text{Total \# of nucleotide mismatches between each primer and sequence}}{\text{Total \# of nucleotides in all primers}}$$

RT-LAMP

All set-up and execution of RT-LAMP reactions were done in an enclosed room using designated pipettes and filter tips. Analysis and imaging took place in separate rooms to prevent contamination. RT-LAMP reactions were executed in a total volume of 25 μ L of 1x isothermic amplification buffer, 1.4 mM dNTPs, 8 mM MgSO₄, 1.6 μ M FIP/BIP, 0.2 μ M F3/B3, 0.4 μ M FL/BL primers, 0.32 U/ μ L *Bst* 2.0, 1 U/ μ L Antarctic Thermolabile UDG, and 0.6 U/ μ L *Warm-Start Reverse Transcriptase* in ddH₂O. A total of 2.0 μ L of patient sample was used per reaction. Addition of the uracil-DNA glycosylase (UDG) reduced crossover contamination from previous reactions. Reactions were set-up on ice, incubated at 63°C for 30 minutes (15–45 minutes have been tested), and then inactivated at 80°C for 10 minutes. In order to optimize visualization of positive reactions, 2 μ L SYBR Green I was added to reactions at a 1:10 dilution. For testing RNA isolated from clinical samples with COVID-19 symptoms, 3 μ L of sample were used and reactions were done for 45 minutes. All experiments were replicated 3–5 times.

qRT-PCR

For testing, a serial dilution of SARS-CoV-2 ssDNA control fragment was made, from 2 ng to 0.06 fg. Nuclease-free ddH₂O was used as a no template control. No template control or SARS-CoV-2 was added to SYBRSelect Master Mix (ThermoFisher), and 10 μ M forward and reverse primers with a total reaction volume of 20 μ L. Primers used were F 5' – ATTTGGTGCCACTTCTGCTGC – 3' and R 5' – TCACTGCCGCTTGTGACCA – 3'. Cycling conditions

were 50 °C for 2 minutes and 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute, performed on a QuantStudio 3 PCR System (Applied Biosystems).

Analysis of RT-LAMP

1:10 SYBR green I (Life Technologies) dilution was made in TAE buffer, then 2 µL of the SYBR dilution was added to the RT-LAMP reactions. The visual change of color (orange to yellow) can be used to identify positive amplifications. In addition, the SYBR green I PCR tubes were imaged under UV light in the Bio-Rad ChemiDoc XRS+ Imaging System as the reaction creates a fluorescent output. One half of the RT-LAMP reaction was electrophoresed along with Invitrogen Low DNA Mass Ladder on a 2% agarose gel in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) at 90 V for 90 minutes. Gels were imaged under UV light using the Bio-Rad ChemiDoc XRS+ Imaging System. Lanes containing a ladder banding pattern were qualified as a positive amplification. Clinical samples were considered positive for RT-LAMP products if they had both a fluorescent signal by excitation of UV light and if they had a laddering pattern when run on a gel.

Results

RT-LAMP is specific and sensitive for SARS-CoV-2

To establish the optimal conditions for RT-LAMP using a SARS-CoV-2 PCR-standard designed from nucleotide 2941–3420 of the SARS-CoV-2 Wuhan-Hu-1 complete genome (MN908947), several primer sets, ranges of incubation temperatures (57–65°C), and incubation times (15–45 mins) were tested. The best amplification results were obtained at 63°C for 30 minutes as indicated by a banding pattern after electrophoresis on a gel (Fig 1). Positive reactions containing SYBR Green I could be observed by naked eye by a color change from orange to yellow (Fig 1-top panel), with a fluorescent light in response to UV excitation (Fig 1- middle panel), or by a laddering pattern of bands after electrophoresis on a gel (Fig 1-bottom panel). The RT-LAMP reaction required all 6 primers to work under the optimized conditions; removing the forward and backward inner primers or the loop primers did not result in a positive result (Fig 1). In order to determine the lower detection limit of the RT-LAMP reaction for SARS-CoV-2, a dilution series ranging from 10.0 ng to 0.06 fg SARS-CoV-2 DNA oligo was amplified (Fig 2). This corresponds to approximately 3.802×10^{10} to 228 copies of virus. The viral load in patients has been reported to range from 641 copies/mL to 1.34×10^{11} copies/mL, with a median of 7.99×10^4 in throat samples and 7.52×10^5 in sputum samples [10]. The limit of detection was estimated to be 0.08 fg, which is approximately equal to 304 viral copies. This dilution series was run in parallel with qRT-PCR using primers that targeted this same region of the SARS-CoV-2 genome; the qRT-PCR Ct values for the dilution series are reported in S1 Table.

SARS-CoV-2 detection in simulated clinical samples with RT-LAMP

To demonstrate the clinical utility of this system for SARS-CoV-2 detection, we spiked various human specimens with SARS-CoV-2, Middle East Respiratory Syndrome (MERS), Betacoronavirus England-1 (BtCoV), or Murine hepatitis virus (MHV) control oligos. MERS, BtCoV and MHV spiked samples were used to test the specificity of the RT-LAMP assay. Spiked specimens included serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs as we wanted to verify that substances present in these samples would not interfere with RT-LAMP. Samples were directly used for RT-LAMP without performing nucleic acid isolation. Only samples containing SARS-CoV-2, but not MERS, BtCoV, or MHV, had positive RT-LAMP reactions indicating specificity of the reaction for SARS-CoV-2 (Fig 3). Furthermore,

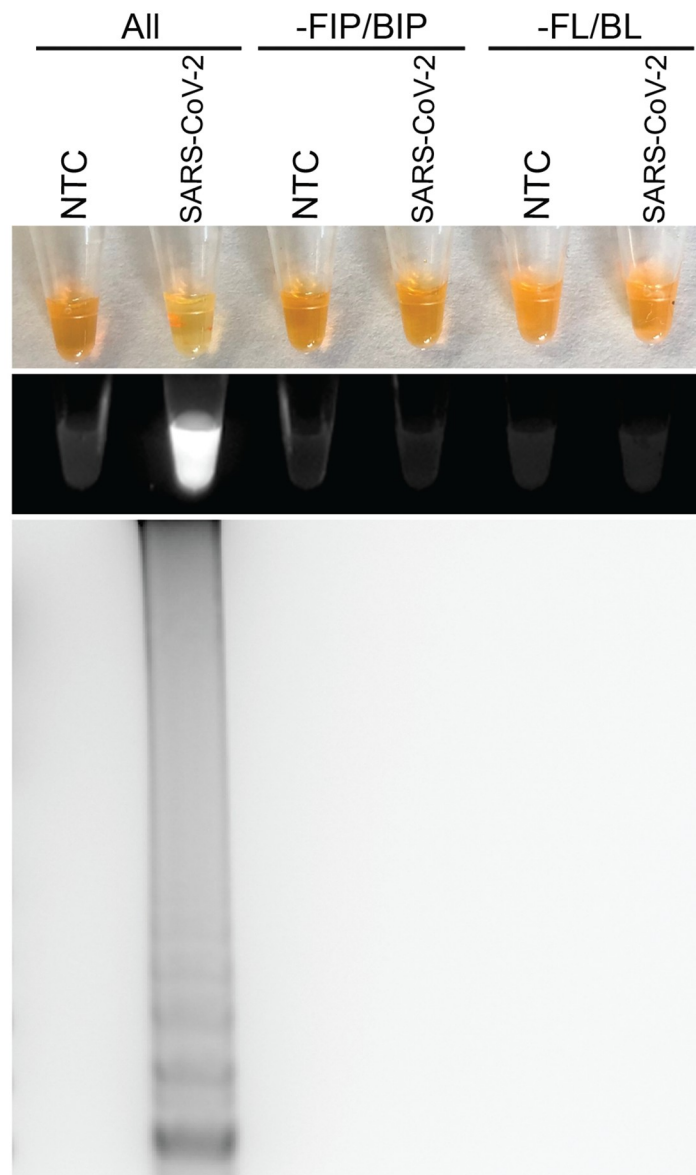


Fig 1. RT-LAMP detection of SARS-CoV-2. (A) SARS-CoV-2 RT-LAMP amplification of SARS-CoV-2 PCR standard (SARS-CoV-2; IDT custom oligo) but not no template control (NTC; negative control) as visualized by addition of SYBR Green I (SYBR) by eye (upper panel), fluorescence (middle panel), or gel electrophoresis (bottom panel). All primers (ALL) are required for effective RT-LAMP reaction. Reactions without FIP and BIP (-FIP/BIP) or FL and BL (-FL/BL) primers resulted in a negative RT-LAMP reaction.

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RT-LAMP could be successfully completed using human serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs.

RT-LAMP is specific for SARS-CoV-2

The sequences of the RT-LAMP primers were also compared to aligned sequences of various strains of Severe Acute Respiratory Syndrome coronaviruses (SARS) or coronaviruses commonly associated with the common cold (human coronavirus strains 229E, NL63, HKU1, or OC43). These other coronaviruses had 27–54% nucleotide mismatch with our RT-LAMP

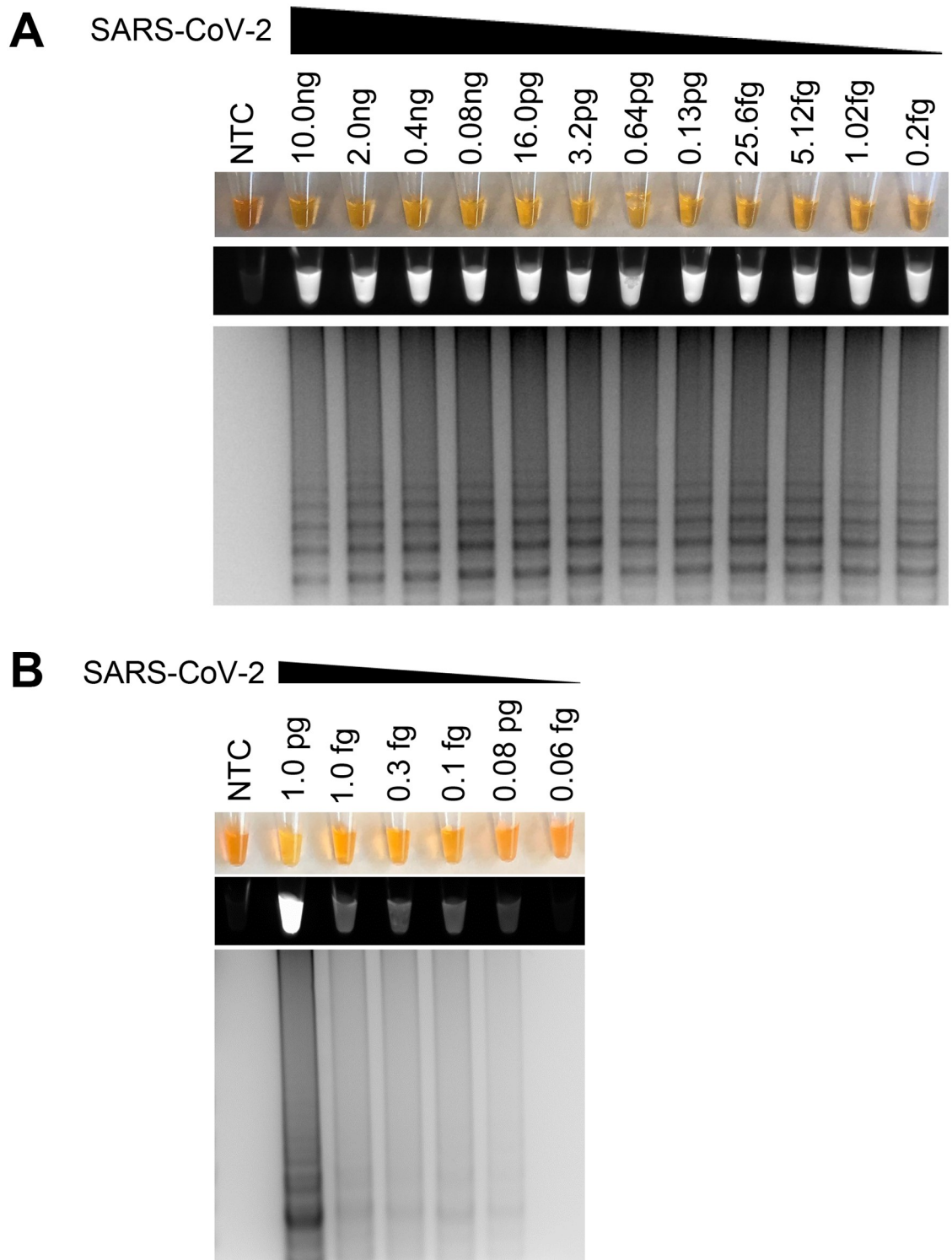


Fig 2. SARS-CoV-2 RT-LAMP sensitivity for SARS-CoV-2. Sensitivity assessment of SARS-CoV-2 RT-LAMP using serial dilutions of SARS-CoV-2 PCR Standard from 10.0 ng/reaction to 0.06 fg/reaction as visualized by addition of SYBR Green I by eye (upper panel), fluorescence (middle panel), or gel electrophoresis (bottom panel). NTC: No template control (negative control).

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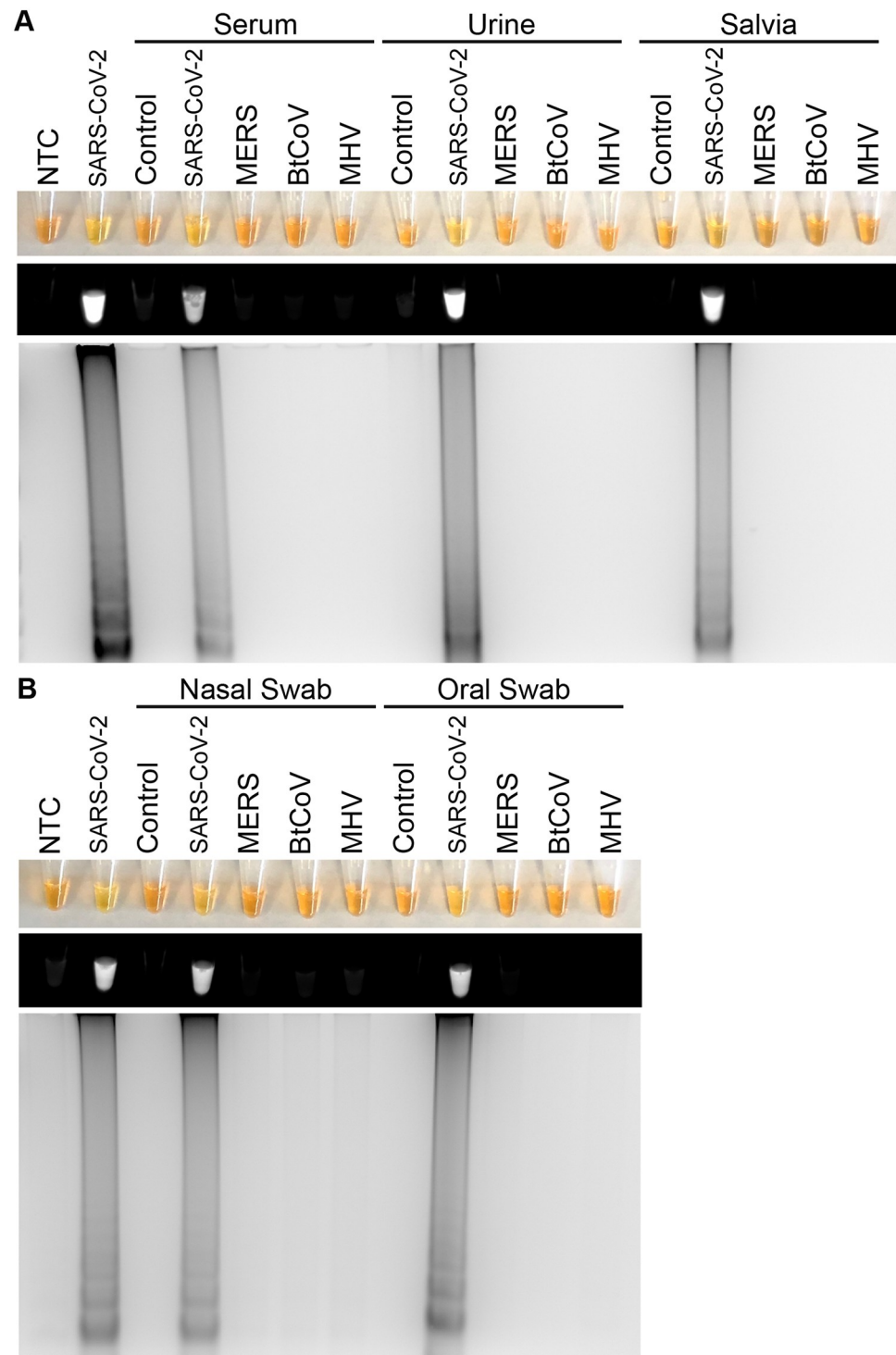


Fig 3. SARS-CoV-2 RT-LAMP specificity for SARS-CoV-2 in simulated patient samples. Specificity assessment of SARS-CoV-2 RT-LAMP in control samples (control) or samples spiked with SARS-CoV-2, MERS, BtCoV, MHV PCR standards (IDT custom oligos) as visualized by the addition of SYBR Green I by eye (upper panel), fluorescence (middle panel), or gel electrophoresis (bottom panel). Types of human samples tested included serum, urine, saliva, nasopharyngeal swabs (nasal swab), or oropharyngeal swabs (oral swab). NTC: No template control (negative control). 3–5 different patient samples were tested for each condition with one representative patient being shown.

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Table 2. RT-LAMP primers alignment with other coronaviruses.

Virus	GenBank ID	% Mismatch
SARS-COV-2	MN908947	0
Bat SARS-like CoV 2015	MG772933.1	27-22
Bat SARS-like CoV 2017	MG772934.1	30-77
Bat SARS CoV RM1/2004	KY417144.1	40-83
SARS CoV ZS-C	AY395003.1	42-60
Civet SARS CoV SZ16/2003	AY304488.1	39-64
SARS CoV	NC_004718.3	40-24
SARS CoV MA15	FJ882957.1	40-24
Middle East Respiratory CoV	NC_019843.3	52-07
Betacoronavirus England 1	NC_038294.1	54-44
Murine hepatitis virus	NC_001846.1	53-25
Human Coronavirus 229E	NC_002645.1	51-48
Human Coronavirus NL63	NC_005831.2	54-44
Human Coronavirus HKU1	NC_006577.2	53-25
Human Coronavirus OC43	NC_006213.1	49-11

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primers, making it highly unlikely they would give a positive RT-LAMP result, supporting the specificity of this assay for SARS-CoV-2 (Table 2). Given that viruses are prone to genetic mutation, we likewise examined if RT-LAMP primers had any mismatch with 27 different isolated strains of SARS-CoV-2 from various locations. The 27 strains were from different regions of China (Hubei, Zhejiang, and Guangdong providences), France, Thailand, Finland, United States, and Australia. The strains were collected and sequenced from 12/24/2019 to 01/29/2020. Thus these 27 strains reflect more than one region and time point. There was 0% mismatch with all the strains examined, suggesting that these RT-LAMP primers would identify all 27 strains of SARS-CoV-2 examined (S2 Table).

RT-LAMP detection of SARS-CoV-2 in clinical specimens

We next sought to determine if RT-LAMP could detect SARS-CoV-2 in samples collected from patients with COVID-19. Patients with COVID-19 symptoms were confirmed positive or negative for SARS-CoV-2 using standard clinical qRT-PCR testing. Using the same RNA from the patients that was used for the qRT-PCR testing, RT-LAMP was in agreement with the qRT-PCR results in 95% (N = 19/20) of the SARS-CoV-2 positive by qRT-PCR patients (Fig 4A), and 90% (N = 18/20) of the SARS-CoV-2 negative by qRT-PCR patients (Fig 4B). Given that spiking nasopharyngeal swabs samples in viral transport media with SARS-CoV-2 control oligos was able to work with RT-LAMP (Fig 3), we next tested to determine if RT-LAMP could detect SARS-CoV-2 directly without RNA isolation in these nasopharyngeal swab samples. RT-LAMP detected 40% (N = 4/10) of SARS-CoV-2 positive by qRT-PCR patients, and 100% (N = 10/10) of the SARS-CoV-2 negative by qRT-PCR patients (Fig 5). The samples that were positive by RT-LAMP all had a high level of viremia, as indicated by the Ct values less than 24 Ct by qRT-PCR. In contrast, all the samples negative by RT-LAMP had a Ct value greater than 24 Ct by qRT-PCR.

Discussion

Given the rapid emergence of SARS-CoV-2 and the severe complications that can result including acute respiratory distress syndrome (ARDS), pneumonia, and even death, improved

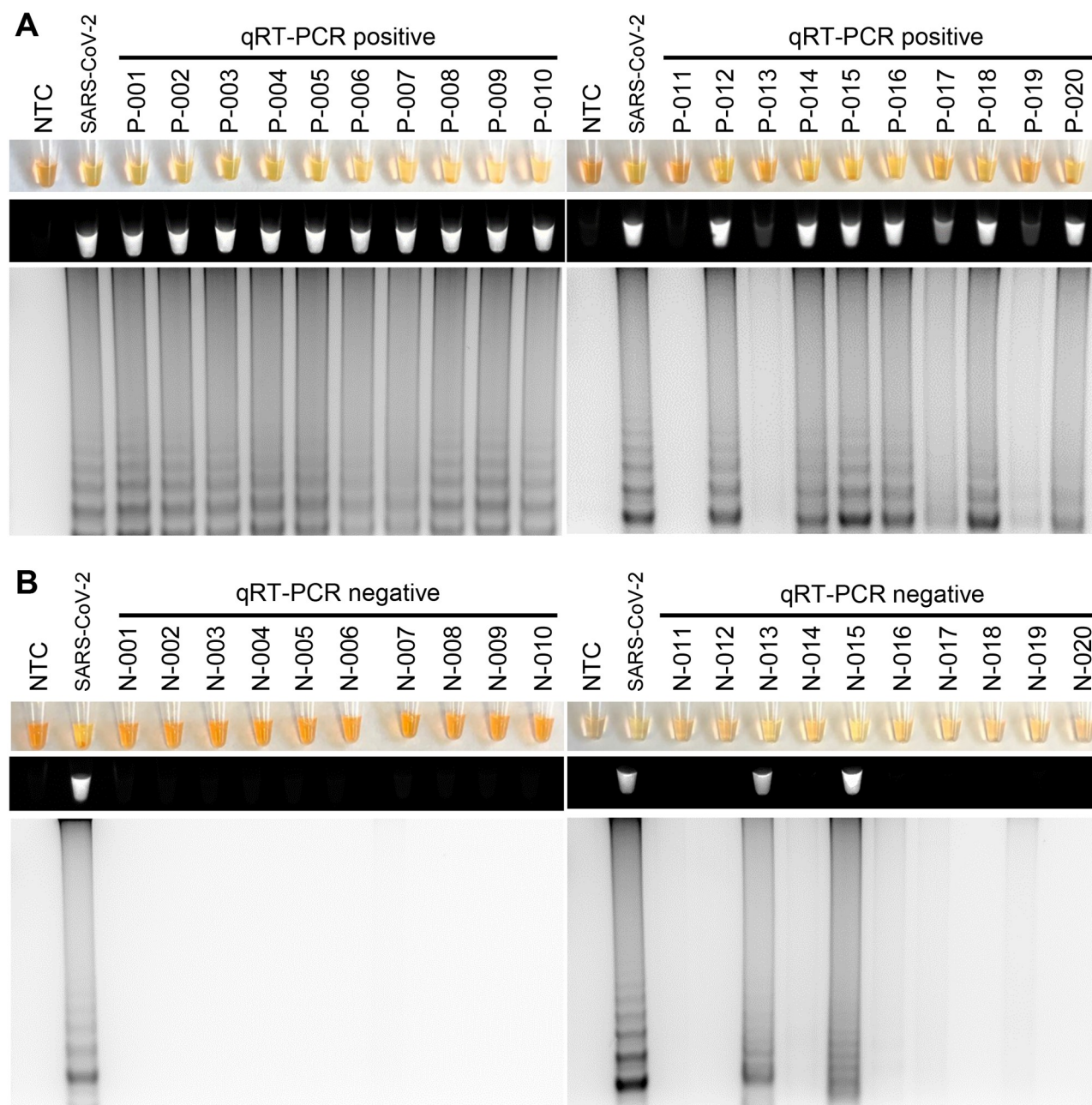


Fig 4. Detection of SARS-CoV-2 with RT-LAMP in patient nasopharyngeal swab samples with RNA isolation. RNA isolated from patients with COVID-19 symptoms with (A) or without (B) SARS-CoV-2 detection by qRT-PCR were tested for SARS-CoV-2 by RT-LAMP. SARS-CoV-2 detection by RT-LAMP was visualized by the addition of SYBR Green I by eye (upper panels), fluorescence (middle panels), or gel electrophoresis (bottom panels).

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diagnostic options that are fast, reliable, easy, and affordable are required. This is critical since SARS-CoV-2 infection can rapidly progress from hospital admission to ARDS in as few as 2 days, and SARS-CoV-2 infection can be fatal [1]. qRT-PCR is specific and sensitive. However, qRT-PCR requires trained clinical laboratory personnel to use specialized equipment in a qualified laboratory setting. Since this virus is spreading rapidly and is highly contagious, centralized labs have had trouble keeping up with testing demands in part due to a significant

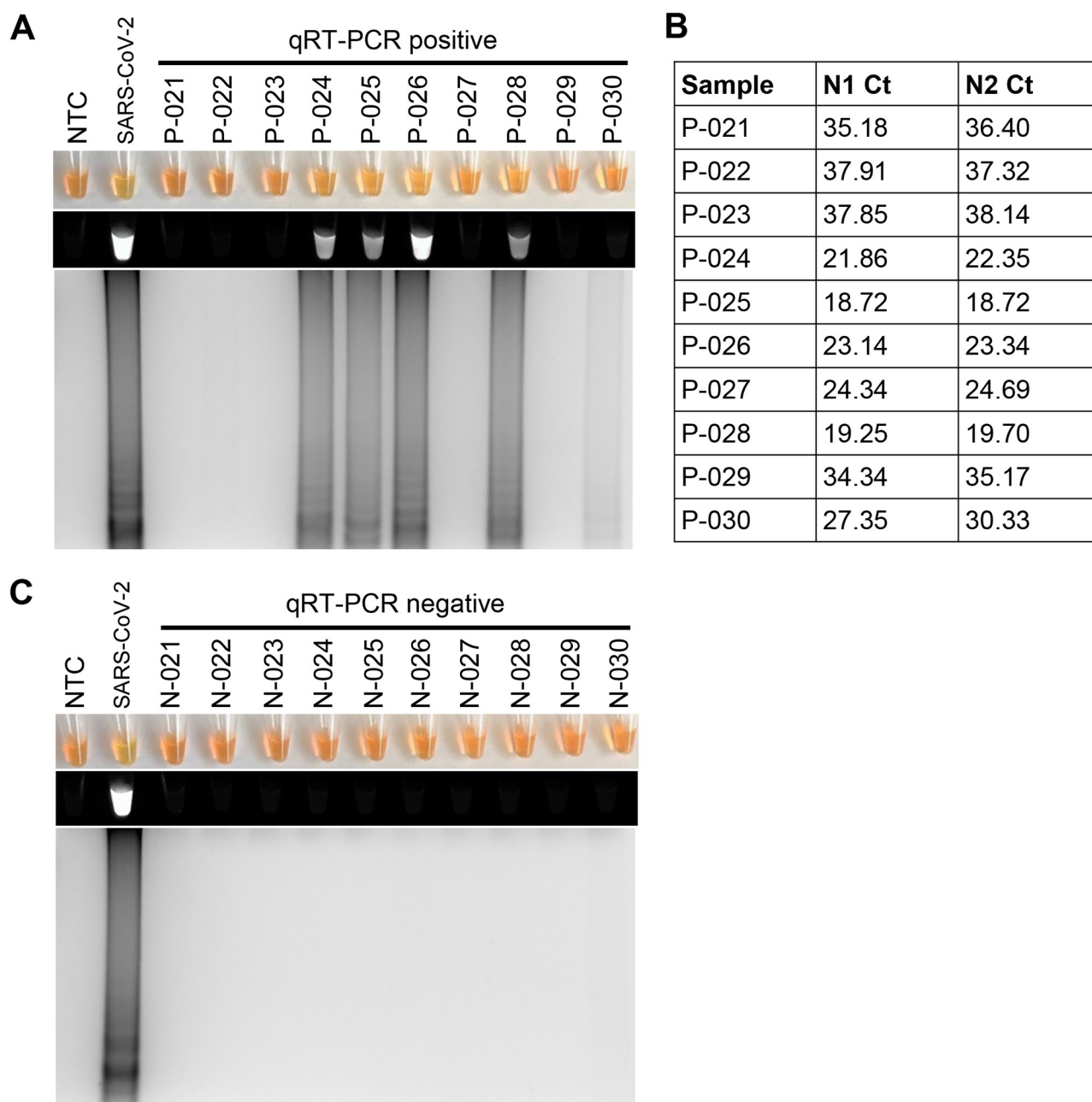


Fig 5. Detection of SARS-CoV-2 with RT-LAMP in patient nasopharyngeal swab samples without RNA isolation. Nasopharyngeal swabs in viral transport media from patients with COVID-19 symptoms with (A) or without (C) SARS-CoV-2 detection by qRT-PCR were tested for SARS-CoV-2 by RT-LAMP. SARS-CoV-2 detection by RT-LAMP was visualized by the addition of SYBR Green I by eye (upper panels), fluorescence (middle panels), or gel electrophoresis (bottom panels). B) Average Ct values from qRT-PCR performed on isolated RNA from the same samples in panel A are listed for target SARS-CoV-2 genes N1 and N2.

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shortage in available qRT-PCR tests despite significant investments by local governments and private industry. This feasibility study demonstrated that RT-LAMP allows rapid detection of SARS-CoV-2 in a variety of common human specimens collected for clinical testing, including serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs.

Currently, clinical testing for SARS-CoV-2 is done by central testing laboratories, which may take one or more days. This study sought to improve upon this by developing a potential point-of-care test. Point-of-care testing has several advantages for SARS-CoV-2. Point-of-care testing should be fast, easy to use by a range of users, low cost, and require little if any laboratory infrastructure. Furthermore, the test needs to be reliable, sensitive, and specific. RT-LAMP meets these requirements and therefore has large value for screening and testing for SARS-CoV-2 in potentially exposed populations. Other groups are working to adopt RT-LAMP for SARS-CoV-2 into alternative readouts. For example, the primers designed by our group along with two other primer sets were adopted for fluorogenic oligonucleotide strand exchange (OSD) probes [11]. This method could allow multiplexing and thus more than one genomic target of SARS-CoV-2 to be included in an assay. Furthermore, this group also demonstrated that the RT-LAMP primers described here and modified for OSD are compatible for RT-LAMP with the Bst DNA polymerase large fragment (Bst-LF), which possesses reverse transcriptase ability as well as DNA polymerase activity. This detected down to 100 SARS-CoV-2 genomes [11] and Bst-LF can be made by bacteria without a purification step, useful for situations with an interrupted supply chain or in settings that are resource poor. RT-LAMP, including the primers described here, are also currently being adopted for LAMP-Seq [12]. This could allow large scale processing using barcoding and next-generation sequencing to pool samples and subsequently identify positive individuals.

RT-LAMP was sensitive for detection of SARS-CoV-2. We were able to detect down to the equivalent of approximately 304 viral copies. Bharda et al have also reported that use of all six of our RT-LAMP primers can detect down to 100 copies of SARS-CoV-2 genomic RNA [11]. The viral load in patients has been reported to range from 641 copies/mL to 1.34×10^{11} copies/mL, with a median of 7.99×10^4 in throat samples and 7.52×10^5 in sputum samples [10]. As such, RT-LAMP is sensitive enough for detection of SARS-CoV-2 in clinical samples.

We sought to determine if this RT-LAMP assay worked in a range of different samples that might be collected in a clinical setting or as a possible non-invasive screening tool. This was important to determine since it may not be feasible to always collect certain specimens. For example, swabs used for nasopharyngeal sampling as well as viral transport media has experienced shortages recently. Although SARS-CoV-2 has been reported to not be detectable in serum of all patients [13], serum may also be difficult to collect from patients who are critically sick or dehydrated, or from elderly, children, and neonates. Furthermore, not all biological samples may be compatible with RT-LAMP. Some sample types may contain chemicals that inhibit nucleic acid assays if the sample is tested directly without first isolating the RNA. RT-LAMP worked in all the human sample types tested when SARS-CoV-2 control oligos were spiked into the samples. We tested this in samples collected from several individuals to control for variation in sample composition across different people. We also demonstrated that RT-LAMP has similar performance to qRT-LAMP in detecting SARS-CoV-2 in RNA isolated from clinical nasopharyngeal samples. Overall, RT-LAMP was positive for 95% ($N = 19/20$) of samples positive by qRT-PCR and RT-LAMP was also negative for 90% ($N = 18/20$) of samples negative by qRT-PCR. The two samples that were positive by RT-LAMP but negative by qRT-PCR could represent false positives, contamination, or increased sensitivity of RT-LAMP compared to qRT-PCR. While RT-LAMP can be prone to false positives, we have taken several precautions to control for this in our assay set-up and false positives will usually not be positive if the samples are tested again. On retesting these samples, they were still positive which suggests that they are not false positives. While we use good laboratory practice to reduce contamination, we cannot exclude this possibility. Lastly, it is possible that RT-LAMP can be more sensitive than qRT-PCR as this has been previously described, although one of the samples that was positive by qRT-PCR was negative by RT-LAMP [14].

We previously demonstrated that RT-LAMP for ZIKV does not require prior RNA isolation from urine or serum samples [4,5]. The use of unprocessed samples could save considerable time and reduce costs. Therefore, we tested RT-LAMP with nasopharyngeal swabs placed in viral transport media directly without an RNA isolation step. In order to detect low levels of viremia, we increased the sample volume added to the RT-LAMP reaction from 2–3 μ L and also increased the RT-LAMP incubation time from 30 to 45 minutes. RNA was also isolated from these samples to verify which samples contained SARS-CoV-2 by qRT-PCR. RT-LAMP detected 40% ($N = 4/10$) of SARS-CoV-2 positive by qRT-PCR patients, and was negative for 100% ($N = 10/10$) of the SARS-CoV-2 samples also negative by qRT-PCR. The samples that were positive by RT-LAMP all had a high level of viremia, as indicated by the Ct values less than 24 Ct by qRT-PCR. This data suggests that the viral transport media may contain inhibitor(s), possibly glucose, that make RT-LAMP less effective. Hence, RT-LAMP was only able to detect SARS-CoV-2 in samples with high levels of virus. For clinical utility, RT-LAMP has better performance in RNA purified samples compared to direct testing of nasopharyngeal swabs placed in viral transport media. It may be that direct testing of nasopharyngeal swabs is possible for clinical utility by RT-LAMP if the nasal swabs are placed in an alternative matrix, such as saline, water, or ideally a preservative that can stabilize the RNA and render the virus inactive. The potential for direct RT-LAMP testing of other sample types, such as saliva, is also possible.

Primers were designed for a conserved span of SARS-CoV-2 sequence that was found in 27 isolated SARS-CoV-2 strains but was a sequence that was also divergent from related coronavirus SARS. Given that all the isolated strains of SARS-CoV-2 thus far have shown very little genetic differences, we anticipate that this RT-LAMP will detect SARS-CoV-2 with the same level of confidence as qRT-PCR. As with any molecular test, including PCR, it is possible that viral evolution may impact primer binding, but this can be monitored as new strains are sequenced. Of the mutations thus far reported as of April 5, 2020 [15], none of the reported mutations corresponded to the sequence targeted by these RT-LAMP primers. This SARS-CoV-2 RT-LAMP assay was highly specific as it did not give a positive result for MERS, BtCoV, and MHV. The RT-LAMP primers also had significant mismatch with numerous strains of SARS (27–40% mismatch) and common cold associated human coronavirus strains 229E, NL63, HKU1, or OC43 (49–54% mismatch), making it highly unlikely to have cross-reactivity with these other respiratory viruses. We tested a range of temperatures from 57°C to 65°C; all the temperatures gave comparable results, indicating that RT-LAMP for SARS-CoV-2 has a large working temperature range with an optimal temperate of 63°C. We also tested a range of incubation times from 15–45 minutes. The optimal time for detection of RT-LAMP products was 30–45 minutes, however SARS-CoV-2 RT-LAMP products were detectable by UV light excitation or banding patterns on gels in as little as 15 minutes.

This study has several limitations. First, SARS-CoV-2 is Biosafety level 3 so our laboratory was unable to work directly with purified virus. As such, most of the experiments presented here used a nucleotide oligo of SARS-CoV-2 corresponding to the GenBank MN908947 sequence. Similarly, we were unable to directly test related coronaviruses and instead used nucleotide oligos from the same region of those viruses. The primers were further evaluated for specificity by BLASTing them to related coronavirus sequences. However, we have demonstrated that RT-LAMP can detect SARS-CoV-2 in RNA isolated from nasopharyngeal swab samples at a similar detection rate with qRT-PCR. Furthermore, RT-LAMP could detect SARS-CoV-2 in some nasopharyngeal swab samples directly without an RNA isolation step. This does represent a proof-of-feasibility for this assay, in that these RT-LAMP primers can detect SARS-CoV-2 in clinical samples and should be highly specific for SARS-CoV-2 and not detect other related viruses. Although RT-LAMP reactions are highly specific, it is not a

quantitative test. Other groups are working at improving the read outs of RT-LAMP assays including the use of smartphone-integrated sensors to make interpretation of the assay even more user-friendly [16]. RT-LAMP reactions can have a higher rate of false positives compared to qRT-PCR. As such, we took several precautions including having a lateral work flow for experiments, using filter pipet tips, and including a Thermolabile Uracil-DNA Glycosylase (UDG) in all reactions to prevent possible carry-over contamination of RT-LAMP products from previous reactions. This study was not powered to determine sensitivity in a clinical population. Lastly, this is a rapidly developing area of study and all the information presented at the time of publication represent the authors' best knowledge at the time. As our understanding of SARS-CoV-2 continues to develop, this information may change.

Conclusions

Here we describe a fast and robust assay for detection of SARS-CoV-2 in 30–45 minutes. This simple assay could be used outside of a central laboratory on various types of biological samples. This assay can be completed by individuals without specialty training or equipment and may provide a new diagnostic strategy for combatting the spread of SARS-CoV-2 at the point-of-risk.

Supporting information

S1 Fig. Original images for all gels reported in figures. NTC = no template control; MWM = molecular weight marker; X = lane not included in final image.
(PDF)

S1 Table. Detection of SARS-COV-2 at different concentrations by qRT-PCR. Data shown as mean $\pm$ standard deviation.
(DOCX)

S2 Table. RT-LAMP primers alignment with different isolated strains of SARS-COV-2.
(DOCX)

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References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*.
2. Tomita N, Mori Y, Kanda H, Notomi T (2008) Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat Protoc* 3: 877–882. <https://doi.org/10.1038/nprot.2008.57> PMID: 18451795
3. Francois P, Tangomo M, Hibbs J, Bonetti EJ, Boehme CC, et al. (2011) Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. *FEMS Immunol Med Microbiol* 62: 41–48. <https://doi.org/10.1111/j.1574-695X.2011.00785.x> PMID: 21276085
4. Bartolone SN, Tree MO, Conway MJ, Chancellor MB, Lamb LE (2018) Reverse Transcription-Loop-mediated Isothermal Amplification (RT-LAMP) Assay for Zika Virus and Housekeeping Genes in Urine, Serum, and Mosquito Samples. *J Vis Exp*.
5. Lamb LE, Bartolone SN, Tree MO, Conway MJ, Rossignol J, et al. (2018) Rapid Detection of Zika Virus in Urine Samples and Infected Mosquitos by Reverse Transcription-Loop-Mediated Isothermal Amplification. *Sci Rep* 8: 3803. <https://doi.org/10.1038/s41598-018-22102-5> PMID: 29491389
6. Chinese SMEC (2004) Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303: 1666–1669. <https://doi.org/10.1126/science.1092002> PMID: 14752165
7. Staroscik A (2012) Copy number calculator for realtime PCR.
8. Shu Y, McCauley J (2017) GISAID: Global initiative on sharing all influenza data—from vision to reality. *Euro Surveill* 22.
9. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402. <https://doi.org/10.1093/nar/25.17.3389> PMID: 9254694
10. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q (2020) Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 20: 411–412. [https://doi.org/10.1016/S1473-3099\(20\)30113-4](https://doi.org/10.1016/S1473-3099(20)30113-4) PMID: 32105638
11. Bhadra S, Riedel TE, Lakhotia S, Tran ND, Ellington AD (2020) High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes. *bioRxiv*: 2020.2004.2013.039941.
12. Schmid-Burgk JL, Li D, Feldman D, Slabicki M, Borrajo J, et al. (2020) LAMP-Seq: Population-Scale COVID-19 Diagnostics Using a Compressed Barcode Space. *bioRxiv*: 2020.2004.2006.025635.
13. Wang W, Xu Y, Gao R, Lu R, Han K, et al. (2020) Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*.
14. Parida MM, Santhosh SR, Dash PK, Tripathi NK, Saxena P, et al. (2006) Development and Evaluation of Reverse Transcription-Loop-Mediated Isothermal Amplification Assay for Rapid and Real-Time Detection of Japanese Encephalitis Virus. *Journal of Clinical Microbiology* 44: 4172–4178. <https://doi.org/10.1128/JCM.01487-06> PMID: 17005741
15. Khailany RA, Safdar M, Ozaslan M (2020) Genomic characterization of a novel SARS-CoV-2. *Gene Rep*: 100682.
16. Becherer L, Borst N, Bakheit M, Frischmann S, Zengerle R, et al. (2020) Loop-mediated isothermal amplification (LAMP)—review and classification of methods for sequence-specific detection. *Analytical Methods*.

RESEARCH ARTICLE

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Proteomic analysis of bladder biopsies from interstitial cystitis/bladder pain syndrome patients with and without Hunner's lesions reveals differences in expression of inflammatory and structural proteins

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Abstract

Background: Interstitial cystitis/bladder pain syndrome is a bladder disease usually characterized by pain, urgency, and frequency. Interstitial cystitis is currently classified into two subtypes, with and without Hunner's lesions. However, the underlying etiology of interstitial cystitis and its subtypes are largely unknown.

Methods: To better understand the biological changes in the bladder of interstitial cystitis/bladder pain syndrome patients, we directly analyzed bladder tissue of interstitial cystitis patients, both those with Hunner's lesions and those without. Proteins in the bladder biopsies were analyzed using nanoscale high-performance liquid chromatography-tandem mass spectrometry. Disease subgroups were compared and significantly expressed proteins were mapped using STRING to determine protein associations and functions.

Results: We found that patients with Hunner's lesions had significant increases in inflammatory and endoplasmic reticulum stress proteins, with a decrease in cellular adhesive proteins, compared to patients without Hunner's lesions. These patients also exhibited a decrease in proteins associated with the Rap1 signaling pathway, which regulates cell proliferation and wound healing. When comparing diseased and non-disease-apparent tissue in patients with Hunner's lesions, diseased tissue exhibited a decrease in ubiquitination proteins.

Conclusions: In summary, there are significant differences in protein expression found in the bladders of interstitial cystitis patients with and without Hunner's lesions, indicating a disturbance in proteins associated with cellular adhesion, proliferation, protein processing, and wound healing.

Keywords: Interstitial cystitis, Proteomics, HPLC, Urinary bladder

Background

Interstitial cystitis (IC) is a disease of the bladder that can be characterized by urinary frequency, urgency, pelvic pressure, and pain in the absence of a urinary tract infection. The projected incidence of IC varies, ranging from 45 per 100,000 to as many as 307 per 100,000 individuals [1, 2]. However, the incidence may be higher than estimated as IC is commonly misdiagnosed. The

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pathogenicity of IC is currently unknown, though multiple theories have been proposed [3]. There are two IC/BPS subtypes, the first is IC/BPS with Hunner's lesions (HIC; also known as Hunner's ulcers), representing 5–10% of IC/BPS patients [4]. HIC is diagnosed via cystoscopy and is characterized by areas of increased inflammation and cellular degradation. HIC patients often have more severe symptoms, including increased pain, sensitivity, and changes in bladder permeability [5]. The second subtype is IC/BPS without Hunner's lesions (NHIC). NHIC is difficult to diagnose given the lack of clear physiological changes; patients may or may not have areas of submucosal glomerulations, or small areas of submucosal bleeding in the bladder wall distinct from Hunner's lesions. As a result, diagnosis is mainly based on exclusion of other conditions with similar symptoms [6].

While IC/BPS is a widely studied urological condition, the molecular differences between healthy patients, NHIC and HIC remain poorly understood. Several studies have illustrated that IC bladders, compared to healthy bladders, display decreased cellular proliferation and cell permeability [7, 8]. Additionally, biomarker studies have identified distinct differences between HIC and NHIC bladders; urine from HIC patients have higher concentrations of inflammatory cytokines and urothelial breakdown products [9]. The differences between diseased and non-disease-apparent (NDA) areas within one bladder also remains unknown. Therefore, the goal of our study is to use nanoscale high performance liquid chromatography coupled to tandem mass spectrometry (nHPLC-MS/MS) on bladder biopsies of diseased and NDA tissue to identify distinct protein differences between NHIC and HIC. This method is highly sensitive, which is beneficial when working with limited sample volume, such as bladder biopsies. This is the first time nHPLC-MS/MS has been used to examine IC bladders directly. Using this innovative approach, we found that HIC patients exhibit increased inflammatory proteins and decreased cellular adhesion proteins in the urothelium, as well as increased ER stress, suggesting a possible difference in disease etiology between HIC and NHIC.

Methods

Sample collection and storage

This study was approved by the institutional IRB (IRB# 2009-218) and written consent for all participants was obtained. Disease state was determined during cystoscopy by a trained urologist. Bladder samples were obtained from 4 female patients with HIC and 4 female patients with NHIC during routine medical cystoscopy. Two biopsies were collected from each patient: one from a diseased site (glomerulations in NHIC patients, Hunner's lesions in HIC patients), and another from an

adjacent non-disease-apparent (NDA) site as determined by an experienced urologist. 5 mm × 5 mm samples of bladder tissue were snap-frozen and stored at − 80 °C. Patients were asked to complete the validated Interstitial Cystitis Symptom Index and Problem Index (ICSI/ICPI) to measure lower urinary tract symptoms (LUTS) and impact on patient quality of life (QOL) [10]. ICSI scores range from 0 to 19 and ICPI scores range from 0 to 16, with higher scores representing more severe symptoms and impact [3].

Sample processing

Deidentified samples were sent to MSBioworks for processing and analysis. Samples were blinded and randomized to the researchers. Samples were washed in 1 × PBS and mechanically disrupted in a Bullet Blender (NextAdvance) with 1.6 mm stainless steel beads in 100 µL of modified RIPA lysis buffer (2% SDS, 150 mM NaCl, 50 mM Tris pH 8, 1X Roche complete protease inhibitor). Samples were incubated at 60 °C for 30 min, then clarified by centrifugation. The protein concentration of each extract was determined by Qubit fluorometry (Invitrogen). 10 µg of each sample was processed by SDS-PAGE using a 10% Bis-Tris NuPage mini-gel (Invitrogen) in the MES buffer system. The migration window (2 cm lane) for each sample was excised into ten equally sized bands, each was processed by in-gel digestion with trypsin using a ProGest robot (DigiLab). Samples were first washed with 25 mM ammonium bicarbonate, followed by acetonitrile. Samples were reduced with 10 mM dithiothreitol at 60 °C, followed by alkylation with 50 mM iodoacetamide at room temperature. Digestion was performed with trypsin at 37 °C for 4 h. Finally, samples were quenched with formic acid and the supernatant was analyzed via nHPLC-MS/MS.

Liquid chromatography and mass spectrometry

Sample supernatants were analyzed by nHPLC-MS/MS with a NanoAcquity HPLC system (Waters) linked to a Q Exactive mass spectrometer (ThermoFisher). Digested proteins were loaded on a Luna C18 resin (Phenomenex) trapping column and eluted over a Luna C18 resin 75 µm analytical column at a flow rate of 350 nL/min. The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 70,000 FWHM for MS and 17,500 FWHM for MS/MS. The fifteen most abundant ions were selected for MS/MS.

Data processing and statistical analysis

Data was analyzed using Mascot with the following parameters: the database used was SwissProt Human (concatenated forward and reverse plus common contaminants), with the enzyme set to Trypsin/P. Fixed

modifications were set to Carbamidomethyl (C), while variable modifications included Acetyl (N-term), Deamidation (N,Q), Oxidation (M), and Pyro-Glu (N-term Q). Mass values were monoisotopic. Peptide and Fragment Mass Tolerances were 10 ppm and 0.02 Da, respectively. 2 max missed cleavages were allowed. After Mascot processing, data was parsed into Scaffold for validation and filtering. Data were filtered using a 1% protein and peptide false discovery rate (FDR) while requiring at least 2 unique peptides per protein. To determine approximate relative abundance of proteins within a given sample and between samples, normalized spectral abundance factor (NSAF) was calculated based on the following equation:

$$\text{NSAF} = (\text{SpC}/\text{MW}) / \sum (\text{SpC}/\text{MW})_N$$

where SpC = Spectral Counts, MW = Protein Molecular Weight (in kDa), and N = Total Number of Proteins. Significant proteins were determined by comparing average NSAF values for diseased and adjacent NDA tissues to generate fold change values, as well as a T-test for significance. Proteins were considered significant if they had a fold change difference ≥ 4 and a p value ≤ 0.05 . Interactions and relationships between these proteins were mapped using STRING. Proteins were connected by function and interaction, with interactions predicted by experimental data, database searches, text mining, and co-expression.

STRING mapping

Significant proteins were uploaded to STRING [11] (string-db.org) and mapped to predict protein–protein interactions, represented by colored lines between protein nodes. These relationships include known interactions determined by experiments (purple) and database mining (teal), predicted interactions via gene neighborhood (green), co-occurrence (blue), or gene fusion (red), or from text mining (yellow), co-expression (black), or protein homology (light blue).

Results

Our group was interested in understanding the differences in protein expression between bladder biopsies from HIC and NHIC patients. These biopsies were processed and analyzed via nHPLC-MS/MS (Fig. 1), resulting in 2,625 unique proteins identified between all patient samples. Patients also completed the ICSI and ICPI questionnaires, with HIC patients reporting higher average scores than NHIC patients (Table 1). This is consistent with what has been previously reported. When comparing protein of interest between subgroups, we focused on proteins that showed a fold change of $\geq 4\times$ and a significant difference between groups (e.g. $p \leq 0.05$).

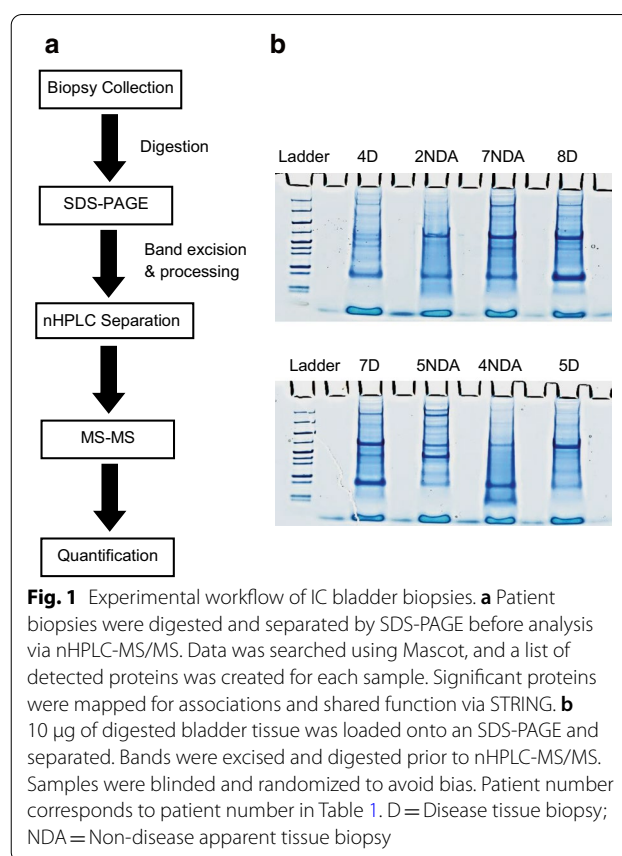


Table 1 Patient demographics

Patient #	Age	Gender	IC subtype	ICSI score	ICPI score
1	60	F	HIC	19	16
2	79	F	HIC	15	16
3	71	F	HIC	N/A	N/A
4	55	F	HIC	18	15
5	36	F	NHIC	10	9
6	63	F	NHIC	19	16
7	43	F	NHIC	11	13
8	53	F	NHIC	15	12

Diseased areas in HIC patients exhibit decreased expression of ubiquitination proteins compared to NDA areas in HIC patients

First, we compared diseased areas of HIC tissue to NDA areas of HIC bladders to determine protein differences. 18 proteins were found to be significantly altered when comparing NDA to diseased HIC tissues. Three of these proteins were linked to ubiquitination (Table 2).

Table 2 Diseased Hunner's IC biopsies compared to non-disease-apparent Hunner's IC biopsies

Accession ID	Gene name	Description	Fold change	p value ($p \leq 0.05$)
		Ubiquitination (down-regulated) 3 of 18 significant proteins	NDA HIC/diseased HIC	
P68036	UBE2L3	Ubiquitin-conjugating enzyme E2 L3	7.565	0.0119
P63279	UBE2I	SUMO-conjugating enzyme UBC9	7.288	0.0347
Q7Z6Z7	HUWE1	E3 ubiquitin-protein ligase	6.997	0.0040

Diseased areas in NHIC patients show minimal changes in protein composition when compared to NDA areas

Comparing diseased areas of NHIC bladder tissue to NDA areas revealed 13 proteins were significant. These proteins function in various stages of peptide and lipid metabolism, as well as partial function in cytoskeleton organization.

Areas of disease in HIC patients show increased expression of inflammatory proteins and decreased expression of cellular adhesion proteins compared to areas of disease in NHIC patients

Next, we compared diseased areas of HIC patients with diseased areas of NHIC patients. 34 proteins were found to be significantly increased when comparing diseased HIC tissue to diseased NHIC tissue. STRING's protein mapping (Fig. 2) revealed that ten of these proteins are involved in immune response and are associated with inflammatory diseases, such as asthma and type I diabetes mellitus, while nine of these proteins are associated with protein processing in the endoplasmic reticulum (ER) (Table 3). In addition, five proteins involved in collagen formation and cellular adhesion were significantly decreased in HIC patients compared to NHIC patients (Table 3).

NDA areas in HIC patients exhibit decreased expression of protein metabolism and Rap1 signaling proteins compared to NDA areas in NHIC patients

Finally, we sought to compare NDA tissues of HIC patients with NDA tissue of NHIC patients. 50 proteins were found to be significantly altered when comparing NDA NHIC tissue to NDA HIC tissue. STRING's protein mapping (Fig. 3) revealed nine proteins involved in protein metabolism and four proteins linked to the Rap1 signaling pathway, which is involved in cell proliferation and adhesion, as well as three proteins involved in the immune response (Table 4).

Discussion

To better understand the underlying mechanisms of disease in IC, we sought to determine differences in protein expression between patients with HIC and NHIC. To

accomplish this, we collected tissue biopsies from diseased and non-disease apparent areas (NDA) of the bladder from four female patients with HIC and four female patients with NHIC. The tissue samples were processed, and nHPLC-MS/MS was used to analyze the protein compositions. We found significant differences in protein expression in bladder biopsies between patients with HIC and NHIC. Diseased HIC tissues showed a decrease in proteins involved in ubiquitination compared to NDA HIC tissues. Diseased NHIC compared to NDA NHIC tissues showed few differences, mainly in lipid and peptide metabolism. Diseased HIC patient tissues, when compared to diseased NHIC tissues, showed an increase in proteins involved with ER stress and inflammatory response, with a decrease in cell adhesion proteins. Finally, NDA HIC patient tissues also showed a decrease in immune cell adhesion proteins compared to NDA NHIC tissues.

Within the same patient and bladder, the diseased HIC tissue samples had decreased expression of ubiquitination pathway proteins compared to NDA HIC tissue samples. Ubiquitination is a posttranslational modification and plays an important role in regulation of multiple protein pathways, including membrane trafficking and protein turnover involving the ER [12]. Ubiquitination is also important for wound healing and angiogenesis, as well as protein metabolism [13]. This data suggests that the diseased areas of HIC bladders are impaired in their ability to identify and label misfolded or damaged proteins for intracellular proteolysis by the proteasome, which may result in the accumulation of damaged proteins, abnormal protein aggregation, and/or inappropriate association with other proteins. This could subsequently influence oxidative and ER stress, inflammation, and apoptosis, as is common for other chronic conditions, especially neurodegenerative disorders, including Alzheimer's, Parkinson's and Huntington's diseases [14].

Comparing diseased and NDA NHIC biopsies revealed 13 significant proteins. These proteins function primarily in peptide (ADE2) and lipid (APOC-III) metabolism, as well as cytoskeletal formation and organization (Spectrin alpha chain). These results imply that there is not as extreme a difference between areas in the same bladder

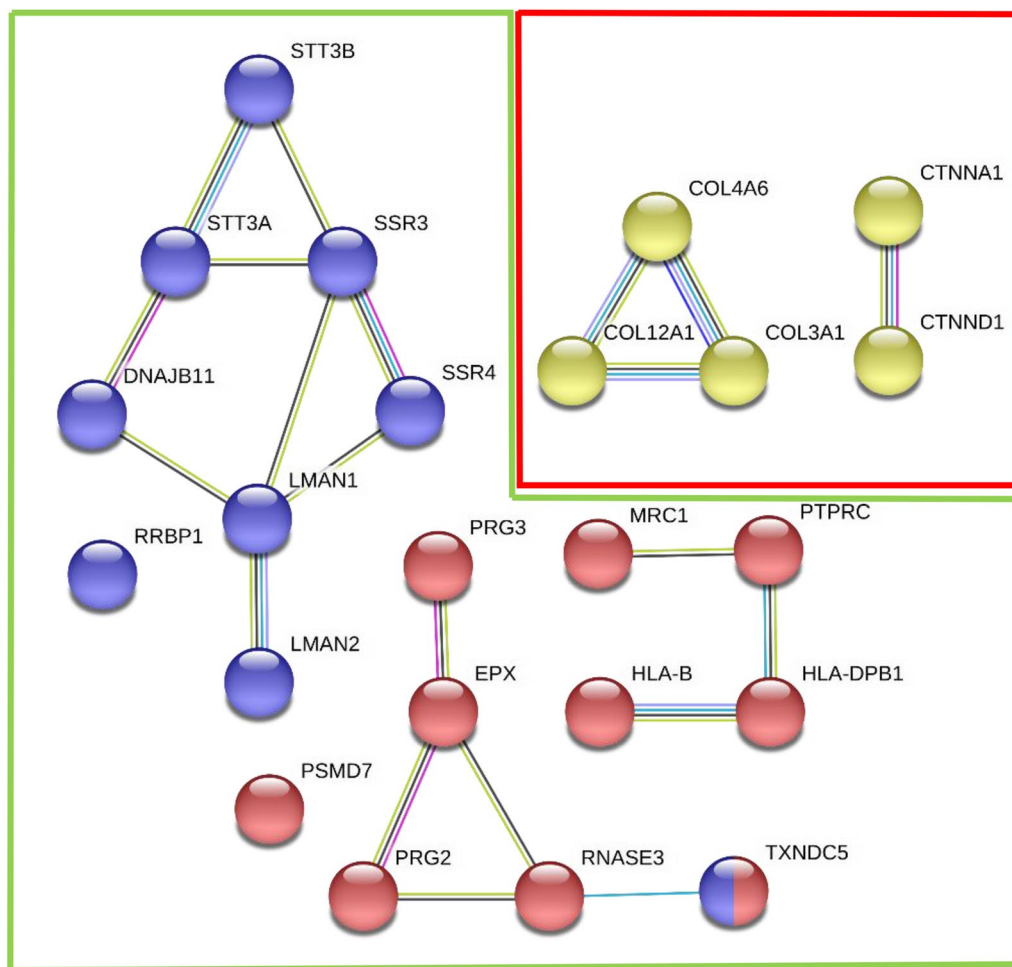


Fig. 2 Diseased HIC samples exhibited increased expression of immune response and ER stress proteins, with a decrease in cellular adhesion proteins. Association map for proteins differentially expressed in diseased Hunner's IC biopsies compared to diseased non-Hunner's IC biopsies. Nodes in the green box represent proteins with increased expression in diseased HIC tissue, while nodes within the red outline represent proteins with decreased expression. Red nodes represent proteins with an immune response function, blue represents ER protein processing/stress response, and yellow represent cellular adhesive proteins. Lines between nodes indicate reported associations as detailed in "Methods" section

of NHIC patients, compared to differences seen in HIC patients. There is also no overlap in significant protein expression between the two comparison groups, which could imply a difference in disease etiology.

Comparing areas of disease in HIC patients to NHIC patients, there was a significant increase in proteins associated with inflammation in HIC. The upregulation of inflammatory proteins may indicate an increased immune response within Hunner's lesions. These proteins have various functions in the mediation of immune processes, including Eosinophil Peroxidase and Tyrosine-Phosphatase C, which had a fold change of 74.7 and 45.9, respectively. These proteins are indicative of increased eosinophil and leukocyte activity. Other immune cell proteins increased in HIC tissue are known to be

expressed by monocytes. This increased expression of immune cells is consistent with prior research, as the literature suggests that HIC may have an immune response component [15, 16].

Diseased HIC bladder tissue also showed an increase in proteins involved with ER stress when compared to diseased NHIC tissues. ER stress is a cellular response to improperly folded proteins or high cellular secretory load and may lead to cell death. An increase in the concentration of ER stress proteins in the biopsies of HIC patients may suggest impaired protein processing, which can lead to inflammation and apoptosis. A study in rats with pro-tamine/lipopolysaccharide-induced IC noted an increase in ER stress biomarkers post-procedure, suggesting a link between IC and ER stress [13]. Diseased HIC tissues

Table 3 Diseased Hunner's IC biopsies compared to diseased non-Hunner's IC biopsies

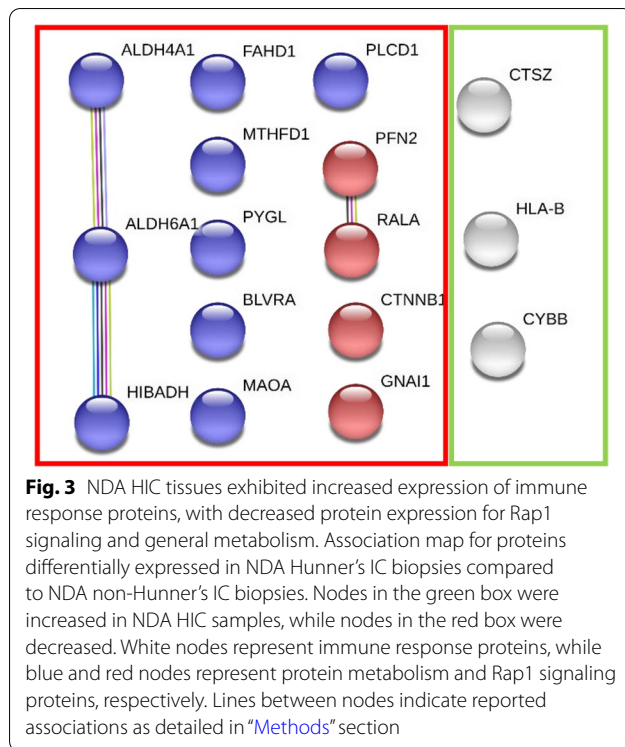
Accession ID	Gene Name	Description	Fold change (FC ≥ 4)	p value ($p \leq 0.05$)
Immune and inflammatory response (up-regulated) 10 of 34 significant proteins			Diseased HIC/diseased NHIC	
P11678	EPX	Eosinophil peroxidase	74.7	0.0184
P08575	PTPRC	Tyrosine phosphatase C	45.9	0.0163
P12724	RNASE3	Eosinophil cationic protein	19.6	0.0072
P13727	PRG2	Bone marrow proteoglycan precursor	13.6	0.0046
P01889	HLA-B	HLA class I histocompatibility antigen, B-41	10.5	0.0011
P51665	PSMD7	26S proteasome non-ATPase regulatory subunit	9.16	0.0481
P04440	HLA-DPB1	HLA class II histocompatibility antigen, DP beta 1 chain	6.77	0.0079
Q8NBS9	TXNDC5	Thioredoxin domain-containing protein 5	5.55	0.0065
P22897	MRC1	Mannose-receptor C-type 1	5.91	0.0303
Q9Y2Y8	PRG3	Proteoglycan 3	4.46	0.0038
ER protein processing & ER stress (up-regulated) 9 of 34 significant proteins				
Q8TCJ2	STT3B	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3B	22.2	0.026
Q9UNL2	SSR3	Translocon-associated protein subunit gamma	13.6	0.0135
Q2P2E9	RRBP1	Ribosome-binding protein 1	11.2	0.0119
Q9UBS4	DNAJB11	DnaJ Homolog subfamily B member 11	11.1	0.0008
P46977	STT3A	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A	8.79	0.0248
P49257	LMAN1	Protein ERGIC-53	5.97	0.0244
Q8NBS9	TXNDC5	Thioredoxin domain-containing protein 5	5.55	0.0065
Q12907	LMAN2	Vesicular integral-membrane protein VIP36	5.27	0.0028
P51571	SSR4	Translocon-associated protein subunit delta	4.04	0.0091
Cellular adhesion (down-regulated) 5 of 42 significant proteins			Diseased NHIC/Diseased HIC	
Q14031	COL4A6	Collagen alpha-6(IV)	12.6	0.0144
P35221	CTNNA1	Catenin alpha-1	9.54	0.0005
O60716	CTNND1	Catenin delta-1	5.69	0.0288
P02461	COL3A1	Collagen alpha-1(III)	4.62	0.0039
Q99715	COL12A1	Collagen alpha-1(XII)	4.46	8.13e-5

also showed decreased expression of cellular adhesive proteins, including multiple collagen (collagen alpha-1, collagen alpha-6) and catenin (catenin alpha-1, catenin delta-1) subtypes. These results could imply a weakening of the urothelial lining of the bladder and loss of the barrier function, exposing underlying cell layers to irritants and possibly explaining in part the pain symptoms of patients. Increased ER stress proteins and decreased cellular adhesion proteins in HIC bladders further suggest that the diseased areas of HIC patients are more disrupted and compromised than the diseased areas of NHIC, and also why focal treatment of these areas in HIC can lead to at least temporary symptom relief.

NDA bladder biopsies of HIC patients showed decreased expression of proteins involved in peptide metabolism and protein processing when compared to NDA biopsies from NHIC patients. These patients also

showed a decrease in proteins related to Rap1 signaling. The Rap1 signaling pathway controls cell proliferation, cell-to-cell adhesion, and cellular junction formation. The decreased expression of these proteins suggests a weakened cell-to-cell connection in the urothelium of HIC patients and possibly an impaired wound healing response, consistent with the chronic course of IC.

The limitations of this study include the limited number of patient samples, the exclusion of male samples, and the lack of healthy controls. The small number of patient samples reflects the difficulty of obtaining human bladder biopsy samples, which requires an invasive procedure and has some risks including infection and pain. The majority of IC patients are females, therefore we focused on female samples to eliminate gender as a variable. There were several advantages of this study. While other groups have analyzed protein concentrations in



bladder tissue from IC patients [17], this is the first time nHPLC-MS/MS has been used for proteomic analysis of IC bladder tissues; nHPLC-MS/MS uses significantly less material compared to normal HPLC, which is important considering how precious these bladder biopsy samples are [18]. This study also directly examined human bladder tissue samples of IC patients, rather than examining human urine or experimental animal models of interstitial cystitis.

Conclusions

Interstitial cystitis is a disease with unknown etiology. We sought to understand the differences in protein expression in the bladder tissue of patients with two IC subtypes, Hunner's IC and Non-Hunner's IC. To do this, we collected biopsies from four female HIC patients and four female NHIC patients, processed the biopsies, and analyzed them using nHPLC-MS/MS. In the same bladder, diseased HIC tissues showed a decrease in ubiquitination proteins compared to NDA tissues. When comparing between IC subtypes, HIC patients showed significantly increased inflammatory response and ER stress proteins, with a decrease in cellular adhesive proteins and proteins associated with the Rap1 signaling pathway, which regulates cellular adhesion and wound healing. These results indicate that the bladder cells of

Table 4 Non-disease-apparent Hunner's IC biopsies compared to non-disease-apparent Non-Hunner's IC biopsies

Accession ID	Gene name	Description	Fold change (FC ≥ 4)	p value ($p \leq 0.05$)
Immune and inflammatory response (up-regulated) 3 of 11 significant proteins			NDA HIC/NDA NHIC	
P30479	HLA-B	Human leukocyte antigen B	15.6	0.0039
P04839	CYBB	Cytochrome b-245 beta chain	8.11	0.0136
Q9UBR2	CTS2	Cathepsin Z	7.88	0.0037
Protein metabolism (down-regulated) 9 of 50 significant proteins			NDA NHIC/NDA HIC	
P30038	ALDH4A1	Delta-1-pyrroline-5-carboxylate dehydrogenase	17.9	0.0252
P06737	PYGL	Glycogen phosphorylase	8.13	0.0316
P31937	HIBADH	3-Hydroxyisobutyrate dehydrogenase	7.73	0.0073
P53004	BLVRA	Biliverdin reductase A	5.57	0.0316
P51178	PLCD1	1-Phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta 1	5.18	0.0093
Q02252	ALDH6A1	Methylmalonate-semialdehyde dehydrogenase	4.66	0.0265
Q6P587	FAHD1	Acylpyruvase FAHD1	4.61	0.0235
P21397	MAOA	Amine oxidase [Flavin-containing] A	4.61	0.0117
P11586	MTHFD1	C-1-tetrahydrofolate synthase	4.28	0.0107
Rap1 Signaling (down-regulated) 4 of 50 significant proteins			NDA NHIC/NDA HIC	
P11233	RALA	Ras-related protein Ral-A	7.11	0.0026
P35080	PFN2	Profilin-2	6.49	0.0124
P63096	GNAI1	Guanine nucleotide-binding protein G(i) subunit alpha-1	4.23	0.0266
P35222	CTNNB1	Catenin beta-1	4.04	0.0118

HIC patients are significantly more disrupted than those of NHIC patients, with increased protein misfolding and decreased cellular adhesion leading to an inflammatory response and painful lesions in the bladder wall which heal slowly. These results are important, as they may lead to a greater understanding of the underlying causes of IC, as well as potential new therapeutic targets for HIC or NHIC.

Abbreviations

D: Disease; IC/BPS: Interstitial cystitis/bladder pain syndrome; HIC: Hunner's interstitial cystitis; NHIC: Non-Hunner's interstitial cystitis; nHPLC-MS/MS: Nano high performance liquid chromatography linked to tandem mass spectrometry; NDA: Non-disease apparent; ICSI: Interstitial Cystitis Symptom Index; ICPI: Interstitial Cystitis Problem Index; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; NSAF: Normalized spectral abundance factor; ER: Endoplasmic reticulum; HLA: Human leukocyte antigen.

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Authors' contributions

EW analyzed and interpreted proteomic data, and was the primary contributor in writing the manuscript. SB assisted in analyzing and interpreting proteomic data, and was a contributor in writing the manuscript. MC was a contributor in writing the manuscript. KP planned the study and collected the samples. LL was a contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Beaumont Health Institutional Review Board (IRB# 2009-218) and written consent for all participants was obtained.

Consent for publication

Not applicable.

Competing interests

No direct or indirect commercial, personal, academic, political, religious or ethical incentive is associated with publishing this article.

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References

- Temml C, Wehrberger C, Riedl C, Ponholzer A, Marszalek M, Madersbacher S. Prevalence and correlates for interstitial cystitis symptoms in women participating in a health screening project. *Eur Urol*. 2007;51(3):803–8 (discussion 809).
- Parsons JK, Kurth K, Sant GR. Epidemiologic issues in interstitial cystitis. *Urology*. 2007;69(4 Suppl):5–8.
- Sant GR. Etiology, pathogenesis, and diagnosis of interstitial cystitis. *Rev Urol*. 2002;4(Suppl 1):S9–15.
- Peeker RFM. Toward a precise definition of interstitial cystitis: further evidence of differences in classic and nonulcer disease. *J Urol*. 2002;167(6):2470–2.
- Slobodov G, Feloney M, Gran C, Kyker KD, Hurst RE, Culkin DJ. Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. *J Urol*. 2004;171(4):1554–8.
- Wein AJ, Hanno PM, Gillenwater JY. Interstitial cystitis: an introduction to the problem. In: Hanno PM, Staskin DR, Krane RJ, Wein AJ, editors. *Interstitial cystitis*. London: Springer; 1990. p. 3–15.
- Keay S, Zhang CO, Kagen DI, et al. Concentrations of specific epithelial growth factors in the urine of interstitial cystitis patients and controls. *J Urol*. 1997;158(5):1983–8.
- Keay S, Zhang CO, Shoenfelt JL, Chai TC. Decreased in vitro proliferation of bladder epithelial cells from patients with interstitial cystitis. *Urology*. 2003;61(6):1278–84.
- Maeda D, Akiyama Y, Morikawa T, et al. Hunner-type (classic) interstitial cystitis: A distinct inflammatory disorder characterized by pancystitis, with frequent expansion of clonal B-cells and epithelial denudation. *PLoS ONE*. 2015;10(11):e0143316.
- Brookes ST, Donovan JL, Wright M, Jackson S, Abrams P. A scored form of the Bristol Female Lower Urinary Tract Symptoms questionnaire: data from a randomized controlled trial of surgery for women with stress incontinence. *Am J Obstet Gynecol*. 2004;191(1):73–82.
- Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607–13.
- Goder V. Roles of ubiquitin in endoplasmic reticulum-associated protein degradation (ERAD). *Curr Protein Pept Sci*. 2012;13(5):425–35.
- Wu S, Yao M. Advances in the research of mechanism of protein ubiquitination in wound healing. *Zhonghua Shao Shang Za Zhi*. 2016;32(2):119–21.
- Huang Q, Figueiredo-Pereira ME. Ubiquitin/proteasome pathway impairment in neurodegeneration: therapeutic implications. *Apoptosis*. 2010;15(11):1292–311.
- Duh K, Funaro MG, DeGouveia W, et al. Crosstalk between the immune system and neural pathways in interstitial cystitis/bladder pain syndrome. *Discov Med*. 2018;25(139):243–50.
- Gamper M, Viereck V, Eberhard J, et al. Local immune response in bladder pain syndrome/interstitial cystitis ESSIC type 3C. *Int Urogynecol J*. 2013;24(12):2049–57.
- Shie JH, Liu HT, Kuo HC. Increased cell apoptosis of urothelium mediated by inflammation in interstitial cystitis/painful bladder syndrome. *Urology*. 2012;79(2):484.e487–413.
- Gama MR, Collins CH, Bottoli CB. Nano-liquid chromatography in pharmaceutical and biomedical research. *J Chromatogr Sci*. 2013;51(7):694–703.

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De Novo Urinary Symptoms Associated With COVID-19: COVID-19-Associated Cystitis

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Laura E. Lamb^{d, e}, Michael B. Chancellor^{d, e, f}

To the Editor

Clinical symptoms that present early in coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have been well-reported. Although the majority number of patients develop mild symptoms, a small percentage can progressively develop acute respiratory distress syndrome and multiple organ dysfunction syndrome resulting in demise [1]. There is an emergence of new symptoms that involve many organ systems, some more subtle than others. As these symptoms may overlap with other common disease processes, it has been difficult to identify and link these symptoms as direct association with COVID-19 as the underlying cause [2].

Recently Mumm and his colleagues have reported increased urinary frequency in COVID-19 patients [3]. We noticed at our tertiary care medical center's COVID-19 clinic that patients reported *de novo* urinary tract symptoms and therefore obtained institutional review board (IRB) approval for a case series. We surveyed urinary symptoms in patients who followed up in the outpatient setting after their hospitalization from COVID-19. All patients had confirmed positive SARS-CoV-2 molecular diagnostic test. Patients filled out their survey responses in the office setting. Urinary symptoms are based on a validated bladder health questionnaire (Overactive Bladder (OAB) Assessment Tool) [4]: The five individual symptom scores range from 0 to 5 based on urinary urgency, urge incontinence, incontinence, frequency, and nocturia. We noted patient's hospital dates to establish length-of-stay (LOS) and the study was conducted from May 22 to June 26, 2020.

We identified 39 COVID-19-positive patients, including seven females and 32 males, who developed *de novo* urinary symptoms without urinary tract infection per standard urine

culture and sensitivity testing. The patients were all outpatients post hospital discharge and did not have fever or other clinical conditions that would require inpatient health care. All patients were African American. Median LOS was 10 days (range 5 - 30). All 39 patients completed the symptom score survey. The median total OAB symptom score in men and women was 18 (ranges 12 - 20 and 15 - 21, respectively) (Table 1). Our case series bring awareness to the possibility of new onset urinary symptoms in COVID-19 patients and the cause of the symptoms remains unsolved. The most remarkable urologic complaints were increased urinary frequency of ≥ 13 episodes/24 h (85%) and nocturia $\geq$ four episodes/night (87%).

In conclusion, in a limited survey of patients at a tertiary care COVID-19 clinic, we found COVID-19 patients, both men and women, may report *de novo* lower urinary tract symptoms. The most bothersome new urinary tract symptoms include urinary frequency and nocturia. Physicians caring for COVID-19 should be aware of COVID-19-associated cystitis (CAC).

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None to declare.

Financial Disclosure

None to declare.

Conflict of Interest

The authors declare that they have no relevant conflict of interest to report.

Informed Consent

Informed consents have been obtained from the patients.

Author Contributions

MC and ND contributed to the formulation. SD, ND, LL, and MC planned the study. RT, ML, ND, and SD collected the data.

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Table 1. Demographics and Symptoms

Demographics	
Gender, n	Male, 32/female, 7
Age (IQR), years	63.5 (56 - 68.75)
Ethnicity, n	African American, 39
LOS (IQR), days	10 (9 - 12)
Symptoms	
Urinary urgency (%)	100%
Urge incontinence (%)	100%
Incontinence (%)	100%
Frequency (%)	100%
Number of times per day, n (%)	
1 - 6	0 (0%)
7 - 8	0 (0%)
9 - 10	1 (2.6%)
11 - 12	5 (12.8%)
13 - 14	26 (66.7%)
15+	7 (17.9%)
Nocturia (%)	100%
Number of times per night, n (%)	
0	0 (0%)
1	0 (0%)
2	0 (0%)
3	5 (12.8%)
4	30 (76.9%)
5	4 (10.3%)

IQR: interquartile range; LOS: length-of-stay.

LL, MC, ND, and SD analyzed the data. All authors involved in writing the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

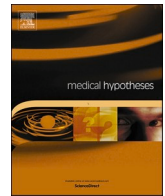
References

1. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. *Am J Emerg Med.* 2008;26(6):711-715.
2. Sighinolfi MC, Rocco B, Mussini C. COVID-19: Importance of the Awareness of the Clinical Syndrome by Urologists. *Eur Urol.* 2020;78(1):e40-e41.
3. Mumm JN, Osterman A, Ruzicka M, Stihl C, Vilsmaier T, Munker D, Khatamzas E, et al. Urinary Frequency as a Possibly Overlooked Symptom in COVID-19 Patients: Does SARS-CoV-2 Cause Viral Cystitis? *Eur Urol.* 2020.
4. Overactive Bladder Assessment Tool. 2018. <https://www.urologyhealth.org/educational-materials/overactive-bladder-assessment-tool-x4801>. Accessed May 1, 2020.



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COVID-19 inflammation results in urine cytokine elevation and causes COVID-19 associated cystitis (CAC)

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ABSTRACT

Coronavirus disease 2019 (COVID-19) causes a wide range of symptoms, including several unexpected symptoms such as loss of taste, skin changes, and eye problems. We recently observed patients with documented COVID-19 develop de novo severe genitourinary symptoms, most notably urinary frequency of ≥ 13 episodes/24 h and nocturia ≥ 4 episodes/night. We call these associated urinary symptoms COVID-19 associated cystitis (CAC). COVID-19 severity is associated with inflammation. We collected urine samples from COVID-19 patients, including patients with CAC, and found elevation of proinflammatory cytokines also in the urine. It has been previously shown that patients with urinary incontinence and ulcerative interstitial cystitis/bladder pain syndrome have elevated urinary inflammatory cytokines compared to normal controls. We therefore hypothesize that CAC, with presentation of de novo severe urinary symptoms, can occur in COVID-19 and is caused by increased inflammatory cytokines that are released into the urine and/or expressed in the bladder.

The most important implications of our hypothesis are: 1) Physician caring for COVID-19 patients should be aware of COVID-19 associated cystitis (CAC); 2) De novo urinary symptoms should be included in the symptom complex associated with COVID-19; and 3) COVID-19 inflammation may result in bladder dysfunction.

Introduction/Background

Coronavirus disease 2019 (COVID-19), caused by infection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus, can cause a wide range of symptoms with the most common being fever, dry cough, and tiredness. Other symptoms may include headache, sore throat, shortness of breath or difficulty breathing, muscle aches, or chills. However, COVID-19 can also result in unexpected symptoms including loss of taste or smell, itchy lesions on hands and feet, swollen eyelids and increase eye discharge, and gastrointestinal symptoms, including a loss of appetite, nausea, vomiting and diarrhea. Recently, Mumm and colleagues reported increased urinary frequency in COVID-19 patients [1]. We also observed confirmed COVID-19 patients develop de novo severe genitourinary symptoms, most notably urinary frequency of > 13 episodes/24 h and nocturia > 4 episodes/night [2]. Some patients also noted pain or discomfort associated with urination.

We have called these associated urinary symptoms COVID-19 associated cystitis (CAC). Since COVID-19 severity is associated with inflammation and increased inflammatory cytokines, we then examined expression of inflammatory cytokines in the urine of these COVID-19 patients. We observed that the urine of confirmed COVID-19 patients had elevated levels of pro-inflammatory cytokines.

The hypothesis/theory

We hypothesize that CAC, with presentation of de novo severe urinary symptoms, can occur in COVID-19 patients and is caused by increased inflammatory cytokines that are released into the urine and/or expressed in the bladder (Fig. 1). We further hypothesize that chronic COVID-19 Associated Cystitis may occur in COVID-19 patients who do not fully recover and have a chronic inflammatory condition ongoing.

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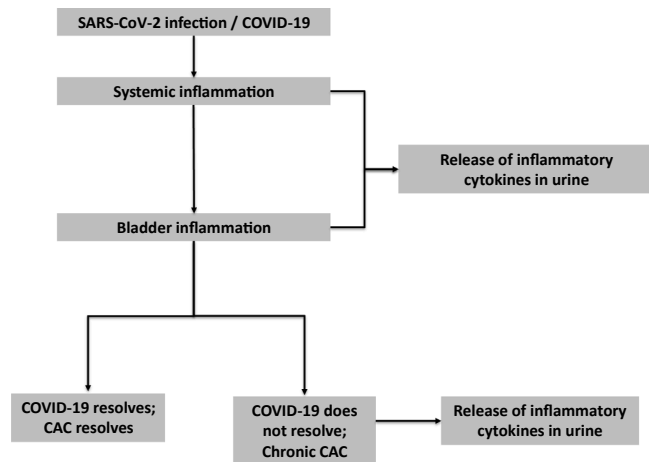


Fig. 1. COVID-19 Associated Cystitis (CAC) Hypothesis.

Evaluation of the hypothesis/idea

Our hypothesis is supported by several lines of evidence. First, Mumm and colleagues as well as our group have observed alterations in bladder function in COVID-19 patients [1]. Mumm et al first observed increased urinary frequency in 7 males out of 57 patients admitted to their COVID-19 wards [1]. All patients tested positive for SARS-CoV-2 in nasopharyngeal swabs and developed pulmonary symptoms detectable on imaging. These patients did not have urinary infections, acute renal injury, nor prostatitis and were negative for urine cultures. These patients reported an average of 13.7 urinary voids per day on the day of admission and 11.6 on day 5; in comparison most people urinate between six and eight times a day. In our observations, all the COVID-19 positive patients (7 females and 32 males; all African American) developed de novo urinary symptoms without urinary tract infection by urine culture [2].

Second, other viral infections are known to result in lower urinary tract symptoms (LUTS), which can include storage symptoms (urgency, urgency incontinence, frequency, and nocturia), voiding symptoms (slow or interrupted stream, hesitancy, or straining to urinate), or post-micturition symptoms (dribble or sensation of incomplete emptying of bladder). For example, Human immunodeficiency virus (HIV) status is an independent risk factor for bothersome LUTS and the odds of severe LUTS are greater in HIV infected men with a history of AIDS [3]. Human T cell lymphotropic virus-1 (HTLV-1) infection can also result in bothersome LUTS including nocturia, urgency, frequency, and dysuria [4]. BK polyomavirus (BKPyV) after allogeneic hematopoietic cell transplantation can cause macroscopic hematuria and cystitis, including urinary frequency, dysuria, gross hematuria, and urinary blood clots [5]. Lastly, UTIs can be caused by viruses including adenovirus and cytomegalovirus (CMV), although viral UTIs are relatively uncommon.

There have been reports that inflammatory cytokines are elevated in the urine in urinary incontinent and interstitial cystitis/bladder pain syndrome patients compared to normal controls [6–11]. For example, in overactive bladder (OAB), urinary cytokines elevated in OAB patients compared to controls included monocyte chemoattractant protein-1 (MCP-1), soluble fraction of the CD40 ligand (sCD40L), macrophage inflammatory protein (MIP-1 β), IL-12p70/p40, IL-5, epidermal growth factor (EGF), growth-related oncogene GRO- α , sIL-2R α , and IL-10 [8]. The bladder tissue of interstitial cystitis patients compared to controls have increased IL-16, IL-18, CTACK, ICAM-1, MCP-3, SCGF β , TRAIL, and VCAM-1 [7]. Urinary cytokines are also elevated in interstitial cystitis with Hunner's lesions, specifically IL-6, IL-8, and GRO [6].

Cystitis, or bladder inflammation, can cause bladder dysfunction [12,13]. Inflammation can occur from pathogenic (bacterial or viral), non-pathogenic (i.e. radiation, medication, chemicals, etc.), or

idiopathic (i.e. interstitial cystitis) causes. Pro-inflammation mediators, including cytokines, can damage and irritate the bladder mucosa, the bladder tissue that is in contact with urine [12,13]. The bladder mucosa is a dynamic sensory structure that can sense bladder fullness and can modulate bladder nerve and muscle function. Inflammation damage to the mucosa can thus result in bothersome lower urinary tract symptoms as the damaged urothelium may activate afferent nerves as well as locally influence bladder muscle function [12,13]. Infection with SARS-CoV-2 in some can result in systemic inflammation and abnormally high immunologic response is associated with more severe COVID-19 symptoms and outcomes, including death [14–16]. This has been referred to as the cytokine storm syndrome or cytokine release syndrome. Increased expression of cytokines, especially IL-6, IL-1 β , C-reactive protein, and TNF- α , are detectable in serum of COVID-19 patients with IL-6 being the best single prognostic indicator of COVID-19 severity [17].

In our observations detailed below, we have observed that several cytokines are elevated and detectable in the urine of COVID-19 patients with CAC. The presence of these cytokines in the urine could impact bladder sensation and thus function, resulting in the observed increased urinary frequency and nocturia. It is also possible that the virus may directly damage the bladder tissue, impacting bladder function. Lastly, impaired kidney function may cause some of the symptoms observed with CAC.

If our hypothesis is correct, we predict that drugs that are useful in helping treat overactive bladder may also be considered in CAC. Given that rest and sleep are important during illness for recovery, improving nocturia and frequency could help improve the speed and quality of recovery of COVID-19 patients. Furthermore, monitoring urinary cytokines may serve as a prognostic indicator for COVID-19 progression and severity when serum collection is not feasible or practical.

Empirical data

Clean catch urine samples were collected from 8 adult patients with normal renal function, 4 with COVID-19 and de novo urinary tract symptoms and 4 age matched normal control without COVID-19 and no history of urinary tract diseases. COVID-19 was diagnosed by SARS-CoV-2 molecular testing and presence of COVID-19 symptoms. IRB approval and written participant consent obtained and urine cultures were negative. Urine samples were analyzed in duplicate for cytokine expression level using a Luminex multiplex assay. The cytokines analyzed included interleukin-6 (IL-6), interleukin-8 (IL-8) and IP-10. The 4 COVID-19 positive patients with CAC were 3 female and 1 male African American, mean age of 68 years (range 51–85). The control were 2 female and 2 male African American, mean age of 66 years (range 50–78). The 4 COVID-19 positive CAC patients had de novo urological symptoms of urinary urgency (4/4), urge incontinence (4/4) and urinary frequency/24 hrs of 11–12 episodes (n = 1), 13–14 episodes (n = 1) and 15 + episodes/24 hrs (n = 2). All COVID-19 patients report de novo nocturia of 3, (n = 1), 4 (n = 2) and 5 episodes/night (n = 1). All COVID-19 positive patients had elevated levels of inflammatory cytokines IL-6, IL-8, and IP-10 in their urine compared to COVID-19 negative patients, with IL-6 and IL-8 being statistically significant (Table 1). There was variance between individuals that may reflect the severity and duration of their disease, and/or the presence of comorbidities. This data supports that COVID-19 patients with CAC have increased inflammatory cytokines in the urine.

Consequences of the hypothesis and discussion

We propose that COVID-19 Associate Cystitis (CAC), with presentation of de novo severe urinary symptoms, can occur in COVID-19 and is caused by increased inflammatory cytokines that are released into the urine and/or expressed in the bladder. The most important implications of our hypothesis are: 1) Physicians and other clinicians caring for COVID-19 patients should be aware of CAC; 2) De novo urinary

Table 1

Cytokine expression in urine of asymptomatic controls (n = 4) and COVID-19 patients with de novo urinary symptoms (n = 4). SEM = Standard error mean. P-value calculated from a one-tailed, two-sample T test with unequal variances.

Patient	COVID-19	SEX	AGE	IL-6 (pg/mL)	IL-8 (pg/mL)	IP-10 (pg/mL)
N1	Negative	F	50	7.02	48.1	0
N2	Negative	M	71	0	1.01	0
N3	Negative	M	78	1.51	1.79	154.89
N4	Negative	F	65	0	2.33	0
AVERAGE ± SEM				2.13 ± 1.67	13.31 ± 11.60	38.72 ± 38.72
P1	Positive	F	57	5.42	329.31	2307.64
P2	Positive	F	80	39.36	260.2	68.96
P3	Positive	M	85	12.81	287.9	527.79
P4	Positive	F	51	21.18	155.06	548.11
AVERAGE ± SEM				19.69 ± 7.30	258.12 ± 37.17	863.13 ± 494.05
p-value				0.046	0.002	0.097

symptoms should be included in the symptoms complex associated with COVID-19; and 3) COVID-19 inflammation may result in bladder dysfunction. We further hypothesize that chronic COVID-19 Associated Cystitis may occur in COVID-19 patients who do not fully recover and have a chronic inflammatory condition ongoing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2020.110375>.

References

- [1] Mumm JN, Osterman A, Ruzicka M, Stihl C, Vilsmaier T, Munker D, et al. Urinary Frequency as a Possibly Overlooked Symptom in COVID-19 Patients: Does SARS-CoV-2 Cause Viral Cystitis? *European urology*. 2020 May 19. PubMed PMID: 32475747. Pubmed Central PMCID: 7236674.
- [2] Dhar N, Dhar S, Timar R, Lucas S, Lamb LE, Chancellor MB. De Novo Urinary Symptoms Associated with COVID-19: COVID-19 Associated Cystitis (CAC). *J Clin Med Res* 2020. In Press.
- [3] Breyer BN, Van den Eeden SK, Horberg MA, Eisenberg ML, Deng DY, Smith JF, et al. HIV status is an independent risk factor for reporting lower urinary tract symptoms. *J Urol* 2011;185(5):1710–5. PubMed PMID: 21420120. Pubmed Central PMCID: PMC3565615. Epub 2011/03/2eng.
- [4] Castro NM, Rodrigues Jr W, Freitas DM, Muniz A, Oliveira P, Carvalho EM. Urinary symptoms associated with human T-cell lymphotropic virus type I infection: evidence of urinary manifestations in large group of HTLV-I carriers. *Urology* 2007; 69(5):813–8. PubMed PMID: 17482910. Epub 2007/05/08. eng.
- [5] Imlay H, Xie H, Leisenring WM, Duke ER, Kimball LE, Huang M-L, et al. Presentation of BK polyomavirus-associated hemorrhagic cystitis after allogeneic hematopoietic cell transplantation. *Blood Adv* 2020;4(4):617–28.
- [6] Lamb LE, Janicki JJ, Bartolone SN, Peters KM, Chancellor MB. Development of an interstitial cystitis risk score for bladder permeability. *PLoS ONE* 2017;12(10): e0185686. PubMed PMID: 29088231. Pubmed Central PMCID: PMC5663335 methods for diagnosing interstitial cystitis. All other authors have declared no conflicts of interest exist. This does not alter our adherence to PLOS ONE policies on sharing data and materials. Epub 2017/11/01. eng.
- [7] Corcoran AT, Yoshimura N, Tyagi V, Jacobs B, Leng W, Tyagi P. Mapping the cytokine profile of painful bladder syndrome/interstitial cystitis in human bladder and urine specimens. *World J Urol* 2013;31(1):241–6. PubMed PMID: 22441309. Pubmed Central PMCID: PMC367457Epub 2012/03/24. eng.
- [8] Tyagi P, Barclay D, Zamora R, Yoshimura N, Peters K, Vodovotz Y, et al. Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. *Int Urol Nephrol*. 2010;42(3):629–35. PubMed PMID: 19784793. Epub 2009/09/29. eng.
- [9] Liu HT, Jiang YH, Kuo HC. Alteration of Urothelial Inflammation, Apoptosis, and Junction Protein in Patients with Various Bladder Conditions and Storage Bladder Symptoms Suggest Common Pathway Involved in Underlying Pathophysiology. *Lower Urinary Tract Symptoms* 2015;7(2):102–7. PubMed PMID: 26663690. Epub 2015/12/15. eng.
- [10] Kuo HC. Potential urine and serum biomarkers for patients with bladder pain syndrome/interstitial cystitis. *Int J Urol*. 2014;21(Suppl 1):34–41. PubMed PMID: 24807491.
- [11] Zhang CO, Li ZL, Kong CZ. APF, HB-EGF, and EGF biomarkers in patients with ulcerative vs. non-ulcerative interstitial cystitis. *BMC Urol* 2005;29(5):7. PubMed PMID: 15862132. Pubmed Central PMCID: PMC1131910.
- [12] Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. *Therapeutic Adv Urol* 2011;3(1):19–33. PubMed PMID: 21789096. Pubmed Central PMCID: 3126088.
- [13] Fry CH, Vahabi B. The Role of the Mucosa in Normal and Abnormal Bladder Function. *Basic & clinical pharmacology & toxicology*. 2016 Oct;119 Suppl 3:57–62. PubMed PMID: 27228303. Pubmed Central PMCID: 5555362.
- [14] Cheung EW, Zachariah P, Gorelik M, Boneparth A, Kernie SG, Orange JS, et al. Multisystem Inflammatory Syndrome Related to COVID-19 in Previously Healthy Children and Adolescents in New York City. *JAMA*. 2020 Jun 8. PubMed PMID: 32511676. Pubmed Central PMCID: 7281352.
- [15] Levy HR. COVID-19 and cytokine storm syndrome2020 07/24/2020]. Available from: <https://www.mlo-online.com/continuing-education/article/21138224/covid19-and-cytokine-storm-syndrome>.
- [16] Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clinical immunology*. 2020;214. PubMed PMID: 32222466. Pubmed Central PMCID: 7102614.
- [17] Liu T, Zhang J, Yang Y, Ma H, Li Z, Zhang J, et al. The potential role of IL-6 in monitoring severe case of coronavirus disease 2019. *medRxiv*. 2020: 2020.03.01.20029769.

20-7529

National Collaborative Crowdsourcing Method of Diagnostic Biomarker Development for Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS)

Laura Lamb, Sarah N. Bartolone, Royal Oak, MI, Joseph J. Janicki, Pittsburgh, PA, Kenneth M. Peters, Royal Oak, MI, ICA Interstitial Cystitis Association, McLean, VA, Nitya Abraham, Melissa Laudano, Bronx, NY, Christopher P. Smith, Houston, TX, Michael B. Chancellor, Royal Oak, MI*

INTRODUCTION AND OBJECTIVE: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) has significant unmet medical needs including the lack of an objective laboratory test, such as a urine based biomarker. National collection of adequate patient samples for IC/BPS including normal controls can be challenging. We developed a novel research model that can engage multiple stakeholders and allow fast and broad participation for biomarker development.

METHODS: After a successful initial pilot effort, we have initiated a national crowdsourced research project engaging the entire United States IC/BPS community in collaboration with the Interstitial Cystitis Association (ICA). Announcement of this study through social media was disseminated by the ICA. YouTube videos describing the scope of the project and participant requirements were posted. Questionnaires were completed online through HIPAA compliant Survey Monkey. Special urine containers with preservatives developed to maintain protein integrity in urine at ambient temperature were sent to consented participants with prepaid return shipping. Urine samples were also collected at three clinical sites (Detroit, New York, and Houston) to compare clinical versus at-home collection.

RESULTS: Public participation was overwhelming and enrollment was closed within several weeks. Overall, men and women of all age groups submitted samples from across the entire United States. Multiplex analysis discovered three proteins that were highly statistically significant different between IC with ulcer vs IC without ulcer and normal control. Strategies that improved return of samples included using US Mail and email reminders to study participants.

CONCLUSIONS: Crowdsourced research toward biomarker development was shown to be feasible and successful in advancing IC/BPS research. Patients with ulcerative IC/BPS have a different profile of urine proteins than normal and non-ulcerative IC patients. Crowdsourced research for biomarker development is a promising new area of pilot clinical research that can be done for reasonable cost, over a shorter time period, and with larger demographic populations being represented.

Source of Funding: We would like to thank Beaumont Urology and the Taubman family for their generous support. This work was supported by the U.S. Army Medical Research Acquisition Activity, through the Congressionally Directed Medical Research Programs, Peer Reviewed Medical Research Program under Award No. W81XWH-19-1-0288. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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No Access | Journal of Urology | Infections/Inflammation/Cystic Disease of the Genitourinary Tract: Kidney & Bladder II (MP29) | 1 Sep 2021

MP29-15 COVID-19 ASSOCIATED CYSTITIS (CAC): INCREASED URINARY SYMPTOMS AND BIOMARKERS OF INFLAMMATION IN URINE IN PATIENTS WITH ACUTE COVID-19

Michael B. Chancellor, Melissa Wills, Ryan Timar, Sarah N. Bartolone, Nevedita Dhar, Sorabh Dhar, Dragana Komnenov, and Laura E. Lamb

[View All Author Information](#)

<https://doi.org/10.1097/JU.0000000000002026.15>

Abstract

INTRODUCTION AND OBJECTIVE:

Emerging evidence suggests that the bladder is one of many organs are targeted by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which contributes significantly to Coronavirus Disease 2019 (COVID-19) morbidity and leads to organ-specific complications. We were the first U.S. group to identify severe and bothersome de novo genitourinary symptoms in patients with confirmed SARS-CoV-2 infection, termed COVID-19 Associate

Cystitis (CAC). We then sought to determine if this was associated with pro-inflammatory cytokines in the urine of patients.

METHODS:

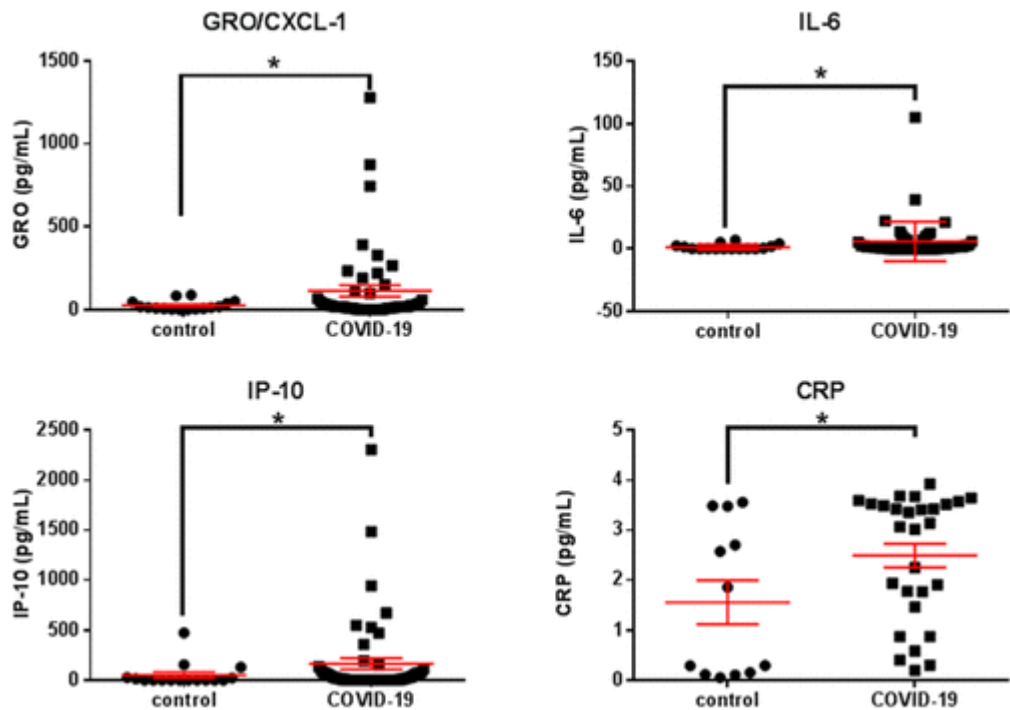
With IRB approval, hospital discharged COVID-19 patients with normal renal function (N=53) and asymptomatic controls (N=12) completed an AUA Urology Care Foundation Overactive Bladder (OAB) Assessment Tool to determine their current urinary symptoms (score of 0 to 25, increasing severity) and provided a urine sample. Urine samples were tested for SARS-CoV-2 using PCR, CRP by ELISA, and pro-inflammatory cytokines using Luminex assays following manufacturer's instructions.

RESULTS:

The median total OAB symptom score in both men and women with COVID-19 was 18 (range 4-21). The median total QOL score for both men and women with COVID-19 was 19 (range 8-24). Median age was 64.5 (range 47-82). Symptoms of CAC include increased urinary frequency, urgency, nocturia, and pain or pressure upon voiding. These symptoms were not due to urinary tract infection, acute renal injury, prostatitis, or urinary retention. These symptoms have caused worsening QOL in CAC patients, and nocturia the most bothersome symptom. The majority of COVID-19 patients did not have virus in their urine. The levels of GRO/CXCL-1, IL-6, IP-10, and CRP were significantly increased in urine samples from COVID-19 patients compared to controls.

CONCLUSIONS:

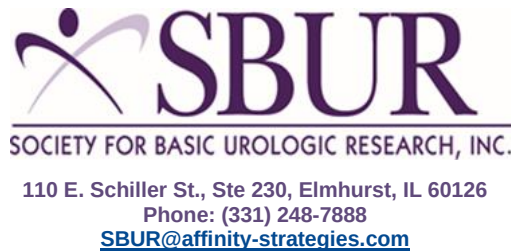
COVID-19 patients reported severe de novo genitourinary symptoms, most notably an increase in urgency, frequency, and nocturia. SARS-CoV-2 infection results in increased expression of proinflammatory biomarkers in the urine. The variance between individuals may reflect the severity and duration of their disease, and/or the presence of comorbidities. This data supports that COVID-19 patients, especially those with CAC, have increased inflammatory cytokines in the urine.



Source of Funding:

None

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Dr. Laura Lamb
Oakland University William Beaumont School of Medicine
Laura.Lamb@Beaumont.org

Dear Dr. Lamb,

On behalf of the Society for Basic Urologic Research (SBUR), I invite you to present your outstanding research on proteomic landscape of bladder diseases at the 2021 SBUR “Spring” Meeting in Las Vegas, Nevada on September 11, 2021.

The theme of this year’s meeting is “From Hype to Reality: Where Technology Meets Biology to Advance Personalized Medicine”. The meeting is conducted in conjunction with the AUA 2021- American Urological Association – Annual Meeting, Fri, Sep 10 – Mon, Sep 13, 2021. Per the AUA guidelines the SBUR is currently planning for a hybrid meeting with in-person and remote audiences.

We invite you to present your work in the following session:
SBUR Breakout Session: Technology Advances toward Personalized Medicine
Topic: Proteomic Determination of Disease State
Time: Saturday afternoon, September 11th

SBUR member speakers donate their time and provide their own travel and registration expenses to attend the meeting. We hope that you would accept our invitation. We will provide you with additional details regarding the meeting as the time draws closer.

Thank you very much in advance for your consideration. I look forward to hearing from you in your earliest convenience.

Sincerely,

Haojie Huang, Ph.D.
SBUR President Elect and Program Chair



SBUR 2021 Afternoon Session

Las Vegas, Nevada

September 11, 2021

Technological Advances toward Precision Medicine

Co-Chaired by Benyi Li, Irfan Asangani, Tanya Stoyanova and Feng Yang

1:00 p.m. **Welcome and Introductory Remarks**

Session Title: Genomics and Transcriptomics

1:05 – 1:25 p.m. **Integrative Molecular Profiling of Benign Prostatic Hyperplasia**
Christopher Barbieri, MD, PhD- Weill Cornell Medicine

1:25- 1:45 p.m. **HOXB13 Regulation of Epigenetic Remodeling and Lipogenic Reprogramming**
Jindan Yu, MD, PhD- Northwestern University

Session Title: Methyloomics and Epigenetics

1:45 – 2:05 p.m. **The Landscape of Methylation in Advanced Prostate Cancer**
David Quigley, PhD- University of California San Francisco

2:05– 2:25 p.m. **LSD1: A Therapeutic Target in Castration-Resistant Prostate Cancer**
Changmeng Cai, PhD- University of Massachusetts Boston

2:25 – 2:35 p.m. **Break**

Session Title: Proteomic Determination of Disease State

2:35 – 2:55 p.m. **The Proteogenomics of Localized Prostate Cancer**
Paul Boutros, PhD, MBA- UCLA Institute of Urologic Oncology

2:55– 3:15 p.m. **Interstitial Cystitis Diagnostic Score Development using Crowdsourcing and Machine Learning Algorithm**
Laura Lamb, PhD- Oakland University William Beaumont School of Medicine

Session Title: Inflammation and Immunological Microenvironment

3:15 – 3:35 p.m. **Mechanisms Regulating the CD8 T-cell Response to Urologic Cancers**
Haydn Kissick, PhD- Emory University Winship Cancer Institute

3:35– 3:55 p.m. **Intersections in Cytokine and Hormonal Signaling: Models Connecting Inflammation to LUTS in the Benign Prostate**
Travis Jerde, PhD- Indiana University School of Medicine

4:00 p.m. **Adjourn**

RETURN TO RICE: A message from the President on planning for the fall semester

FREE WEBINAR SERIES | APRIL 16-JUNE 18, 2020

Frontiers in Bioengineering Research

Thursdays, 3 to 4:30 p.m. CT | Open to all members of the bioengineering and biomedical research community.

Organized by the Rice University Department of Bioengineering.

As the COVID-19 pandemic unfolds across the globe, these unprecedented times have caused the cancellation of in-person seminar series, conferences and workshops that our students would participate in and engage with to enhance their educational goals. Rice Bioengineering has organized this webinar series to help our trainees stay up-to-date with frontiers of bioengineering research

and to continue facilitating communication between our students and colleagues. All academics, professionals and students at all levels in the field and related fields are invited to attend.

This weekly series of live 90-minute webinar presentations features invited speakers discussing cutting-edge research currently being performed in the bioengineering field.

JOIN THE WEBINAR

Week 1

Synthetic Biology Approaches for COVID-19 Diagnostics

Thursday, April 16, 3-4:30 p.m. CT

Moderator(s)

- JEFFREY TABOR, RICE UNIVERSITY

Speaker(s)

- HOWARD SALIS, PENN STATE UNIVERSITY | “Sprinting from Concept to Clinic: A Massively Parallel COVID-19 Diagnostic Assay for Simultaneous Testing of 19200 Patient Samples”
- KEITH PARDEE, UNIVERSITY OF TORONTO | “Responding to COVID-19: Developing Gene Circuit-based SARS-CoV-2 Sensors for Local and Sustained Diagnostic Capacity”

Week 3**COVID-19 Diagnosis and Critical Care**

Thursday, April 30, 3-4:30 p.m. CT

Moderator(s)

- REBECCA RICHARDS-KORTUM, RICE UNIVERSITY

Speaker(s)

- Laura Lamb, Oakland University | “Developing a Point-of-Risk Test for novel Coronavirus 2019”
- MARIA ODEN and MATTHEW WETTERGREEN, Rice University | “ApolloBVM: A ventilation for COVID-19 patients”

JOIN THE WEBINAR

Rebecca Richards-Kortum

Malcolm Gillis University Professor, Professor of Bioengineering, Director, Rice 360° Institute for Global Health, Rice University



Dr. Richards-Kortum's research and teaching focus on developing low-cost, high-performance technology for low-resource settings. She is known for providing vulnerable populations in the developing world access to life-saving health technology, focusing on diseases and conditions that cause high morbidity and mortality, such as cervical and oral cancer, premature birth, and malaria. Richards-Kortum is also leading a multi-institutional team to develop a package of 17 life-saving neonatal technologies, designed for low-resource settings while providing the same efficacy a related technologies used in North America, but at a fraction of the cost.

Laura Lamb

Assistant Professor and Director, Translational Urology Research, Oakland University



Dr. Lamb is an enterprising scientist with over ten years of experience in urology, oncology, cellular and molecular biology, with expertise in cell signaling. She has experience identifying and validating new molecular targets for therapeutic application and biomarker discovery with a diverse background in bladder, prostate, mammary gland, cervix, and skin biology. Dr. Lamb enjoys teaching and mentoring students at all levels as well as educating the public and policymakers on how research and science impact their lives.



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CONGRESS**

AGENDA

SEPTEMBER 21 - 25, 2020

GLOBAL MULTIDISCIPLINARY CONGRESS ON COVID-19

<https://eutranslationalmedicine.org/eustm-2020>

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EUSTM-2020 on Covid-19



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DAY 1: 21 SEPTEMBER 2020

9:00 (CET)

Coronaviruses: History and Biology

Prof. Susan R. Weiss

Penn Center for Research on Coronaviruses & Emerging Pathogens,
University of Pennsylvania, USA

11:00 (CET)

Genome-wide CRISPR screen reveals host genes that regulate SARS-CoV-2 infection

Prof. Craig B. Wilen

Yale School of Medicine Departments of Laboratory Medicine
& Immunobiology, New Haven, USA

14:00 (CET)

Lung under attack by COVID-19-induced cytokine storm: pathogenic mechanisms and therapeutic implications

Dr Corrado Pelaia

University "Magna Græcia" of Catanzaro, Italy

16:00 (CET)

Laboratory Biosafety in the Time of COVID-19

Dr Michael Baclig

St. Luke's Medical Center

17:30 (CET)

COVID 19 Risk factors and therapies in Italy: results from the CORIST collaboration

Prof. Licia Iacoviello

IRCCS Neuromed, Pozzilli & University of Insubria Varese, Italy

10:00 (CET)

Spatiotemporal analysis of micro-epidemics of influenza and COVID-19

Prof. Eleftherios Mylonakis

Alpert Medical School of Brown University, Rhode Island
Hospital & The Miriam Hospital, USA

13:00 (CET)

SARS-CoV-2 mechanisms of severe outcomes

Dr Jeremy W Prokop

Department of Pharmacology & Toxicology,
Michigan State University, Grand Rapids, USA

15:00 (CET)

Baricitinib as a potential drug for COVID-19: evidence from a multicenter trial and translational research studies

Dr Delia Goletti

National Institute for Infectious Disease L. Spallanzani, Rome, Italy

17:00 (CET)

Allosteric site of SARS-CoV-2 main protease as drug target in the presence of its emerging mutations

Prof. A. Ozlem Tastan Bishop

Department of Biochemistry and Microbiology,
Rhodes University, South Africa

17:45 (CET)

Poster Talk: Obstacles to mental health screening in hospitalized COVID-19 patients

Erin Reed

Yale School of Medicine New Haven, USA

DAY 2: 22 SEPTEMBER 2020

9:00 (CET)

Optimally Pooled COVID-19 Testing

Prof. Dor Ben-Amotz

Purdue University, Department of Chemistry West Lafayette, IN, USA

11:00 (CET)

Lessons learned from animal models of COVID-19

Prof. Ravi Mahalingam

University of Colorado Anschutz Medical Campus, USA

14:00 (CET)

Asymptomatic transmission during the COVID-19 pandemic and implications for public health strategies

Dr Hanalise V Huff

Boston Children's Hospital, Harvard Medical School, USA

16:00 (CET)

Allelic Variations in ACE2 and Natural Resistance against COVID-19

Dr Mushtaq Hussain

Dow University of Health Sciences, Karachi, Pakistan

17:30 (CET)

Poster Talk: SARS-CoV-2 diagnostic markers – Missing clues to the bigger picture

Sundararaj Stanleyraj Jeremiah

Yokohama City University, Fukuoka, Japan

10:00 (CET)

Controlling the second Covid-19 wave by Mandatory Mobile Contact Tracing (MMCT) Containment and Time Dependent Doubling Time Control

Prof. Antonio Bianconi

Rome International Center for Materials Science, Superstripes, Roma, Italy

13:00 (CET)

Bioengineering tools to speed up the discovery and preclinical testing of vaccines for SARS-CoV-2 and therapeutic agents for COVID-19

Prof. Dr. Manuela Teresa Raimondi

Politecnico Di Milano, Milano, Italy

15:00 (CET)

Stem cells from gestational tissues: a promising tool to restore the body's own potential to fight SARS-CoV-2 infections

Dr Bruna Corradetti

Houston Methodist Research Institute, USA

17:00 (CET)

Innovative Therapeutic Interventions for SARS-CoV-2 – exploiting metabolic synergy

Prof. Rushika Perera

Arthropod-borne & Infectious Diseases Laboratory
Colorado State University, USA



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DAY 3: 23 SEPTEMBER 2020

9:00 (CET)

Internet Knowledge Mining tools and A.I. For Rapid Response to New Pandemics with Proposals for Synthetic Vaccines and Therapeutics. The Example of COVID19

Prof. Dr Barry Robson

Ingine Inc. Cleveland Ohio, USA

9:30 (CET)

The renin angiotensin system (RAS) involvement in SARS-CoV-2 infection

Prof. Rafael Franco

Prof. Rafael Franco

11:00 (CET)

Molecular basis of COVID-19 comorbidities

Prof. Antonio Facchiano

Istituto Dermatologico dell'Immacolata (IDI-IRCCS), Rome, Italy

14:00 (CET)

Gut-lung axis in COVID-19

Dr Busra Aktas

Burdur Mehmet Akif Ersoy University, Burdur, Turkey

17:00 (CET)

Covid-19, The King of 2020: lessons learned and still learning from a tertiary care centre in Chennai, India

Prof. Padma Srikanth

Department of Microbiology, Sri Ramachandra Institute, India

17:30 (CET)

Poster Talk: Explainable approach for COVID-19 identification via CT-Scans

Eduardo Almeida Soares

Lancaster University, UK

10:00 (CET)

Epidemiological monitoring and comparative analysis of non-pharmaceutical interventions using a non-Markovian modelling framework

Prof. Mircea T. Sofonea

University of Montpellier, France

13:00 (CET)

Screening Possible Drug Molecules for Covid-19: A Computational Study based on Docking Studies & Molecular Dynamics to Design New Inhibitor Candidates

Dr. Manos Vlasios

Department of Health and Life Sciences, University of Nicosia, Nicosia, Cyprus

16:00 (CET)

Generation of SARS-CoV-2 S1 spike glycoprotein putative antigenic epitopes in vitro by intracellular aminopeptidases

Dr Efstratios Stratikos

National Center for Scientific Research Demokritos, Greece

DAY 4: 24 SEPTEMBER 2020

9:00 (CET)

Vesicular stomatitis virus vectored vaccine for SARS-CoV-2

Prof. Sean P. J. Whelan

Department of Molecular Microbiology, Washington University
School of Medicine, St. Louis, USA

11:00 (CET)

Pulse Therapy with Corticosteroids and Intravenous Immunoglobulin in the Management of Severe COVID-19. Unusual Treatment with Intriguing Results

Prof. Mikhail Sheianov

Burnasyan Federal Medical Biophysical Center, Moscow, Russia

14:00 (CET)

Complement and neutrophil-mediated immunothrombosis: lessons from COVID-19

Prof. Panagiotis Skendros

Democritus University of Thrace (DUTH),
University Hospital of Alexandroupolis, Greece

16:00 (CET)

How Molecular Modelling Could help in the COVID19 Crisis

Dr. Muhamed Amin

University of Groningen, Groningen, Netherlands

17:30 (CET)

Poster Talk: The use of Computational Docking to estimate the effects of Montelukast on potential viral main protease catalytic site

Dr Salman Mansoor

Sligo University Hospital, Ireland

10:00 (CET)

Potential connections of COVID-19 and prostate cancer – the TMPRSS2 story

Prof. Jianyu Rao

UCLA Medical Center, Santa Monica, LA, USA

13:00 (CET)

Targeting the Master Regulator of Cellular Redox Homeostasis, Nrf2, in SARS-CoV-2 Infection

Prof. Adela Cota-Gomez

Division of Pulmonary Sciences and Critical Care Medicine,
University of Colorado, USA

15:00 (CET)

Early prediction of COVID-19 in Cuba using the SEIR model

Prof. Dr Luis Beltrán Ramos-Sánchez

University of Camagüey, Camagüey, Cuba

17:00 (CET)

Weighing the Impact of Obesity on COVID-19 in Low- to Middle-Income Countries

Dr Dicky L. Tahapary

Faculty of Medicine Universitas Indonesia (RSCM-FKUI), Indonesia

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DAY 5: 25 SEPTEMBER 2020

9:00 (CET)

Developing a Point-of-Risk Molecular Test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Dr Laura Lamb

Oakland University William Beaumont School of Medicine,
Rochester Hills, Michigan, USA

10:00 (CET)

In silico drug discovery for COVID-19 using gene expression profiles of mouse infected by MHV and those of human cell lines infected by SARS-CoV-2

Prof. Y-H. Taguchi

Department of Physics, Chuo University, Tokyo, Japan

11:00 (CET)

SARS-CoV-2 Associated Nephropathy in the Pandemic Era

Prof. Maurizio Salvadori

Department of Nephrology and Transplantation,
University of Florence, Florence, Italy

13:00 (CET)

Machine learning analysis of single-cell gene regulatory networks reveals potential mechanisms of action of antimalarials against SARS-CoV-2

Prof. James Cai

Texas A&M University, USA

14:00 (CET)

COVID-19 pathophysiology may be driven by a loss of inhibition of the Renin-Angiotensin-Aldosterone System

Dr Johan Lundberg

Karolinska University Hospital, Stockholm, Sweden

15:00 (CET)

The Clinically Validated Viral Superinfection Therapy (SIT) Platform Technology May Mitigate Severe Cases of COVID-19 Infections

Dr Tibor Bakács

Hungarian Academy of Sciences & HepC Inc, Hungary

16:00 (CET)

Vaccine and Drug Designing for COVID-19 - Research and Prevention Strategies

Dr Syed Sikander Azam

Quaid-i-Azam University, Islamabad, Pakistan

17:00 (CET)

The higher the altitude the less COVID-19: Decreased incidence, transmission capacity of the virus and severity at altitude in the American continent

Christian Arias Reyes

Faculty of Medicine, Université Laval, Québec, QC, Canada

17:30 (CET)

Poster Talk: Homologous Proteins in Drosophila melanogaster for SARS-CoV-2/Human Interactome

Sanya Shabbir

Dow University of Health Sciences, Karachi, Pakistan

17:45 (CET)

Poster Talk: Filipino next generation nurses as frontliners amid COVID-19 pandemic: A Husserlian phenomenology study

Dr Agnes Valencia-Raymundo

Manila central University College of nursing, Caloocan City, Philippines



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11 a.m. to 12:30 p.m. - Health Disparities: How COVID Affects Marginalized Populations

1 to 3:30 p.m. - Exploring the LEAD Certificate Program and Graduates' Applied Leadership Activities

4 to 5 p.m. - Antecedents and Consequences of Positive Team Work

5:30 to 7 p.m. - Residents as Teachers (RAT) Presentation: Professional Opportunities and Challenges in Medical Care

Thursday, May 20: Research Day

9 to 9:40 a.m. - Project MedWell: A Longitudinal Sleep, Stress & Activity Tracking Initiative to Improve Medical Student Wellness

10 to 10:55 a.m. - Identifying Genomic Modifiers of Cardiovascular Disease in Mice

11 to 11:55 a.m. - Biomarkers of Neurodegenerative Disease

12 to 12:55 p.m. - Cerebrovascular Impact of Chronic Smoking and Vaping: Mechanistic Insights, Biological Targets, and Potential Countermeasures

1 to 1:55 p.m. - Improving Diagnostic Testing for Interstitial Cystitis, Radiation Cystitis, and COVID-19

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10th Annual William Davidson Medical Education Week at OUWB School of Medicine

Medical Education Week highlights innovative research and advancements in medical education at **[Oakland University William Beaumont School of Medicine](#)**.

Its purpose is to honor faculty members who contribute to the improvement of medical education. The diverse and multifaceted program includes presentation of poster abstracts and prominent speakers from the medical education field who are invited to share insight and expertise.

From: [Translational Medicine Conference](#)
To: [Lamb, Laura, PhD](#)
Subject: Guest Speaker Invitation Translational Medicine Conference
Date: Saturday, October 24, 2020 1:02:06 PM

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Dear Dr. Lamb,

Following on from our chairperson Maedbh's recent correspondence with you via LinkedIn, I would like to briefly explain to you about our society and its upcoming conference, as well as our core aims and objectives, where we feel your work perfectly ties in with and exemplifies our ideology surrounding Translational Medicine and its exciting potential in today's world.

The UCC Society of Translational Medicine is an exciting new student-led opportunity focused on encouraging students to actualise their potential in creating change in the world of human health through translational medicine. Our principal objective is to spark interest and support the next generation of students to bridge the gap between medical science and clinical needs. Translational Medicine is a multi-disciplinary, highly collaborative approach to improving human health and longevity which works to convert laboratory discoveries into clinical applications. This is called the "bench-to-bedside" approach. Translational medicine strives to enhance healthcare by facilitating rapid and successful translation of scientific knowledge to clinical implementation. This modern approach has emerged from the increasing need to eradicate strategies and the evolution of disease treatment and prevention being "lost in translation". To do this we must equip people with translational skills - science and clinical practice - in order to lift the silence between disciplines and promote inter-institutional and entrepreneurial collaboration. To achieve this objective, TM Society operates various programmes and initiatives to educate, spark interest, and disseminate knowledge of developments in TM to students. We want to provide a fertile environment of innovation for students that will transform research into reality and advance human health.

Our flagship event; the Translational Medicine Conference, will be held virtually on **Thursday, January 28th** and we cordially invite you to be a guest speaker. This conference is the first in Ireland that will be a platform for the cooperation and interaction

among clinicians, scientists, academia, industry, government, funding and regulatory agencies, investors, and policymakers from various medical-related backgrounds. The conference will represent a unique multidisciplinary platform to discuss the latest findings, challenges, solutions, and advancements in healthcare. Our vision for this conference is that speakers will give a presentation on their work, where they see the industry going, and their hopes for the future. As many of the attendees will be students, we want to hear the paths you took to get to where you are today to serve as a guide for future generations.

On behalf of all members of the University College Cork's Translational Medicine Society, we take a huge interest in your work, personally, I listened to your talk on the development of a novel point of risk molecular test for Covid at the EUSTM congress recently and I was in awe of the potential that this test holds. In particular, with the impending flu season, Covid tests are only going to increase in demand, and having a test that could potentially provide results in less than half an hour could prove absolutely pivotal in keeping hospitals afloat this winter with the impending flu season adding to Covid-19's burden on our public health service. As a society, we all feel quite passionately about this novel test which you and your team are developing at OUWB as well as its profound potential, and thus we would be thrilled to hear you speak about its development at our conference next year. We hope to hear from you soon.

Kind regards,

Paddy O'Brien,
Vice Public Relations Officer