Case No. 84986

In the Supreme Court of Nevada

STARR SURPLUS LINES INSURANCE CO.,
Petitioner,

v.

THE EIGHTH JUDICIAL DISTRICT COURT OF THE STATE OF NEVADA, IN AND FOR THE COUNTY OF CLARK et al.,
Respondents,

and

JGB VEGAS RETAIL LESSEE, LLC,
Real Party in Interest.

ON PETITION FOR A WRIT OF MANDAMUS OR, IN THE ALTERNATIVE, PROHIBITION
EIGHTH JUDICIAL DISTRICT COURT CASE NO. A-20-816628-B

REQUEST FOR JUDICIAL NOTICE OF THE RESTAURANT LAW CENTER, BLOOMIN' BRANDS, INC., TREASURE ISLAND, LLC, AND CIRCUS CIRCUS LV, LP

MICHAEL S. LEVINE*
LORELIE S. MASTERS
HUNTON ANDREWS KURTH LLP
2200 Pennsylvania Avenue, NW
Washington, DC 20037
Tel: (202) 955-1851
mlevine@HuntonAK.com
lmasters@HuntonAK.com

RENEE M. FINCH
MESSNER REEVES LLP
8945 West Russell Road, Suite 300
Las Vegas, NV 89148
Tel: (702) 363-5100
rfinch@messner.com

**Pro Hac Vice Motion to be Filed*

CHRISTOPHER J. CUNIO
NICHOLAS D. STELLAKIS
HUNTON ANDREWS KURTH LLP
60 State Street, Suite 2400
Boston, MA 02109
Tel: (617) 648-2747
nstellakis@HuntonAK.com

Attorneys for Amici Curiae, Restaurant Law Center, Bloomin' Brands, Inc., Treasure Island, LLC, and Circus Circus LV, LP

COUNSEL PRESS · (800) 3-APPEAL PRINTED ON RECYCLED PAPER

REQUEST FOR JUDICIAL NOTICE OF THE RESTAURANT LAW CENTER, BLOOMIN' BRANDS, INC., TREASURE ISLAND, LLC, AND CIRCUS CIRCUS LV, LP

In accordance with Nev. R. App. P. 27 and NRS 47.150, the Restaurant Law Center, Bloomin' Brands, Inc., Treasure Island, LLC, and Circus Circus LV, LP, hereby request that this Court take judicial notice of exhibits that are publicly available.

Specifically, amici ask that the Court take judicial notice of documents publicly filed in the case of Treasure Island, LLC v. Affiliated FM Ins. Co., 2:20-cv-00965-JCM-EJY (D. Nev. Oct. 29, 2021). These documents are found on the public docket in that case at ECF 205-1 and were submitted in support of Treasure Island, LLC's Motion for Partial Summary Judgment. The documents consist of expert reports and deposition testimony of Drs. Alex LeBeau, Joseph Lewnard, and Angela Rasmussen.

Amici also as that the Court take judicial notice of a document filed in the case of Baylor College of Medicine v. Underwriters at Lloyd's Syndicates, No. 2020-53316 (Harris County, Texas, District Court). The document, the Expert Report of Peter J. Hotez, M.D., Ph.D., was filed as

Exhibit 10 to Plaintiff's Combined Response to Insurers' Two Latest Motions for Summary Judgment.

These materials are appended here to and numbered RJN001-RJN110.

Amici do not believe it is necessary for this Court to grant a request for judicial notice because "[i]t is not unusual for an amicus curiae brief to include factual material that is outside the record." Puentes v. Wells Fargo Home Mortgage, Inc., 72 Cal. Rptr. 3d 903, 911 (2008) (quoting Eisenberg et al., Cal. Practice Guide: Civil Appeals and Writs (The Rutter Group 2007) ¶ 9:210.1, p. 9–54.2). Nevertheless, this request is submitted in an abundance of caution.

Judicial notice of a fact is appropriate if it is "[c]apable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned." NRS 47.130(2)(b). The contents of the federal docket squarely fit this, as anyone with an internet connection can readily verify what appears there. Of course, the veracity of those contents is likely to be vigorously contested by Starr Surplus Lines Insurance Co., but their existence cannot be denied. It is a matter of public record that eminent scientists have opined that COVID-19 and

SARS-CoV-2 cause physical loss or damage to property. Whether they are right will be tested at trial. But the fact that they have said so is something that this Court should know and not something that Starr should want to hide from the Court.

Date: September 30, 2022

Respectfully submitted,

/s/ Renee M. Finch

Renee M. Finch
Messner Reeves LLP
8945 W. Russell Road, Suite 300
Las Vegas, NV 89148
(702) 363-5100
rfinch@messner.com

Michael S. Levine*
Lorelie S. Masters
Hunton Andrews Kurth LLP
2200 Pennsylvania Ave., NW
Washington, DC 20037
(202) 955-1851
lmasters@HuntonAK.com
mlevine@HuntonAK.com

Christopher J. Cunio
Nicholas D. Stellakis
Hunton Andrews Kurth LLP
60 State Street Suite 2400
Boston, MA 02109
(617) 648-2747
nstellakis@HuntonAK.com

Counsel for Amici

*Pro hac vice motion to be filed.

CERTIFICATE OF SERVICE

Pursuant to NRAP 25, I certify that on this 30th day of September 2022, the foregoing Request for Judicial Notice was e-filed with the Clerk of the Supreme Court of the State of Nevada and services were executed to the below counsel via the Court's Electronic Filing System pursuant to NEFCR 9:

Daniel F. Polsenberg, Esq.
Joel D. Henriod, Esq.
Abraham G. Smith, Esq.
LEWIS ROCA ROTHGERBER
3993 Howard Hughes Parkway
Suite 600
Las Vegas, NV 89169

Amy M. Samberg, Esq.
Lee H. Gorlin, Esq.
CLYDE & CO US LLP
7251 W. Lake Mead Blvd.
Suite 430
Las Vegas, NV 89128

Attorneys for Petitioner Starr Surplus Lines Insurance Company

Bradley Schrager, Esq.
WOLF RIFKIN SHAPIRO
SCHULMAN & RABKIN, LLP
3773 Howard Hughes Pkwy
Suite 590 South
Las Vegas, NV 89169
bschrager@wrslawyers.com

Mark T. Ladd, Esq.
COHEN ZIFFER FRENCHMAN
& MCKENNA LLP
1350 Avenue of the Americas
New York, NY 10019
mladd@cohenziffer.com

Attorneys for Real Party in Interest JGB Retail Vegas Lessee, LLC

VIA E-MAIL ONLY:

The Honorable Judge Mark Denton
EIGHTH JUDICIAL DISTRICT COURT DEPARTMENT NO. 13
Regional Justice Center, Courtroom 16D
200 Lewis Avenue
Las Vegas, Nevada 89155
Dept13lc@clarkcountycourts.us
Trial Court Judge

Case No. 84986

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EIGHTH JUDICIAL DISTRICT COURT CASE NO. A-20-816628-B

EXHIBITS TO REQUEST FOR JUDICIAL NOTICE OF THE RESTAURANT LAW CENTER, BLOOMIN' BRANDS, INC., TREASURE ISLAND, LLC, AND CIRCUS CIRCUS LV, LP

MICHAEL S. LEVINE*
LORELIE S. MASTERS
HUNTON ANDREWS KURTH LLP
2200 Pennsylvania Avenue, NW
Washington, DC 20037
Tel: (202) 955-1851
mlevine@HuntonAK.com
lmasters@HuntonAK.com

RENEE M. FINCH
MESSNER REEVES LLP
8945 West Russell Road, Suite 300
Las Vegas, NV 89148
Tel: (702) 363-5100
rfinch@messner.com

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CHRISTOPHER J. CUNIO
NICHOLAS D. STELLAKIS
HUNTON ANDREWS KURTH LLP
60 State Street, Suite 2400
Boston, MA 02109
Tel: (617) 648-2747
nstellakis@HuntonAK.com

Attorneys for Amici Curiae, Restaurant Law Center, Bloomin' Brands, Inc., Treasure Island, LLC, and Circus Circus LV, LP

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1.	Excerpts from deposition transcript of Alex LeBeau dated April 14, 2021 *found in Appendix of Exhibits to Motion for Partial Summary Judgment, Treasure Island, LLC v. Affiliated FM Ins. Co., 2:20-cv-00965-JCM-EJY (D. Nev. Oct. 29, 2021)*	RJN001—RJN014
2.	Excerpts from deposition transcript of Angela Rasmussen dated April 13, 2021 *found in Appendix of Exhibits to Motion for Partial Summary Judgment, Treasure Island, LLC v. Affiliated FM Ins. Co., 2:20-cv-00965-JCM-EJY (D. Nev. Oct. 29, 2021)*	RJN015—RJN038
3.	Rebuttal/Supplementary Report of Angela L. Rasmussen, Ph.D. dated December 7, 2020 *found in Appendix of Exhibits to Motion for Partial Summary Judgment, Treasure Island, LLC v. Affiliated FM Ins. Co., 2:20-cv-00965-JCM-EJY (D. Nev. Oct. 29, 2021)*	RJN039—RJN054
4.	Expert Witness Report of Joseph Lewnard, Ph.D. dated November 6, 2020 *found in Appendix of Exhibits to Motion for Partial Summary Judgment, Treasure Island, LLC v. Affiliated FM Ins. Co., 2:20-cv-00965-JCM-EJY (D. Nev. Oct. 29, 2021)*	RJN055—RJN075
5.	Expert Report of Peter J. Hotez, MD, Ph.D. dated March 18, 2022 *found in Plaintiff's Combined Response to Insurer's Two Latest Motions for Summary Judgment, Baylor College of Medicine v. Underwriters at Lloyd's Syndicates, No. 2020-53316 (Harris County, Texas, District Court Mar. 18, 2022)*	RJN076—RJN110

UNITED STATES DISTRICT COURT
DISTRICT OF NEVADA

CASE NO. 2:20-cv-00965-JCM-EJY

TREASURE ISLAND, LLC,
Plaintiff,
vs.
AFFILIATED FM INSURANCE COMPANY,
Defendant.
_____/

Remote via Zoom
Wednesday April 14, 2021
9:03 a.m. - 1:36 p.m.

VIDEOTAPED/VIDEOCONFERENCE DEPOSITION

OF ALEX LEBEAU, PH.D.

Taken before Darline M. West,

Registered Professional Reporter, Notary Public

in and for the State of Florida At Large,

pursuant to Notice of Taking Deposition filed

by the Defendant in the above cause.

- - -

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A. Yes. Based on your last statement, you included surfaces and air, which I was responding to. So if you want to separate them out, that's fine.

Q. Okay. Thank you.

And I think you said that these articles support the notion that the presence of the virus changed the character of either the surface or the air; is that correct?

A. Yes. These articles show that the virus -- the SARS-CoV-2 impacted the surface -- impacted surfaces.

Q. And how does SARS-CoV-2 impact surfaces?

A. SARS-CoV-2 can impact surfaces through release in aerosols, and those aerosols can settle onto surfaces and adhere to those surfaces. They can also be deposited by larger respiratory droplets, and, again, deposit and adhere physically onto those surfaces.

Q. And how do aerosols adhere to surfaces?

A. Aerosols adhere following release from a COVID-19 positive individual for SARS-CoV-2. After they've been present in the air for minutes to hours, settle on surfaces through gravitational forces electrostatic forces or simple just diffusion onto those surfaces, impacting them.

Q. And how does an aerosol that settles on a surface through gravity impact the surface?

A. It impacts it by changing the character of that surface.

Q. And how does it change the character of the surface?

A. Whereas before COVID-19, that surface did not contain any settled SARS-CoV-2 aerosols. It now contains, adhered to, physically impacting those SARS-CoV-2 aerosols.

Q. So the change that you're describing is from no virus on the surface to virus on the surface; is that correct?

MR. CUNIO: Objection.

THE WITNESS: It is stating that before SARS or COVID-19, there was no SARS-CoV-2 on that surface. Now there are SARS-CoV-2 adhering to that surface following deposition from gravitational forces or electrostatic forces and impacting that surface and -- impacting that surface.

BY MS. WANG:

Q. And what is the mechanism or chemistry by which the -- the virus adheres to surfaces?

A. Sure. Again, from generation of aerosols

and asymptomatic, and that surface contamination occurred in rooms occupied by surface -- people who were classified as being asymptomatic by the time they vacated their cabins. And because they were guests on there and they stayed in rooms on there and the Treasure Island also contains guest rooms, that it is a comparable comparison to show what may have happen in a room where you have an asymptomatic individual.

Q. Do you know whether the surface samples taken at the -- on the Diamond Princess Cruise Ship were taken before cleaning or after cleaning?

A. I don't recall the specifics. If you have the study, I'll be happy to take a look at it.

Q. Do you believe that -- strike that.

We talked a few minutes ago about how aerosols containing the virus, droplet containing the virus can settle on surfaces changing the character of those surfaces.

Do you believe that change in character of those surfaces is damage?

MR. CUNIO: Objection.

THE WITNESS: I believe that those surface deposits and surface deposits and settled aerosols impact and adhere to those

surfaces and put them in a condition that they were not previously in.

BY MS. WANG:

Q. And the -- the -- the condition created by the presence of surface deposits in aerosols is detrimental, correct, in your opinion?

A. Those surface deposits and settled aerosols that are released in facility do impact those surfaces in a negative way.

Q. And is that because, in your opinion, the presence of surface deposits and aerosols can lead to transmission to humans?

A. Those deposits which are administrative controls and engineering controls were designed to reduce the impacts of viral materials on the surfaces and air at Treasure Island and reduce the transmission of COVID-19 to uninfected individuals.

Q. That -- that wasn't my question, unfortunately.

My question was: A moment ago you testified that the surface deposits in aerosols impact the property in a negative way. Do you recall that?

A. Yes.

Q. And my question is: Is it your opinion

that the impact is negative because the presence of surface deposits and aerosols on surfaces can lead to fomite transition?

A. The impacts of viral materials on surfaces and the air and subsequently can lead to viral transition, but it's also changing the air and surfaces at the facility.

Q. And the change in the air and the surfaces is -- is -- as I think you testified earlier, whereas before COVID-19, those surfaces and air did not have viral particle -- SARS-CoV-2 viral particles in the air or on those surfaces; whereas, now, the air contains particles, viral particles, and the surfaces may have viral particles on them, correct?

MR. CUNIO: Objection.

THE WITNESS: Before COVID-19 was around, the air did not contain -- the surfaces did not contain SARS-CoV-2 viral materials; whereas, now, it contains aerosols released from COVID-19-positive individuals, and surfaces are physically impacted by SARS-CoV-2 viral material.

BY MS. WANG:

Q. And are you aware of any study or article addressing physical transformation of services due to

the virus? And by "physical transformation," I mean, a chemical alteration, degradation, discoloration, or anything of that nature.

MR. CUNIO: Objection.

THE WITNESS: No. The articles that I have identified show that there is a physical presence impacting the surface of SARS-CoV-2 physically impacting the surface with viral material, and air is physically impacted with the material -- with released aerosols.

BY MS. WANG:

Q. Have you ever done risk assessment or trial hygiene consultation concerning mold?

A. Yes. I have evaluated mold effects on facilities.

Q. And you're a mold assessor licensed in the State of Florida, correct?

A. Correct.

Q. And is it true that mold can subsist from being attached to surfaces?

MR. CUNIO: Objection.

THE WITNESS: I guess I -- I don't understand your question.

Q. You talked about some technology that was installed to avoid touching; is that correct?

A. Yes, that's correct. There was an electric eye installed on -- on doors in the facility.

Q. Okay. Now, fast forwarding quite a bit away into your deposition. You talked about -- or you said something to the effect of one person with COVID-19 on property will impact the property, and then you said many people with COVID-19 on property will impact the property.

Do you recall that testimony?

A. I recall having that discussion, yes.

Q. Okay. And just very briefly, would you explain how one person with COVID-19 on property impacts the property?

A. Sure. There's one COVID-19-positive individual there. While they are there, they are releasing aerosols into the air while they talk, while they breathe, when they -- excuse me, cough. From an individual, one individual, that takes between ten and 20 breaths a minute, releasing that every minute, those aerosols into the air or whether they sneeze or cough releasing those larger droplets will impact the facility, will -- will change the characteristics of the air and change the character

of the surfaces they are physically impacting.

Q. Okay. And just focussing on the characteristics of the air, does it change the content of the air?

A. Yes. So, as -- I think I mentioned earlier as before, if you have -- before COVID-19, there was no aerosols containing SARS-CoV-2 in the air; afterwards there are. Industrial hygiene thinks of air as in -- industrial hygienists think of air in terms of volume. So you can look at a piece of air, section of air, as a cubic meter. And you can imagine that before SARS-CoV-2 or before COVID-19, there was no SARS-CoV-2 in that cubic meter of air, because it was not present in our population; whereas, now, after COVID-19, there are people releasing aerosols into the air, and now that cubic meter of air, that volume of air now contains aerosolized SARS-CoV-2.

Q. Now, where -- where Treasure Island sits on that spectrum, whether there is one person with COVID-19 on the property or many people with COVID-19 on the property, was that within the scope of your charge in this case?

A. I'd say it -- as far as one versus many, it would -- it would be in regards to showing that one

COVID-19 on surfaces and the air of occupied spaces at and in the vicinity of Treasure Island?

A. No, it doesn't change my opinion.

Q. Okay. And similarly, whether there was one person or many people at Treasure Island with COVID-19, does that change your Opinion 3, Treasure Island has reopened with administrative and engineering controls in accordance with best practices to mitigate and control the impact of COVID-19 on surfaces and the air of occupied spaces at the location?

A. No, it does not change my opinion.

Q. Now, moving forward again, I want to talk a little bit about the table-cleaning hypothetical. Do you remember that?

A. Yes.

Q. Okay. So you were asked a number of questions about cleaning and/or disinfecting a table. Do you recall those questions?

A. Yes.

Q. Okay. So we're gonna assume that there is a person with COVID-19 -- strike that.

We're gonna assume there is a person that has COVID-19 on the property depositing SARS-CoV-2 viral material on that table.

That's a reasonable assumption for that hypothetical, right?

A. I think it's safe to assume if they're on property, they're depositing that material, yes.

Q. And -- and you've talked about the aerosolized process and the fomite process that results from shedding, correct?

A. Correct.

Q. Now, we have -- we're assuming in our hypothetical that we have a person and that person has deposited SARS-CoV-2 viral materials on that table. Okay? And they're at Treasure Island. Okay?

You're aware of the fact that Treasure Island is 2.1 million square feet of facility, correct?

A. Yes. Approximately, yes.

Q. How would an employee or staff at Treasure Island know where that one table with deposited viral material is on the facility?

MS. WANG: Objection.

THE WITNESS: I don't know. Such a large facility with a number of different rooms and spaces, I don't know.

BY MR. CUNIO:

Q. And presumably that person is, under this

hypothetical, who deposited the viral material that that table is moving around the casino.

How do we know -- how would an employee or staff at Treasure Island know what other tables at the facility that that individual deposited viral material on?

MS. WANG: Objection.

THE WITNESS: I don't know. The facility is, like I said, is so large with different areas. That individual would be shedding and spreading that wherever they're going at the facility.

BY MR. CUNIO:

Q. In fact, there is no way of tracing a person -- there's no technology that exists for tracing a person who's shedding viral material, correct, that you know of?

A. Not that I know of. And that's why I said the cleaning and -- and doing that at Treasure Island is pretty much impractical just 'cause of size and scope.

Q. Okay. So let's continue with this assumption, though.

This person has deposited viral material on a table and is expelling viral material. If the

staff was fortunate enough to find that one table, what would happen if it was cleaning that table with aerosolized COVID-19 in the vicinity?

MS. WANG: Objection.

THE WITNESS: If the table was clean and disinfected and there was still aerosols of SARS-CoV-2 in the environment, even after the cleaning, they could be deposited on that surface.

BY MR. CUNIO:

Q. Okay. And what would happen if another individual with COVID-19 came in the vicinity of that table?

A. Again, they would likely deposit materials into the air and onto that surface.

Q. I think this is part of the reason why you said it's impractical to simply clean to address COVID-19; is that correct?

A. Yes. Because of the size and scope and because individuals are continually shedding and spreading this at the facility. So if there's aerosols that exist in the air from minutes to hours from an individual, and that surface is clean for one moment, minutes after, seconds after more aerosols could be deposited on that surface and re-impact --

physically re-impact that surface with SARS-CoV-2 viral material.

Q. So SARS-CoV-2 viral material is not something that can simply be cleaned up at any given point in time at the Treasure Island, 2.1 million-square-foot facility, correct?

MS. WANG: Objection.

THE WITNESS: The size and scope make it impractical to do at the facility.

BY MR. CUNIO:

Q. Okay. And then just towards the afternoon, you were asked a number of times whether the engineering and administrative controls made Treasure Island safe. Do you remember those questions?

A. Yes.

Q. Okay. And I think you kept saying something to the effect of the administrative and engineering controls reduced the impact on property and reduced the risk caused by that impact, something to that effect. Do you recall that?

MS. WANG: Objection.

THE WITNESS: Yes. I remember talking about mitigation and the risk.

BY MR. CUNIO:

Q. Okay. And did I summarize your testimony

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	)	No. 2:20-cv-00965-JCM-EJY
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AFFILIATED FM INSURANCE COMPANY,	)	
Defendant.	)	

VIDEOTAPED DEPOSITION UPON ORAL EXAMINATION

of

ANGELA L. RASMUSSEN, Ph.D.

APRIL 13, 2021

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(866)624-6221
www.MagnaLS.com
File No. 704309
REPORTED REMOTELY BY: Karen M. Grant, WA CCR No. 2155

So the PCR test is testing for the RNA, not infectious virus. But it's also important to note that infectious virus -- well, PCR tests will not come out positive unless there was infectious virus there at some point.

Q. (By Ms. Wang) All right. Let's -- let's turn to your opinions, and they're summarized on page 4 of the report. And then -- and then, of course, the report discusses them one at a time, so feel free to refer to your report --

A. Okay.

Q. -- if you'd like.

So your first opinion is that COVID-19 physically damages property, with persons shedding the virus into the air and onto surfaces, resulting in tangible, demonstrable, and detectable physical alteration and transformation of air and surfaces.

So, first of all, I'd like to know whether "tangible, demonstrable, detectable physical alteration" was a phrase that you developed or whether those were words provided to you by someone?

A. That was a phrase that I developed.

Q. And where did you -- what were the sources for that phrase, or you just made it up?

A. I used words that I felt describes the situation

best.

Q. And the word "alteration," what do you mean by alteration?

A. Change, which I think is the definition of alteration, unless we're talking about tailoring.

Q. I don't think (inaudible).

And are you aware of any evidence that physical alteration or change of property occurred at Treasure Island's hotel and casino?

MR. CUNIO: Objection.

A. So yes. There were people with COVID on the property. Those people, if they're infected with SARS-Coronavirus-2, will shed virus into their environment, both the air and onto surfaces. The presence of that virus mediates a change, so -- so that's what I was referring to. And the physical presence of that virus mediates a change.

Q. (By Ms. Wang) And when you say "mediates," what do you mean? You mean results in a change?

A. It results in a change.

Q. Okay. And -- and the change in that instance is that the surfaces at Treasure Island didn't have virus particles on them, and then when infectious people were in the property, they did have virus particles on them. In that way, they changed from not having virus to having virus; is that --

Q. Okay. And can seasonal influenza virus also be detected on surfaces?

A. It can.

Q. Can it be -- I don't know what the right word is. Aerosolized?

A. It can.

Q. And do you believe that prior to COVID-19, if there were people who had the flu at Treasure Island, that the property would be altered because the influenza virus was on surfaces and in the air?

A. Well, yes, it would be altered.

Would that cause damage? That is a bit of a different question. While seasonal influenza can also be lethal, its infection fatality rate is considerably lower than SARS-Coronavirus-2.

And furthermore, not only is there a vaccine for influenza, which is very well understood, to reduce disease severity in people who get that vaccine, but the vast majority of the population has a preexisting immunity to influenza, which may not completely prevent infection from other strains of seasonal flu, but often does have a mitigating effect on disease severity because there's enough cross-reactivity with your immune system that it can reduce the risk further of seasonal influenza.

And then finally, there's also several drugs that

can be used to treat influenza, and it's readily diagnosed, including with rapid antigen tests.

So influenza, yes, would alter the property, but there are a number of mitigating factors that -- that would make that alteration less damaging.

Q. Thank you.

Is there any distinction between cleaning and disinfecting?

A. Yes. Although, this is probably a question more for an industrial hygienist if we're talking about the technical terms.

The difference, though, largely, between cleaning and disinfecting is that cleaning is just removing dirt, and disinfecting is treating with a chemical or a substance or some other method of actually rendering pathogens noninfectious.

Many cleaning agents, however, are also disinfectants. So, for example, detergent can disinfect a number of different pathogens, including SARS-Coronavirus-2. The detergent itself will disrupt the lipid envelope on the surface of the virus particle and render it noninfectious.

So "cleaning" and "disinfection" are often used interchangeably just because many cleaning agents are also disinfectants.

Q. Okay. And is there a way to ameliorate

Yeast are fungi and can be considered a type of mold.

But I think that -- that the answer, probably, to the question that you're asking is no. I am not a mycologist. And even though I have worked with yeast as a laboratory organism, I have not worked with -- with pathogenic molds or fungi that -- that I think you're asking about.

Q. Okay. Do you believe that viral particles on surfaces interact in any way with those surfaces?

A. No.

Like do you mean a chemical reaction?

Q. Well, in any -- in any fashion. For example, it's my understanding that certain types of mold will attach to a surface and use the surface for nutrition, which I consider to be an interaction, but viral particles do not do the same kind of thing. Is that your understanding?

MR. CUNIO: Objection.

A. So I'd like to unpack this a little bit. Now, I do have a Ph.D. in microbiology, so I have studied mold and bacteria as part of my training and education.

The interaction that you're discussing is essentially a chemical interaction where the mold or fungus is using a substrate for nutrition. That is not the only type of interaction that even a fungus or a bacteria will have with a substrate.

So mold and some types of bacteria can form what are called spores, and those are essentially like seeds that are essentially dormant. They don't do anything. If you had mold spores of a pathogenic fungus or if you had anthrax spores on a surface, that spore itself would only be interacting with the surface in a physical way, not a chemical way, in which it was using that as a -- as a substrate, a chemical substrate to get nutrition.

However, that would be, in the case of anthrax spores, very damaging because that, in turn, similar to a virus, could be inhaled, and that would -- that would then cause pulmonary anthrax, which is very deadly. And in that spore form, it's similar to SARS-Coronavirus-2 in how it would be interacting with that surface. It's physically interacting with the surface by being present on it. But unlike an inert particle, like a piece of dust, it can go on then to biologically damage somebody who is exposed to it.

So I would consider that type of interaction between a mold or a bacteria, or bacterial spore, to be a physical interaction, despite the fact that that is not using that as a growth substrate or a nutritional source.

Q. (By Ms. Wang) The physical interaction is that the viral particle is sitting on the surface?

A. Correct. It's physically present on the surface.

Q. Okay. But it doesn't get nutrition from the

Q. And the first rebuttal opinion is that you don't agree with Dr. Roberts' statement, SARS-CoV-2 being similar to other respiratory viruses, correct?

A. That's correct.

Q. And in fact, your opinion is, it's nothing like other respiratory viruses, because it's so highly contagious and potentially deadly; is that --

A. That's --

Q. -- correct?

A. That is correct.

Q. I'm summarizing, but that's the gist of it?

A. Well, and in addition to that, as it goes on to say, at the time that this was written, there was no currently available vaccine and no confirmed natural immunity.

Q. Right. And of course now there is starting to be a publicly available vaccine, correct?

A. Correct.

Q. And does that change your opinion as to whether SARS-CoV-2 is nothing like other respiratory viruses?

A. Well, as a virologist, SARS-CoV-2 is nothing like many other respiratory viruses, since coronaviruses and influenza viruses, for example, are very, very distinct.

It's certainly -- the availability of the vaccine - vaccines, I should say - does mitigate the threat

of COVID-19 transmission beginning when those vaccines were available. When I wrote this report, however, they were not. And talking about cases that were occurring as far back as January and February 2020, obviously there was no vaccine available then.

So while SARS-Coronavirus-2, as we get closer to the herd-immunity threshold, may become more similar to other respiratory viruses, in terms of the public health burden and our ability to mitigate to the severity and to reduce transmission in the population, when I wrote this and before that, it was nothing like any respiratory virus that was circulating.

Q. It's still considered a highly contagious disease, correct?

A. Correct.

Q. And it's still potentially deadly, although perhaps less so than it was; is that correct?

A. For vaccinated people, it is less deadly. For nonvaccinated people, actually, one of the variants that is currently circulating, B117, the variant first detected in the U.K., is actually considered to be more pathogenic. It increases your risk of hospitalization and death compared to other variants that are circulating.

Q. And is it also still the case that there is no confirmed natural immunity?

workers, for example?

A. Correct. Correct.

Q. And of course they're open now, right?

A. I believe so, yes.

Q. And -- and again, you don't have any information as to whether they are addressing health concerns, how they are addressing health concerns at Treasure Island --

A. I don't -- I don't know the specifics of the mitigation measures that they've employed.

Q. Yeah. Okay.

Okay. And rebuttal opinion No. 2 is essentially that there is nothing typical about SARS-CoV-2. It's quite different from other coronaviruses or respiratory pathogens, correct?

A. That's correct.

Q. And how does -- or does the fact that SARS-CoV-2 is not typical relate to whether or not it alters property, or does it?

A. So yes, it does relate to how it alters property, because, as I pointed out in response to Dr. Roberts' contention that a virus on a surface or in the air is like a particle of dust, what makes SARS-Coronavirus-2 atypical is its ability to infect people and to cause COVID-19, which is something unique to SARS-Coronavirus-2.

That is what makes it atypical and different from

other things that may physically alter the environment by being present. They don't present the same safety risk that SARS-Coronavirus-2 does. In other words, if you -- if you are present on a property that has dust floating in the air and on surfaces, that dust isn't going to infect and potentially kill you.

Q. I see.

A. And for that matter, nor is that dust going to go on and infect somebody else.

Q. But wouldn't you agree that, for example, influenza virus particles could infect people and, you know, be quite dangerous?

A. They could, but --

MR. CUNIO: Go ahead. Objection.

A. Seasonal influenza has a much lower infection fatality rate and case fatality rate than SARS-Coronavirus-2.

In addition, as I mentioned earlier, seasonal influenza, there are vaccines that protect against it. We know that there is cross-reactivity from prior exposure to influenza, as well as to prior vaccinations, that also provide partial protection and ameliorate disease severity, and there are therapeutics that can be used to treat influenza virus infection. So influenza does not present the same risk that SARS-Coronavirus-2 does.

Q. Sure.

A. -- look over this. It's been a long day, and this is my first time, so I just want to make sure that I'm not missing anything.

Q. Take your time.

A. (Perusing.) Okay. Starting off on the first paragraph of "Rebuttal of Dr. Rasmussen," Dr. Roberts says, "Dr. Rasmussen makes multiple claims that the presence of the SARS-Coronavirus-2 virus results in 'tangible, demonstrable, and detectable physical alteration and transformation to the air and surfaces and rendering them dangerous transmission vehicles" --

THE COURT REPORTER: I'm sorry. You'll have to read a little bit slower, Doctor.

THE WITNESS: Sure. Sorry.

A. Dr. Rasmussen makes multiple claims that the presence of the SARS-Coronavirus-2 virus results in 'tangible, demonstrable, and detectable physical alteration and transformation to the air and surfaces and rendering them dangerous transmission vehicles for the potentially deadly disease.' She did not, however, provide direct evidence that tangible, demonstrable, and detectable physical alterations and transformation did in fact occur at Treasure Island locations nor did she define those terms."

So I'm happy to define those terms, as well as say

why I am certain that I don't actually need to provide direct evidence that tangible, demonstrable, and detectable physical alterations and transformation did occur.

So let me start by defining those terms. Tangible, it means it's physically present. Demonstrable means you could test for it. It can be demonstrated by a variety of different types of evidence. Detectable, that means it could be detected by a molecular diagnostic assay, even though that didn't occur here.

We've already talked about what physical alterations means, but the environment, the physical environment, is altered when the virus is physically present. That creates a transmission risk, which is the transformation that I'm referring to.

I don't need -- why I don't feel that I need to demonstrate that the virus is there, presumably Dr. Roberts means by PCR, or environmental testing, because I have seen ample evidence that suggests SARS-Coronavirus-2 is prevalent in the community. There were sick guests. There are employees who (inaudible) seropositive. They had antibodies. That means they were infected.

THE WITNESS: Can you -- do you need me to slow down, Karen?

THE COURT REPORTER: You glitched out again there, I'm sorry.

MR. CUNIO: It glitched out right after "guests."

A. There were sick guests. There were employees who tested positive for antibodies, meaning they had previously been infected with SARS-Coronavirus-2. There were many employees that tested positive for SARS-Coronavirus-2 antibodies in May, suggesting that many of them could have been actively infected with SARS-Coronavirus-2 while they were working.

And finally, this property is huge and gets a lot of traffic from many different places outside of the community in Las Vegas. That means that people certainly could have been traveling to Las Vegas, entering the property, and people almost certainly did. When I read Dr. Lewnard's analysis of this, that confirmed what I already knew from these multiple lines of evidence, so I disagree with Dr. Roberts that I failed to demonstrate this.

Q. (By Ms. Wang) Okay. So I certainly appreciate that you disagree with pretty much everything that's going to be in his report, but what I'm wondering is whether there are particular aspects that you're critical of that we have not touched on today. And believe it or not, I think just about everything -- well, I think everything in the answer that you just gave, which I understand is heartfelt, we did touch on already.

So just in the interest of time, I just want to see if there's anything in here that we haven't discussed at all that you would like to say about -- about Dr. Roberts' rebuttal. And I know you don't agree with it. I'm not --

A. Yes.

Q. I have no illusions about that, so there's no need to say: I don't agree with, you know, each point in there. I can assume that. But just wondering if there's anything in particular that we haven't already discussed that you want to point out.

A. So just looking through here, we have discussed many of these issues --

Q. Yeah.

A. -- with regard to, you know, cruise ships and Treasure Island and how similar are they, things like that. I think you know how I feel.

But there is one point that I feel that I have not adequately made, and that has to do with the second paragraph, in which Dr. Roberts is talking about how detection of viral genetic material cannot use to -- cannot be used to conclude that viable infective virus -- which actually is not the correct term. The correct term would be infectious virus -- is present, as genetic material can persist after a virus had been inactivated.

We did discuss this, but one very important point

that I would like to make and that I haven't really expanded on is that while it is true that a PCR test does not detect infectious virus, the test that you would use to detect infectious virus, which is called either a plaque assay or a Median Tissue Culture Infectious Dose assay, it is not feasible to do those. They are not routinely done. They -- that is not required to demonstrate that infectious virus is present on a property.

And here is why. So they're technically very difficult to do with environmental samples, anyways, but they also have to be done in a BSL-3 laboratory, which is a high-containment laboratory. There is one high-containment laboratory in, BSL-3 laboratory, in Clark County. It seems that Dr. Roberts is not aware of this.

But that laboratory would be used for urgent public health needs, so that would be things like testing antibodies, looking for reinfection, doing experiments to understand what's going on in Clark County. That would not be used to do environmental testing at Treasure Island or any other business. Those facilities are in high demand, as you might imagine, right now during the pandemic. There is no way that you could conduct testing for viable virus.

And here's the other reason why you don't even need to: Because, as I said at the very beginning of this deposition, viral RNA does not appear out of nowhere. It

comes because it is shed from people who are infected, when they are contagious, so they will be shedding a mixture of noninfectious and infectious virus.

You can detect that by PCR and you can make the assumption that, regardless of the fact that you're not detecting infectious virus, there was a person who put that virus there who was shedding infectious virus. That's how I know with certainty that people in the community shedding the virus on Treasure Island are causing damage to the property by physically depositing virus there, both infectious and noninfectious, and that that damaged the property by creating risk for any person who might be occupying that property.

I just want to make that very clear, because Dr. Roberts seems to think that the lack of PCR testing data and the fact that the PCR assay does not test for infectious virus is indicative that there was -- that we failed to prove that SARS-Coronavirus-2 was on the property.

I completely disagree with that assertion. And I don't think that a plaque assay or a TCID50, the Median Tissue Culture Infectious Dose assay, are necessary to demonstrate that, and it again shows his lack of experience in the field of virology.

MS. WANG: Okay. I have no further questions.

MR. CUNIO: Okay. I have a few. Is it okay

if I just jump right in, Joyce, or do you --

MS. WANG: Fine by me.

MR. CUNIO: Sure.

EXAMINATION

BY MR. CUNIO:

Q. So we start of started, Dr. Rasmussen, and ended at the same place, which is talking about the words "tangible, demonstrable, detectable," et cetera.

MR. CUNIO: And actually, Juliette, I think you need to give me back control for a minute --

MS. MADRID: Okay. Sure.

MR. CUNIO: -- please.

Q. (By Mr. Cunio) You'll recall, Dr. Rasmussen, that there was discussion about these terms at the beginning of your deposition.

A. Yes.

Q. And you indicated that those were terms that you chose to utilize in your report, or reports, correct?

A. Correct.

Q. And going to Exhibit 286, which is your first report, obviously those terms appear in a number of places, but I'm going to go to your summary of opinions, which is on page 4 of Exhibit 286. Can you see that?

A. Yeah. Hang on. I don't know why I always have to

do this funny thing where I put the page number in and then switch to a different document and then switch back. But, yes, I can see it now.

Q. It does get hung up.

Okay. So the first series of questions is, I just want it to be clear because, you know, over the course of five and a half hours, things get put in different places in the transcript, and I kind of want to bring them all back to one place. I want to walk you through a series of questions that are going to begin with: Is SARS-CoV-2 at Treasure Island? And then I'm going to go through it, okay?

A. Okay.

Q. Is SARS-CoV-2 at Treasure Island tangible?

A. Yes.

Q. And we've already discussed what tangible means, right?

A. It means physically present.

Q. And is SARS-CoV-2 at Treasure Island demonstrable?

A. Yes.

Q. And we've discussed what demonstrable means, correct?

A. Yes. It means you can demonstrate that in a number of different ways.

Q. Okay. And is -- strike that.

Is SARS-CoV-2 at Treasure Island comparable to

dust?

A. No.

Q. Okay. A brief explanation of that. Why not?

A. The brief explanation of that is that dust does not infect you and cause COVID-F19 and potentially kill you when you breathe it in or are exposed to it.

Q. Is SARS-CoV-2 at Treasure Island something that can be simply cleaned and/or disinfected and eradicated from the property?

A. In theory, yes. In reality, no. There are far too many surfaces to be able to clean all of them before they could have virus reintroduced onto them.

Furthermore, while the air can be cleaned, in part, I don't know what Treasure Island's ventilation system looks like. But it's doubtful that Treasure Island has installed an air disinfection or filtration system on par with a hospital ICU, and that is really -- you would need a combination of those two things. But again, you wouldn't be able to clean all of the air on Treasure Island. It is a huge property.

You would not be able to clean all the surfaces, because virus would be constantly dropping out of the air, as shown in Figure 1 of my report, onto those surfaces, especially if people are on the property touching those surfaces and putting more virus into the air.

That's the problem. If you could disinfect this by freezing time at any one moment and clean every single surface and completely recycle all the air and filter it in the facility, theoretically this is possible. Obviously we can't stop time. This is the real world, and people are continuing to come on this property, people are continuing to reintroduce SARS-Coronavirus-2 to the property, so infinite cleaning and disinfection and ventilation and air purification will not fully remove the risk.

Q. Now I want to ask you a couple questions switching the terminology a little bit. I want to talk about the impact of SARS-CoV-2 and COVID-19 at Treasure Island, okay?

A. Sure.

Q. Is the impact physical at Treasure Island?

A. Yes, the impact is physical. The virus is physically present. The risk that it poses means that people cannot use the property for its intended -- its intended use. It means that people cannot safely occupy the property. And it is the physical deposition of the virus onto the property that is causing the impact.

Q. Is the impact of SARS-CoV-2 and COVID-19 at Treasure Island temporary?

A. It is not temporary, because the rate of people entering and exiting the property and interacting with one another means that there will be regular reintroductions of

the virus, given the prevalence in the community and even outside the community because, after all, Treasure Island is a property that attracts a number of guests from outside of the Clark County community. So it is not temporary, because there is constant reintroduction and then spread, once on the property.

Q. Is the impact of SARS-CoV-2 and COVID-19 at Treasure Island something that can be simply cleaned up and/or disinfected?

A. No, for the exact reasons that I just mentioned. There are so many surfaces, there is so much volume of air, and there are so many people coming in and out on (inaudible) that it is impossible in the real world to effectively clean and disinfect the property.

Q. I think you glitched out there at the end. You said "people on and off the property," and then the last piece of that didn't come through clearly. Do you remember what you said?

A. Yeah. So, I mean, I'll break it down to even more simple language. The bottom line is that it is not feasible in the real world to clean and disinfect the property to -- to completely eliminate the risk.

And people are coming in and out of the property. Those people can become infected on the property by viruses there. Then they will go on to deposit virus on the

property. In addition, people out in the community are getting infected and coming onto the property and continuing to shed virus and physically alter and damage the property.

So it is not temporary and it is not something that you can mitigate just by cleaning.

Q. Is the impact of SARS-CoV-2 and COVID-19 at Treasure Island comparable to the virus that causes the flu?

A. No. Influenza, seasonal influenza, is less deadly than SARS-CoV-2. It is preventable by a vaccine. And while there are vaccines now for SARS-CoV-2, there were not until essentially December of 2020. And even now, there are still communities in which people cannot access those vaccines.

In addition, people have a long history with having influenza. Most people have had Influenza A virus at some point in their life. They will have residual immunity that may be somewhat cross-protective, which can ameliorate the severity. We do not have that existing cross-protective immunity against SARS-Coronavirus-2.

And finally, there are drugs, oseltamivir and zanamivir, that can be used to treat influenza infection. We do not have drugs that are effective at treating SARS-Coronavirus-2. The only antiviral that we have, remdesivir, does not really make a difference, in terms of patients' ultimate outcome. Monoclonal antibodies are hard to get and they -- they also only marginally improve

people's outcome. And the only other treatments we have are for people who already have severe COVID-19 and are at high risk of death.

So there's -- there's really no comparison to influenza, other than the fact that they're both respiratory viruses. These are different pathogens that cause different disease for which there are different interventions. And the bottom line is, the interventions available for influenza significantly mitigate the risk that that virus poses compared to SARS-Coronavirus-2.

Q. Okay. And you referenced Figure 1 in your first report a number of times. Again, your first report is Exhibit 286. Figure 1 is titled "The Cycle of Property Again by Persons with COVID-19 in Air and On Surfaces."

Briefly, could you explain the role of property in transmission of COVID-19?

A. Yeah. Could you just remind me which page that is on, Chris? Because --

Q. Sure.

A. -- I need to navigate to it in AgileLaw.

Q. Sure. It's on -- I can show it to you, I think. Yeah, I can show it to you. It's page 11 of your report, and I can go to it and share the screen here. "Show Witness This Page." You should be looking at Figure 1 of your first report, which is Exhibit 286, and we're on page 11.

Rebuttal/Supplementary Report

Angela L. Rasmussen, Ph.D.

December 7, 2020

CONFIDENTIAL—subject to the Protective Order entered in this case

EXHIBIT
287

Rebuttal/Supplemental Report

I incorporate the materials considered and the opinions expressed in my original report dated November 6, 2020. I have attached an updated curriculum vitae as Exhibit 1 hereto. The opinions below are based on my knowledge, skill, training, education and materials considered. I hold these opinions to a reasonable degree of scientific certainty.

Dr. Roberts offers three opinions in his November 6, 2020 report:

> (1) There is no evidence that environmental surfaces are physically altered as a result of viral particles being deposited on them.
>
> (2) Public health actions that involve closing or reducing occupancy in business establishments, as well as recommendations to clean/disinfect frequently touched surfaces such as door handles, handrails, and/or elevator buttons, are typically intended by public health officials to reduce or eliminate the person-to-person spread of SARS-CoV-2 virus.
>
> (3) The plaintiff in this matter has not provided objective evidence that SARS-CoV-2 virus was present on the premises during the pandemic.

I offer the following in response to his first two opinions:

Regarding Dr. Roberts' Opinion 1: In reaching his opinion, Dr. Roberts likens SARS-CoV-2 to "all other respiratory viruses" because it "may also attach to aerosol particles (*e.g.,* dust) or fall onto surfaces for some time." He states that SARS-CoV-2 "[v]irions on surfaces are therefore comparable to dust particles deposited from the air."

SARS-CoV-2, however, is nothing like "all other respiratory viruses" because it causes COVID-19, a highly contagious disease and potentially deadly disease for which there is currently no publicly available vaccine and no confirmed natural immunity. SARS-CoV-2 is at least as contagious as other pandemic influenza viruses and other highly pathogenic betacoronaviruses such as SARS-CoV and MERS-CoV[1], and more contagious than seasonal influenza and other coronaviruses and rhinoviruses which cause the common cold[2–4]. Mortality caused by COVID-19 is considerably higher than for common colds or seasonal influenza[1–4], as well. Furthermore, patients who recover from acute COVID-19 have a high rate of chronic symptoms, now known as "long haulers" or "long COVID," which persist long after the acute infection has resolved[5]. While this is an emerging area of research, it is clear that SARS-CoV-2 can cause debilitating injury after even mild symptomatic disease, and in this regard is very dissimilar to common colds or to dust particles in the air or on surfaces.

Moreover, SARS-CoV-2 virions are not remotely comparable to "dust particles deposited from the air." SARS-CoV-2 virions, which are physically intact virus particles with complete structural features such as capsid, surface glycoproteins, and envelope packaging of the viral

genome within, are physical structures shed by persons infected with COVID-19 as indicated my original report. These virions persist in the air and on surfaces and physically transform and alter them into dangerous transmissions mechanism for COVID-19. As a result, the property becomes a veritable petri dish for COVID-19 and unfit for its ordinary function—here, a casino visited by thousands of people each day. Complete closure of the facility is an appropriate way to eliminate the physical impact, alteration and damage to the property from viral particles in the air and on surfaces. Alternatively, operating with personal protective equipment and administrative and engineering controls, is a way to mitigate and control the physical impact, alteration and damage from viral particles in the air and on surfaces.

Dr. Roberts' report suggests a fundamentally incorrect understanding of virology, specifically as that pertains to SARS-CoV-2 replication, transmission, and pathogenesis. Structurally intact virus particles called virions were incorrectly defined by Dr. Roberts as the "complete infective form of a virus." This is a definition given by dictionary.com, but it is not consistent with how it is defined in virology. Virions are defined as physically intact virus particles existing outside the host cell where viral replication occurs, independent of whether or not they are infectious[6]. Virions can contain genomes with deletions or mutations that render them defective and non-infectious, or they can be unable to infect a cell because of ambient chemical or environmental conditions. Virions are physical structures, and as such, they physically alter the property.

Non-infectious virions are not rare for any viral pathogen, and they do not disprove the presence of infectious virions capable of transmitting to others and cause an infection. In fact, they confirm the property damage that has occurred, as non-infectious particles are only produced by an infected person who is also shedding infectious virions. Most RNA viruses, like coronaviruses such as SARS-CoV-2, produce many non-infectious virions during the course of infection, a characteristic measured by the particle-to-plaque-forming unit (PFU) ratio. While the particle-to-PFU ratio can vary greatly from host to host, for SARS-CoV-2, studies in cell culture suggest that thousands on non-infectious virions are shed for every infectious virion[7], yet these cultures still produce high titers of infectious virus. Thus SARS-CoV-2 virions are not "comparable to dust particles deposited from the air," as the shedding of non-infectious virions is always accompanied by shedding of infectious virions, and infectious virus in the air and on surfaces present a significant risk of transmission of a severe, debilitating, and potentially lethal communicable disease. Therefore, the presence of SARS-CoV-2-infected people guarantees that they will be shedding infectious virus onto surfaces and into the air, causing physical damage to the property and rendering the property unfit and unsafe for occupation.

Regarding Dr. Roberts' Opinion 2: There is absolutely nothing "typical" about SARS-CoV-2 and COVID-19 (which is precisely why it is called a novel coronavirus), as it is substantially different from other emergent betacoronaviruses or other common respiratory pathogens. This is demonstrated by the international community's response thereto (including unprecedented governmental mandated shutdowns and wide-scale stay-at-home orders). Moreover, the "public health actions" including closure, reduction in capacity, and cleaning that Dr. Roberts' refers to are designed to eliminate (in the case of closure) or mitigate and control (in the case of the alternatives) SARS-CoV-2 virions that persist in the air and on surfaces and physically transform those mediums and physically damage the property consistent with my statement above and as specified in my original expert report.

Furthermore, as SARS-CoV-2 persists in the air, particularly in enclosed, low-humidity environments such as indoor, air-conditioned spaces, cleaning and surface disinfection are unlikely to fully eliminate virus from the premises. As demonstrated by Figure 1 in Section II, Exhibit A of my original expert report, virus in the air will be deposited on surfaces where it can remain infectious for days depending on ambient environmental conditions. People who are infected on the property will continually shed infectious virus and continually damage the property, rendering these mitigation efforts largely futile.

Infection with SARS-CoV-2 results in replication and amplification of the virus, with eventual shedding and transmission to others. As cleaning and disinfection are not automated processes, and transmission is most likely to occur from presymptomatic individuals as described in my original expert report, the staff who would carry out the cleaning and disinfection protocols could inadvertently further damage the property if they were unknowingly infected and shedding virus prior to symptom onset. In January through March 2020, the potential for aerosol transmission was not well understood, and as a result the cleaning measures recommended by public health officials was not sufficient to fully mitigate transmission risks and thus offset potential damage caused by infected persons on the property.

I reserve the right to supplement or amend my opinion as new data emerges and/or I am provided with additional documents or data.

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Exhibit 1.

CURRICULUM VITAE

Angela L. Rasmussen, Ph.D.

117 E. Louisa St
Box 448
Seattle, WA 98102
Twitter: @angie_rasmussen
angelarasmussen.org
angierasmussen@gmail.com
ar1692@georgetown.edu

Education

University of Washington, Seattle, WA 2009-2012
Department of Microbiology
Postdoctoral Fellowship

Columbia University Graduate School of Arts & Sciences, Coordinated Doctoral Program in Biomedical Sciences, New York, NY 2003-2009
Department of Microbiology
Ph.D, Microbiology, 2009
M.Phil., Microbiology, 2006
M.A., Microbiology, 2005

Smith College, Northampton, MA 1996-2000
B.A., Biological Sciences

Awards, Honors, and Invited Lectures

Keynote speaker 32nd Annual Holiday Conference, Hospital for Special Surgery	2020
Invited speaker/panelist, Cell Press Beijing Online Conference	2020
Invited speaker, The Scientist Webinar Series	2020
Invited speaker/honoree, Department of Biological Sciences, Smith College	2020
Invited panelist, ASM Virtual Journal Club	2020
Invited speaker, NYC Health System Special Pathogens	2020
Invited speaker, Memorial Sloan Kettering Grand Rounds: Advanced Topics in Infectious Disease	2020
Elemental 50 Experts to Follow in a Pandemic	2020
Invited speaker, MJH Life Sciences, COVID-19 Fact or Fiction?	2020
Invited speaker, MJH Life Sciences, Battling Dual Threats: Flu and COVID-19	2020
Member, MJH Life Sciences COVID-19 Coalition	2020
Invited speaker, MJH Life Sciences, COVID-19: Race for a Vaccine	2020

Invited speaker, COVID-19 Lessons Learned and Best Practices Dialogue, 2020
DTRA and the Republic of Philippines Department of Health
Invited panelist, Pandemic Preparedness: A Roadmap for Future Outbreaks, 2020
Center for Global Development
Invited speaker, Breaking Science Writing, Johns Hopkins University 2020
Invited speaker, COVID-19 Seminar Series, HHMI Janelia 2020
Invited speaker, Coronavirus Preparedness Summit 2020
Invited speaker, Host Responses to Viral Pathogens, UC-Riverside 2020
Calderone Junior Faculty Prize, Columbia Mailman School of Public Health 2019
Invited speaker, Hot Topics in Emerging Pathogens, New York University 2018
Invited speaker, Institute of Systems Genetics, New York University 2016
Invited speaker, Mini-Medical School, University of Washington 2016
Invited speaker/honoree, Department of Biological Sciences, Smith College 2012
Invited speaker, Faculty of Veterinary Medicine, Udayana University, Denpasar, 2012
Bali, Indonesia
Elected to Sigma Xi 2000
Margaret Wemble Brigham Award for Excellence in Microbiology or Immunology Research,
Smith College 2000
Blakeslee Fellowship, Smith College 1999
Elizabeth Drew Memorial Prize for best short fiction, Smith College 1997

Please see angelarasmussen.org for a full record of press clippings and non-scientific writing

Experience

Non-Resident Affiliate **2020-present**
Center for Global Health Science and Security, Georgetown University, Washington, DC

- *Affiliate member collaborating closely with GHSS center faculty and staff*
- *Virology lead of the Viral Emergence Research Initiative (VERENA) Consortium, a multi-disciplinary research effort to study the ecology, evolution, and emergence potential of novel viral pathogens using machine learning and advanced analytical approaches, with an emphasis on open data and equity.*
- *Leading several studies including host response to SARS-CoV-2 in animal models and studying sex bias to emerging viruses in human and wildlife hosts*
- *Contribute to VERENA workshops and seminar series*
- *Continuing collaborations established in my prior academic positions, studying SARS-CoV-2, MERS-CoV, Ebola virus, influenza virus, Lassa virus, Crimean-Congo hemorrhagic fever virus, and other emerging viruses of critical importance to public health and biosecurity.*

Consultant and Writer **2020-present**
Self-employed

- *Provide expert advice on virology and public health practices for a variety of clients in the field of law, marketing, pharmaceutical development, education, and public health.*
- *Write on current scientific state of the art for major media outlets including Forbes, Slate, The Guardian, Leapsmag, and Foreign Affairs*

- *Leveraging my considerable social media platform (~177,000 Twitter followers) to maximize engagement with the public for scientific communication, including providing guidance regarding best practices during a pandemic, critiquing and clarifying policy positions, and educating about the current state of the art in virology and immunology research.*

Associate Research Scientist (Junior Faculty) 2016-2020
Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY

- *Principal Investigator on a FastGrant award to use transcriptomics and machine learning approaches to study host responses to SARS-CoV-2 infection in rhesus macaques. We also used classification approaches combined with functional analysis to predict infection and identify host-directed drugs with potential as antiviral therapeutics.*
- *Lead scientist and project manager for a project grant within the Center for Research on Discovery and Diagnostics (CRDD), a U19 Center of Excellence for Translational Research. This project employs systems biology approaches to develop host response signatures with diagnostic or prognostic value.*
- *Principal Investigator on a cooperative agreement with the Defense Advanced Research Projects Agency (DARPA) to investigate host responses associated with tolerance to infection with Ebola virus and MERS-CoV*
- *Lead scientist on contracts with the Defense Threat Reduction Agency (DTRA), and the National Biodefense and Countermeasures Center (NBACC) in the Department of Homeland Security (DHS), investigating the host transcriptional response to infection with multiple emerging pathogens with significant relevance to biodefense (Ebola and Burkholderia pseudomallei).*
- *Directly supervise a veterinarian-scientist performing all high-containment work on BSL-4 pathogens as a special volunteer at the Rocky Mountain Laboratories Integrated Research Facility*
- *Directly supervise technicians and bioinformaticians*
- *Write grants, establish collaborations, and obtain support for an independent research program.*
- *Coordinate with international team of investigators to transfer samples, manage personnel, write grant proposals, and publish manuscripts.*
- *Drive scientific studies with integral roles in project conceptualization, experimental design, data collection and analysis, and authorship of original research manuscripts. These studies use a systems biology-based approach and analysis on zoonotic viral pathogens, primarily those that cause hemorrhagic fever (Ebola virus, Marburg virus, Lassa virus, Lujo virus, Hantaan virus), respiratory disease (influenza, MERS-CoV), and emerging arboviruses (dengue virus, SFTSV, Heartland virus, Powassan virus, Rift Valley fever virus).*

Research Assistant Professor 2014- 2016
Department of Microbiology, University of Washington, Seattle, WA
Katze Laboratory

- *Lead scientist and project manager on three U19 program projects totaling $7.2 million in direct costs. Also responsible for reporting, personnel management, resource allocation, and funding renewal of these programs.*
- *Write grants and establish collaborations.*
- *Coordinate with international team of investigators to transfer samples, manage personnel, write grant proposals, and publish manuscripts.*
- *Responsible for all efforts involving emerging or highly pathogenic viruses, specializing in highly pathogenic emerging viruses.*
- *Drive scientific studies with integral roles in project conceptualization, experimental design, data collection and analysis, and authorship of original research manuscripts. These studies focused on emerging pathogens including filoviruses, MERS-CoV, dengue virus, H7N9 influenza virus, bunyaviruses, and arenaviruses.*
- *Guest lecturer during spring quarter graduate-level virology lecture courses.*
- *Mentor postdoctoral fellows and junior scientists in the laboratory.*

Scientific Project Manager 2012-2014
Department of Microbiology, University of Washington, Seattle, WA
Katze Laboratory

- *Management of Katze lab efforts contributing to three large, multi-institutional research grants (PNWRCE, CETR, Systems ImmunoGenetics), including personnel, resources, scientific contributions, programmatic reporting, compliance, and funding renewal.*
- *Coordination with other researchers worldwide for sample procurement, data acquisition, and analysis.*

Senior (Postdoctoral) Fellow 2009-2012
Department of Microbiology, University of Washington, Seattle, WA
Katze Laboratory
Principal Investigator: Professor Michael G. Katze, Ph.D.

- *Systems biology-based analysis of infection and pathogenesis of hepatitis C virus in both human liver transplant recipients and experimental models of HCV replication.*
- *Use of both systems approaches (transcriptomics, proteomics, metabolomics) and traditional molecular, biochemical, cellular, and virologic techniques.*

Graduate Research Associate 2003-2009
Department of Microbiology, Columbia University, New York, NY
Principal Investigator: Professor Vincent R. Racaniello, Ph.D.
Dissertation: "Development of a mouse model of rhinovirus infection."

- *Development of a mouse model of rhinovirus infection by isolating and characterizing host range variants capable of enhanced replication in mouse cells.*
- *Maintained Racaniello laboratory mouse colony.*

Graduate Technology Fellow 2006-2008
Columbia Technology Ventures

- *Performed numerous analyses to support intellectual property and technology transfer at Columbia University, including evaluating inventions for commercial viability, patent searches, scientific literature searches, and identification of potential licensees.*

Research Associate 2000-2003
Xcyte Therapies, Inc., Seattle, WA

- *Developed T-cell expansion technologies for large-scale lymphocyte cultivation in the context of samples collected from patients with hematological malignancies*
- *Performed a variety of functional and characterization studies to support preclinical development of T-cell immunotherapies for renal cell carcinoma, prostate cancer, B-cell chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma.*

Blakeslee Fellow/Student Researcher 1998-1999
Smith College, Northampton, MA
Principal Investigator: Professor Christine A. White-Ziegler, Ph.D.
Areas of Concentration: Microbiology, Microbial Genetics
Special Studies Project: "Mutational Analysis of rimJ"

- *Mutational analysis of rimJ, a gene involved in transcriptional thermoregulation of Escherichia coli Pap fimbrial gene expression.*

Intern 1998
XOMA (US) LLC, Berkeley, CA

- *Comparison of rBPI$_{21}$, a recombinant antibacterial peptide, to Polymyxin B as inhibitors of lipopolysaccharide-mediated proinflammatory cytokine secretion*

Academic Service
Member, Editorial Board, *mSphere*, 2020-present
Guest Editor, "Host Factors in Viral Infection," *Viruses*, 2020-present
Member, Editorial Advisory Board, *Cell Reports*, 2020-present
Member, WHO Ad Hoc Expert Group on Preclinical Models of COVID-19 Disease. February 2020-present.
Reviewer, Tick Borne Disease Panel, FY20 Peer Review Tick Borne Disease Research Program (TBDRP), CDMRP, August 2020-October 2020.
Reviewer, Viral Infectious Disease Panel, FY20 Peer Review Medical Research Program (PRMRP) Discovery Award, CDMRP, May 2020-July 2020.
Reviewer, Fondazione Cariplo, Call to support the development of collaborations for the identification of therapies, diagnostic tools, protective equipment and analysis systems to help fight the Coronavirus emergency and other potential future viral emergencies, April-May 2020.
Reviewer, Flavivirus RA-S-IN Panel, FY20 Military Infectious Diseases Research Program (MIDRP), intramural research program study section, December 2019-January 2020.

Steering Committee, Public Health 2035: Developing a Bold Vision for Our Second Century, Columbia Mailman School of Public Health, October 2019-present

Reviewer, Emerging Infectious Diseases Panel, Congressionally Directed Medical Research Programs (CDMRP), FY20 Peer Review Medical Research Program (PRMRP) Focused Program Award, July-September 2019.

Member, NIH Advisory Committee to the Director Working Group on Changing the Culture to End Sexual Harassment, 2019-present.

Reviewer, National Aeronautics and Space Administration (NASA), HERO Inflammation-Immunology study section, 2018.

Reviewer, National Aeronautics and Space Administration (NASA), Space Biology study section, 2018.

Topic editor, "Host-pathogen interactions during arboviral infections," *Frontiers in Cellular Infection and Microbiology.* 2018-2019.

Guest editor, "Host Responses to Viral Infection," *Vaccines*. 2017.

Member, Institutional Biosafety Committee, University of Washington, November 2014-March 2016.

Reviewer, Pre-Dengue Panel, Congressionally Directed Medical Research Programs (CDMRP), FY15 Peer Review Medical Research Program (PRMRP), July 2015.

Reviewer, Lethal Virus Countermeasures Panel, US Army Medical Research and Materiel Command, FY16 Military Infectious Diseases Research Program (MIDRP), Joint Program Committee-2, intramural research program study section, March 2015.

Panelist, NIH Pathways to Prevention (P2P): Advancing the Research on Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), 2014.

Reviewer, National Aeronautics and Space Administration (NASA), Space Biology study section, 2014.

Publications

M. Aminian, T. Ghosh, A. Peterson, **A.L. Rasmussen**, S. Stiverson, K. Sharma, and M. Kirby. ***Early prognosis of respiratory virus shedding in humans***. *Scientific Reports*, under review.

K. Escandón, A.L. Rasmussen, I.I. Bogoch, E.J. Murray, and K. Escandón. ***COVID-19 and false dichotomies: time to change the black-or-white messaging about health, economy, SARS-CoV-2 transmission, and masks***. *Travel Medicine and Infectious Disease,* under review.

D. Gurdasani, L. Bear, D. Bogaert, R.A. Burgess, R. Busse, R. Cacciola, Y. Charpak, T. Colbourn, J. Drury, K. Friston, V. Gallo, L.R. Goldman, T. Greenhalgh, Z. Hyde, K. Kuppalli, M.S. Majumder, J.M. Martin-Moreno, M. McKee, S. Michie, E. Mossialos, A. Nouri, C. Pagel, D. Pimenta, S. Popescu, V. Priesemann**, A.L. Rasmussen,** S. Reicher, W. Ricciardi, K. Rice, J. Silver, T.C. Smith, C. Wenham, R. West, G. Yamey, C. Yates, H. Ziauddeen. ***The UK needs a sustainable strategy for COVID-19.*** *The Lancet*, online. DOI: 10.1016/S0140-6736(20)32350-3. November 9, 2020.

A.L. Rasmussen, K. Escandón, and S.V. Popescu. ***Facial Masking for Covid-19***. *New England Journal of Medicine*, online. 10.1056/NEJMc2030886. October 23, 2020.

K. Alexanderson, C. Althaus, N. Alwan, L. Bear, R.A. Burgess, S. Ashworth, R. Beale, N. Bhadelia, D. Bogaert, R. Busse, C. Carlson, Y. Charpak, T. Colbourn, A. Costello, J. Dowd, J. Drury, I. Eckerle, J. Fellay, D. Fisman, K. Friston, V. Gallo, L.R. Goldman, T. Greenhalgh, D. Gurdasani, A. Hamdy, W.P. Hanage, E.B. Hodcroft, D. Hunter, Z. Hyde, D. Ingleby, P. Kellam, M. Kelly-Irving, K. Khunti, I. Kickbusch, D. King, F. Krammer, K. Kuppalli, A. Leyland, M. Lipsitch, M.S. Majumder, J. Mamo, J.M. Martin-Moreno, M. McKee, P. McLaren, A. McNally, S. Michie, M. Mills, M. Mina, J. Moran-Gilad, E. Mossialos, S. Neil, A. Nouri, A. Odone, C. Pagel, A. Phelan, D. Pillay, D. Pimenta, S. Popescu, V. Priesemann, **A.L. Rasmussen**, S. Reicher, W. Ricciardi, K. Rice, H. Rutter, G. Scally, C. Signorelli, J. Silver, T. Smith, D. Sridhar, A. Staines, C. Swanton, R.P. Walensky, K. Watkins, C. Wenham, R. West, G. Yamey, K. Yates, H. Ziauddeen. ***The John Snow Memorandum***, online www.johnsnowmemo.com; originally published ***Scientific consensus on the COVID-19 pandemic: we need to act now***. *Lancet* 396(10260): E71-E72. DOI: 10.1016/S0140-6736(20)32153-X. October 15, 2020.

C. Muñoz-Fontela, W.E. Dowling, S.G.P. Funnell, P.S. Gsell, X.R. Balta, R. Albrecht, H. Andersen, R. Baric, M.W. Carroll, Q. Chuan, I. Crozier, K. Dallmeier, L. de Waal, E. de Wit, L. Deland, E. Dohm, P. Duprex, D. Falzarano, C. Finch, M.B. Frieman, B. Graham, L. Gralinski, B. Haagmans, G. Hamilton, A.L. Hartman, S. Herfst, W. Klimstra, I. Knezevic, J. Kuhn, R. Le Grand, M. Lewis, W.-C. Liu, P. Maisonnasse, A.K. McElroy, V. Munster, N. Oreshkova, **A.L. Rasmussen**, J. Rocha Pereira, B. Rockx, E. Rodríguez, T. Rogers, F.J. Salguero, M. Shotsaert, K. Stittelaar, H.J. Thibaut, C.-T. Tseng, J. Vergara-Alert, M. Beer, T. Brasel, J.F.W. Chan, A. García-Sastre, J. Neyts, S. Perlman, D. Reed, J.A. Richt, C.J. Roy, J. Segalés, S. Vasan, A.M. Henao-Restrepo, and D.H. Barouch. ***Animal models for COVID-19.*** *Nature*, online. DOI: 10.1038/s41586-020-2787-6. Sept. 2020.

N.D. Grubaugh, W.P. Hanage, and **A.L. Rasmussen.** ***Making sense of mutation: what D614G means for the COVID-19 pandemic remains unclear.*** *Cell,* S0092-8674(20)30817-5. July 2020. K. Kuppalli and **A.L. Rasmussen**. ***A glimpse into the eye of the COVID-19 cytokine storm.*** *EBioMedicine* 55: 102789. May 7, 2020. PMID: 32388462.

A. Price, A. Okumura, E. Haddock, F. Feldmann, K. Meade-White, P. Sharma, M. Artami, W.I. Lipkin, D.W. Threadgill, H. Feldmann, **A.L. Rasmussen**. ***Transcriptional Correlates of Tolerance and Lethality in Mice Predict Ebola Virus Disease Patient Outcomes.*** *Cell Reports* 30(6): 1702-1713. Feb 11, 2020. PMID: 32049004.

A.G. Goodman and **A.L. Rasmussen**. ***Host-Pathogen Interactions During Arbovirus Infection***. *Frontiers in Cellular and Infection Microbiology* 9:77. March 26, 2019. PMID: 30972308.

A. Price, A. Caciula, C. Guo, B. Lee, J. Morrison, A. Rasmussen, W.I. Lipkin, and K. Jain. ***DEvis: An R package for aggregation and visualization of differential expression data***. *BMC Bioinformatics* 20(1): 110. March 4, 2019. PMID: 30832568.

A.L. Rasmussen. ***Host Factors Involved in Ebola Virus Replication.*** *Current Topics in Microbiology and Immunology* 419: 113-150. 2018. PMID: 28710692

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M. Dutta, S.J. Robertson, A. Okumura, D.P. Scott, J. Chang, J.M. Weiss, G.L. Sturdevant, F. Feldmann, E. Haddock, A.I. Chiramel, S.S. Ponia, J.D. Dougherty, M.G. Katze, **A.L. Rasmussen**, and S.M. Best. ***A Systems Approach Reveals MAVS Signaling in Myeloid Cells as Critical for Resistance to Ebola Virus in Murine Models of Infection.*** *Cell Reports* 18(3): 816-829. January 17, 2017. PMID: 28099857. PMCID: PMC5289750.

A.L. Rasmussen. ***Host Factors in Ebola Infection.*** *Annual Reviews in Genetics and Genomics* 17: 333-351. August 31, 2016. PMID: 27147086.

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A.L. Rasmussen. ***Probing the Viromic Frontiers.*** *mBio* 6(6): e01767-15. November 10, 2015. PMID: 26556279. PMCID: PMC4659475.

A. Okumura, **A.L. Rasmussen**, P. Halfmann, F. Feldmann, A. Yoshimura, H. Feldmann, Y. Kawaoka, R.N. Harty, and M.G. Katze. ***Suppressor of Cytokine Signaling 3 Is an Inducible Host Factor That Regulates Virus Egress during Ebola Virus Infection.*** *Journal of Virology* 89(20): 10399-406. October 15, 2015. PMID: 26246577. PMCID: PMC4580175.

K.J. Lubick, S.J. Robertson, K.L. McNally, B.A. Freedman, **A.L. Rasmussen**, R.T. Taylor, A.D. Walts, S. Tsuruda, M. Sakai, M. Ishizuka, E.F. Boer, E.C. Foster, A.I. Chiramel, C.B. Addison, R. Green, D.L. Kastner, M.G. Katze, S.M. Holland, A. Forlino, A.F. Freeman, M. Boehm, K. Yoshii, and S.M. Best. ***Flavivirus Antagonism of Type I Interferon Signaling Reveals Prolidase as a Regulator of IFNAR1 Surface Expression.*** *Cell Host Microbe* 18(1): 61-74. July 8, 2015. PMID: 26159719. PMCID: PMC4505794.

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A.L. Rasmussen, N. Tchitchek, D. Safronetz, V.S. Carter, C.M. Williams, E. Haddock, M.J. Korth, H. Feldmann, and M.G. Katze. ***Delayed inflammatory and cell death responses are associated with reduced pathogenicity in Lujo virus-infected cynomolgous macaques.*** *Journal of Virology*: 89(5): 2543-52. March 1, 2015. PMID: 25520505. PMCID: PMC4325716.

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genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. *Science* 346(6212): 987-91. November 21, 2014. PMID: 25359852. PMCID: PMC4241145.

N. Tchitchek, D. Safronetz, **A.L. Rasmussen**, C. Martens, K. Virtaneva, S.F. Porcella, H. Feldmann, H. Ebihara*, and M.G. Katze*. ***Assembly of an Expressed Sequence Tag Library Derived from Syrian Hamster (Mesocricetus auratus) Organs***. *PLoS ONE* 9(11):e112617. November 14, 2014. PMID: 25398096. PMCID: PMC4232415.

D. Falzarano, E. de Wit, F. Feldmann, **A. L. Rasmussen**, A. Okumura, X. Peng, M.J. Thomas, N. van Doremalen, E. Haddock, L. Nagy, R. LaCasse, T. Liu, J. Zhu, J.S. McLellan, D.P. Scott, M.G. Katze, H. Feldmann, and V.J. Munster. ***Infection with MERS-CoV causes lethal pneumonia in the common marmoset.*** *PLoS Pathogens*. 10(8): e1004250. August 21, 2014. PMID: 25144235. PMCID: PMC4140844.

E. de Wit, **A.L. Rasmussen**, F. Feldmann, T. Bushmaker, C. Martellaro, E. Haddock, A. Okumura, S.C. Proll, J. Chang, D. Gardner, M.G. Katze, V.J. Munster, and H. Feldmann. ***Influenza virus A/Anhui/1/2013 (H7N9) replicates efficiently in the upper and lower respiratory tract of cynomolgus macaques.*** *mBio* 5(4): e01331-14. August 12, 2014. PMID: 25118237. PMCID: PMC4145683.

D. Falzarano, E. de Wit, **A.L. Rasmussen**, F. Feldmann, A. Okumura, D.P. Scott, D. Brining, T. Bushmaker, C. Martellaro, A.G. Benecke, M.G. Katze, V.J. Munster, and H. Feldmann. ***Combined interferon-α2b and ribavirin treatment of rhesus macaques improves clinical outcome following infection with Middle East respiratory syndrome coronavirus (MERS-CoV).*** *Nature Medicine* 19(10): 1313-1317. October 2013. PMID: 24013700. PMCID: PMC4093902.

E. de Wit, **A.L. Rasmussen**, D. Falzarano, T. Bushmaker, F. Feldmann, D.L. Brining, E.R. Fischer, C. Martellaro, A. Okumura, J. Chang, D. Scott, A.G. Benecke, M.G. Katze, H. Feldmann, and V.J. Munster. ***Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques.*** *Proceedings of the National Academy of Sciences* 110(41):16598-603. October 8, 2013. PMID: 24062443. PMCID: PMC3799368.

A.L. Rasmussen*, I.-M. Wang*, M.C. Shuhart*, S.C. Proll, Y. He, R. Cristescu, C. Roberts, V.S. Carter, C.M. Williams, D.L. Diamond, J.T. Bryan, R. Ulrich, M.J. Korth, L.V. Thomassen, and M.G. Katze. ***Chronic Immune Activation is a Distinguishing Feature of Liver and PBMC Gene Signatures from HCV/HIV Coinfected Patients and May Contribute to Hepatic Fibrogenesis.*** *Virology* 430(1): 43-52. August 15, 2012. PMID: 22608059. PMCID: PMC3371131.

A.L. Rasmussen*, N. Tchitchek*, N.J. Susnow, A.L. Krasnoselsky, D.L. Diamond, M.M. Yeh, S.C. Proll, M.J. Korth, K.-A. Walters, S. Lederer, A.M. Larson, R. L. Carithers Jr., A. Benecke, and M.G. Katze. ***Early Transcriptional Programming Links Progression to Hepatitis C Virus-Induced Severe Liver Disease in Transplant Patients.*** *Hepatology* 56(1): 17-27. July 2012. PMID: 22278598. PMCID: PMC3349763.

D.L. Diamond, A.L. Krasnoselsky, K. E. Burnum, M.E. Monroe, B. Webb-Robertson, J.E. McDermott, M.M. Yeh, S. Strom, S.C. Proll, S.E. Belisle, **A.L. Rasmussen**, K. Walters, J.M. Jacobs, M.A. Gritsenko, D.G. Camp, R. Bhattacharya, J.D. Perkins, R.L. Carithers Jr., I.W. Liou, A.M. Larson, K.M. Waters, R.D. Smith, and M.G. Katze. ***Proteome and Computational Analyses Reveal New Insights into the Mechanisms of Hepatitis C Virus-Mediated Liver Disease***. *Hepatology* 56(1): 28-38. July 2012. PMID: 22331615. PMCID: PMC3387320.

J.E. McDermott, D.L. Diamond, C. Corley, **A.L. Rasmussen**, M.G. Katze, and K.M. Waters. ***Topological Analysis of Protein Co-Abundance Networks Identifies Novel Host Targets Important for HCV Infection and Pathogenesis.*** *BMC Systems Biology* 6: 28, April 30, 2012. PMID: 22546282. PMCID: PMC3383540.

A.L. Rasmussen, D.L. Diamond, J.E. McDermott, X. Gao, T.O. Metz, M.M. Matzke, V.S. Carter, S.E. Belisle, M.J. Korth, K.M. Waters, R.D. Smith, and M.G. Katze. ***Systems Virology Identifies a Mitochondrial Fatty Acid Oxidation Enzyme, DCI, Required for HCV Replication and Likely Pathogenesis.*** *Journal of Virology*, 85(22): 11646-11654. 15 November 2011. PMID: 21917952. PMCID: PMC3209311.

A.L. Rasmussen and V.R. Racaniello, ***Selection of Rhinovirus 1A Variants Adapted for Growth in Mouse Lung Epithelial Cells.*** *Virology* 420(2): 82-88, 25 November 2011. PMID: 21943827. PMCID: PMC3205939.
M. Bonyhadi, M. Frohlich, **A. Rasmussen**, C. Ferrand, L. Grosmaire, E. Robinet, J. Leis, R.T. Maziarz, P. Tiberghien, and R.J. Berenson. **In vitro *engagement of CD3 and CD28 Corrects T Cell Defects in Chronic Lymphocytic Leukemia.*** *Journal of Immunology* 174(4): 2366-2375. 15 Feb 2005. PMID: 15699173.
*Authors contributed equally to this work

Manuscripts in Preparation

A. Price, P. Sharma, N. van Doremalen, A. Okumura, V.J. Munster, E. de Wit, and **A.L. Rasmussen**. *Host response signatures linked to SARS-CoV-2 pathogenesis in rhesus macaques*. Expected submission: November 2020

N. van Doremalen, A. Price, N. Bhanu, R. Wu, P. Sharma, A. Okumura, M. Artami, B.A. Garcia, V.J. Munster, and **A.L. Rasmussen**. ***Integrated host response profiles define severity in a mouse model of MERS coronavirus.*** Expected submission: December 2020.

A. Price, R.W. Cross, P. Sharma, C.B. Woolsey, K.N. Agans, B. Lee, J. Garcia, A. Oleynik, A. Gokden, M. Artami, W.I. Lipkin, T.W. Geisbert, and **A.L. Rasmussen**. ***Differential Ebola virus disease severity after conjunctival exposure.*** Expected submission: January 2021.

O.M. Allicock, E. Haddock, A. Okumura, A. Price, F. Feldmann, D.W. Hawman, H. Feldmann, and **A.L. Rasmussen**. ***Host responses distinguishing outcome in a cynomolgus macaque model of Crimean-Congo hemorrhagic fever.*** Expected submission: February 2021.

A. Okumura, P. Sharma, N. Bhanu, R. Wu, E. Haddock, F. Feldmann, K. Meade-White, M. Artami, D.W. Threadgill, H. Feldmann, B.A. Garcia, and A.L. Rasmussen. ***Proteomic and metabolomic signatures of tolerance to Ebola virus infection.*** Expected submission: February 2021.

Research Funding

CURRENT
FastGrants (Rasmussen)
Longitudinal study of COVID-19 progression in non-human primate models
$50,000
The goal of this project is to identify the biological basis for COVID-19 progression in a rhesus macaque model of SARS-CoV-2 pathogenesis.
Role: PI

PENDING
NSF BII (Carlson)
VERENA: Viral Emergence Research Initiative
$200,000
This award supports the development of the VERENA consortium as a research institute, with the institute application projected for submission in 2021.
Role: Co-PI, virology team leader

NIH/NIAID R01 (Rasmussen)
Sex-specific host responses in Ebola virus pathogenesis
$2,198,322
The goal of this project is to use the Collaborative Cross model of Ebola virus disease to study the genetic basis for sex biases in disease severity and define the role of sex hormones in pathogenesis.
Role: PI

Canadian Institute of Health Research (Kindrachuk/University of Manitoba)
Mechanisms of asymptomatic Ebola virus testicular infections
The goal of this project is to characterize the basis for testicular persistence of Ebola virus infection and elucidate mechanisms of male-to-female sexual transmission.
Role: Subaward

EXPERT WITNESS REPORT

Joseph Lewnard, PhD

6 November 2020

SCOPE OF ENGAGEMENT

I was engaged by Hunton Andrews Kurth in the matter Treasure Island LLC v. Affiliated FM Insurance Company, which is currently pending in Federal Court in Nevada. I was asked to give opinions within my area of expertise, epidemiology, on the following topics:

1. *From a statistical standpoint, whether individual(s) with COVID-19 was/were on site prior to March 18, 2020?*

2. *What was the purpose of the Nevada Governor's closure order, and was it appropriate at the time?*

I am being compensated $475/hr. for my work on this report and $475/hr. for deposition and trial testimony.

In answering these questions, my opinions are based on my knowledge, skill, training, education, and materials considered (identified herein). I hold the opinions stated below to a reasonable degree of scientific certainty. The materials I considered in connection with this report are identified in the footnotes herein.

SUMMARY OF QUALIFICATIONS

I am a tenure-track Assistant Professor of Epidemiology at the School of Public Health of the University of California, Berkeley, holding secondary affiliations in the Division of Infectious Diseases & Vaccinology and the Center for Computational Biology (College of Engineering). I earned a PhD in Epidemiology of Microbial Diseases from Yale University in 2017 and held appointments as a postdoctoral fellow, and later Research Associate, at Harvard TH Chan School of Public Health in the Center for Communicable Disease Dynamics.

I study the transmission dynamics and control of infectious disease agents. My work has centrally involved the development and application of mathematical and statistical modeling methods to address such questions, with a focus on respiratory pathogens including influenza and *Streptococcus pneumoniae*. I have led studies published in pre-eminent scientific and medical journals including, among others, *Nature*, *Science*, *JAMA*, *The BMJ*, and *Proceedings of the National Academy of Sciences*, and my research has received extramural support from the National Institutes of Health, The Bill & Melinda Gates Foundation, Pfizer, Merck, the Wellcome Trust, the World Health Organization, the US-Israel Binational Science Foundation, and other funders. In 2019, I was named a Kavli Fellow of the US National Academy of Sciences.

Since early 2020 I have devoted substantial research attention to SARS-CoV-2 epidemiology through quantitative, field-based, and laboratory research. Significant recent outputs of this work listed below include (A) the first large scale study of SARS-CoV-2 epidemiology in a low/middle income country setting, including the largest (to date) contact tracing study quantifying risk of spread associated with differing types of interactions; (B) a high-profile study of the descriptive epidemiology of the first epidemic wave on the US west coast, based on comprehensive health

records of >9 million individuals in a comprehensive care cohort; and (C-E) authoritative reviews summarizing the state of knowledge regarding the scientific and bioethical principles around non-pharmaceutical interventions to mitigate COVID-19 burden, and methods for modeling spread. Parallel to this work, I am leading a study of SARS-CoV-2 infection prevalence and risk factors among farm workers in Monterey County, California, and a laboratory-based study of respiratory co-infections among individuals with and without symptoms, found to shed or not to shed SARS-CoV-2. My full CV is appended.

A. Laxminarayn R, Wahl B, Dudala SR, Gopal K, Mohan C, Neelima S, Jawahar. Reddy KS, Radhakrishnan J, **Lewnard JA**. Epidemiology and transmission dynamics of COVID-19 in two Indian states. *Science* 2020; doi:10.1126/science.abd7672.
B. **Lewnard JA**, Liu VX, Jackson ML, Schmidt MA, Jewell BL, Flores JP, Jentz C, Northrup GR, Mahmud A, Reingold AL, Petersen M, Jewell NP, Young S, Bellows J. Incidence, clinical outcomes, and transmission dynamics of severe coronavirus disease 2019 in California and Washington: prospective cohort study. *BMJ* 2020; doi:10.1136/bmj.m1923.
C. **Lewnard JA**, Lo NC. Scientific and ethical basis for social-distancing interventions against COVID-19. *Lancet Infect Dis* 2020; doi:10.1016/S1473-3099(20)30190-0.
D. Jewell NP, **Lewnard JA**, Jewell BL. Predictive mathematical models of the COVID-19. Pandemic: underlying principles and value of projections. *JAMA* 2020; doi:10.1001/jama.2020.6585.
E. Jewell NP, **Lewnard JA**, Jewell BL. Caution warranted: using the Institute for Health Metrics and Evaluation (IHME) model for predicting the course of the COVID-19 pandemic. *Ann Intern Med* 2020; doi:10.7326/M20-1565.

SUMMARY OF OPINIONS

It is my professional opinion that:

1. With near certainty (i.e., probability greater than 99.9%), an individual with COVID-19 was on site at Treasure Island ("TI") on or before March 18, 2020. I reach this conclusion from statistical analysis independent of any test results of employees or guests.

2. The purpose of the Nevada Governor's closure order was to address and remedy re-introduction of persons with COVID-19 on-site, and to avoid the ramifications of such re-introduction to the property. It was appropriate at the time.

FULL OPINIONS WITH SUPPORT

1. *With near certainty (i.e., probability greater than 99.9%), SARS-CoV-2 had been introduced to the physical premises of TI by March 19.*

The volume of guests entering TI over a 76-day period from January 1 to March 18, 2020 was approximately 329,000 persons, with an average of 7,000 present on any given day.[1] From this information, we may reasonably estimate the likelihood of a person with COVID-19 (henceforth termed a "case") being present on-site any particular day, as well as the cumulative probability of a COVID-19 case on-site on or before March 18, under differing and empirically falsifiable assumptions about the background prevalence of infection in the population.

Consider that a proportion *p* (with $0 < p < 1$) of individuals who may enter the casino are COVID-19 cases; thus *p* designates the probability that each individual entering the casino on a given day will introduce this communicable disease to the premises, by virtue of being a case, and its complement $1 - p$ is the probability that each individual is not a case and therefore does not introduce the disease to the premises. The probability that nobody, among a total of *n* individuals, is a COVID-19 case is the union of each individual not being a case, and may be computed as $(1 - p)^n$. The probability of at least one case, among *n* individuals, is thus $1 - (1 - p)^n$.

Precise determinations of infection prevalence over the first months of the pandemic (to provide inputs for the parameter *p*) are not widely available owing to inadequacies in test

[1] TI Complaint.

availability and limited indications for testing of only individuals with severe symptoms, known contact, travel histories to Wuhan or other severely affected settings, etc. I therefore explored a range of values for *p* to inform analyses, informed by a review of published prevalence studies detailed here. Barret et al. estimated 0.4% prevalence in a general (non-healthcare worker) sub-sample of individuals in New Jersey from March 24-April 7,[2] and Fassett et al. estimated 0.45% prevalence among pregnant women in Southern California;[3] samples of pregnant women have often been used as "sentinels" for epidemiological surveillance in the community during outpatient antenatal care visits. Monitoring asymptomatic travelers repatriated to Greece from the UK, Spain, and Turkey between March 20-25, Lytras estimated prevalence ranging from 3.6-6.3%;[4] this population is worthy of consideration as many guests of TI were international travelers, including from regions affected by COVID-19. In a general population sample in Indiana from April 25-29, Menachemi et al. estimated a prevalence of 1.74%.[5] Last, after successful implementation of mitigation measures, Riley et al. estimated that prevalence of infection in a general population sample in the UK reached 0.13%.[6] Serological testing of for evidence of previous infection (via titers of immune globulin G [IgG] against SARS-CoV-2) among TI employees revealed 12.4% (42/340) cumulative infection prevalence as of June 4; assuming a 7-day period of infectiousness for each case,[7] the mean prevalence of infection over a 155-day period of January 1 to June 4 in this group would be 0.558%. Assuming negligible case numbers in the first weeks of 2020, the mean prevalence of infection over the 110-day period of February 15 to June 4 in this group would be 0.786%.

[2] Barrett et al., *medRxiv* 2020; https://doi.org/10.1101/2020.04.20.20072470.
[3] Fassett et al., *Am J Perinatol* 2020; https://doi.org/10.1055/s-0040-1714060.
[4] Lytras et al., *J Trav Med* 2020; https://doi.org/10.1093/jtm/taaa054.
[5] Menachemi et al., *MMWR* 2020; https://doi.org/10.15585/mmwr.mm6929e1.
[6] Riley et al., *medRxiv* 2020; https://doi.org/10.1101/2020.07.10.20150524.
[7] He et al., *Nature Med* 2020; https://doi.org/10.1038/s41591-020-0869-5.

Applying inputs in the range of these estimates to the formula derived above, we see that the probability of no COVID-19 cases being present on-site at TI—both among 7,000 guests present on a given day, and among 329,000 guests arriving between January 1 and March 18—rapidly becomes negligible as we consider background prevalence estimates in the empirically observed range (**Table 1**). Even applying estimates around one order of magnitude below the lowest values observed (i.e. 0.01% prevalence), we obtain ≥50% probability of infection being present at TI on any given day, and negligible probability of evading introductions for the whole period. Thus, *it is my professional opinion that COVID-19 case(s) was/were on site on or before March 18, 2020, with near certainty (probability >99.0%).*

Table 1: Probabilities of no introduction of SARS-CoV-2

Background prevalence of infection (*p*)	Probability of no introduction of SARS-CoV-2 to TI	
	On a given day (n=7,000 guests)	Over a 76-day period (n=329,000 guests)
0.01%	49.7%	(5.14×10^{-13})%†
0.02%	24.7%	(2.63×10^{-27})%
0.05%	3.02%	(3.47×10^{-70})%
0.1%	(9.09×10^{-2})%	(1.11×10^{-141})%
0.2%	(8.20×10^{-5})%	(8.87×10^{-285})%
0.5%	(5.78×10^{-14})%	Too small to compute
0.558%*	(9.75×10^{-16})%	Too small to compute
0.786%*	(1.02×10^{-22})%	Too small to compute
1%	(2.79×10^{-29})%	Too small to compute
2%	(3.82×10^{-60})%	Too small to compute

*Mean period prevalence estimates corresponding to antibody detection prevalence among returning employees.
†Values are expressed using scientific notation for brevity. This quantity is equivalent to 0.000000000000514%.

2. *The purpose of the Nevada Governor's closure order was to address and remedy re-introduction of persons with COVID-19 on-site, and to avoid the ramifications of such re-introduction to the property. It was appropriate at the time.*

In assessing this issue, first I considered whether TI had other options as of March 18, 2020, short of closure, to remedy the circumstance of COVID-19 case(s) being, or having recently been, present on-site, and for preventing the arrival of further COVID-19 cases, and next whether it would be possible for TI to continually mount necessary interventions to address continued COVID-19 re-introductions at the rate with which they would be expected to occur.

As of March 18, 2020, no effective strategies were available to TI to (1) identify COVID-19 cases among individuals present on-site; or (2) to effectively screen persons entering TI to halt (or significantly reduce) re-introductions. Peer-reviewed findings were available as early as January, 2020[8] demonstrating that screening persons for symptoms, absent implementation of further measures to ascertain who may be a case, would fail to detect as many as 70-90% of COVID-19 cases at risk of transmitting infection once entering the premises, owing to the fact that such transmission may occur prior to or in the total absence of clinically manifest symptoms. The lack of widely-available, rapid, accurate tests to detect SARS-CoV-2 shedding among persons entering TI as of March (and to this date) moreover made direct testing impossible. This shortage is well documented; the first Emergency Use Authorization (EUA) for a diagnostic test was issued only on 29 February, 2020,[9] and most testing was conducted by state laboratories, with substantial

[8] Gostic et al., *eLife* 2020; https://doi.org/10.7554/eLife.55570 (published 24 February; initially published as a pre-print on January 30 at https://doi.org/10.1101/2020.01.28.20019224).
[9] US Food and Drug Administration. Coronavirus Disease 2019 (COVID-19) Emergency Use Authorizations for Medical Devices: *In Vitro* Diagnostic EUAs. https://www.fda.gov/medical-

numbers of tests submitted for confirmatory testing by CDC until March 14.[10] As of March 18, all EUAs issued allowed for testing only in laboratories certified to perform high-complexity tests. Further, the American Society for Microbiology addressed the present and anticipated long-term shortage of necessary materials for widespread testing in a position paper dated March 10, 2020.[11]

In light of the inability to effectively identify COVID-19 cases as of March 18 based on these facts, I next considered the question of whether the Nevada Governor's closure orders was needed to remedy COVID-19 introduction and mitigate future re-introduction, which could lead to onward transmission on-site of this communicable disease.

With an arrival rate of 329,000 guests over a 76-day period between January 1 and March 18, the average frequency of new SARS-CoV-2 introductions to TI occurring (with background prevalence of the range considered above of 0.01% to 2%) would be between once every 2.31 days (obtained by dividing 76 days by 0.0001 times 329,000) and once every 16.6 minutes (obtained by dividing 76 days by 0.02 times 329,000; **Table 2**). Based on the size of TI and the likelihood for guests and workers to transit through multiple common areas,[12] I considered that appropriate cleaning and sanitation of surfaces, plumbing and HVAC systems, and all other potential sources of exposure

devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#imft1 (accessed 4 November, 2020).

[10] US Centers for Disease Control and Prevention. Coronavirus Disease 2019 (COVID-19) Cases, Data & Surveillance: Previous US Viral Testing Data. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/previous-testing-in-us.html (accessed 4 November, 2020).

[11] American Society of Microbiology. ASM Expresses Concern about Coronavirus Test Reagent Shortages. https://asm.org/Articles/Policy/2020/March/ASM-Expresses-Concern-about-Test-Reagent-Shortages (accessed 4 November, 2020).

[12] TI floor plans and schematics.

may take a minimum of 24 or 48 hours, during which time functions would need to be suspended to allow cleaning to take place.

Assuming a 48-hour cleaning, we see that even at the at the lowest background prevalence considered (0.01%), re-introduction would be expected before cleaning could be completed in over one half of instances; with a 24-hour cleaning, re-introduction would be expected before cleaning could be completed in roughly one in three instances. At more realistic estimates of the background prevalence of infection based on the prevalence estimates referenced in my above opinion, the probabilities of evading re-introduction before cleaning can be completed are too small to compute numerically. These findings indicate that TI would, in effect, need to remain continuously closed in order to address introductions of COVID-19 at the rate they would be expected to occur.

Table 2: Estimated frequencies of introduction of SARS-CoV-2

Background prevalence of infection (*p*)	**Dynamics of introduction and re-introduction**		
	With 329,000 guests entering over 76 days	Probability 48h cleaning can be completed before SARS-CoV-2 reintroduction†	Probability 24h cleaning can be completed before SARS-CoV-2 reintroduction†
0.01%	2.31 days	42.1%	64.9%
0.02%	1.16 days	17.7%	42.1%
0.05%	0.462 days (11.1 hours)	1.32%	11.5%
0.1%	0.231 days (5.54 hours)	(1.74×10^{-2})%	1.32%
0.2%	0.112 days (2.77 hours)	(3.02×10^{-6})%	(1.74×10^{-2})%
0.5%	0.0462 days (1.11 hours)	Too small to compute	(3.98×10^{-8})%
0.558%*	0.0414 days (59.6 minutes)	Too small to compute	(3.23×10^{-9})%
0.786%*	0.029 days (42.3 minute)	Too small to compute	(1.71×10^{-13})%
1%	0.0231 days (33.3 minutes)	Too small to compute	Too small to compute
2%	0.0116 days (16.6 minutes)	Too small to compute	Too small to compute

*Mean period prevalence estimates corresponding to antibody detection among returning employees
†Computed assuming exponentially-distributed inter-arrival times with means as presented in the second column from left.

In light of these considerations, *it is my professional opinion that the purpose of the Nevada Governor's order was to address and remedy re-introduction of persons with*

COVID-19 on-site, and to avoid the ramifications of such re-introduction to the property.

It was reasonable at the time.

Joseph A. Lewnard, PhD
Division of Epidemiology, School of Public Health
University of California, Berkeley
Berkeley, California, USA
Tel.: +1 (510) 664-4050
E-mail: jlewnard@berkeley.edu

Appointments

University of California, Berkeley
Assistant Professor 2018–
Division of Epidemiology, School of Public Health
Division of Infectious Diseases & Vaccinology, School of Public Health
Center for Computational Biology, College of Engineering

Center for Communicable Disease Dynamics, Harvard TH Chan School of Public Health
Research associate 2018
Postdoctoral research fellow, laboratory of Marc Lipsitch 2017–18

Education

PhD, Epidemiology of Microbial Diseases, Yale University 2017
MPhil, Epidemiology of Microbial Diseases, Yale University 2016
BA, 1^{st} Class Hons., Geography and Music, McGill University (Canada) 2013

Grants

Active

Pfizer, Inc. 61775823. Assessing the role of *Streptococcus pneumoniae* in SARS-CoV-2 infection and disease progression (PI).
Total costs for project period: $472,180.
Project period: Nov. 2020–Oct 2021.

National Institute of General Medical Sciences. MIDASNI2020-3 (under U24GM132013-02S2). Transmission and severity of SARS-CoV-2 in India (PI).
Total costs for project period: $98,226.
Project period: Oct 2020–Sep 2021.

National Institute of Allergy and Infectious Diseases. R01-AI14812701A1: Emerging methods and applications for test-negative studies of infectious disease interventions (co-I).
Total costs for project period: $1,777,419.
Project period: Jul 2020–Jun 2024.

US-Israel Binational Science Foundation. Analyzing the causes for mumps re-emergence in vaccinated populations, combining epidemiology with statistical and transmission modeling (co-PI).
Total costs for project period: $320,000
Project period: Oct 2020–Sep 2024

Merck, Inc. Effectiveness and cost effectiveness of MMR3 vaccination for mumps outbreak mitigation (PI).
Total costs for project period: $141,613
Project period: Jun 2020–May 2021

Pfizer, Inc. Changes in antimicrobial prescribing for otitis media in pneumococcal conjugate vaccine era (PI).
Total costs for project period: $214,364
Project period: May 2020–Apr 2022

Innovative Genomics Institute (UC Berkeley). Community-based surveillance of SARS-CoV-2 transmission among California farmworkers (co-PI).
Total costs for project period: $275,000
Project period: Jun 2020–Dec 2020

Berkeley Population Center. Characterizing the early transmission dynamics and clinical spectrum of SARS-CoV-2 in the United States (PI).
Total costs for project period: $15,000
Project period: Jun 2020—Dec 2020.

Koret Berkeley-Tel Aviv Initiative. Computational methods for integrating sequence and epidemiological data to model transmission of infectious diseases (co-PI).
Total costs for project period: $20,000
Project period: July 2020—Jun 2021.

National Institute of Allergy and Infectious Diseases. R01-AI148336: Integrating epidemiologic and environmental approaches to understand and predict *Coccidioides* exposure and coccidioidomycosis emergence (co-I).
Total costs for project period: $3,800,000.
Project period: Dec 2019–Nov 2023

Wellcome Trust. 219741/Z/19/Z. Impact of *Shigella*, rotavirus, and other enteric vaccines on etiology-specific diarrhea, antibiotic use, and exposure of subclinical infections to antibiotics among children in low-resource settings (co-I).
Total costs for sub-award: $55,594
Project period: Feb 2020–Nov 2022

UC Berkeley. Dr. E. Dowdle Fund: Causal inference for susceptibility to recurrent tuberculosis (PI).
Total costs for project period: $140,000
Project period: Jul 2019–Jun 2023

Bill & Melinda Gates Foundation. OPP1190803: Modeling the value of vaccines in reducing the burden of antimicrobial resistance (co-I).
Total costs for sub-award: $158,998
Project period: Dec 2018–Aug 2021

International Symposium on Pneumococci and Pneumococcal Diseases/Pfizer, Inc. Robert Austrian Research Award (PI).
Total costs for project period: $25,000
Project period: Apr 2018–*unrestricted*

Completed

World Health Organization. Criteria for the assessment of modeling studies in WHO guidelines development (PI).
Total costs for project period: $24,500
Project period: Mar 2019–Dec 2019

CDC California Emerging Infections Program. Scope of impact for vaccines against Group A Streptococcus in the United States (co-I).
Total costs for sub-award: $14,000
Project period: Jul 2018–Dec 2018

Pfizer, Inc. Modulation of susceptibility to otitis media by early-life infection (PI).
Total costs for project period: $190,126
Project period: Mar 2017–Oct 2018

Publications

Articles in peer-reviewed journals

Original research

[40] **Lewnard JA**, Givon-Lavi N, Dagan R. Effectiveness of pneumococcal conjugate vaccines against community-acquired alveolar pneumonia attributable to vaccine-serotype *Streptococcus pneumoniae* among children: a case-control study. *Clin Infect Dis* 2020 (in press).

[39] Cheng Q, Collender PA, Heaney AK, Li X, Dasan R, Li C, **Lewnard JA**, Zelner J, Liang S, Chang HH, Waller LA, Lopman BA, Yang C, Remais JV. Towards a simulation framework for optimizing infectious disease surveillance: an information theoretic approach for surveillance system design. *PLoS Comp Biol* 2020 (in press). Pre-print from *medRxiv*: doi:10.1101/2020.04.06.20048231.

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[35] **Lewnard JA**. Uses of pathogen detection data to estimate vaccine direct effects in case-control studies. *J Roy Soc Interface* 2020;**17**:20200161. doi:10.1098/rsif.2020.0161.

[34] **Lewnard JA**, Rogawski McQuade ET, Platts-Mills JA, Kotloff KL, Laxminarayan R. Incidence and etiology of clinically-attended, antibiotic-treated diarrhea among children under five years of age in low- and middle-income countries: evidence from the Global Enteric Multcenter Study. *PLoS Negl Trop Dis* 2020;**14**:e0008520. doi:10.1371/journal.pntd.0008520.

[33] Bennett A, Pollock L, Bar-Zeev N, **Lewnard JA**, Jere KC, Lopman, BA, Iturriza-Gomara M, Pitzer VE, Cunliffe NA. Community transmission of rotavirus infection in a vaccinated population in Malawi: a prospective household cohort study. *Lancet Infect Dis* 2020 (in press). Pre-print from *medRxiv*: doi:10.1101/2020.04.05.20036574.

[32] **Lewnard JA**, Liu VX, Jackson ML, Schmidt MA, Jewell BL, Flores JP, Jentz C, Northrup GR, Mahmud A, Reingold AL, Petersen M, Jewell NP, Young S, Bellows J. Incidence, clinical outcomes, and transmission dynamics of severe coronavirus disease 2019 in California and Washington: prospective cohort study. *BMJ* 2020;**369**:m1923. doi:10.1136/bmj.m1923.

- Accompanying editorial: Anesi GL, Halpern SD, Delgado MK. Covid-19 related hospital admissions in the United States: needs and outcomes. *BMJ* 2020; **369**:m2082. doi:10.1136/bmj.m2082.

[31] **Lewnard JA**, King LM, Fleming-Dutra KE, Link-Gelles R, Van Beneden CA. Incidence of pharyngitis, sinusitis, acute otitis media, and outpatient antibiotic prescribing preventable by vaccination against group A *Streptococcus* in the United States. *Clin Infect Dis* 2020; doi:10.1093/cid/ciaa529.

- Accompanying editorial: Tanz RR, Shulman ST. Antimicrobial stewardship: a potentially important benefit of a group A *Streptococcus* vaccine in areas with low rates of acute rheumatic fever. *Clin Infect Dis* 2020; doi:10.1093/cid/ciaa533.

[30] **Lewnard JA**, Lo NC, Arinaminpathy N, Frost I, Laxminarayan R. Childhood vaccines and antibiotic use in low and middle income countries. *Nature* 2020; **581**:94–99. doi:10.1038/s41586-020-2238-4.

- Accompanying editorial: Dempsey LA. Vaccines versus antibiotics. *Nature Immunol* 2020; **21**: 596. doi:10.1038/s41590-020-0701-x.

[29] **Lewnard JA**, Whittles LK, Rick AM, Martin JM. Naturally-acquired protection against upper respiratory symptoms involving group A *Streptococcus* in a longitudinal cohort study. *Clin Infect Dis* 2020; doi:10.1093/cid/ciaa044.

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[27] Head JR, Collender PA, **Lewnard JA**, Skaff NK, Peng Y, Ohringer A, Cheng Q, Baker J, Li C, Liang S, Yang C, Hubbard A, Lopman BA, Remais JV. Early evidence of inactivated enterovirus 71 vaccine impact against hand, foot, and mouth disease in a major center of ongoing transmission in China, 2011–2018: a longitudinal surveillance study. *Clin Infect Dis* 2020; doi:10.1093/cid/ciz1188.

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[25] **Lewnard JA**, Givon-Lavi N, Dagan R. Dose-specific effectiveness of 7- and 13-valent pneumococcal conjugate vaccines against vaccine-serotype *Streptococcus pneumoniae* colonization in children. *Clin Infect Dis* 2019; doi:10.1093/cid/ciz1164

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[20] **Lewnard JA***, Tähtinen PA*, Laine MK, Lindholm L, Jalava J, Huovinen P, Lipsitch M, Ruohola A. Impact of antimicrobial treatment for acute otitis media on carriage dynamics of penicillin-susceptible and penicillin–non-susceptible *Streptococcus pneumoniae*. *J Infect Dis* 2018; **218**:1356–66. doi:10.1093/infdis/jiy343. (**contributed equally*)
- Accompanying editorial: Flasche S, Atkins KE. Balancing benefits and risks of antibiotic use. *J Infect Dis* 2018; **218**: 1351–3. doi:10.1093/infdis/jiy344.

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- Accompanying editorial: Hall RH. Curbing cholera. *Sci Transl Med* 2018; **10**:eeat9483. doi:10.1126/scitranslmed.aat9483

[18] **Lewnard JA**, Givon-Lavi N, Tähtinen PA, Dagan R. Pneumococcal phenotype and interaction with nontypeable *Haemophilus influenzae* as determinants of otitis media progression. *Infect Immun* 2018; **86**:e00727-17. doi:10.1128/IAI.00727-17.
- Accompanying editorial: Pelton SI. Deconstructing progression from pneumococcal colonization to disease. *Infect Immun* 2018; **86**:e00225-18. doi:10.1128/IAI.00225-18.

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- Awarded Kenneth Rothman Prize for the best paper published in *Epidemiology* in 2017 (link).

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Reviews (peer-reviewed)

[3] **Lewnard JA**, Reingold AL. Emerging challenges and opportunities in infectious disease epidemiology. *Am J Epidemiol* 2019; **188**:873–82. doi:10.1093/aje/kwy264

[2] **Lewnard JA**, Hanage WP. Making sense of differences in pneumococcal serotype replacement. *Lancet Infect Dis* 2019; **19**:e213–20. doi:10.1016/S1473-3099(18)30660-1.

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Comments and correspondence (not peer-reviewed)

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Lewnard JA, Lo NC. Scientific and ethical basis for contact-reducing interventions against COVID-19. *Lancet Infect Dis* 2020; doi:0.1016/S1473-3099(20)30190-0.

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Submitted manuscripts

[8] **Lewnard JA***, Bruxvoort K*, Fischer H, Hong V, Grant LR, Jodar L, Gessner BD, Tartof SY. Prevention of coronavirus disease 2019 among older adults receiving pneumococcal conjugate vaccine. Under review.

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[6] Andrejko K, Ratnasiri B, Hausdorff WP, Laxminarayan R, **Lewnard JA**. Antimicrobial resistance in pediatric *Streptococcus pneumoniae* isolates amid global implementation of pneumococcal conjugate vaccines: a systematic review and meta-regression analysis. Under review.

[5] Miller AC, Foti NJ, **Lewnard JA**, Jewell NP, Guestrin C, Fox EB. Mobility trends provide a leading indicator of changes in SARS-CoV-2 transmission. Pre-print from *medRxiv*: doi:10.1101/2020.05.07.20094441.

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[3] **Lewnard JA**, Cowley LA. An empirical Bayes method for serotype case-carrier ratios, with an application to Group B streptococcus. Revised. Pre-print from *bioRxiv*: doi:10.1101/421412.

[2] Fu H, **Lewnard JA**, Frost I, Laxminarayan R, Arinaminpathy N. Estimating the vaccine-avertable burden of multi-drug-resistant tuberculosis: a modelling study. Submitted.

[1] Miller AC, Hannah L, Futoma J, Foti NJ, Fox EB, D'Amour A, Sandler M, Saurous RA, **Lewnard JA**. Statistical deconvolution for inference of infection time series. Submitted. Pre-print from *medRxiv*: doi:10.1101/2020.10.16.20212753

Science outreach

[1] **Lewnard JA**. The Olympics won't spread Zika around the world. *The Conversation*, 25 July 2016. http://bit.ly/2au5kr9.

Awards and fellowships

Visiting Fellow, Center for Disease Dynamics, Economics, and Policy	2020–23
Kavli Fellow, US National Academy of Sciences	2019
Emerging Leaders in Biosecurity Fellow, Center for Health Security, Johns Hopkins Bloomberg School of Public Health	2018
Wilbur Downs Award, Yale School of Medicine ($7,500)	2015
Science Undergraduate Research Award, McGill Faculty of Science ($5,600, *1 per department per year*)	2013
Undergraduate Award, Canadian Association of Geographers (*1 per institution per year*)	2013

Teaching

Students supervised

University of California, Berkeley

PhD committee service
Kristin Andrejko (epidemiology), *Primary dissertation supervisor*
Graham Northrup (computational biology), *Dissertation co-supervisor*
Sean Wu (epidemiology), *Dissertation committee member*
Shelley Facente (epidemiology), *Dissertation committee member*
Christopher Hoover (environmental health sciences), *Dissertation committee member*

MPH capstone theses supervised

Nicholas Murdock, Tyler Chervo, Karen Click	2021
Lauren Linde	2020
Arti Kundu, Drew Wodecki, Whitney Mgbara	2019

PhD qualifying examinations administered

Jennifer Head (epidemiology), Joanna Vinden (IDV), Cheyenne Butcher (epidemiology)	2020
Nicholas Lo (IDV), Whitney Mgbara (ESPM), Kieran O'Brien (epidemiology), Mary Horton (epidemiology), Christopher Hoover (epidemiology)	2019

Harvard TH Chan School of Public Health

Angel Rollo (undergraduate), *summer research prcject*	2018
Wilma Figueroa (undergraduate), *summer research prcject*	2018
Veronica Wang (undergraduate), *summer research prcject*	2017
Winston Kunkel (undergraduate), *summer research prcject*	2017

Courses taught

University of California, Berkeley

As primary instructor

PH253B Epidemiology and control of infectious diseases	2019–
PH293 Advanced doctoral seminar in epidmeiology	2020–

As guest instructor

PH260E Molecular epidemiology of infectious diseases	2019

Fundaçao Oswaldo Cruz

International course in molecular epidemiology	2018

Yale University

Developing research proposals for the Downs Fellowship in public health, *Primary instructor.*	2015–16
Quantitative methods in infectious disease epidemiology, *Graduate teaching fellow.*	2014–16
Introduction to public health surveillance, *Graduate teaching fellow.*	2016
Introductory statistics, *Head graduate teaching fellow.*	2015

Presentations

Engagements at external academic institutions

[14]

Harvard TH Chan School of Public Health, October, 2020
[13] University of Texas at Austin, October, 2020
[12] Mathematical Sciences Research Institute. Berkeley, California. August, 2020.
[11] Koret Berkeley-Tel Aviv Initiative in Computational Biology Symposium. Tel Aviv University, Tel Aviv, Israel. June, 2019
[10] University of Bath, Bath, United Kingdom. February, 2019
[9] Imperial College, London, London, United Kingdom, September, 2018
[8] London School of Hygiene and Tropical Medicine, London, United Kingdom, September, 2018
[7] University of California, San Francisco. San Francisco, California. February, 2019
[6] University of Minnesota School of Public Health. Minneapolis, Minnesota. February, 2018
[5] Boston University School of Public Health, Boston, Massachusetts. February, 2018
[4] Rollins School of Public Health, Emory University, Atlanta, Georgia. February, 2018
[3] Langone School of Medicine, New York University, New York, New York. February, 2018
[2] The Broad Institute, Cambridge, Massachusetts. May, 2017
[1] Columbia Mailman School of Public Health, New York, New York. October, 2016

Policy and industry engagements

[7] Kaiser Permanente. March, 2020–.
[6] Pfizer Advisory Board. December, 2019.
[5] Pfizer Advisory Board. September, 2019.
[4] WHO Advisory Committee on vaccines against antimicrobial resistance, London, United Kingdom. February, 2019.
[3] Merck, Upper Gwynned, Pennsylvania. November, 2018.
[2] Pfizer, Collegeville, Pennsylvania. September, 2017.
[1] Biomedical Advanced Research and Development Authority (Office of the Assistant Secretary of Preparedness and Response, US Dept. Health and Human Services), Washington, DC. November, 2014.

Scientific conferences

[18] American Society for Microbiology Microbe Conference. June, 2020. *Invited oral presentation; canceled due to COVID-19.*
[17] Vaccines for Enteric Diseases. October, 2019. *Contributed oral presentation.*
[16] Korean-American Kavli Frontiers of Science Symposium. June, 2019. *Poster.*
[15] National Foundation for Infectious Diseases Annual Conference on Vaccinology Research. April, 2019. *Invited oral presentation.*
[14] Bay Area Ecology and Evolution of Infectious Diseases Meeting. March, 2019. *Invited oral presentation.*
[10–13] International Symposium on Pneumococci and Pneumococcal Diseases, Melbourne, Australia. April, 2018. *Contributed oral presentations (4).*
[9] Epidemics 6, Sitges, Spain. December, 2017. *Poster.*
[7–8] Infectious Diseases Society of America IDWeek, San Diego, USA. October, 2017. *Contributed oral presentation, Poster.*
[6] American Society for Microbiology (ASM) Microbe, New Orleans, USA. June, 2017. *Poster.*
[5] Twelfth International Rotavirus Symposium, Melbourne, Australia. September, 2016. *Poster.*
[4] International Symposium on Pneumococci and Pneumococcal Diseases, Glasgow, UK. June, 2016. *Contributed oral presentation.*
[3] Ecology and Evolution of Infectious Diseases, Ithaca, USA. June, 2016. *Poster.*
[2] Vaccines for Enteric Diseases, Edinburgh, UK. July, 2015. *Poster.*
[1] Palestinian-Israeli Collaborative Research Conference, Jerusalem, Israel. May, 2015. *Oral presentation.*

Service

Service to profession

Grant review

Swiss National Science Foundation 2020
Wellcome Trust, UK 2019

Editorial service

Reviewing Editor, *eLife* 2020–
Guest Editor, *PLoS Negl Trop Dis* 2019

Manuscript peer review

AIDS and Behavior, *American Journal of Epidemiology*, *American Journal of Tropical Medicine and Hygiene*, *Annals of Internal Medicine*, *BMC Infectious Diseases*, *The BMJ*, *BMJ Global Health*, *Clinical Infectious Diseases*, *Emerging Infectious Diseases*, *Epidemics*, *Epidemiologic Methods*, *Epidemiology and Infection*, *Expert Review of Vaccines*, *Innate Immunity*, *International Journal of Epidemiology*, *International Journal of Infectious Diseases*, *International Journal of STD and AIDS*, *JAMA*, *Journal of Infectious Diseases*, *The Lancet*, *The Lancet Infectious Diseases*, *The Lancet Global Health*, *The Lancet Public Health*, *mSphere*, *Nature Medicine*, *Pathogens and Global Health*, *PLoS Biology*, *PLoS Computational Biology*, *PLoS Medicine*, *PLoS Neglected Tropical Diseases*, *PLoS ONE*, *Proceedings of the National Academy of Sciences of the USA*, *Public Health Nutrition*, *Science*, *Science Translational Medicine*, *Scientific Reports*, *Sexually Transmitted Infections*, *Vaccine*

Institutional service

University of California, Berkeley

UC Office of the President, Systemwide Testing and Tracing Task Force 2020–
Chancellor's Committee on Public Health and Testing 2020–
Epidemiology graduate admissions committee 2019–

Yale Graduate School of Arts and Sciences

Epidemiology and Public Health Departmental Representative, Yale Graduate Student Assembly 2014–16
Professional ethics facilitator, Yale Graduate School of Arts and Sciences 2015–16

CAUSE NO. 2020-53316

BAYLOR COLLEGE OF MEDICINE,	§	
	§	
Plaintiff,	§	
	§	**IN THE DISTRICT COURT OF**
vs.	§	
	§	
UNDERWRITERS AT LLOYD'S SYNDICATES: LLOYD'S SYNDICATE 1967 SUBSCRIBING TO POLICY B0180PG1922227; SOMPO INTERNATIONAL FOR AND ON BEHALF OF ENDURANCE WORLDWIDE INSURANCE LTD., NEON LLOYD'S OF LONDON SYNDICATE NO. 2468, UNICORN UNDERWRITING LIMITED, HCC INTERNATIONAL INSURANCE COMPANY PLC FOR AND ON BEHALF OF HOUSTON CASUALTY COMPANY (UK BRANCH), CHUBB UNDERWRITING AGENCIES LIMITED FOR AND ON BEHALF OF SYNDICATE 2488, TALBOT UNDERWRITING LTD FOR AND ON BEHALF OF LLOYD'S UNDERWRITER SYNDICATE NO. 1183 TAL, and QBE EUROPEAN OPERATIONS PLC	§	**HARRIS COUNTY, TEXAS**
	§	
	§	**295th JUDICIAL DISTRICT**
Defendants.	§	

EXPERT REPORT OF PETER J. HOTEZ, MD, PhD

RJN076

Background and Qualifications

1. My name is Dr. Peter J. Hotez. My address is One Baylor Plaza; Houston, Texas 77030.

2. I am the Dean of the National School of Tropical Medicine and Professor of Pediatrics and Molecular Virology & Microbiology at Baylor College of Medicine where I am also the Co-Director of the Texas Children's Center for Vaccine Development and Texas Children's Hospital Endowed Chair of Tropical Pediatrics. I am also University Professor at Baylor University, Fellow in Disease and Policy at the James A Baker III Institute for Public Policy, Senior Fellow at the Scowcroft Institute of International Affairs at Texas A&M University, Faculty Fellow with the Hagler institute for Advanced Studies at Texas A&M University, and Health Policy Scholar in the Baylor Center for Medical Ethics and Health Policy.

3. As head of the Texas Children's Center for Vaccine Development, I lead a team and product development partnership for developing new vaccines for a number of diseases, including SARS/MERS/SARS-2 coronavirus. Our COVID-19 vaccine in partnership with Biological E based in India has been authorized for emergency use in India for adults and now children.

4. I obtained my undergraduate degree in molecular biophysics from Yale University in 1980; my Ph.D. degree in biochemistry from Rockefeller University in 1986; and my M.D. from Weil Cornell Medical College in 1987.

5. I am an elected member of the National Academy of Medicine and the American Academy of Arts & Sciences. In 2011, I was awarded the Abraham Horwitz Award for Excellence in Leadership in Inter-American Health by the Pan American Health Organization of the World Health Organization. In 2014-2016, I served in the Obama Administration as US Envoy, focusing on vaccine diplomacy initiatives between the US Government and countries in the Middle East and North Africa. In 2018, I was appointed by the US State Department to serve on the Board of Governors for the US Israel Binational Science Foundation, and am frequently called upon to testify before the US Congress. I have served on infectious disease task forces for two consecutive Texas Governors. In 2017 I was named by FORTUNE Magazine as one of the 34 most influential people in health care; in 2018, I received the Sustained Leadership Award from Research!America; in 2019, I received the Ronald McDonald House Charities Award for Medical Excellence; in 2021, I was selected as a recipient of the 2021 American Medical Association Scientific Achievement Award by the Association of American Medical Colleges

and American Medical Association; and in 2022, Congresswoman Lizzie Fletcher nominated my colleague Dr. Maria Elena Bottazzi and me for the Nobel Peace Prize for our work on a Covid vaccine that is safe, effective, and more affordable to manufacture than the others, enabling low-income countries a more meaningful opportunity to vaccinate large swaths of their populations. Also in 2022, StatNews a Boston Globe subsidiary named me among the top 46 leaders and change makers in the life sciences.

6. I have advised many public and private institutions across Texas, the nation, and the world—including Baylor College of Medicine—regarding how COVID-19 is transmitted, how best to contain its spread, and the efficacy and safety of the vaccines that have been developed to combat it. I am a frequent guest on CNN, MSNBC, BBC, NPR and other news outlets. I have also served on two Texas Governors task forces for infectious diseases.

Viruses in General

7. A virus is an organized collection of macro-molecules that causes illness by attaching to and entering human cells for replication. A virus is not made up of cells and is incapable of replicating outside a human or animal host. There is a debate in the scientific community about whether viruses are microorganisms. Because of their dependence on host cells, the generally prevailing view, and the one that I share, is that viruses are non-living, and therefore not microorganisms.[1]

SARS-CoV-2

8. SARS-CoV-2 is the virus that causes COVID-19. While it is now commonly called "coronavirus," it is one of several types of viruses known as coronaviruses. Coronaviruses are single-stranded RNA viruses that replicate through mRNA intermediates.[2] Some coronaviruses are associated with the common cold and not considered severe. Important exceptions include SARS which emerged from Southern China in 2002, and MERS from the Arabian Peninsula in

[1] https://www.ncbi.nlm.nih.gov/books/NBK279387/

[2] Using the gRNA template, RNA synthesis by the RTC starts with producing both a full-length genome complement (the anti-genome) and a set of minus-strand sgRNAs, which are derived from the gRNA region downstream of ORF1a and ORF1b (the replicase gene). Whereas the anti-genome serves as a template to produce new gRNA, the minus-strand sgRNAs direct the synthesis of a nested set of subgenomic mRNAs (sg-mRNAs) (discussed later). Although transcription is defined principally as the synthesis of RNA from a DNA template, in this Review we use the term to describe the synthesis of sg-mRNAs from RNA templates, to conform with the terminology used in the coronavirus literature. https://www.nature.com/articles/s41580-021-00432-z

2012. In December 2019, a new coronavirus emerged that caused the severe respiratory and multisystem disease we know as COVID-19. SARS-CoV-2 is more contagious—and more lethal—than the upper respiratory coronaviruses that cause the common cold.

9. The genetic material that makes up SARS-CoV-2 is stored inside an outer layer of proteins and lipids. This outer layer or "envelope" contains proteins made up of amino acids that bond with human cells during infection. During infection, the distinctive "spike protein" interacts with enzymes on the cell membrane of the host to gain entrance to its cells.

10. Individuals can become infected with SARS-CoV-2 by inhaling viral particles contained in respiratory droplets and aerosols in the air or by touching mucous membranes in the nose, mouth, or eyes after contact with contaminated surfaces or objects, known as fomites. How likely the virus is to be transmitted from one person to the next depends in large part on the specific physical conditions of the environment in which the virus exists.

How SARS-CoV-2 Affects Indoor Environments

11. There have been many studies over the past two years on precisely how SARS-CoV-2 is transmitted. These studies examine how the virus is emitted by its host; how it behaves in the air and on surfaces; and how indoor spaces can be physically adapted to mitigate the impacts of COVID-19. I have reviewed reports detailing the results of those studies and have attached them to this declaration. *See* Exhibits A-D.

12. Infected individuals release large respiratory droplets and/or smaller aerosols from their noses and mouths when exhaling, talking, coughing, or sneezing. Those droplets, can remain suspended in the air in indoor environments for long periods of time and can travel tens of meters in a typical indoor space, eventually settling on surfaces and the floor. The air essentially holds and carries the droplet nuclei, which can infect others if inhaled by another person in the vicinity. How far the aerosols and droplets containing viral matter remain in the air and how far they travel in an indoor environment before settling on the floor or other surfaces depends on the temperature, air circulation, and level of humidity in the space.

13. Viral particles in the air in inside environments eventually settle onto floors and surfaces. Surfaces such as door knobs, railings, and other high-touch points can also become contaminated when infected individuals shed viral particles

directly onto them. The amino acids found in the spike protein of the virus can interact via electrostatic or hydrogen bonds or van der waals forces on different surfaces. These forces can be influenced by changes or alterations in pH, surface chemistry and materials, relative humidity, and temperature. Individuals who come into contact with contaminated surfaces can become infected by transferring the viral particles to their noses, mouths, or eyes.

14. How long the virus adheres to a surface and remains capable of infecting a new host who is exposed to the virus through these surfaces depends on the physical properties of the surface it is on, specifically how porous the surface is, the temperature in the room, relative humidity, and other environmental conditions. The virus persists longer in cooler, drier environments. But for the most part, the studies indicate viral particles retain their infectivity on surfaces for hours or up to several days.

15. Indeed, the most effective ways to prevent the spread of the virus in indoor spaces involve changing the physical environment of the buildings by adapting them to provide sufficient and effective ventilation, such as by supplying clean outdoor air and minimizing recirculation of air; supplementing general ventilation with airborne infection controls such as local exhaust, high efficiency air filtration, and germicidal ultraviolet lights; sanitizing surfaces; and implementing measures to help promote social distancing, such as erecting signage, and minimizing the number of people permitted to gather in indoor spaces. Of course, screening, testing, masking, hand-washing, and other social behaviors are also helpful to contain infection.

COVID-19 on BCM's Insured Property

16. BCM's insured properties—Baylor Clinic, Baylor Main Campus, Family & Community Medicine—River Oaks, Family & Community Medicine—Upper Kirby, Greenway Plaza, Jamail Specialty Care Center; McNair Campus, Neurosensory—Jones Tower—consist of clinical spaces, classrooms, and research facilities.

17. I have consulted with Dr. James McDeavitt, the leader of BCM's Incident Command Center and COVID-response team, and have reviewed internal data regarding the number of persons physically present on the insured properties during the coverage period. The data I reviewed includes the COVID-19 report cards and other materials showing the number of individuals on BCM's insured

properties during the coverage period. I have also reviewed the infection rates in the Houston community between March and October 2020.

18. Based on the conversations I have had about BCM's in-person operations during the coverage period, my personal knowledge of BCM's response to COVID-19, and the data I have reviewed pertaining to that time, it is my opinion that there were in-person activities conducted at BCM's insured properties during the coverage period at volumes that make it almost statistically certain that infected individuals shedding viral particles were physically present in and on BCM's insured properties during the coverage period.

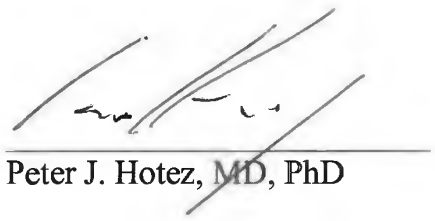
19. Based on my review of scientific studies detailing how the virus interacts with surfaces and persists in the air in indoor spaces; my own understanding of the virus as a scientist, physician, and public health expert, and my personal knowledge of BCM's COVID-19 experience and response, it is my opinion that, during the coverage period, individuals infected with COVID-19 emitted microdroplets containing viral matter that remained in the air and on the surfaces of BCM's insured property and caused direct physical loss or damage to the buildings, fixtures, and equipment at those properties by altering the physical properties of the air and surfaces inside those buildings and limiting or halting their use.

20. The direct physical loss or damage to BCM's insured property occasioned by the virus caused BCM to limit the number of people—patients, healthcare providers, educators, students, and researchers—who could access its insured property. That contraction of in-person activities caused BCM to incur losses of revenue across all three of its missions—health care, research, and education. I understand those losses will be the subject of a separate expert report.

21. The direct physical loss or damage to BCM's insured property occasioned by the virus also caused BCM to incur extra expense associated with the purchase of personal protective equipment, hand sanitizer, cleaning and sanitation supplies; expanded janitorial services; the purchase of signage to facilitate social distancing; the installation of plexiglass and other protective barriers; costs associated with air quality testing; costs of refitting clinical, laboratory, and classroom spaces for social distancing; expense associated with setting BCM employees up to work remotely; expense associated with establishing a telehealth infrastructure for BCM patients; expense associated with establishing and staffing the Incident Command Center to facilitate communication with the BCM community; expense associated with establishing screening protocols and temperature checks for essential workers and visitors; expense associated with

establishing testing sites for employees; expense associated with transitioning Simulation and Anatomy labs to a virtual platform; expense associated with ensuring adequate clinical training for medical students; and expense associated with the urgent embalming of all cadavers in BCM's willed-body program. The specific expenses associated with each of these items will be addressed in a separate report.

SIGNED on March 18, 2022 in Harris County, Texas.



Peter J. Hotez, MD, PhD

3.18.22

Date

Exhibit A

RJN083

Clinical Infectious Diseases

INVITED COMMENTARY

It Is Time to Address Airborne Transmission of Coronavirus Disease 2019 (COVID-19)

Lidia Morawska[1] and Donald K. Milton[2]

[1]International Laboratory for Air Quality and Heath, WHO Collaborating Centre, Queensland University of Technology, Brisbane, Australia, and [2]Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, Maryland, USA

Keywords. airborne transmission; airborne infection spread; coronavirus; COVID-19; SARS-CoV-2 virus.

We appeal to the medical community and to the relevant national and international bodies to recognize the potential for airborne spread of coronavirus disease 2019 (COVID-19). There is significant potential for inhalation exposure to viruses in microscopic respiratory droplets (microdroplets) at short to medium distances (up to several meters, or room scale), and we are advocating for the use of preventive measures to mitigate this route of airborne transmission.

Studies by the signatories and other scientists have demonstrated beyond any reasonable doubt that viruses are released during exhalation, talking, and coughing in microdroplets small enough to remain aloft in air and pose a risk of exposure at distances beyond 1–2 m from an infected individual ([1–4]). For example, at typical indoor air velocities [5], a 5-μm droplet will travel tens of meters, much greater than the scale of a typical room, while settling from a height of 1.5 m to the floor. Several retrospective studies conducted after the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) epidemic demonstrated that airborne transmission was the most likely mechanism explaining the spatial pattern of infections [6]. Retrospective analysis has shown the same for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [7–10]. In particular, a study of video records from a Chinese restaurant observed no evidence of direct or indirect contact between the infected diner and other parties at the three tables where diners became infected [10]. In their review of video records from the restaurant, they observed no evidence of direct or indirect contact between the 3 parties. Many studies conducted on the spread of other viruses, including respiratory syncytial virus (RSV) [11], Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [12], and influenza [2, 4], show that viable airborne viruses can be exhaled [2] and/or detected in the indoor environment of infected patients [11, 12]. This poses the risk that people sharing such environments can potentially inhale these viruses, resulting in infection and disease. There is every reason to expect that SARS-CoV-2 behaves similarly, and that transmission via airborne microdroplets [10, 14] is an important pathway. Viral RNA associated with droplets <5 μm has been detected in air [14], and the virus has been shown to maintain infectivity in droplets of this size [13]. Other viruses have been shown to survive equally well, if not better, in aerosols compared to droplets on a surface [15].

The current guidance from numerous international and national bodies focuses on hand washing, maintaining social distancing, and droplet precautions. Most public health organizations, including the World Health Organization (WHO) [16], do not recognize airborne transmission except for aerosol-generating procedures performed in healthcare settings. Hand washing and social distancing are appropriate but, in our view, insufficient to provide protection from virus-carrying respiratory microdroplets released into the air by infected people. This problem is especially acute in indoor or enclosed environments, particularly those that are crowded and have inadequate ventilation [17] relative to the number of occupants and extended exposure periods (as graphically depicted in Figure 1). For example, airborne transmission appears to be the only plausible explanation for several superspreading events investigated that occurred under such conditions [10], and others where recommended precautions related to direct droplet transmissions were followed.

The evidence is admittedly incomplete for all the steps in COVID-19 microdroplet transmission, but it is similarly incomplete for the large droplet and fomite modes of transmission. The airborne transmission mechanism operates in parallel with the large droplet and fomite routes [16] that are now the basis of guidance. Following the precautionary principle, we must address every

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Correspondence: L. Morawska, International Laboratory for Air Quality and Heath, 2 George Street, Brisbane, QLD 4001, Australia (l.morawska@qut.edu.au).

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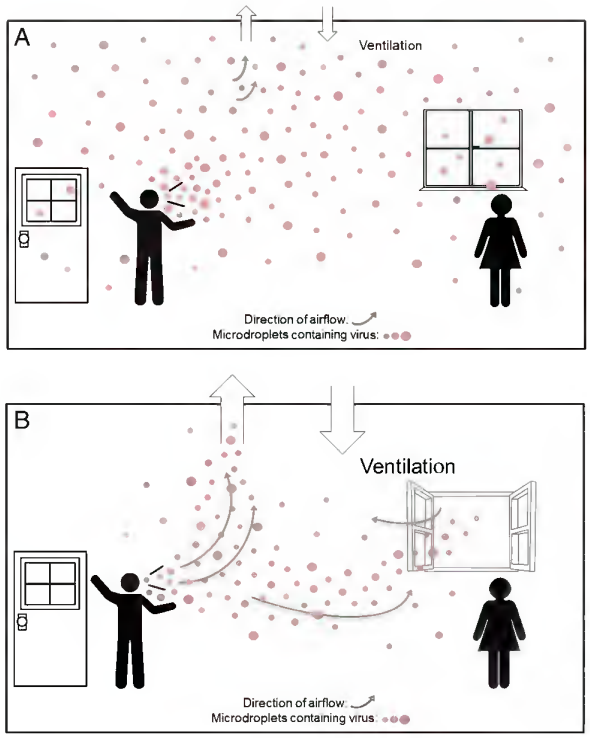

Figure 1. Distribution of respiratory microdroplets in an indoor environment with (*A*) inadequate ventilation and (*B*) adequate ventilation.

potentially important pathway to slow the spread of COVID-19. The measures that should be taken to mitigate airborne transmission risk include:

- Provide sufficient and effective ventilation (supply clean outdoor air, minimize recirculating air) particularly in public buildings, workplace environments, schools, hospitals, and aged care homes.
- Supplement general ventilation with airborne infection controls such as local exhaust, high efficiency air filtration, and germicidal ultraviolet lights.
- Avoid overcrowding, particularly in public transport and public buildings.

Such measures are practical and often can be easily implemented; many are not costly. For example, simple steps such as opening both doors and windows can dramatically increase air flow rates in many buildings. For mechanical systems, organizations such as ASHRAE (the American Society of Heating, Ventilating, and Air-Conditioning Engineers) and REHVA (the Federation of European Heating, Ventilation and Air Conditioning Associations) have already provided guidelines based on the existing evidence of airborne transmission. The measures that we propose offer more benefits than potential downsides, even if they can only be partially implemented.

It is understood that there is not as yet universal acceptance of airborne transmission of SARS-CoV2; but in our collective assessment there is more than enough supporting evidence so that the precautionary principle should apply. In order to control the pandemic, pending the availability of a vaccine, all routes of transmission must be interrupted.

We are concerned that the lack of recognition of the risk of airborne transmission of COVID-19 and the lack of clear recommendations on the control measures against the airborne virus will have significant consequences: people may think that they are fully protected by adhering to the current recommendations, but in fact, additional airborne interventions are needed for further reduction of infection risk.

This matter is of heightened significance now, when countries are reopening following lockdowns: bringing people back to workplaces and students back to schools, colleges, and universities. We hope that our statement will raise awareness that airborne transmission of COVID-19 is a real risk and that control measures, as outlined above, must be added to the other precautions taken, to reduce the severity of the pandemic and save lives.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Exhibit B

RJN087

COVID-19 transmission—up in the air

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October 29, 2020
https://doi.org/10.1016/S2213-2600(20)30514-2

For more on **SARS-CoV-2 transmission by fomites** see Correspondence *Lancet Infect Dis* 2020; published online Sept 20. https://doi.org/10.1016/S1473-3099(20)30678-2

For more on **airborne transmission and aerodynamic behavior** see https://science.sciencemag.org/content/370/6514/303.2

For more on **particle sizes of infectious aerosols** see **Viewpoint** in *Lancet Respir Med* 2020; **8:** 914–24

For more on the **WHO advice for the public** see https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public

For the **statement on airborne transmission of COVID-19** see https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa939/5867798

For more on the **CDC brief on potential airborne transmission** see https://www.cdc.gov/coronavirus/2019-ncov/more/scientific-brief-sars-cov-2.html

For more on **CDC guidance of face masks on public transport** see https://www.cdc.gov/quarantine/masks/mask-travel-guidance.html

As we approach the end of 2020, and a year since the outbreak of COVID-19 began, cases are increasing again. We have learnt a lot about SARS-CoV-2 and our ability to test for and manage COVID-19 has improved, but ongoing debate remains about how SARS-CoV-2 is transmitted.

Respiratory viruses are transmitted in three main ways. First, contact transmission, where someone comes into direct contact with an infected person or touches a surface that has been contaminated. Second, through droplet transmission of both large and small respiratory droplets that contain the virus, which would occur when near an infected person. Third, through airborne transmission of smaller droplets and particles that are suspended in the air over longer distances and time than droplet transmission.

During the initial stages of the pandemic there was concern about surface transmission. However, latest research suggests that this is unlikely to be a major route of transmission as although SARS-CoV-2 can persist for days on inanimate surfaces, attempts to culture the virus from these surfaces were unsuccessful.

Infection control guidelines have stated that most respiratory virus transmission occurs from large infected droplets produced by coughing, sneezing, and breathing in close proximity to another person. This understanding has led to social distancing being the cornerstone of public health advice, but confusion exists as to the safe distance required between people to reduce transmission with the WHO suggesting 1 m and the CDC and NHS saying 2 m. For social distancing to be effective, infective respiratory particles would need to fall to the ground or be in low enough concentrations at 2 m from the source to not cause transmission. Studies and guidelines have historically used a threshold of 5 μm to differentiate between large and small particles, but researchers are now suggesting that a size threshold of 100 μm better differentiates aerodynamic behaviour of particles, and particles that would fall to the ground within 2 m are likely to be 60–100 μm in size. Investigators have also measured particle sizes of infectious aerosols and have shown that pathogens are most commonly found in small particle aerosols (<5 μm), which are airborne and breathable.

Initially it was thought that airborne transmission of SARS-CoV-2 was unlikely, but growing evidence has highlighted that infective microdroplets are small enough to remain suspended in the air and expose individuals at distances beyond 2 m from an infected person. This knowledge is also corroborated by investigation of spread of cases between people who were not in direct or indirect contact, suggesting that airborne transmission was the most likely route. In July, over 200 scientists published a statement calling for international bodies to recognise the potential for airborne spread of COVID-19 as they were concerned that people would not be fully protected by adhering to the current recommendations.

On Oct 5, 2020, the CDC updated their COVID-19 webpage to say that there is growing evidence that COVID-19 infection can occur from airborne exposure to the virus under certain circumstances. Cases of transmission from people more than 2 m apart have occurred but in enclosed spaces with poor ventilation, and typically with extended exposure to an infected person of more than 30 min. The CDC have been clear to point out that most infections are spread through close contact and that airborne transmission is not the primary route of transmission.

Whether droplet or airborne transmission is the main route, the risk of infection is known to be much lower outside where ventilation is better. As winter approaches in the northern hemisphere, the opportunity to socialise and exercise outdoors becomes more challenging and concerns are growing over the increased risk of transmission of COVID-19. Public health guidance now needs to advise people how to navigate risk in indoor settings and wearing facemasks is becoming mandatory in many countries for travelling on public transport, indoor shopping, and gatherings. Facemasks and shields offer protection from larger droplets but their effectiveness against airborne transmission is less certain. Advice on spending time indoors should also focus on improved ventilation and avoiding crowded spaces.

As 2021 draws near, people are getting tired of the disruption the pandemic has brought to their lives and their willingness to adhere to strict rules and lockdowns might wane. As cases of COVID-19 increase globally, we need to more fully understand the transmission routes. It is crucial that we embrace new research and do not rely on recommendations based on old data so that clearer and more effective infection control guidance can be provided in the face of pandemic fatigue.

■ *The Lancet Respiratory Medicine*

Exhibit C

RJN089

Aerosol persistence in relation to possible transmission of SARS-CoV-2

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Scott H. Smith, G. Aernout Somsen, Cees van Rijn, Stefan Kooij, Lia van der Hoek, Reinout A. Bem, and Daniel Bonn

RJN090

Physics of Fluids ARTICLE scitation.org/journal/phf

Aerosol persistence in relation to possible transmission of SARS-CoV-2

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Scott H. Smith,[1] G. Aernout Somsen,[2] Cees van Rijn,[1] Stefan Kooij,[1] Lia van der Hoek,[3] Reinout A. Bem,[4] and Daniel Bonn[1,a)]

AFFILIATIONS

[1]Van der Waals-Zeeman Institute, Institute of Physics, University of Amsterdam, 1098 XH Amsterdam, The Netherlands
[2]Cardiology Centers of the Netherlands, 1073 TB Amsterdam, The Netherlands
[3]Laboratory of Experimental Virology, Department of Medical Microbiology, Amsterdam UMC, Location AMC, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands
[4]Department of Pediatric Intensive Care, Emma Children's Hospital, Amsterdam University Medical Centers, Location AMC, 1105 AZ Amsterdam, The Netherlands

Note: This paper is part of the Special Topic, Flow and the Virus.
[a)]**Current address:**: Institute of Physics, University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands.
Author to whom correspondence should be addressed: d.bonn@uva.nl. **Tel.:** +31205255887

ABSTRACT

Transmission of SARS-CoV-2 leading to COVID-19 occurs through exhaled respiratory droplets from infected humans. Currently, however, there is much controversy over whether respiratory aerosol microdroplets play an important role as a route of transmission. By measuring and modeling the dynamics of exhaled respiratory droplets, we can assess the relative contribution of aerosols to the spreading of SARS-CoV-2. We measure size distribution, total numbers, and volumes of respiratory droplets, including aerosols, by speaking and coughing from healthy subjects. Dynamic modeling of exhaled respiratory droplets allows us to account for aerosol persistence times in confined public spaces. The probability of infection by inhalation of aerosols when breathing in the same space can then be estimated using current estimates of viral load and infectivity of SARS-CoV-2. The current known reproduction numbers show a lower infectivity of SARS-CoV-2 compared to, for instance, measles, which is known to be efficiently transmitted through the air. In line with this, our study of transmission of SARS-CoV-2 suggests that aerosol transmission is a possible but perhaps not a very efficient route, in particular from non-symptomatic or mildly symptomatic individuals that exhibit low viral loads.

INTRODUCTION

Respiratory droplets form the most important carrier of SARS-CoV-2 virions and may infect humans by direct inhalation or indirectly through hand or object contact. During the current COVID-19 pandemic, numerous explosive local outbreaks, so-called super-spreading events, in public spaces or health care settings have raised concerns of aerosol transmission of SARS-CoV-2. Aerosols, or microdroplets, are formed and exhaled during loud speaking, singing, sneezing, and coughing. As infected persons (initially) may have none or mild symptoms, an aerosol transmission route of SARS-CoV-2 may have tremendous impact on health care strategies to prevent the spreading of COVID-19 in public spaces. Importantly, SARS-CoV-2 viral particles have been detected in microdroplets, which may spread in exhaled air during breathing, talking, singing, sneezing, or coughing by an infected individual.[1–12]

Microdroplets form aerosol clouds, which have a relatively long airborne time,[13] and may thus pose an important threat to community spread of COVID-19. However, to what extent microdroplets in practice result in infections with the SARS-CoV-2 virus remains a topic of intense debate.[14–21]

Next to virus and host factors, this type of viral transmission through aerosols depends strongly on droplet properties and behavior.[22,23] In order to aid in the development of effective preventive

strategies for SARS-CoV-2 transmission, in this study we measure and model respiratory droplet physics to predict the importance of community SARS-CoV-2 transmission by the aerosol route.

RESULTS AND DISCUSSION

Size distribution

We measure size distributions of droplets in aerosols released when speaking or coughing using laser diffraction (Malvern Spraytech®) and consistently find a double-peaked drop size distribution for coughing, and a single-peak drop size distribution for speech, which can be described by a distribution corresponding to a normal liquid spraying process,[23] as shown in Fig. 1. A previous study[7] showed that age, sex, weight, and height have no statistically significant effect on the aerosol composition in terms of the size and number of droplets. We tested seven healthy volunteers (five male, two female) and found that the variability in drop production by coughing between the different emitters was relatively small, except for one person, who produced 17 times more liquid volume than the others. It has been suggested that if such a person would be infected with SARS-CoV-2, he or she could become a so-called "super-spreader" due to the high number of droplets emitted.[2,12]

Using a precision balance, the volumes of saliva/mucus produced by the high emitter when coughing or speaking into a small plastic bag were measured by weighing before and after a single cough or saying "Stay Healthy" for ten times.[24] Averaging over 20 experiments, we find that a single cough yields a liquid weight of 0.07 ± 0.05 g, whereas speaking ten times produces a weight of 0.003 ± 0.001 g.

Size distributions of droplets from aerosols released when speaking or coughing were measured using laser diffraction employing Malvern Spraytech with a 300 mm lens. In this configuration, drop sizes between 0.2 μm and 2 mm can be measured. Speaking and coughing is done directly into the laser beam, and data acquisition is done in the "fast acquisition" mode so that there is no dead time and the drop size distribution is measured before evaporation. For coughing, the volumetric distribution measured using laser diffraction shows that on average, 98% ± 1% of the volume of the spray is contained in the large drops (100 μm–1000 μm). For the small aerosol droplets, this amounts to ~20×10^6 microdroplets produced in a single cough and ~7×10^6 for speech. For COVID-19, thus from symptomatic patients, the viral RNA load in the undiluted oral fluid or sputum has been found to be 10^4–10^6 copies/ml.[25–28] During infection, there are major changes in viral load, and the rate at which these changes happen could be related to the severity of the COVID-19 symptoms. While in some cases, very high viral loads up to ~10^{11} copies/ml have been reported,[26] a relation with the severity of the symptoms has not been firmly established so far. As such, following Ref. 25 to avoid underestimation, we used a number of 7×10^6 copies/ml in respiratory samples in our primary analysis. The total number of virus particles present in the total volume of only the microdroplets is then 10^4, implying that only one in 2000 aerosol droplets contains a virus particle.

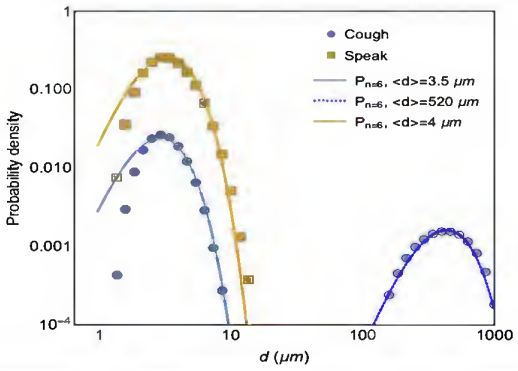

FIG. 1. Measured drop size distributions of droplets produced when coughing (circles) and speaking (squares). Solid lines are fits with gamma distributions, where P denotes the probability density and n is a measure for the width of the gamma distribution, see Ref. 23 for details.

Persistence of aerosols

The persistence of these aerosol droplets in the air is of the greatest concern regarding community transmission of SARS-CoV-2 in public spaces. This airborne time is governed by evaporation and gravity-driven sedimentation toward the floor. The latter can be explained by balancing the forces of gravity ($F = mg$) and air drag ($F = 6\pi\eta RU$, with η being the air viscosity, R being the droplet radius, and U being the falling velocity), from which it follows that a droplet with a radius of 5 μm will take 9 min to reach the ground from an initial height of 1.5 m. This time will even increase by the evaporation of the liquid phase of the droplet. Sputum droplets are known to consist for 1%–10% of their volume of solid solutes.[29] Consequently, they will not evaporate completely but leave a "solid" core residue. For microdroplets smaller than 10 μm in radius, the contraction to the solid core having half of the original droplet size (i.e., ~10% of the initial volume) happens within a second in quiescent air with a relative humidity (RH) of 50%,[29] and a droplet half the size stays airborne four times longer.

A laser light sheet was used to track microdroplets similar to those produced by coughing and speaking. To mimic small respiratory droplets, droplets were generated with a Rayleigh jet nozzle chip (Medspray®) yielding the same droplet size distribution as droplets from a typical cough. To achieve this, we use a mixture of 1% glycerol and 99% ethanol; within a second, ethanol evaporates, yielding polydisperse non-evaporating droplets of glycerol with a median mass aerodynamic diameter (MMAD) of 5 ± 3 μm, similar to the microdroplets produced by coughing or speaking. The number of drops passing through the laser sheet suspended in the center of our $2 \times 2 \times 2$ m^3 experimental chamber was analyzed by processing of the images using a home-built Python algorithm that detects the illuminations caused by the droplets. Typical results are shown in Fig. 2 and capture the reduction in the number of droplets over time due to coupled effects of sedimentation, horizontal displacement, and evaporation. The smallest air currents will make the aerosol concentration rather homogeneous. This was verified by measuring aerosol concentrations at different locations in the room.

FIG. 2. (a)-(d) Laser-illuminated aerosol droplets at different times after initial spraying. Initially (a), droplets have a maximum sedimentation velocity of about 2 cm/s, corresponding to droplets of about 25 μm in diameter. In the 16 min frame (d), the fastest moving droplet has a sedimentation velocity of at most 1 mm/s, corresponding to a droplet of about 4 μm–5 μm in diameter.

If these aerosol droplets are a vector of transmission for the SARS-CoV-2 virus, how the number of droplets decreases as a function of time will have a significant influence on the potential airborne transmission of SARS-CoV-2. To predict the evolution in the number of microdroplets, the evaporation and sedimentation can be accounted for to calculate the number of airborne aerosol particles with knowledge of the initial droplet size distribution.

A simple model for the persistence

The evaporation of a spherical droplet in an environment with a known relative humidity (RH) can be evaluated using the diffusion model presented and validated in Ref. 30. The rate of change in the mass of the droplet, $m_d(t)$, is given by

$$\frac{\partial m_d(t)}{\partial t} = 4\pi R^2(t) D_{va} \left.\frac{\partial C(r,t)}{\partial r}\right|_{r=R(t)}, \tag{1}$$

where $R(t)$ is the radius of the droplet, D_{va} is the diffusivity of water vapor in air, and $C(r, t)$ is the water vapor concentration along direction r. Assuming that the droplets are sufficiently spaced and that the relative humidity of the air in which they are falling through does not change, the final term can be written as

$$\left.\frac{\partial C(r,t)}{\partial r}\right|_{r=R(t)} = (C(r=\infty) - C(R(t),t)\left(\frac{1}{R(t)} + \frac{1}{\sqrt{\pi D t}}\right). \tag{2}$$

The water vapor concentration at the surface of the droplet [i.e., $r = R(t)$] is given by the equilibrium vapor pressure, ρ_{vap}, of the environment and, very far away from the droplet surface [i.e., $r \gg R(t)$], is given by the product of the RH of the environment and ρ_{vap}, resulting in

$$\frac{\partial m_d(t)}{\partial t} = 4\pi R^2(t) D_{va} \rho_{vap} (RH - 1)\left(\frac{1}{R(t)} + \frac{1}{\sqrt{\pi D t}}\right). \tag{3}$$

Assuming that the solids (salt, proteins, and possibly virus particles) constitute a "spherical core" of the droplet, the mass, m_d, of the droplet at any time is given by

$$m_d(t) = \frac{4\pi}{3} R_0^3 \rho_s + \frac{4\pi}{3}\left(R^3(t) - R_0^3\right)\rho_w, \tag{4}$$

where ρ_s is the density of the solid found in human mucus/saliva (i.e., 1500 kg/m^3) from Ref. 31 and ρ_w is the density of liquid water. Differentiating Eq. (4) with respect to time and combining the result with Eq. (3) give a non-separable differential equation for the evolution in size of the droplet due to evaporation, where evaporation stops when the droplet is completely composed of the solid fraction or when $R(t) = R_0$,

$$\frac{\partial R(t)}{\partial t} = \frac{\rho_{vap} D_{va}}{\rho_w} (RH - 1)\left(\frac{1}{R(t)} + \frac{1}{\sqrt{\pi D_{va} t}}\right). \tag{5}$$

For the purpose of the following calculations, it is taken that the solid core R_0 of each droplet is half of the initial size $R(t = 0)$ and corresponds to an initial density of ~1080 kg/m^3 for the water–solute mixture. Figure 3 displays solutions to Eq. (5) for the largest microdroplet sizes and shows the influence of the RH on the evaporation kinetics of a 10 μm droplet. Within 1 s, the evaporation of the small micro-droplets is complete, resulting in a solid core.

Due to the fact that the evaporation occurs quickly, the dominant mode of decline in suspended droplets is sedimentation. As we will show below, the exponential decay in the number of drops that we observe can be quantitatively accounted for by taking only the sedimentation of already evaporated droplets into account. At all times, the droplets are assumed to be vertically falling at their terminal velocities described by Stokes flow,

$$\frac{\partial h(R(t),t)}{\partial t} = \frac{2(\rho_d(t))}{9\eta} g R^2(t). \tag{6}$$

This describes the rate of change in the height, $h(R(t), t)$, through which the droplet has fallen where ρ_a is the density of air and g is the acceleration due to gravity. By solving Eqs. (5) and (6) numerically, the progressive evaporation and sedimentation of the droplets are coupled and comparable to models presented in Refs. 5 and 32. For

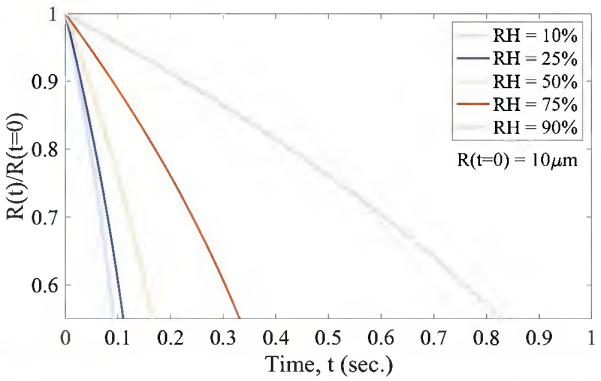

FIG. 3. Influence of the relative humidity (RH) on the evaporation kinetics of a droplet with $R(t = 0) = 10\ \mu m$.

the framework presented herein, how the number of droplets in a given volume evolves can be predicted, allowing the persistence calculations in Fig. 4 to be made. For this calculation, it is assumed that the droplets of each size class have a uniform random initial height in the volume in which they progressively sediment. From the particle size distribution, the total number of particles of each size class, $N(R(t = 0))$, initially in the volume can be obtained. The evolution in the total number of particles for each size class is then directly given by $N(R(t), t) = N(R(t = 0))\frac{h(R(t),t)}{h_{sys}}$, taking $h(R(t), t)$ to always be smaller than the system height h_{sys} in which dispersion experiments are made and the computational domain height in which the sedimentation and evaporation of the droplets are calculated. The total number of droplets in the system, N_{total}, at any time t is then the discrete summation of this number over all particle sizes, n, for which $h(R(t), t) < h_{sys}$.

Figure 4 shows that the derived system of equations and model system can directly predict the persistence of the aerosol particles with knowledge of the system size, initial size distribution of the aerosol droplets, and relative humidity. Explicit calculation shows that the half-life reduces nearly 50% when the relative humidity is 100%—corresponding to conditions in which there is no evaporation of aerosol. As expected, when no evaporation occurs, the droplets fall faster through the system due to their nominally larger size and higher terminal velocities. The decrease in the number of microdroplets in the system due to the effects of a higher relative humidity then implies a lower-likelihood of aerosol mediated transmission of CoV-2, which corresponds to other studies[33,34] that show that higher relative, and absolute, humidity environments may lead to lower infectivity rates of influenza and other respiratory infections. Based on these results, a more general model can be derived to explain the exponential decline in droplets. Given a number N_o of drops with diameter D, and in view of the experimental results, it is reasonable to assume that the decrease in time will be exponential: $N(D, t) = N_o e^{-\alpha D^2 t}$, with α being an empirical constant independent of the droplet diameter D. A good estimate is $\alpha \cong \rho g/18\eta h$, with h being a typical sedimentation height. The life time of a microdroplet is then characterized by the exponent in Eq. (6), given by $t_{life} \equiv 1/\alpha D^2$.

In case of droplets with a varying size distribution, we collect the different droplet sizes and obtain $N_{total}(D, t) = \sum_{i=1}^{i=n} N_i e^{-\alpha D^2 t}$.

Figure 4 compares our predictions for droplet persistence results with our own results and those reported by others.[12] It shows that the model accurately captures the exponential decline in the number of droplets over time for both experiments and suggests the decline is, to a small extent, influenced by the evaporation of the droplets (i.e., the relative humidity of the environment) but dominated by the sedimentation. Additionally, from Fig. 4, it can be concluded that the time to half the original number of droplets in the system (i.e., the half-life) is between 5.5 min and 7 min. These lines are not fits but outcomes of the

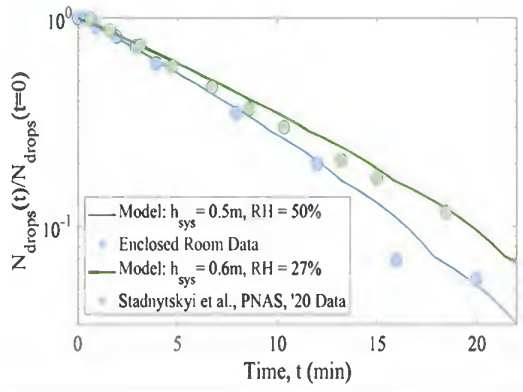

FIG. 4. Normalized number of droplets as a function of time as determined experimentally (blue circles) compared to the data of Ref. 24 (green circles). Solid lines are model outcomes for both sets of data, with input parameters relative humidity (RH) and system height (h_{sys}).

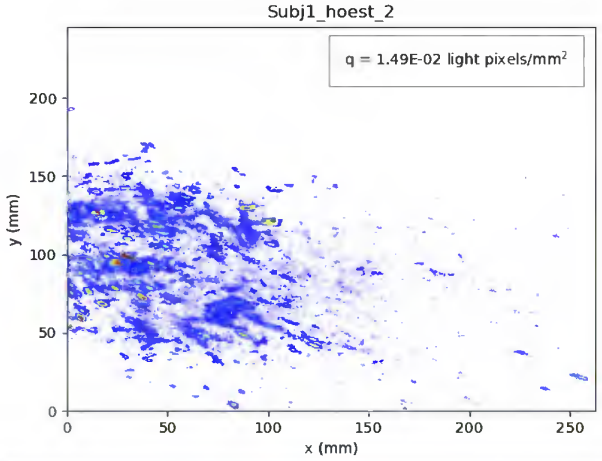

FIG. 5. Picture and movie of the droplets produced by coughs of a high emitter. Multimedia view: https://doi.org/10.1063/5.0027844.1

derived analytical model. The small discontinuities are a result of using, in the model, the original binned values of initial droplet sizes and discretely modeling the particles. Using continuous distributions for the initial droplet sizes systematically removes the discontinuities.

Estimate of infection risk

This then allows us to estimate how many virus particles one would inhale while inside a room where an infected person coughed a single time. The highest probability of infection occurs when a person enters a poorly ventilated and small space where a high emitter has just coughed and inhales virus-carrying droplets. We model coughing in our $2 \times 2 \times 2$ m^3 unventilated space that could represent, e.g., a restroom. The drop production by coughing was found to be very similar for six out of the seven emitters. We find peak values of $1.18 \pm 0.09 \times 10^3$ pixels that light up in the field of view of our laser sheet (21×31 cm^2). This directly corresponds to the volume of emitted droplets;[12] the high emitter produced $1.68 \pm 0.20 \times 10^4$ lit up pixels, more than an order of magnitude larger. Figure 5 (Multimedia view) shows the cough of the "superemitter."

Based on these numbers and the earlier measured volume and drop size, we can calculate the amount of virus inhaled by a person entering and staying in the same room where an infected person produced the droplets as a function of entrance delay and residence time. As detailed above, the calculation assumes a viral load of 7×10^6 copies/ml of saliva.[33] We also assume a single inhalation volume of 0.0005 m^3 (tidal volume 6 ml/kg body weight for an adult man) and a normal respiratory rate of ~16 inhalations/min.[35] In Fig. 6, we compare the results for the high emitter with those for a regular (low) emitter on the basis of the amount of light scattered from droplets produced by a single cough.

The number of virus particles needed to infect a single individual, N_{inf}, needs to be considered to translate these findings into risk of infection. This obviously also depends on factors such as the vulnerability/susceptibility of the host; in addition, as detailed in Ref. 36, the respiratory infectivity for SARS-CoV-2 is not yet well known. In the absence of data on SARS-CoV-2, the most reasonable assumption is that the critical number of virus particles to cause infection is comparable to that for other coronaviruses, including SARS-CoV-1, and influenza virus. In that case, $N_{inf} \sim 100$–1000, which corresponds to ~10 PFU to 100 PFU where PFU denotes

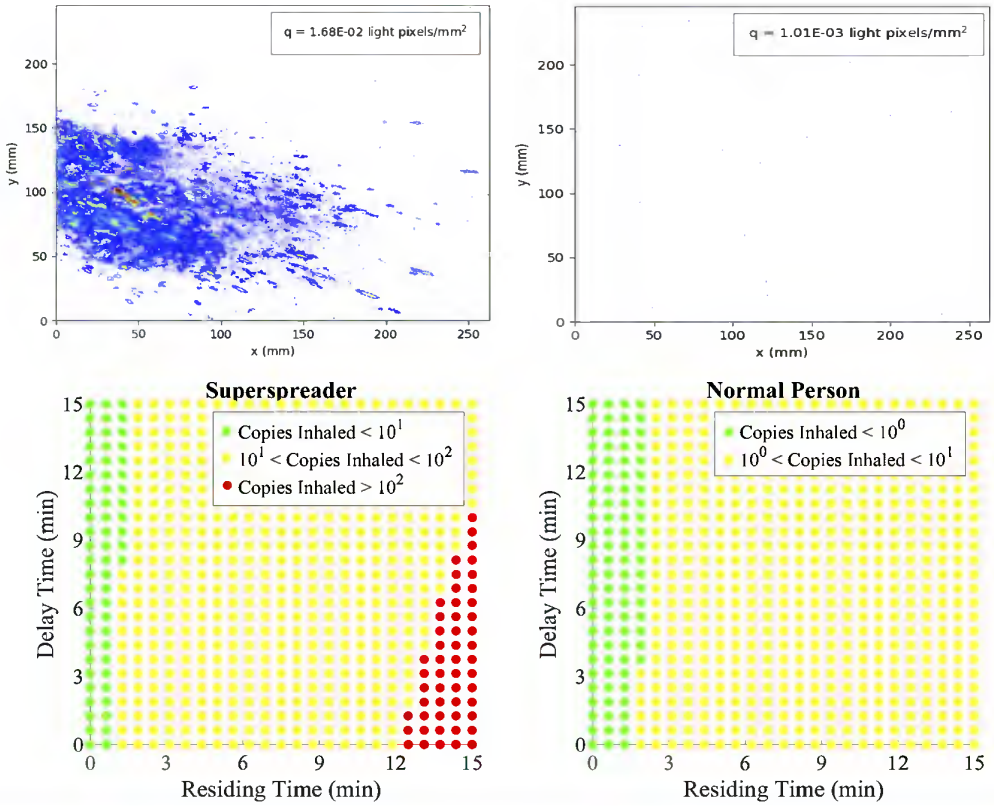

FIG. 6. Instantaneous pictures of the droplets produced by coughs of a high emitter (a) and a normal emitter (b) as detected with laser sheet imaging. The cough volumes allow us to estimate the number of inhaled virus particles as a function of (i) the delay between the cough and a healthy person entering the room and (ii) the time the healthy person spends in the room [(c) and (d)].

plaque forming units, the standard way of expressing whether a virus is infectious or not.[36–38] If we adopt a conservative approach and assume the upper limit of this range ($N_{inf} \sim 100$), we find that our unventilated $2 \times 2 \times 2$ m^3 space contaminated by a single cough is relatively safe for residing times less than 12 min due to the low virus content of the aerosol particles. Additionally, the maximal number of inhaled viral copies by a person entering the room after the high emitter has coughed is ~120 ± 60, where the error margin comes from variation in relative volume of small and large drops produced by a cough. If the infected person is a regular emitter, the probability of infecting the next visitor of the confined space by means of a single cough for any delay or residence time is therefore rather low. For speech, due to the low volumes emitted, this probability is even smaller. Nevertheless, prolonged speaking produces very large numbers of aerosols that could result in droplet accumulation to levels far higher than that in coughing or sneezing, thereby leading to an increased risk. Our small non-ventilated room can be looked upon as a "worst-case": in better ventilated, large rooms, aerosols become diluted very rapidly.[13] The methods described here do allow for a complete modeling of the probability of infection also for other types of rooms, with different particle inputs and ventilation characteristics. This should be a useful starting point for many hydrodynamic-based simulations of SARS-CoV-2 transmission that are currently being performed.[7,9,20]

CONCLUSION

Our dynamic modeling of transmission of SARS-CoV-2 in confined spaces suggests that aerosol transmission is not a very efficient route, in particular from non-symptomatic or mildly symptomatic individuals that are likely to have low virus content in their saliva. Highly infected people having a large viral load in their saliva and superspreaders producing lots of aerosols are likely far more dangerous. Comparing aerosol transmission to other transmission routes, it is useful to realize that the large droplets that are believed to be responsible for direct and nosocomial infections may contain about 500 virus particles per droplet and are thus likely to also be very important in a mixed transmission model.

A limitation to our study is that we cannot easily take changes in virus viability inside microdroplets into account, which depend on the local microenvironment of the aerosol gas clouds as produced under different circumstances.[39] However, viable SARS-CoV-2 in aerosols can be found after several hours,[40] and as such, this limitation will not likely affect our main conclusion. Importantly, our results do not completely rule out aerosol transmission. It is likely that large numbers of aerosol droplets, produced by continuous coughing, speaking, singing, or certain types of aerosol-generating medical interventions, can still result in transmission, in particular in spaces with poor ventilation.[13] Our model explains the rather low reproduction number of SARS-CoV-2 in environments where social distancing is practiced compared to the reproduction numbers of other "true" airborne pathogens.[22,41,42] For a "true" airborne virus such as measles, the R-factor is 12–18, whereas the current best estimate for SARS-CoV-2 is about 2.5.[43] This suggests that direct droplet transmission and fomite transmission are relatively more important ways of transmission than airborne transmission, for which R-values are generally (very) high. The calculation presented here allows us to do a risk estimation based on what we now know about the virus; in the case of new insights, the parameters of our model can be readily modified to incorporate these. The interpretation of the associated risk is necessarily subjective; what is acceptable as an infection probability is beyond the scope of this paper.

ACKNOWLEDGMENTS

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None of the authors declare any conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request. Part of this study included measurements from subjects, which were approved by a local ethical committee (AMC2020_098/NL73585.018.20).

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Exhibit D

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Perspective

Surface Chemistry Can Unlock Drivers of Surface Stability of SARS-CoV-2 in a Variety of Environmental Conditions

Edris Joonaki,[1,*] Aliakbar Hassanpouryouzband,[2] Caryn L. Heldt,[3,4] and Oluwatoyin Areo[3,4]

SUMMARY

The surface stability and resulting transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), specifically in indoor environments, have been identified as a potential pandemic challenge requiring investigation. This novel virus can be found on various surfaces in contaminated sites such as clinical places; however, the behavior and molecular interactions of the virus with respect to the surfaces are poorly understood. Regarding this, the virus adsorption onto solid surfaces can play a critical role in transmission and survival in various environments. In this article, we first give an overview of existing knowledge concerning viral spread, molecular structure of SARS-CoV-2, and the virus surface stability is presented. Then, we highlight potential drivers of the SARS-CoV-2 surface adsorption and stability in various environmental conditions. This theoretical analysis shows that different surface and environmental conditions including temperature, humidity, and pH are crucial considerations in building fundamental understanding of the virus transmission and thereby improving safety practices.

The Bigger Picture

Challenges and opportunities

- The novel SARS-CoV-2 can be recognized on various surfaces in a contaminated site, and its stability in different environmental conditions has been reported.
- The literature suffers from the lack of fundamental understanding of molecular drivers of the virus-surface interactions, and the chemistry that occurs on the solid surfaces and its effects on the virus adsorption and stability is still in its nascent stages because of the complexity of the phenomena.
- The roles of fluids pH values, surface chemistry, relative humidity, and temperature in the virus adsorption and desorption phenomena and persistence of SARS-CoV-2 on surfaces should be explored, and experimental scientists need to unravel the molecular drivers implicated in this new coronavirus transmission from the surfaces in different environmental conditions.

MOLECULAR STRUCTURE OF NOVEL SARS-CoV-2 AND ITS SURFACE-ACTIVE SPECIES

Coronavirus genomes are comparatively large for RNA viruses and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) encodes an extensive complement of nonstructural proteins (e.g., 3-chymotrypsin-like [3CL] protease, papain-like protease, etc.) as well as structural proteins as follows: spike (S) glycoprotein, envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) phosphoprotein.[1] The SARS-CoV-2 spike (S) glycoprotein exhibits ~76% amino acid sequence identity with the SARS-CoV S (Urbani strain) and ~80% identity with S proteins of bat SARSr-CoV ZXC21 and ZC45.[1,2] CoV S glycoproteins form club-shaped trimers and decorate the viral membrane,[3] giving coronavirus virions their characteristic morphology. As a substantial component of the outer surface of the virion, S likely plays a critical role in adsorption of viruses onto the solid surfaces under various environmental conditions.[4] For further clarification, Figure 1A depicts a central slice through an electron micrograph of mouse hepatitis virus, which exhibits the presence of S on the virion surface. Figures 1B and 1C also illustrate the structure of the SARS-CoV-2 S glycoprotein, its surface-active species, and potential intermolecular interactions among them during virus assembly.

Determination of the adsorption and stability of SARS-CoV-2 on various surfaces and under environmental conditions relevant to outbreak settings and zones is vital for amending safety measures for health care professionals, along with responding to the questions about SARS-CoV-2 public transmission. In the following sections we

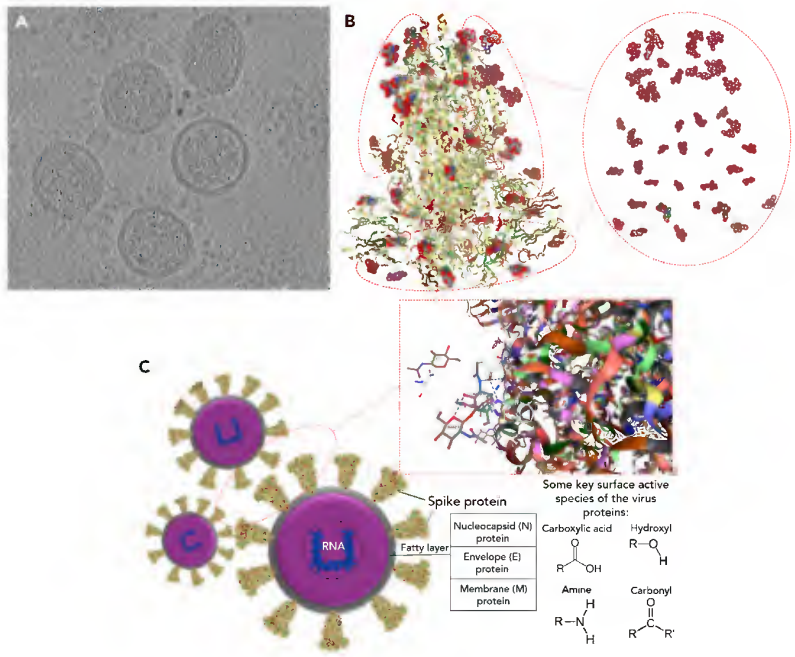

Figure 1. Structural Chemistry and Identifying Surface-Active Species of the Virus

(A) A central slice through a cryo-EM tomogram of mouse hepatitis virus for showing the existence of S on the outer surface of virions.

(B) Structure of the SARS-CoV-2 spike glycoprotein with highlighting corresponding functional groups (protein database [PDB]: 6VXX). Grey, blue, and red spheres are carbon, nitrogen, and oxygen atoms, respectively. The molecular structure is colored based on hydrophobicity; colored from red (hydrophilic) to green (hydrophobic). It utilizes the experimentally attained hydrophobicity scale that relied on whole-residue free energies of transfer ΔG (kcal/mol) from water to 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) interface.

(C) Model representation of SARS-CoV-2 with respective proteins assembly. Key molecular interactions among proteins on the surface of the virus particle are shown as gray dash lines, "hydrophobic interactions," and blue dash lines, "hydrogen bonding (–O–H···O)," (PDB: 6VYB). Some key surface-active moieties of SARS-CoV-2 are denoted as hydroxyl, amine, carbonyl, and carboxylic acid functional groups.

discuss mechanisms of virus adsorption onto different solid surfaces considering the aforementioned knowledge of the structure of viral surface glycoproteins in which amino acid functional groups play a critical role in the adhesion process.

SURFACE STABILITY OF NOVEL CORONAVIRUS ON VARIOUS SURFACES—ADSORPTION PERSPECTIVE

Investigations of the stability of both SARS-CoV-2 and SARS-CoV-1 in air and on different solid surfaces including stainless steel, plastic, copper, and cardboard have calculated their persistence kinetics utilizing a Bayesian regression model.[5] It has been shown that SARS-CoV-2 was more stable on plastic and stainless steel surfaces than on copper and cardboard pieces, and the presence of virus was detected up to ~72 h on certain solid surfaces. The longest persistence of viability for SARS-CoV-2 was found to be on stainless steel and plastic, which showed low-level viability after 72 h. No viable SARS-CoV-2 was detected after ~4 and ~24 h on copper and cardboard, respectively. After 3 h, viable virus was still detected in aerosols. These experimental data imply that fomite transmission of SARS-CoV-2 is likely, given that the virus can stay viable and infectious for h or up to several days on solid surfaces.

[1]TÜV SÜD UK National Engineering Laboratory, Scottish Enterprise Technology Park, East Kilbride, South Lanarkshire, G75 0QF, United Kingdom

[2]School of Geosciences, University of Edinburgh, King's Buildings, West Main Road, Edinburgh, EH9 3JW, United Kingdom

[3]Health Research Institute, Michigan Technological University, Houghton, MI 49931, USA

[4]Department of Chemical Engineering, Michigan Technological University, Houghton, MI 49931, USA

*Correspondence: edris.joonaki@tuv-sud.co.uk

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Generally, some viruses can retain their activities and transmission in the environment for a long period of time, possibly because of the adsorption process on surfaces.[6,7] It can be inferred that building a fundamental understanding of the molecular interactions between viruses, i.e., the outer surface of proteins, and solid surfaces is crucial for controlling environmental transmission and designing removal processes and treatment strategies. The quantity of adsorbed virus is influenced by multiple factors, including characteristics of the virus's outer surface proteins such as surface charge, size, stability, and steric conformation.[8] These are properties of the amino acid composition and post-translational modifications such as addition of carbohydrate moieties.[9] Likewise, substrate surface chemistry and environmental conditions are also key. Therefore, to determine the ability of the virus to adsorb to a surface, both the virus and the surface need to be well characterized and an understanding of how these change with environmental conditions is key.

Viruses adsorb to surfaces through two main mechanisms, van der Waals (mainly mineral surfaces[10]) and, more importantly, electrostatic interactions (charged surfaces in the presence of ions and or not neutral pH[11–13]). These two forces dictate the adsorption of viruses to surfaces. Although the interplay between these two forces is difficult to separate, indications of the interactions can be determined from past data. Viruses tend to be more hydrophobic than proteins,[14] thus they are attracted to metal surfaces because of mainly van der Waals interactions as well as hydrophobic effects.[15] However, their ability to maintain the virus's viability and allow it to remain infectious is more of a function of the humidity and temperature,[16] thus the surface energy of the water molecules plays a large role in the interaction between a virus particle and a surface.

SARS-CoV-2 virions can be adsorbed onto metal surfaces (e.g., gold and stainless steel) in addition to hydroxyl functional group- and oxygen-containing substrates (e.g., wood, cotton, paper, and glass) depending on the surface chemistry and environmental conditions (e.g., bulk fluid pH, surface charge, temperature, etc).[17] Hydrogen bonding plays a key role in the adsorption of viruses to the hydroxyl-containing surfaces and in the presence of an aqueous phase thin film layer.[17] The strength of the bond to the surface would be high in the presence of –O–H···O bonding, particularly in pH environments where the carboxylic acid on the virus is deprotonated (typically above a pH of 4[18]). At neutral pH, most viral particles have a net negative charge because they have an isoelectric point below 7.[19] However, due to the large size of virus particles and their large variety of surface proteins, there are still multiple patches of positive and negative charge in the pH range where viruses are stable (typically from pH 5–8[20]). Therefore, $-NH_2$, $-NH_3^+$, $-COOH$, and $-COO^-$ groups of amino acids in the SARS-CoV2 S protein drive adsorption onto the solid surfaces through double electrostatic interactions between the virion's ionized surface-active species and the oppositely charged surfaces, as well as hydrogen bonding based on the surface characteristics. For example, at neutral pH values, the negatively charged virus particles would be adsorbed significantly less on a stainless-steel surface because of electrostatic repulsion,[21] given that both virion and substrate surface have negative charges. With augmentation of the cation concentration, however, the repulsion would be decreased,[22] and the quantity of adsorbed viruses would increase. Figure 2 presents the potential molecular interactions between the SARS-CoV-2 viral proteins and solid surfaces at different pH values and fluid chemistries. As denoted in Figure 2A, at pH values below the isoelectric point, the overall charge of SARS-CoV-2 could be positive, given that both the carboxylate and amine groups on the outer surface are protonated, and hydrogen bonding would be formed to hydroxyl-containing surfaces such

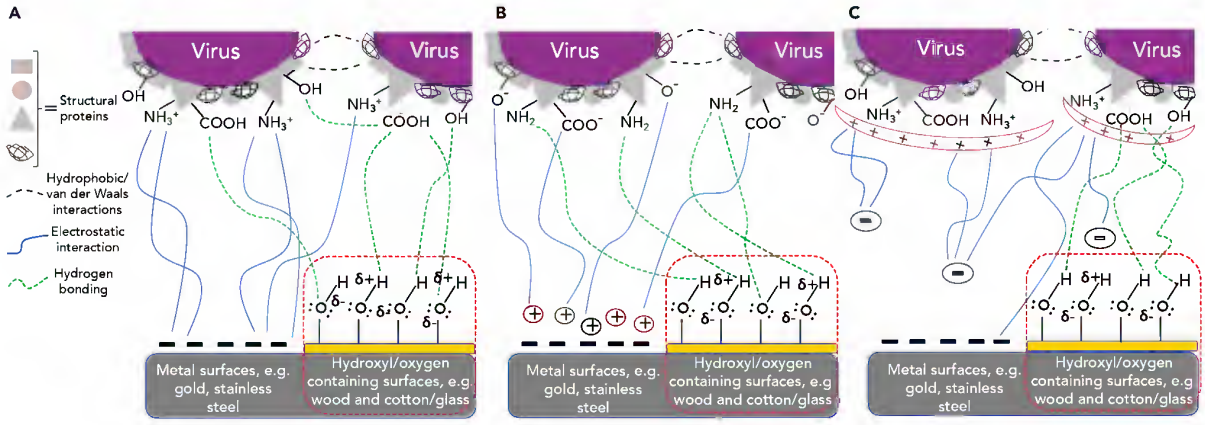

Figure 2. Molecular Interactions at SARS-CoV-2 Viral Interfaces in Different Environmental Conditions

Model of the potential molecular interactions among viruses and between virus and different solid surfaces having negative surface charge and/or hydroxyl functional groups at (A) relatively low pH environment, below the isoelectric point; (B) relatively high pH condition, above isoelectric point in presence of external ions (e.g., salts); and (C) way below the isoelectric point in the presence of potential chemistries (for removal from surface purposes) with negative surface charge.

as wood, cotton, or paper. At pH environments above the isoelectric point (Figure 2B), the outer surface of virions would be deprotonated and therefore negatively charged and cannot be adsorbed on the surface with the same charge. Accordingly, lower virus adsorption onto the surfaces would occur at higher pH values. Instead, they can interact strongly with divalent and/or monovalent cations[23] if they existed in a brine electrolyte solution (more details are presented in the following section). The charge and counterions from the electrolyte could lead to thinner double layer and lower repulsion forces, and again hydrogen bonding formed to surface hydroxyl groups,[24] which results in promoting the virus adsorption process. The gold surface of an electrode in the quartz crystal microbalance (QCM) biosensor, which works on the basis of the oscillating frequency alteration,[25] could be employed for monitoring of the virus surface adsorption and desorption phenomena with or without the presence of negatively charged surface active species in the liquid phase.

3- THE EFFECTS OF HUMIDITY AND TEMPERATURE ON THE VIRUS ADSORPTION PHENOMENON

The functionality of viruses is not only based on their host cell but also on the different environments faced before and during the transmission process.[26] Coronaviruses have been shown to have different inactivation kinetics at different humidities.[27] Therefore, an understanding of the effect of humidity on surface stability and adsorption of this novel virus is crucial. Water molecules in the liquid phase condense from its vapor phase on various surfaces (e.g., between virus particles and solid substrates) and create liquid bridges with a curvature, which is related to the relative humidity "h" expressed by the Kelvin equation[28] (Equation 1). When the solid surfaces are fixed in one dimension, as for structures with a high aspect ratio, the interface curvature radii "r" is determined by:

$$r = \gamma\left(\frac{V_m}{RT\ln(h)}\right) \qquad \text{(Equation 1)}$$

where γ is the water surface tension, V_m is the water molar volume, R is the gas constant, T is the temperature. The water roundel radius $|r|$ increments with h and would be infinite at full hydration with relative humidity of 100%.

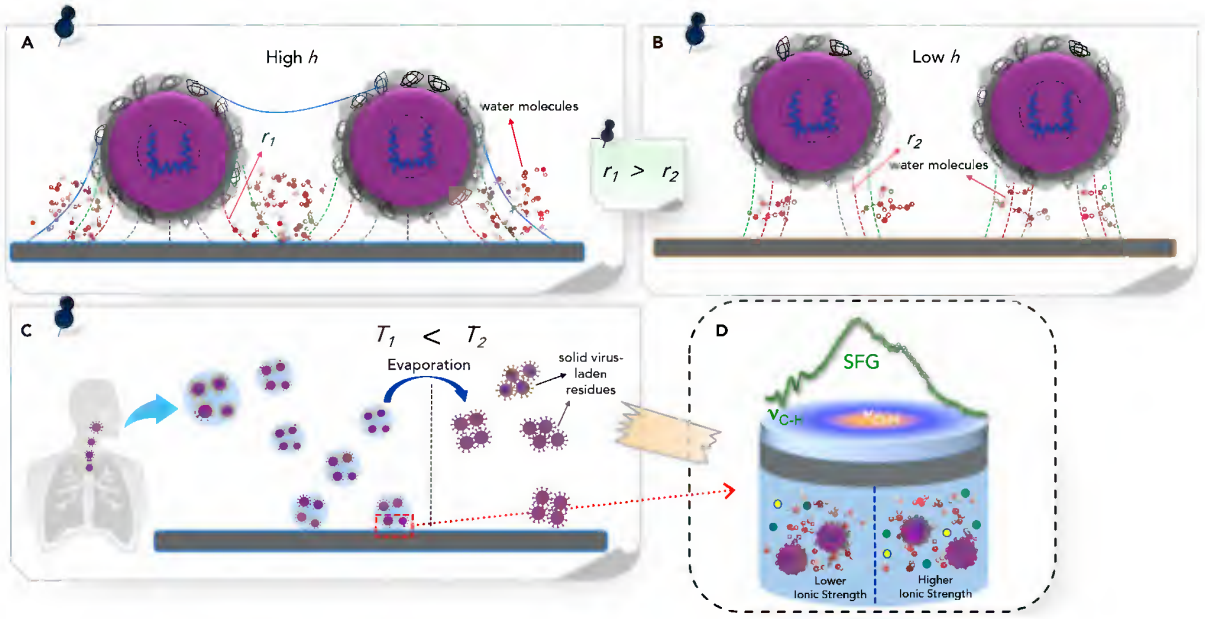

Figure 3. The Effects of Humidity and Temperature on SARS-CoV-2 Surface Stability

(A and B) The schematic diagram of the SARS-CoV-2 particles onto the (A) hydrophilic and (B) hydrophobic surfaces at environments with high and low relative humidity. The detailed molecular structure of S glycoproteins on the outer surface of the virus are not depicted for the sake of lucidity.

(C) The effect of temperature on exhaled virus-laden microdroplets that change to solid residues because of the temperature increase.

(D) Potential application of SFG spectroscopy for monitoring of changes in the hydrogen-bonding network strength due to the changes in ionic strength of the aqueous phase.

On the water-coated surfaces, the virus particles would establish strong interactions with the hydrophilic surface in the presence of a thin film water layer, mainly through hydrogen bonding[29] between water and the virus outer surface protein molecules. Water molecules can also fill the gaps between the virus particles that are spaced closer than the $|r|$ value defined by the relative humidity. On hydrophobic surfaces, the roundel expands less. Therefore, a thin layer of water can be created around the virions; however, the lunule might not be unified to bridge the gap between two virus particles. Figure 3 illustrates this phenomenon.

Capillary forces are also present at high relative humidity, which might vary on the bare substrate and on the virus. Thus, both the solid surface and the virus could be separated by one or multiple water stratums. The presence of either mono- or di-valent cations in the liquid phase[30] (linked by formation of cationic complexes with the hydroxyl groups of the solid surfaces) can result in substitution of the remaining half shell of water ligands at mono- and/or divalent cations by the hydroxyl and carboxylate functional groups[31] of the virus surface, completion of the cation bridging process, and augmentation of adsorbed amount of virus on the surface accordingly. The hydrogen-bond interactions of the interfacial water molecules with the aforementioned surface-active species of the virions, which can be strengthened or weakened by changing the aqueous phase ionic strength, can be monitored by using vibrational sum-frequency generation (vSFG) spectroscopy.[32,33] vSFG spectroscopy is a reliable technique for molecular-level characterization of aqueous interfaces,[34,35] including viral interfaces. This tool can probe the C–H stretches of the alkyl tails as well as the O–H stretching continuum of the hydrogen-bond network in the electrical double layer medium in the presence of the ions.[36]

On the other hand, as discussed, ion-specific interactions at charged interfaces could greatly affect the virus surface adsorption, protonation and/or deprotonation of the surface-active moieties of the virions; the charge densities and potentials of the viral interface, and the structuring of interfacial components in the electrical double layer. These ion-specific interactions can be probed by utilizing the second harmonic generation (SHG) spectroscopy technique,[37] a non-linear optical facility like SFG spectroscopy. The interdependence of the SHG response on the electrostatic potential has resulted in the application of this powerful technique as an optical voltmeter,[38,39] which can be employed for monitoring of the electrical double layer at the solid surface-aqueous phase-virus interfaces as well as quantifying the relative permittivity in the virus-surface gap. Figure 3D depicts potential application of SFG spectroscopy for probing alterations in the strength of hydrogen bonding network because of the variations in the aqueous phase ionic strength.

According to Equation 1, the water roundel radii $|r|$ decreases as temperature increases, which means that at higher temperatures the described complexes and molecular interactions are disturbed, lower water bridging would occur, and a reduced quantity of virus would be adsorbed onto solid surfaces. This theoretical analysis might explain previous observations[27] that higher temperature inactivates coronaviruses on stainless steel. In low temperature environments, the enveloped virions stay infectious for a number of days, as denoted in laboratory experiments.[27] Human lungs acting as reservoirs of respiratory viral infections can disperse microdroplets through sneezing and coughing, and the virions can even be exhaled during the speaking and breathing.[40–42] The bigger virus-laden microdroplets precipitate and adsorb onto the solid surface; however, the water contained within these virus-laden microdroplets can vaporize and form residues as solid phase or droplet nuclei at higher temperature conditions[16,43] (Figure 3C).

It is worth noting that conditions of too-high humidity can create a greater water network around the virus,[44] thus providing interactions with the proteins and the lipid bilayer that promote degradation. As the temperature increases, the movement of the water molecules increase and therefore also promote interaction and degradation.

POTENTIAL APPLICATIONS OF VARIOUS EXPERIMENTAL TECHNIQUES IN SARS-CoV-2 CHARACTERIZATION AND UNDERSTANDING OF VIRUS TRANSMISSION FROM SURFACES

Atomic force microscopy (AFM) and AFM-based methods, including AFM-infrared-spectroscopy (AFM-IR), are powerful techniques for characterization of a wide range of biological species and can help us to understand molecular interactions that occur at viral interfaces.[45] They can monitor the molecular interactions among the biomolecules including protein-protein interaction, antibody-antigen interaction,[46] and ligand-receptor interactions[47] without disrupting the virion.

Nanoindentation measures the rupture force of a virus capsid under force and gives information on the chemical bond strength between viral capsid proteins.[48] To understand the chemical force between a virus particle and a cell, AFM tips have been functionalized with a single viral particle and then used to probe the interaction of the probe and a cell.[49] AFM can also be used to measure how viral chemistry changes in different liquid environments.[45]

A practical example of using the AFM technique for studying of interactions of an enveloped virus with a solid surface is presented in Figure 4. Figure 4A shows how

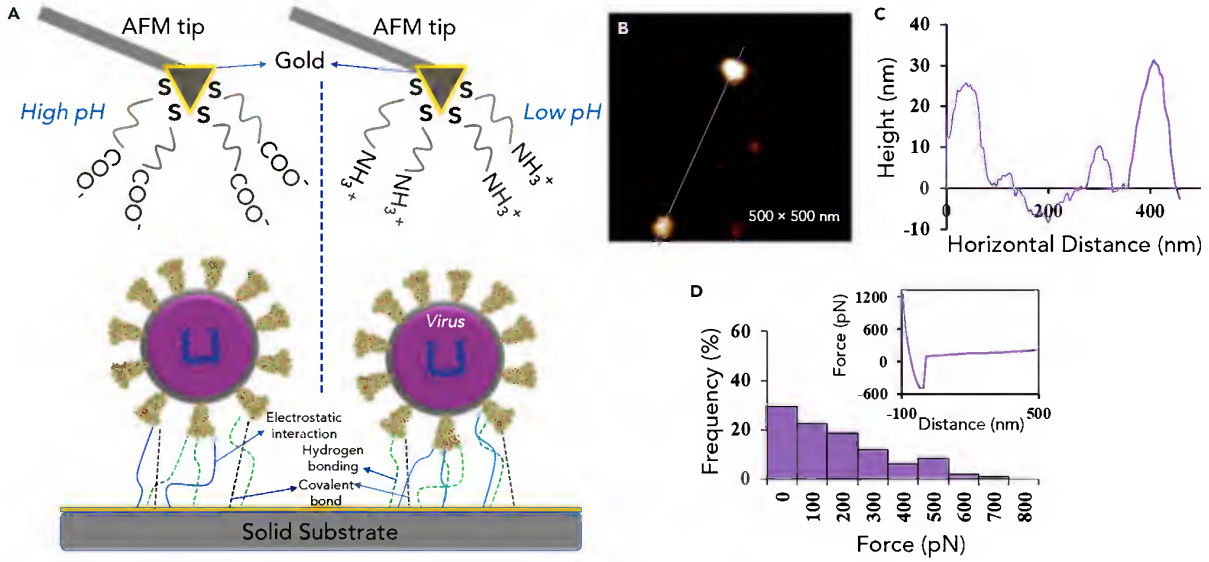

Figure 4. The AFM Technique to Investigate the Interactions of the Virus with a Solid Surface

(A) Overview of AFM probe functionalization and virus adsorption onto a solid surface. AFM tips can be negatively or positively charged through either deprotonation of carboxyl group leading to carboxylate anion formation at high pH environment or protonation of amine functional group at low pH environment, respectively.

(B) AFM to measure virus surface chemistry. AFM image of bovine viral diarrhea virus (BVDV) attached to a glass slide and the hydrophobicity was measured with a methyl-terminated AFM tip.

(C) Corresponding height image of the line in (B). The virus was found to be the size of known BVDV particles.

(D) The frequency of different forces found from pulling on BVDV particles with a methyl-terminated AFM tip. The histogram represents >300 force-distance curves using a combination of 3 different functionalized tips and 3 different batches of virus. The spring constant for the tip was 0.07–0.15 N/m. The mean force was 221 pN. The insert is a representative force distance curve. BVDV is an enveloped virus with size range of 40–60 nm and related to the hepatitis C human virus.

modified AFM tips can be functionalized with different chemistries to determine the viral isoelectric point and other surface chemistry interactions at the viral interface. Shown in Figure 4B, virus particles are covalently bound to a surface. The virus particles maintain their size and shape (Figure 4C), demonstrating that their structure is maintained through the chemical bonding process. The virus is pulled with functionalized AFM tips and the rupture force is compared to measure how different chemistries (i.e., functionalization) interact with the viral surface. The charge of the viral surface changes with solution conditions,[50] as already discussed, and surface hydrophobicity can also be explored (Figure 4D).

Cryogenic-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) are other revolutionary techniques for structural biologists that can be used to inform our understanding of virus structure and the drivers of viral self-assembly.[51–54] In the last decade, cryo-EM and cryo-ET have been employed to determine coronavirus S glycoprotein ectodomain structures in both the pre- and post-fusion conformations, generating snapshots of the fusion reaction at the beginning and end points of the process.[55–57] The atomic models generated by these techniques can inform our deeper understanding of the charge-distribution of the outer surface of SARS-COV-2 and describe structural changes to proteins that take place at different pH and ionic strength conditions,[58] which play a crucial role in the virus molecular interactions with solid surfaces. Furthermore, a newly developed methodology enables us to employ liquid-cell transmission electron microscopy (LCTEM) to complement the conventional Cryogenic transmission electron microscopy (cryo-TEM) for

characterization of nanoscale solvated biological soft matter as well as illustration of dynamics, growth kinetics, and reactivity of nanomaterials.[59] Staining approaches, which can overcome the contrast issues because of the ultra-low electron doses,[60] make LCTEM an adequate technique with potential utility in monitoring of virus interactions with surfaces.

In addition to vSFG and SHG spectroscopies described in section 3, which are recently developed non-linear optical techniques, new windows into the quantification of the hydrogen-bonding network strengths in the viral aqueous phase might be opened by employing Raman spectroscopy to realize the inter- and intramolecular details of water dynamics[61] to complement our understanding of virus surface adsorption under high relative humidity conditions. Moreover, surface-enhanced Raman spectroscopy (SERS) can be used to identify the proteins of enveloped viruses, like SARS-CoV-2, that are adsorbed onto solid surfaces.[62]

As a newly developed approach for high relative humidity conditions, integration of the second-order non-linear spectroscopic technique SHG with quantified adsorbed mass in nanoscale from piezoelectric QCM measurements with dissipation monitoring (QCM-D)[63] could be used for simultaneously determination of the charge per virus adsorbed onto the solid surface and probing the relative permittivity in the viral interfacial area of the electrical double layer.

We need to consider that spectroscopy methods might be a challenge with viral solutions because of their low concentration and difficulty in obtaining high-purity viral stocks. Viral concentrations are often measured in infectious particles per milliliter and laboratory samples typically range from 10^3–10^{10} particles per mL. These are attomolar to subnanomolar concentrations, and some analytical techniques[64] struggle to detect these low viral concentrations. The other major challenge is virus purity. Viruses used for study are either grown in lab cell cultures or derived from natural sources. Regardless, the viruses are a very small percentage of the organic matter in the original sample. Purification techniques need to maintain viral viability, which requires both the structural integrity of the capsid and envelope to remain intact, as well as minimal changes to the chemical surface of the virus.[65] The most common purification technique, ultracentrifugation, exposes the virus to either a high-ionic-strength cesium chloride solution or a high-osmotic-strength sucrose solution. An alternative option is to use iodixanol gradients, given that iodixanol is iso-tonic and non-toxic. However, almost all ultracentrifugation steps have a low yield. Most other laboratory purification techniques struggle to maintain viable virus with the high yield and high purity needed for many spectroscopic techniques to measure virus adhesion.

Figure 5 presents the recently developed techniques discussed in this article for study of virus structure and assembly, molecular interactions at viral interfaces, and direct analysis of solid surfaces.

CONCLUSIONS AND OUTLOOK

In this Perspective, we have highlighted the adsorption characteristics and molecular interactions of the SARS-CoV-2 outer surface proteins on solid surfaces from different points of view, which is critical to understanding the virus transmission process and taking necessary actions to tackle it. As discussed, some important factors influencing the virus adsorption phenomenon include the following: surface-active moieties of the viral proteins, hydrophilic or hydrophobic characteristic of the solid surface, pH of the bulk fluid, relative humidity, and temperature of the environment.

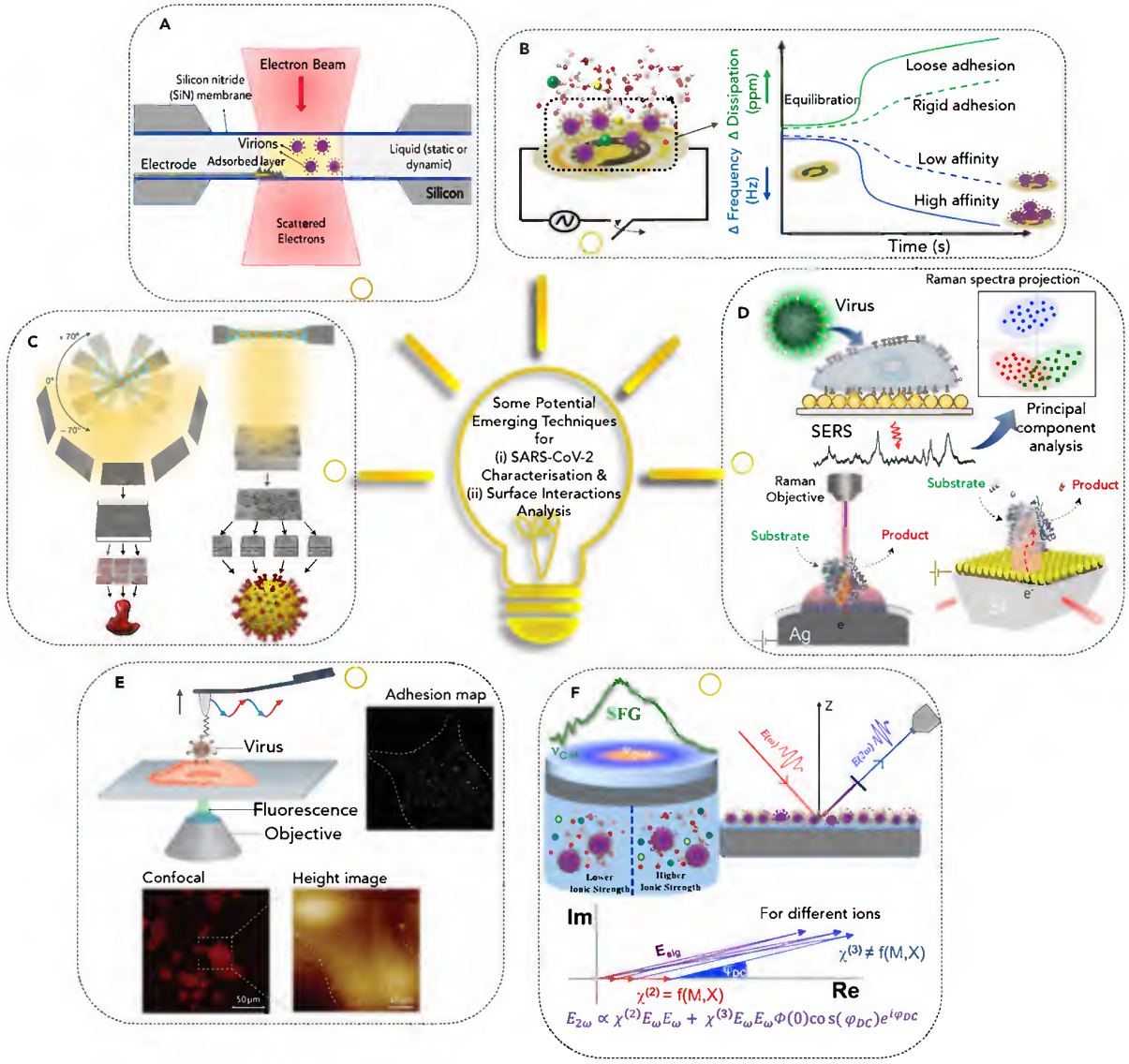

Figure 5. Some Emerging Techniques That Can Be Employed for SARS-CoV-2 Structural Characterization and Surface Interactions Analysis

(A) Schematic diagram of the LCTEM technique and its application for imaging of the virions.

(B) QCM-D technique, quantity of adsorbed virions and rigidity of binding can be determined through alterations in frequency and dissipation, respectively.

(C) Cryo-EM and cryo-ET techniques workflow, from data acquisition to 3D model, reprinted from Luque et al.[51] Copyright 2020 Springer Nature.

(D) SERS to identify cells infected with newly emerging viruses (top), adapted from Lim et al.[62] Copyright 2019 American Chemical Society. SERS on self-assembled monolayer (SAM)-functionalized Ag electrode (bottom left), infrared absorption spectro-electrochemistry on SAM-coated Au electrode (bottom right), reprinted from Kornienko et al.[66] Copyright 2019 American Chemical Society.

(E) Mapping of virus-binding events on living cells by using AFM technique, adapted from Alsteens et al.[45] Copyright 2017 Springer Nature.

(F) Overview of SFG (top left) and SHG (top right) spectroscopies for viral interface studies, and argand diagram for ion-specific SHG responses (bottom), modified from Boamah et al.[37] Copyright 2019 American Chemical Society.

Our theoretical analysis throughout this study has demonstrated that the SARS-CoV-2 can be adsorbed onto surfaces and remain stable within a range of pH values from acidic to basic environments at moderate temperature. On the basis of the Kelvin

equation, it is expected that the SARS-CoV-2 would be less stable in higher temperature conditions. Accordingly, it is anticipated that the rate of transmission and infection will be lower during the summer months than in winter months. Further experimental research studies on this topic are needed to confirm our hypothesis and propositions we have made. The preservation of the virus shape and structure after condensation and/or evaporation processes in the air and/or water and the viral electrostatic surface properties of this new virus should also be explored.

Moreover, application of certain biophysical and biochemical techniques to the structural analysis of viral capsid proteins could be of significant utility for identification of their molecular assembly main drivers as well as development of enhanced viral assemblies. Attaining this valuable information on the SARS-CoV-2 structure and assembly is necessary for helping scientists globally with antiviral drug and vaccine design to combat against COVID-19, given that the development of antiviral drugs is a promising direction for mitigating this new worldwide health threat.

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AUTHOR CONTRIBUTIONS

E.J. conceived the idea presented in the manuscript, undertook the theoretical analyses, and wrote the manuscript. A.H. contributed to the writing of the manuscript and the theoretical analyses and edited the final manuscript. C.L.H. contributed to the writing of the manuscript and supervised the AFM experiments. O.A. conducted the AFM experiments. All the authors discussed the results and contributed to the final manuscript.

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CERTIFICATE OF SERVICE

Pursuant to NRAP 25, I certify that on this 30th day of September 2022, the foregoing Exhibits to Request for Judicial Notice was e-filed with the Clerk of the Supreme Court of the State of Nevada and services were executed to the below counsel via the Court's Electronic Filing System pursuant to NEFCR 9:

Daniel F. Polsenberg, Esq.
Joel D. Henriod, Esq.
Abraham G. Smith, Esq.
LEWIS ROCA ROTHGERBER
3993 Howard Hughes Parkway
Suite 600
Las Vegas, NV 89169

Amy M. Samberg, Esq.
Lee H. Gorlin, Esq.
CLYDE & CO US LLP
7251 W. Lake Mead Blvd.
Suite 430
Las Vegas, NV 89128

Attorneys for Petitioner Starr Surplus Lines Insurance Company

Bradley Schrager, Esq.
WOLF RIFKIN SHAPIRO
SCHULMAN & RABKIN, LLP
3773 Howard Hughes Pkwy
Suite 590 South
Las Vegas, NV 89169
bschrager@wrslawyers.com

Mark T. Ladd, Esq.
COHEN ZIFFER FRENCHMAN
& MCKENNA LLP
1350 Avenue of the Americas
New York, NY 10019
mladd@cohenziffer.com

Attorneys for Real Party in Interest JGB Retail Vegas Lessee, LLC

VIA E-MAIL ONLY:

The Honorable Judge Mark Denton
EIGHTH JUDICIAL DISTRICT COURT DEPARTMENT NO. 13
Regional Justice Center, Courtroom 16D
200 Lewis Avenue
Las Vegas, Nevada 89155
Dept13lc@clarkcountycourts.us
Trial Court Judge