CLINICAL STUDY PROTOCOL SYNOPSIS

Laparoscopic surgery in the COVID-19 era: myths and reality. A multicentre observational study (LAPCOV1 study)

IMP:	
Protocol #:	TBC
Protocol Version, Date:	Version 1.0, dd mmm 2020
Development Phase:	
EudraCT #:	TBC
Sponsor:	TBC
Medical Monitor:	TBC

PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

Laparoscopic surgery in the COVID-19 era: myths and reality. A multicentre observational study.

Protocol #:	
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IMP:	
Development Phase:	N/A
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This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation guidelines for current Good Clinical Practice (ICH-GCP) and applicable regulatory requirements. Compliance with ICH-GCP standards provides assurance that the rights, safety, and wellbeing of study patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

____________________________________ ______________________
Principal Investigator's signature Date (dd-mm-yyyy)

Ahmed R. Ahmed
Principal Investigator's name (printed)

PROTOCOL SYNOPSIS

Study Title:	Laparoscopic surgery in the COVID-19 era: myths and reality. A multicentre observational study.
Protocol #:	TBC
EudraCT #:	N/A
Phase:	N/A
Scientific Rationale:	As the COVID-19 pandemic increases, the need to perform emergency surgeries on COVID-19 patients will increase. The global surgical community has adopted a very cautious approach with regards to laparoscopic surgery during the COVID-19 pandemic, with most specialist organisations and surgical colleges, recommending the avoidance of performing laparoscopic surgery both in elective and also in emergency situations (1, 2, 3, 4, 5, 6). The recommendations have been mainly based on hypothesis suggesting that laparoscopic surgery is regarded as an aerosol generating procedure (AGP), which can result in the release of airborne particles (aerosols). AGPs can create a risk of airborne transmission of infection that usually spread by droplet transmission. Despite the concern regarding aerosolisation, there is currently no evidence of viral transmission during laparoscopy. The hypothetical risks of aerosolisation during laparoscopic surgery stem from the fact that surgical smoke is formed when energy-generating devices raise the intracellular temperature, causing tissue vaporisation. Previous studies have shown that the concentration of smoke particles in the operating room is significantly higher in laparoscopic surgery compared to traditional open surgery. Furthermore surgical smoke generated during laparoscopic surgery may carry small viral particles, such as HIV, hepatitis, and human papillomavirus, suggesting that SARS-Cov2 should not be an exception (7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Table 1 demonstrates that SARS-Cov2 is comparable in size to some of the aforementioned viruses. On the other hand, surgery in patients with HIV and hepatitis B and C has been ongoing for decades, without documented increased risk of transmission from the surgical plume or laparoscopic pneumoperitoneum to surgeons, anaesthetists or operating room personnel. In fact, when possible, laparoscopic techniques that significantly minimize exposure of surgeons to blood-borne pathogens is far preferred to laparotomy . Furthermore, **specific evidence relating to the presence of SARS-CoV-2 virus in the peritoneal cavity and its transmission during laparoscopic surgery is so far lacking**.

Name	**Size (microns) µm**
Bacteria	0.30 µm
HIV	0.12 µm
Coronavirus	0.06-0.14 µm
Hepatitis C Virus	0.06 µm
Human Papilloma Virus	0.05 µm
Hepatitis B Virus	0.04 µm
Hepatitis E Virus	0.03 µm
Hepatitis A Virus	0.02 µm

Table 1: Biological material sizes (19, 20, 21, 22)

Furthermore in some fields of surgery such as weight loss surgery, hernia surgery, laparoscopic surgery carries clear advantages over open surgery where morbidity rates are much higher when operations are performed open.

Despite the above, the hypothetical risks from aerosol transmission are resulting in many surgeons globally adopting open surgery over minimally invasive surgery, leading to increased morbidity, hospital stay (including the need for already scarce ICU care) and recovery (5, 17, 18). Furthermore patients presenting with acute surgical disease are facing delays in receiving surgery based on the same assumption of transmission of COVID-19 to healthcare professionals during laparoscopic surgery. Examples include perforated appendicitis, acute cholecystitis, perforated peptic ulcer , and potential complicated diverticulitis being managed with antibiotics and xray guided drainage rather than undergoing early laparoscopic intervention/washout+drainage which would be the usual standard of care in non-COVID-19 times.

During laparoscopic surgery, carbon dioxide is insufflated into the peritoneal cavity. This creates space which allows the laparoscopic surgeon to perform minimally invasive surgery through laparoscopic ports. Usually 3 or 4 ports are used. All ports have a valve at the instrument insertion site which allows the passage of instruments in/out of the port with minimal gas leak. All ports also have side gas vents with taps (Luer lock type) that are usually in the closed position (See Figure 1). However one or more port side vent can be opened for the following reasons:

(i) to allow gas into the peritoneal cavity (at the beginning of the case and usually left open throughout the case to ensure an inflow of CO2 to maintain the set pressure inside the peritoneal cavity),
(ii) to allow any smoke out created during the surgery from energy device usage (a port tap is opened transiently during the case to allow any smoke which obscures view to escape), and
(iii) to allow gas out at the end of the case (this is to remove any CO2 and smoke out at the the end of the case as these would otherwise irritate the peritoneal lining causing pain as well as continued distension of the abdomen).

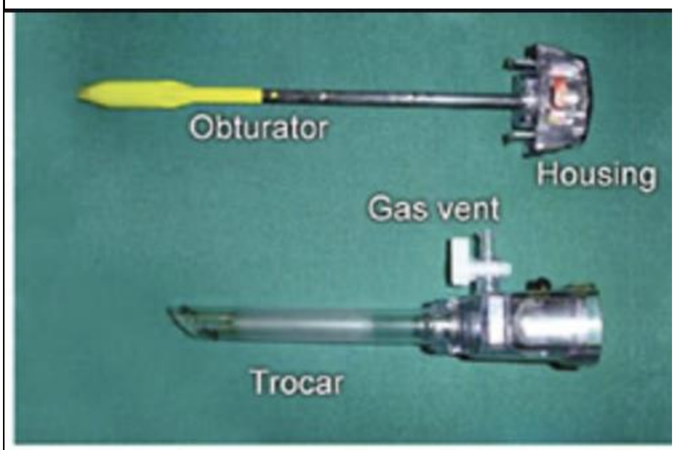

Figure 1: Laparoscopic port

Therefore during laparoscopic surgery, in the absence of any precautionary measures, it is possible to get intraperitoneal gas and smoke escape into the operating room environment during (ii) and (iii) above especially as the air inside the peritoneal cavity is at higher than atmospheric pressure (10-15mmHg). Chun et al have demonstrated that after using laparoscopic electrical and ultrasonic equipment (for dissection / haemostasis) for 10 minutes, the particle concentration of the smoke in laparoscopic surgery was significantly greater than in open surgery.

Hence this has led some to hypothesize that that surgical smoke produced during laparoscopic surgery increases the risk of SARS-CoV-2 exposure (1, 16). Furthermore the passage of laparoscopic instruments in and out of ports (despite the valve inside the ports to prevent escape of gas) may also result in some gas/smoke leakage.

Objectives:	**Primary objective:** • To analyse the presence of SARS-CoV-2 virus in the gas and smoke released from laparoscopic ports at various time intervals during laparoscopic surgery. **Secondary objectives:** • To analyse the presence of SARS-CoV-2 virus in the air inside the peritoneal cavity during laparoscopic surgery. • To analyse the presence of SARS-CoV-2 virus in the peritoneal lining from tissue biopsy. • To assess the infectivity of the above captured virus particles by subjecting them to in vitro cell culture.
Rationale for Study Design:	The novel nature of the COVID-19 pandemic presents many challenges to laparoscopic surgeons. Despite efforts to limit elective surgery during the COVID-19 pandemic, urgent and emergent procedures must still be performed. For many of these urgent procedures, laparoscopy may offer the best surgical approach and outcomes for the patient. Currently patients rarely are being offered laparoscopic surgery, and instead many surgeons feel laparoscopic procedures should be avoided, and laparotomy procedures be universally employed when a patient is COVID-19 positive or status is unknown due to concerns about aerosolization of viral particles via the pneumoperitoneum. We strongly disagree with this premise. SARS-CoV-2 virions are approximately 0.06-0.12 microns in size and are most commonly transmitted as larger (> 20 microns) respiratory water droplets. The virus may also be aerosolized and transmitted in smaller droplets (< 10 microns) in gas suspension. To date, no studies have looked for SARS-CoV-2 in surgical smoke, and even if found, it is not known whether these viral particles are infectious. There is therefore an urgent need to conduct an observational study to investigate if SARS-Cov2 RNA is aerosolised during laparoscopic surgery in COVID-19 patients and if so how great the risk of viral disease transmission is from performing laparoscopy. If the current observational study detects SARS-Cov2 aerosolisation, we have planned an interventional study using smoke evacuation and filtration systems that remove virus particles from any gas/smoke from the peritoneal cavity that is released at the end of surgery or sucked out during surgery which would then allow laparoscopic surgery to be conducted safely.

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<td>Study design:</td>
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This is a multicentre observational study to assess SARS-Cov2 in the operating room during laparoscopic surgery on COVID-19 positive patients.
Preoperative assessment:
This will be performed as standard of care as for any patient presenting to hospital for emergency or elective laparoscopic surgery. Since most if not all hospitals have ceased elective laparoscopic surgery, the majority of subjects will be undergoing emergency laparoscopic procedures (appendicectomy, bariatric complications, perforated peptic ulcer repair, urgent cholecystectomy, ruptured ectopic pregnancy, hemorrhagic ovarian cysts, or ovarian torsion).
Surgical patients requiring laparoscopic surgery that are COVID-19 positive or suspected will be recruited into this study and undergo consent for the research.
Preoperative COVID-19 screening and diagnosis:
Currently it is standard of care for surgery patients who are candidates for surgery (emergent or elective) to undergo SARS-CoV-2 screening by rRT-PCR and/or CT chest.
<blockquote>
Prior to surgery upper (nasopharangeal and oropharangeal swabs) respiratory tract specimens will be collected for all patients and specimens will be sent for rRT-PCR testing (standard of care). All samples will be collected according to WHO guidelines and this should be done by the COVID team in the hospital (30). If swabs are not taken preoperatively, the swabs will be taken within 48 hours of surgery. As rRT-PCR results are usually take 24-48 hours, they should only be used for retrospective confirmation of the diagnosis, and will not delay the surgery nor the recruitment into this study.
Prior to surgery patients will normally undergo CT scanning of the chest (standard of care). A finding of bilateral patchy shadows and ground glass opacity is highly suspicious for COVID-19 (28).
</blockquote>
Once a patient has been identified as meeting the inclusion criteria for the study (COVID-19 positive/suspected and planned for laparoscopic surgery), the admitting team will contact the research team via dedicated mobile phone number which is carried 24/7 by the CI. The CI will then arrange for a member of the research team to attend surgery to collect the samples.
Intraoperative SARS-CoV-2 isolation and analysis
The research team member on arrival will identify themselves to the admitting team and donn full PPE (in accordance with the host institution) and will be present in the operating room from the beginning of the case to collect the air samples using the Coriolis. The Coriolos air sampler (see Figure
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2 below) is a commonly used portable air sampling device and is effective in detecting small particles and viruses (31,33).

Figure 2: Coriolis air biosampler

Biosample 1: Intraperitoneal SARS-CoV-2 sampling via Coriolis

The Coriolis Biological Air Sampler will be used to collect intraperitoneal SARS-CoV-2 in surgical gas/smoke. A sterile 25mm, 5m extension tube will be used to connect the laparoscopic port to the Coriolos. The Coriolos will be set at the maximum rate of 300 l/min. Intraperitoneal sampling will be take place 3 times during the surgery.

1-At the start of surgery once the target pneumoperitoneum is achieved, the gas vent tap on the port is opened, **then** the Coriolos is switched on for **1 minute**. The gas vent tap will then be turned off and the sample of gas collected (now in a cone filled with 10ml of liquid) is removed from the Coriolis
2-Midway through the surgery (approximately after 30min depending on the surgery), the process is repeated.
3-Near the end of the surgery, just prior to full desufflation of the peritoneal cavity, the process is repeated.

Biosample 2: Intraperitoneal SARS-CoV-2 sampling via peritoneal biopsy

A single peritoneal biopsy will be obtained at laparoscopy from all subjects. This will be taken with a standard laparoscopic grasper and placed in a sterile pot. This peritoneal biopsy will be sent for SARS-CoV-2 testing. Furthermore it has been proven that for SARS-CoV-2, the ACE-2 is a vital receptor for entry

	(34). ACE-2 receptors are highly expressed in the gastrointestinal system, but few data are available on ACE-2 receptors presence in the peritoneum / GI tract (35). The density of ACE-2 receptors in the peritoneum lining will be analysed. Lastly, a full tissue biopsy of the target organ will be obtained where the nature of the operation permits resection of tissue (eg. appendicitis, cholecystitis, small bowel resection, ovarian cyst) <u>Biosample 3: Extra-abdominal SARS-CoV-2 sampling via Coriolis</u> A. The Coriolis Biological Air Sampler will be placed within 1 metre and at the same height as the operating table. Sampling will be take place 3 times during the surgery. 1-Immediately after CO2 insufflation. The Coriolis will be placed as close as possible to the abdomen and must be within 1 metre. The Coriolis will be switched on for 10 minutes at a rate of 300 l/min. The sample of air collected (now in a cone filled with 10ml of liquid) is then removed from the Coriolis. 2- 10 minutes* after using an energy device that generates smoke or if no energy device is used, midway through the surgery. The Coriolis will be placed as close as possible to the abdomen and must be within 1 metre. The Coriolis will be switched on for 10 minutes at a rate of 300 l/min. The sample of air collected (now in a cone filled with 10ml of liquid) is then removed from the Coriolis. **The choice of sampling 10 minutes after using an energy device is because a previous study has shown that the concentration of particles in the air during laparoscopic surgery, increases significantly after 10 minutes of using energy devices (15).* 3- On desufflation of the peritoneal cavity which is triggered by opening the gas vent tap on one or more laparoscopic ports. Desufflation is thought to be an aerosol generating process (1). The Coriolis will be placed as close as possible to the abdomen and must be within 1 metre. The Coriolis will be switched on for 10 minutes at a rate of 300 l/min. The sample of air collected (now in a cone filled with 10ml of liquid) is then removed from the Coriolis. <u>Biosample 4: Extra-abdominal SARS-CoV-2 sampling via Filter cassettes (37-mm) air sampler will be attached to 4 AirCheck Touch 220-5000TC pumps.</u> Filter cassettes are another way of collecting virions in air and have been used previously to collect SARS-CoV (36). Pumps will be switched on at the beginning of surgery (before the first incision is made) and will be switched off after 10 minutes after the closure of the skin wound. Four filter cassettes (37mm) with pumps will be used as follows: 1- One filter cassette will be placed near the operating room exit door

	2- One filter cassette will be placed within 1 metre distance of the operating table and at same height as operating table. 3- One filter cassette will be placed at a separate location (preferable opposite to cassette 2 above) but also 1 metre distance of the operating table and at same height as operating table in order to maximise detection. 4- One filter cassette will be placed outside the operating room as a control. Immediately after collection, all the biosamples will be placed on ice and either (i) transported the same day to a Level-3 containment laboratory or (ii) stored at a temperature of −80°C (37) and transported within 24 hours to a Level-3 containment laboratory for analysis for detectibility and viability of SARS-Cov2. The biosamples will also then be subjected to cell culturing to assess potential viral infectivity. **[Further details of the laboratory process for identifying then cell culture for SARS-Cov2 to be added here]**
Sample Size:	10 patients will be recruited for this study. *Why 10 patients?* Kwak et al analysed the presence of hepatitis B virus in surgical smoke, emitted during laparoscopic surgery. To our knowledge this is the only study that has analysed viral aerosolization, during laparoscopic surgery. The study recruited 11 patients, who are hepatitis B positive (3). HBV RNA was detected in 10 of the 11 cases. Despite the low sample size, the high detectibility rate was sufficient to conclude that HBV is detectable in surgical smoke. The HBV size is comparable to that of Coronavirus (Table 1). Thus, we decided to recruit 10 patients for our study. It is important to highlight that recruiting large number of patients to produce high power values is not possible during the COVID-19 era, since most operations (except emergencies) have been cancelled at hospitals across the country. We expect recruiting patients who are COVID-19 positive and needing to undergo laparoscopic surgery will not be simple. However the study has full backing by the Association of Laparoscopic Surgeons of Great Britain and Ireland who will promote the study and encourage recruitment and the study will also be part of the NIHR portfolio.

Coordinating Study Centre	Medical oversight for this this study will be provided by the Chief Investigator Mr Ahmed R Ahmed
Endpoints	**Primary endpoint:** SARS-Cov2 RNA detection in operating room air. **Secondary endpoints:** 1. SARS-Cov2 RNA detection in peritoneal gas. 2. SARS-Cov2 RNA detection in peritoneal biopsy/specimen biopsy 3. ACE-2 receptor density in peritoneal biopsy 4. The infectivity of any detected SARS-Cov2 in operating room air
Study Population:	COVID-19 confirmed or suspected cases, undergoing laparoscopic surgery will be eligible to enter the study provided they fulfil all eligibility criteria.
Inclusion criteria:	1. Patients undergoing laparoscopic surgery (or planned laparoscopic surgery) 2. Provision of signed written informed consent 3. Age 18 to 69 years 4. Positive laboratory test for SARS-CoV-2 (if not available at operation time then taken within 48 hours of surgery) OR CT positive for COVID-19
Exclusion criteria	1. Open surgery patients or cases where laparoscopy is converted to open surgery within 5 minutes of starting surgery. 2. Laparoscopic surgery, where smoke evacuators/filters/airseal trocars have been used. 3. Concurrent treatment with other agents with actual or possible direct acting antiviral activity against SARS-CoV-2
Study Duration Per Patient:	The study duration for each patient is the length of the laparoscopic procedure.

References:

1- Zheng MH, Boni L, Fingerhut A. Minimally invasive surgery and the novel coronavirus outbreak: lessons learned in China and Italy. Ann Surg. 2020;(In Press).

2- Ficarra V, Novara G, Abrate A, et al. Urology practice during COVID-19 pandemic. Minerva Urol Nefrol. 2020. doi:10.23736/S0393-2249.20.03846-1

3- Kwak HD, Kim S-H, Seo YS, Song K-J. Detecting hepatitis B virus in surgical smoke emitted during laparoscopic surgery. Occup Environ Med. 2016;73(12):857 LP - 863. doi:10.1136/oemed-2016-103724

4- Sawchuk WS, Weber PJ, Lowy DR, Dzubow LM. Infectious papillomavirus in the vapor of warts treated with carbon dioxide laser or electrocoagulation: detection and protection. J Am Acad Dermatol. 1989;21(1):41-49.

5- https://www.sages.org/recommendations-surgical-response-covid-19/.

6- https://www.augis.org/wp-content/uploads/2020/04/2nd-Update-Intercollegiate-General-Surgery-Guidance-on-COVID-19-6-April-.._.pdf

7- Capizzi PJ, Clay RP, Battey MJ. Microbiologic activity in laser resurfacing plume and debris. Lasers Surg Med. 1998;23(3):172-174.

8- Hensman C, Baty D, Willis RG, Cuschieri A. Chemical composition of smoke produced by high-frequency electrosurgery in a closed gaseous environment. An in vitro study. Surg Endosc. 1998;12(8):1017-1019.

9- Johnson GK, Robinson WS. Human immunodeficiency virus-1 (HIV-1) in the vapors of surgical power instruments. J Med Virol. 1991;33(1):47-50.

10- Gloster HMJ, Roenigk RK. Risk of acquiring human papillomavirus from the plume produced by the carbon dioxide laser in the treatment of warts. J Am Acad Dermatol. 1995;32(3):436-441.

11- Liu Y, Song Y, Hu X, Yan L, Zhu X. Awareness of surgical smoke hazards and enhancement of surgical smoke prevention among the gynecologists. *J Cancer*. 2019;10(12):2788–2799. Published 2019 Jun 2. doi:10.7150/jca.31464

12- Ferenczy A, Bergeron C, Richart RM. Human papillomavirus DNA in CO2 laser-generated plume of smoke and its conse-quences to the surgeon. Obstet Gynecol. 1990;75:114

13- Taravella MJ, Weinberg A, May M, Stepp P. Live virus survives excimer laser ablation. Ophthalmology. 1999;106:1498–9.

14- Baggish MS, Poiesz BJ, Joret D, Williamson P, Refai A. Presence of human immunodeficiency virus DNA in laser smoke. Lasers Surg Med. 1991;11:197–203.

15- Kubo H. Medical engineering, electrical instruments and medical gas, guideline for surgical practice (in Japanese). Nihon Shujutsu Igaku Kaishi. 2013;34:s98–11.

16- Li C-I, Pai J-Y, Chen C-H. Characterization of smoke generated during the use of surgical knife in laparotomy surgeries. J Air Waste Manag Assoc. 2020;70(3):324-332.

17- Snyman L, Makulana T, Makin JD. A randomised trial comparing laparoscopy with laparotomy in the management of women with ruptured ectopic pregnancy. S Afr Med J. 2017;107 (3):258-63.

18- Aarts JW, Nieboer TE, Johnson N et al. Surgical approach to hysterectomy for benign gynaecological disease. Cochrane Database Syst Rev 2015;8:Cd003677.

19- Cascella M, Rajnik M, Cuomo A, et al. Features, Evaluation and Treatment Coronavirus (COVID-19) [Updated 2020 Mar 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/ <https://www.ncbi.nlm.nih. gov/books/NBK554776.

20- Fisher, Bruce; Harvey, Richards P.; Champe, Pamela C. (2007). Lippincott's Illustrated Reviews: Microbiology Lippincott's Illustrated Reviews. Hager-stown, MD: Lippincott Williams & Wilkins. P. 3. ISBN 978-0-7817-8215-9.

21- IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Human Papillomavirus. Lyon (FR): International Agency for Research on Cancer; 2007. (IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, No. 90.) 1, Human Papillomavirus (HPV) Infection. Available from: https://www. Ncbi.nlm.nih.gov/books/NBK321770/

22-Hepatitis C Virus—proteins, diagnosis, treatment and new approaches for vaccine development. Hossein Keyvani, Mehdi Fazlalipour, Seyed Hamid Reza Monavari, Hamid Reza Mollaie. Asian Pac J Cancer Prev. 2012; 13(12): 5931-5949.

23- Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020 Apr 1.

24- Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med. 2020;382(12):1177–1179.

25- Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020; published online Feb 24.

26- Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W.
Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis. 2020 Mar 19.

27- Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395(10223):497-506.

28- Ng M-Y, Lee EY, Yang J, Yang F, Li X, Wang H, et al. Imaging Profile of the COVID-19 Infection: Radiologic Findings and Literature Review. Radiology: Cardiothoracic Imaging. 2020;2(1):e200034

29- Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. Eurosurveillance. 2020;25(5):2000062.

30- Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases.

https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

31- Mbareche H, Veillette M, Bilodeau GJ, Duchaine C. Bioaerosol Sampler Choice Should Consider Efficiency and Ability of Samplers To Cover Microbial Diversity. Appl Environ Microbiol. 2018;84(23):e01589-18.

32-Hogan CJ Jr, Kettleson EM, Lee MH, Ramaswami B, Angenent LT, Biswas P. Sampling methodologies and dosage assessment techniques for submicrometre and ultrafine virus aerosol particles.
J Appl Microbiol. 2005;99(6):1422-34.

33-Willeke K, Lin X, Grinshpun SA. Improved aerosol collection by combined impaction and centrifugal motion. Aerosol Sci Technol 1998;28:439–56.

34- Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020).

35- Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2 [published online ahead of print, 2020 Mar 3]. *Gastroenterology*. 2020;. doi:10.1053/j.gastro.2020.02.055.

36- Ong SWX, Tan YK, Chia PY, et al. Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient [published online ahead of print, 2020 Mar 4]. *JAMA*. 2020;e203227.

37- Salem E, Hägglund S, Cassard H, et al. Pathogenesis, Host Innate Immune Response, and Aerosol Transmission of Influenza D Virus in Cattle. *J Virol*. 2019;93(7):e01853-18. Published 2019 Mar 21. doi:10.1128/JVI.01853-18