

## Opinion Paper

Giuseppe Lippi\*, Ana-Maria Simundic<sup>a</sup> and Mario Plebani<sup>a</sup>

# Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19)

<https://doi.org/10.1515/cclm-2020-0285>

Received March 9, 2020; accepted March 10, 2020; previously published online March 16, 2020

**Abstract:** A novel zoonotic coronavirus outbreak is spreading all over the world. This pandemic disease has now been defined as novel coronavirus disease 2019 (COVID-19), and is sustained by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As the current gold standard for the etiological diagnosis of SARS-CoV-2 infection is (real time) reverse transcription polymerase chain reaction (rRT-PCR) on respiratory tract specimens, the diagnostic accuracy of this technique shall be considered a foremost prerequisite. Overall, potential RT-PCR vulnerabilities include general preanalytical issues such as identification problems, inadequate procedures for collection, handling, transport and storage of the swabs, collection of inappropriate or inadequate material (for quality or volume), presence of interfering substances, manual errors, as well as specific aspects such as sample contamination and testing patients receiving antiretroviral therapy. Some analytical problems may also contribute to jeopardize the diagnostic accuracy, including testing outside the diagnostic window, active viral recombination, use of inadequately validated assays, insufficient harmonization, instrument malfunctioning, along with other specific technical issues. Some practical indications can hence be identified for minimizing the risk of diagnostic errors, encompassing the improvement of diagnostic accuracy by combining clinical

evidence with results of chest computed tomography (CT) and RT-PCR, interpretation of RT-PCR results according to epidemiologic, clinical and radiological factors, recollection and testing of upper (or lower) respiratory specimens in patients with negative RT-PCR test results and high suspicion or probability of infection, dissemination of clear instructions for specimen (especially swab) collection, management and storage, together with refinement of molecular target(s) and thorough compliance with analytical procedures, including quality assurance.

**Keywords:** coronavirus; COVID-19; diagnosis; reverse transcription polymerase chain reaction (RT-PCR).

## Introduction

Less than a decade after the last human outbreak caused by a zoonotic coronavirus, the Middle East respiratory syndrome (MERS) in 2012, a novel bat coronavirus spillover has emerged in China, and is now spreading all over the world. This new outbreak, defined as novel coronavirus disease 2019 (COVID-19) by the International Committee on Taxonomy of Viruses [1], is sustained by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. According to the most recent statistics of the World Health Organization (WHO), COVID-2 has already been diagnosed in as many as 118,000 people from 115 countries worldwide, causing nearly 4300 deaths [2]. Although SARS-CoV-2 infection seems to occur with mild, influenza-like symptoms in the vast majority of subjects, in 10%–15% of COVID-19 patients (especially the older and those with important co-morbidities), the disease may progress into a severe form of interstitial pneumonia, which may then evolve toward acute respiratory distress syndrome (ARDS) and death in 2%–5% of cases [2].

Although diagnostic errors can occur almost always and everywhere in healthcare [3], the vulnerability of laboratory medicine services is enormously magnified when the staff is forced to work in high-throughput settings,

<sup>a</sup>Ana-Maria Simundic and Mario Plebani share senior authorship in this work.

\*Corresponding author: Prof. Giuseppe Lippi, Section of Clinical Biochemistry, Department of Neuroscience, Biomedicine and Movement, University of Verona, Piazzale LA Scuro, 37134 Verona, Italy, Phone: +39-045-8124308, Fax: +39-045-8122970, E-mail: giuseppe.lippi@univr.it

Ana-Maria Simundic: Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia

Mario Plebani: Department of Laboratory Medicine, University Hospital of Padova, Padova, Italy. <https://orcid.org/0000-0002-0270-1711>

driven to facing high workloads and under severe pressure, as is now materializing in many worldwide facilities due to the exponential growth of SARS-CoV-2-positive cases needing extensive healthcare support [4]. The clinical and economical consequences of diagnostic errors are always significant [5], but in the case of infectious outbreaks, especially when these assume the relevance of pandemic disease such as COVID-19, the repercussions are unquestionably amplified. The generation of false-positive or false-negative test results not only jeopardizes the health of the individual patient, but may also derange and disrupt the efficacy of public health policies, emergency plans and restrictive measures established by national and international authorities for containing the outbreak. A false-positive result not only may lead to unnecessary treatment of uninfected individuals, but may also cause enormous societal problems when attributed to people working in essential public services (health and social care operators, police officers, firefighters and so forth), as it might undermine the workforce available for facing the emergency. On the other hand, a false-negative result accredited to a patient who is instead infected with SARS-CoV-2 may then potentially contribute to foster human-to-human transmission and further spread the virus within the community due to non-timely application of isolation and/or restrictive measures, as well as for failure to identify other potentially infected people (household and/or close contacts).

Laboratory medicine plays an essential role for diagnosing and managing many human pathologies [6], thus including infectious diseases and COVID-19 [7]. As the current gold standard for the etiological diagnosis of SARS-CoV-2 infection is (real time) reverse transcription polymerase chain reaction (rRT-PCR) on respiratory tract specimens [8–10], the diagnostic accuracy of this technique shall be considered a foremost prerequisite. Therefore, the aim of this article is to provide a personal overview on the potential preanalytical and analytical vulnerabilities of RT-PCR testing for diagnosing SARS-CoV-2 infection (Table 1).

## Preanalytical errors

There is now incontrovertible evidence that the preanalytical phase is the major source of errors in laboratory testing, when used for either diagnostic [11] or research [12] purposes. Virology is not an exception, whereby many potential preanalytical errors are similar to those occurring in other different diagnostic areas, whilst others can be classified as specific. Among the former category, the

**Table 1:** Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19) using (real time) reverse transcription polymerase chain reaction (rRT-PCR).

Preanalytical	
General	
	– Lack of identification/misidentification
	– Inadequate procedures for specimen (e.g. swab) collection, handling, transport and storage
	– Collection of inappropriate or inadequate material for quality or volume
	– Presence of interfering substances
	– Manual (pipetting) errors
Specific	
	– Sample contamination
	– Testing in patients receiving antiretroviral therapy
Analytical	
	– Testing carried out outside of the diagnostic window
	– Active viral recombination
	– Use of non-adequately validated assays
	– Lack of harmonization of primers and probes
	– Instrument malfunctioning
	– Insufficient or inadequate material
	– Non-specific PCR annealing
	– Misinterpretation of expression profiles

safety and quality of RT-PCR testing may be endangered by lack of identification or misidentification of patient and/or sample, collection of inappropriate or inadequate material (for quality or volume), inaccurate conditions of sample transportation and storage (e.g. injury exposure, unreliable cold chain, prolonged transportation time), presence of interfering substances (e.g. release of cellular components that may interfere with the assay due to whole blood freezing, use of inappropriate additives) [13–15], as well as by a number of procedural issues occurring during sample preparation, thus including pipetting errors during manual sample preparation or aliquoting, cross-contamination and sample mismatch, among others [16]. The leading specific problems that may plague the quality of RT-PCR assays include sample contamination (even trace amounts of external DNA may jeopardize test results) and testing carried out in patients receiving antiretroviral therapy, which may then generate false-negative results [15].

## Specimen collection and transportation

Among the various preanalytical issues that have been portrayed in the previous paragraph, those related to specimen collection are particularly significant and deserve specific focus. Although no detailed reference

procedures have been provided as yet by the WHO for collecting respiratory material (i.e. lower, but especially upper respiratory specimens) for diagnosing SARS-CoV-2 to the best of our knowledge, the US Centers for Disease Control and Prevention (CDC) recommends that nasopharyngeal and oropharyngeal material shall be collected using swabs with a synthetic tip (e.g. as nylon or Dacron) and an aluminum or plastic shaft (other lower respiratory tract specimens could be collected, when available or feasible) [17]. The recommended procedure for collecting a quality nasopharyngeal specimen entails inserting the swab into the nostril parallel to the palate, maintaining the swab in place for few seconds for enabling secretion absorption and immediate placement of the swab into a sterile tube, containing 2–3 mL of viral transport media. The procedure for collecting oropharyngeal (e.g. throat) specimens entails swabbing the posterior pharynx, avoiding the tongue, and immediate placement of the swab into another separate sterile tube, also containing 2–3 mL of viral transport media. Failure to comply straightforwardly with the recommended procedures (e.g. use of wrong swabs, inappropriate absorption of diagnostic material, insertion into inadequate vials, contamination, and so forth) may be a significant cause of diagnostic errors, as clearly reported for other viral diseases [18, 19].

## Diagnostic accuracy

Several assays have been developed so far for diagnosing SARS-CoV-2 infection. One of the most popular seem to be that originally proposed by the Charité-Universitätsmedizin Berlin Institute of Virology [20], and then endorsed

by the WHO [21], along with that developed by the CDC [22], whose essential characteristics are summarized in Table 2, as reported in the respective websites [21, 22]. In the former case, the *E* gene assay is used as the first-line screening tool, then followed by confirmatory testing with an RNA-dependent RNA polymerase gene (*RdRp*) assay. The *N* gene assay can eventually be analyzed as an additional confirmatory assay. As regards the CDC test, the first panel, encompassing three *N* gene primer/probe sets, is designed for both universal detection of SARS-like coronaviruses (one primer/probe set), as well as for specific detection of SARS-CoV-2 (two primer/probe sets). An additional primer/probe set for detecting human RNase *P* gene (*RP*) in control samples and clinical specimens is included in the panel. Many other assays have then been developed by independent research institutes and in vitro diagnostic companies around the world, as summarized elsewhere [4].

According to recent evidence, the diagnostic accuracy of many of the currently available RT-PCR tests for detecting SARS-CoV-2 may be lower than optimal (i.e. <100%). Xie et al. first described the case of five out of 167 patients (3.0%) with chest computed tomography (CT) evidence of COVID-19, who initially tested negative for SARS-CoV-2 RT-PCR [23]. Interestingly, repeated swab tests carried out during hospitalization gradually turned to be positive in all such patients, with a mean interval period for positivity of  $5.0 \pm 2.7$  days. Ai et al. carried out another study, including 1014 suspected COVID-19 cases who underwent multiple RT-PCR testing and chest CT [24]. Overall, 88% (888/1014) of patients had positive chest CT scans, whilst RT-PCR positivity was found in 59% (601/1014) of all cases. As many as 34.7% of patients with positive chest

**Table 2:** Comparison of the (real time) reverse transcription polymerase chain reaction (rRT-PCR) diagnostic assay of the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) for diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

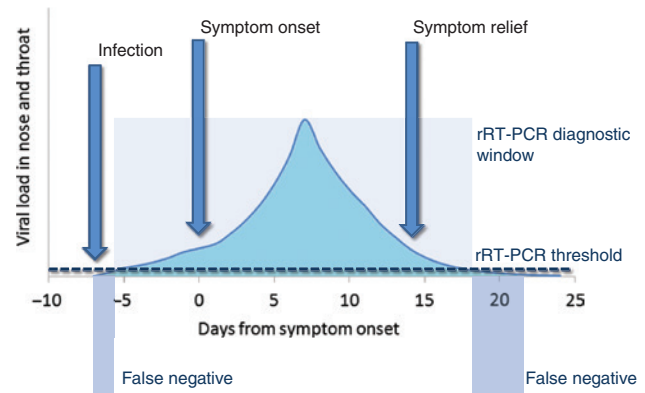
Test	Molecular targets	Scope	Limit of blank	Reference specimens	Storage conditions
WHO	E gene	First-line screening	3.9 copies × reaction	Nasopharyngeal AND oropharyngeal swab or wash in ambulatory patients, lower respiratory specimens (sputum and/or endotracheal aspirate or bronchoalveolar lavage)	≤5 days: 2–8 °C >5 days: ≤70 °C (dry ice)
	RdRp gene	Confirmatory testing	3.6 copies × reaction		
	N gene	Additional confirmatory testing	N/A		
CDC	N1/2/3 gene	Combined assay	1.0–3.2 copies/μL	Nasopharyngeal AND oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage and nasopharyngeal wash/aspirate or nasal aspirate	≤4 days: 4 °C >4 days: ≤70 °C
	RNase P gene	Control assay	N/A		

E gene, envelop gene; N gene, nucleocapsid gene; RdRp gene, RNA-dependent RNA polymerase gene; RNase P gene, human RNase P gene.

CT findings had negative RT-PCR results of throat swab samples. According to clinical history and serial CT features, 11.6% and 16.6% of all patients with initially negative RT-PCR results were finally considered as probable or highly likely COVID-19 cases. Importantly, as many as 93% of all patients whose RT-PCR became positive for SARS-CoV-19 after an initially negative test result had CT features suggestive of COVID-19, with a mean interval period of  $5.1 \pm 1.5$  days for turning positive.

The fact that RT-PCR testing may be initially negative in patients with SARS-CoV-2 infection, especially in those who will later develop overt COVID-19, is not really surprising considering the probable kinetics of SARS-CoV-2 infection. Reliable evidence suggests that the incubation period of SARS-CoV-2 is around 6 days (interquartile range [IQR], 2–11 days) [25], and that the median period between symptom onset and hospital admission is 7 days (IQR, 4–8 days) [26], whilst the median period of symptom duration is around 13 days (IQR, 5–24 days), slightly longer in patients with severe disease (16 days; IQR, 10–20 days) [27]. Convincing information is also accumulating from China and abroad in support of the evidence that human-to-human contagion may be relatively rare, but not impossible, during the non-symptomatic phase of SARS-CoV-2 infection, whereby the virus could be occasionally transmitted during incubation by patients with brief and non-specific illness, including children, in whom the severity of COVID-19 is usually milder than in adults [28–31]. This is supported by evidence that high viral loads, more in the nose than in the throat, can be detected soon after symptom onset, when the patient has not received a diagnosis of COVID-19 and has not been isolated, but can also be found in asymptomatic patients [32]. It is also worth mentioning here that virus shedding in some patients may continue for some days after symptom relief and recovery [33, 34]. Notably, a very recent study showed that the communicable period (expressed as first time of SARS-CoV-2 positive to date of virus clearance) in patients infected by SARS-CoV-2 was 6 days (IQR, 2–12 days) in subjects without symptoms compared to 12 days (IQR, 12–14) in those who became instead symptomatic [35].

Therefore, combining this epidemiologic evidence with the analytical sensitivity of the currently used RT-PCR assays, it is not surprising that at least two gray zones could be identified, potentially plagued by false SARS-CoV-2 negativity attributable to the low viral loads especially in asymptomatic or mildly symptomatic patients (Figure 1). The former would correspond to the initial phase of infection, when the patient is still completely asymptomatic or only mildly symptomatic. Virus shedding may have already



**Figure 1:** Correspondence between development of viral load during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, clinical course and positivity of (real time) reverse transcription polymerase chain reaction (rRT-PCR) assays.

initiated during this period, thought its extent would probably be too low to be identified by some RT-PCR assays (Figure 1). The second period would instead reflect the tail of SARS-CoV-2 infection, when there is symptom relief. In this final phase of the infection, virus shedding may still persist, though remaining below the analytical sensitivity of some RT-PCR assays (Figure 1).

Another important concern, which has recently been highlighted, is the risk of active recombination and mutations, which are attributable to the error-prone RNA-dependent RNA polymerases of coronaviruses. Shen et al. recently found a remarkable level of viral diversity in some infected patients, accounting for a median number of 4 intra-individual viral variants, which is suggestive of the rapid evolution of SARS-CoV-2 [36]. In another study, Yi detected as many as five different SARS-CoV-2 haplotypes, a fact that usually reflects active genetic recombination [37]. Such a viral evolution not only would explain the heterogeneity observed in intra-individual immune response, virulence, pathogenicity and transmissibility [38, 39], but the risk of mutation rate changes may also compromise the accuracy of RT-PCR detection.

Beside these microorganism-related issues, and like other areas of diagnostic testing [40], the accuracy of RT-PCR can be substantially plagued by lack of harmonization (of primers and probes) [41], as well as by a variety of technical and analytical errors, as summarized in detail elsewhere [13–15]. In brief, these typically encompass instrument malfunctioning (including inappropriate PCR cycling conditions), use of insufficient or inadequate material, non-specific annealing of PCR to homologous sequences, misinterpretation of expression profiles and so forth.

## Conclusions

Although the COVID-19 emergency, which has now become pandemic, is remarkably harnessing the usage of laboratory resources for diagnosing SARS-CoV-2 infection, the safety and quality of RT-PCR testing remain of paramount importance for providing accurate and interpretable results, irrespective of whether the tests are carried out using conventional laboratory analyzers or with portable molecular diagnostic instrumentation (Table 3) [42]. Unfortunately, it cannot be excluded that the quality of RT-PCR testing for detecting SARS-CoV-2 could be jeopardized by a number of preanalytical and analytical factors. Some of these are common to other diagnostic areas (e.g. identification errors, collection, handling and storage of the specimen, sample quality, performance of the assay or of the equipment), whilst others are very specific and shall hence be more distinctively pursued (e.g. virus-specific diagnostic window, sample contamination, incorrect nucleotide incorporation, non-specific PCR annealing and so forth) (Table 1).

The occurrence of discrepant results between chest CT and RT-PCR described in some studies, along with the evidence that virus shedding may still occur at undetectable levels in the very early and late phases of SARS-CoV-2 infection (Figure 1), would lead us to conclude that RT-PCR test results shall always be interpreted in a broader context. The evidence emerged from preliminary studies, demonstrating that asymptomatic (subclinical)

**Table 3:** Practical indications to minimize the risk of diagnostic errors in identifying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

---

Combine results of SARS-CoV-2 RT-PCR infection with
– Clinical and epidemiologic evidence (probability of exposure, signs, symptoms, negative diagnostic tests especially for other respiratory illnesses)
– Chest computed tomography (CT; most frequently appear with ground-glass opacities, consolidation with or without vascular enlargement, air bronchogram signs, interlobular septal thickening)
Recollect and test upper respiratory specimens in patients with negative RT-PCR test results and high suspicion or probability of SARS-CoV-2 infection
Provide clear instructions on how nasopharyngeal and oropharyngeal swabs shall be correctly collected, managed and stored
Thorough compliance with assay procedures, including quality assurance
Validate extensively RT-PCR assay before clinical usage
Further refinement of molecular target(s)

---

rRT-PCR, (real time) reverse transcription polymerase chain reaction.

COVID-19 patients may show very early but paradigmatic CT changes even before positive RT-PCR [23, 24, 43], would also support the advice that the most efficient strategy for diagnosing COVID-19 in suspected patients shall encompass a combination of SARS-CoV-2 RT-PCR with clinical and epidemiologic evidence (probability of exposure, signs, symptoms, negative diagnostic tests especially for other respiratory illnesses) and chest CT findings, whilst repeated respiratory specimens shall be collected (daily or, at least, every other day) and tested by RT-PCR in patients with initially negative results and high suspicion (or probability) of having COVID-19. This practice has also been recently endorsed by the US Food and Drug Administration (FDA), which concluded that a negative RT-PCR test result does not completely rule out SARS-CoV-2 infection and shall not be used as single element for patient management decisions, and re-testing shall be considered in consultation with public health authorities [44]. Clear instructions on how the specimens, especially nasopharyngeal and oropharyngeal swabs, shall be collected, managed and stored before testing shall then be provided to the healthcare personnel [45]. The assay procedures must be thoughtfully followed, including standard confirmatory testing and test report guidelines, and quality assurance carried out to validate each analytical session [46]. External quality assessment (EQA) schemes shall be established as soon as possible for purposes of monitoring analytical quality and harmonizing the assays. Despite the urge to provide high throughput and short turnaround time for diagnosing SARS-CoV-2 infection, extensive validation of RT-PCR assay is compellingly needed to enable the adoption of the most appropriate public health measures on individual and population bases. Finally, further refinement of molecular target(s) would also be needed, in order to identify regions of viral genome that may enable to reach the highest possible diagnostic accuracy [46, 47].

**Research funding:** None declared.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Competing interests:** Authors state no conflict of interest.

## References

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020. doi: 10.1038/s41564-020-0695-z. [Epub ahead of print].

2. World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report – 48. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>. Last accessed, March 9, 2020.
3. Lippi G, Plebani M. A Six-Sigma approach for comparing diagnostic errors in healthcare-where does laboratory medicine stand? *Ann Transl Med* 2018;6:180.
4. Sheridan C. Coronavirus and the race to distribute reliable diagnostics. *Nat Biotechnol* 2020. Doi: 10.1038/d41587-020-00002-2.
5. Lippi G, Plebani M, Graber ML. Building a bridge to safe diagnosis in health care. The role of the clinical laboratory. *Clin Chem Lab Med* 2016;54:1–3.
6. Lippi G, Plebani M. A modern and pragmatic definition of Laboratory Medicine. *Clin Chem Lab Med* 2018;56:1846–63.
7. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. *Clin Chem Lab Med* 2020;58:1131–4.
8. Lippi G, Plebani M. The novel coronavirus (2019-nCoV) outbreak: think the unthinkable and be prepared to face the challenge. *Diagnosis (Berl)* 2020;7:79–81.
9. Pang J, Wang MX, Ang IY, Tan SH, Lewis RF, Chen JI, et al. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. *J Clin Med* 2020;9. pii: E623. doi: 10.3390/jcm9030623.
10. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res* 2020;7:4.
11. Lippi G, Simundic AM, European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). The EFLM strategy for harmonization of the preanalytical phase. *Clin Chem Lab Med* 2018;56:1660–6.
12. Lippi G, von Meyer A, Cadamuro J, Simundic AM. PREDICT: a checklist for preventing preanalytical diagnostic errors in clinical trials. *Clin Chem Lab Med* 2020;58:518–26.
13. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev* 2006;19: 165–256.
14. Matos TR, de Rie MA, Teunissen MB. Research techniques made simple: high-throughput sequencing of the T-cell receptor. *J Invest Dermatol* 2017;137:e131–8.
15. van Zyl G, Maritz J, Newman H, Preiser W. Lessons in diagnostic virology: expected and unexpected sources of error. *Rev Med Virol* 2019;29:e2052.
16. Lippi G, Lima-Oliveira G, Brocco G, Bassi A, Salvagno GL. Estimating the intra- and inter-individual imprecision of manual pipetting. *Clin Chem Lab Med* 2017;55:962–6.
17. Centers for Disease Control and Prevention. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19). <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>. Last accessed, March 9, 2020.
18. Irving SA, Vandermause MF, Shay DK, Belongia EA. Comparison of nasal and nasopharyngeal swabs for influenza detection in adults. *Clin Med Res* 2012;10:215–8.
19. Spencer S, Gaglani M, Naleway A, Reynolds S, Ball S, Bozeman S, et al. Consistency of influenza A virus detection test results across respiratory specimen collection methods using real-time reverse transcription-PCR. *J Clin Microbiol* 2013;51:3880–2.
20. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020;25. doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
21. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim guidance 2 March 2020. <https://apps.who.int/iris/rest/bitstreams/1271387/retrieve>. Last accessed, March 9, 2020.
22. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. <https://www.fda.gov/media/134922/download>. Last accessed, March 9, 2020.
23. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing. *Radiology* 2020:200343. doi: 10.1148/radiol.2020200343. [Epub ahead of print].
24. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology* 2020:200642. doi: 10.1148/radiol.2020200642. [Epub ahead of print].
25. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. *Euro Surveill* 2020;25. doi: 10.2807/1560-7917.ES.2020.25.5.2000062.
26. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *J Am Med Assoc* 2020. doi: 10.1001/jama.2020.1585. [Epub ahead of print].
27. Young BE, Ong SW, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *J Am Med Assoc* 2020. doi: 10.1001/jama.2020.3204. [Epub ahead of print].
28. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med* 2020;382:970–1.
29. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. *J Am Med Assoc* 2020. doi: 10.1001/jama.2020.2565. [Epub ahead of print].
30. Tong ZD, Tang A, Li KF, Li P, Wang HL, Yi JP, et al. Potential presymptomatic transmission of SARS-CoV-2, Zhejiang Province, China, 2020. *Emerg Infect Dis* 2020;26. doi: 10.3201/eid2605.200198. [Epub ahead of print].
31. Kam KQ, Yung CF, Cui L, Lin Tzer Pin R, Mak TM, Maiwald M, et al. A well infant with coronavirus disease 2019 (COVID-19) with high viral load. *Clin Infect Dis* 2020. pii: ciaa201. doi: 10.1093/cid/ciaa201. [Epub ahead of print].
32. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 2020. doi: 10.1056/NEJMc2001737. [Epub ahead of print].
33. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR test results in patients recovered from COVID-19. *J Am Med Assoc* 2020. doi: 10.1001/jama.2020.2783. [Epub ahead of print].
34. Ling Y, Xu SB, Lin YX, Tian D, Zhu ZQ, Dai FH, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J (Engl)* 2020. doi: 10.1097/CM9.0000000000000774. [Epub ahead of print].

35. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci China Life Sci* 2020. doi: 10.1007/s11427-020-1661-4. [Epub ahead of print].
36. Shen Z, Xiao Y, Kang L, Ma W, Shi L, Zhang L, et al. Genomic diversity of SARS-CoV-2 in Coronavirus Disease 2019 patients. *Clin Infect Dis* 2020. pii: ciaa203. doi: 10.1093/cid/ciaa203. [Epub ahead of print].
37. Yi H. 2019 novel coronavirus is undergoing active recombination. *Clin Infect Dis* 2020. pii: ciaa219. doi: 10.1093/cid/ciaa219. [Epub ahead of print].
38. Sun P, Qie S, Liu Z, Ren J, Li K, Xi J. Clinical characteristics of 50466 hospitalized patients with 2019-nCoV infection. *J Med Virol* 2020. doi: 10.1002/jmv.25735. [Epub ahead of print].
39. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). *J Gen Intern Med* 2020. doi: 10.1007/s11606-020-05762-w. [Epub ahead of print].
40. Lippi G, Betsou F, Cadamuro J, Cornes M, Fleischhacker M, Fruekilde P, et al. Preanalytical challenges – time for solutions. *Clin Chem Lab Med* 2019;57:974–81.
41. Spackman E, Cardona C, Muñoz-Aguayo J, Fleming S. Successes and short comings in four years of an international external quality assurance program for animal influenza surveillance. *PLoS One* 2016;11:e0164261.
42. Zidovec Lepej S, Poljak M. Portable molecular diagnostic instruments in microbiology: current status. *Clin Microbiol Infect* 2019. pii: S1198-743X(19)30502-6. doi: 10.1016/j.cmi.2019.09.017. [Epub ahead of print].
43. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;395:514–23.
44. Food and Drug Administration. New York SARS-CoV-2 Real-time RT-PCR Diagnostic Panel – February 29, 2020. <https://www.fda.gov/media/135662/download>. Last accessed, March 9, 2020.
45. World Health Organization. Collecting, preserving and shipping specimens for the diagnosis of avian influenza A (H5N1) virus infection. [https://www.who.int/csr/resources/publications/surveillance/CDS\\_EPR\\_ARO\\_2006\\_1.pdf](https://www.who.int/csr/resources/publications/surveillance/CDS_EPR_ARO_2006_1.pdf). Last accessed, March 9, 2020.
46. Sharfstein JM, Becker SJ, Mello MM. Diagnostic testing for the novel coronavirus. *J Am Med Assoc* 2020. Doi:10.1001/jama.2020.3864.
47. Kim S, Kim D, Lee B. Insufficient sensitivity of RNA dependent RNA polymerase gene of SARS-CoV-2 viral genome as confirmatory test using Korean COVID-19 cases. Preprints 2020, 2020020424 (doi: 10.20944/preprints202002.0424.v1).