COMMENTARY

Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring

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In December 2019, an acute febrile illness with severe respiratory distress syndrome started to occur in Wuhan, Hubei Province, China.[1-3] Most patients developed patchy to diffuse infiltration of the lungs radiographically[2] (Figure 1). It was found that most early cases were linked to a local fresh seafood market, the Huanan Seafood Market, although some—particularly later cases—were not.[2] Initial investigations identified a novel bat coronavirus as a possible culprit, which was subsequently confirmed by detection of viral sequences by multiple teams independently, and by virus isolation from the lower respiratory tracts of patients.[3-5] The novel virus has been named novel coronavirus (nCoV)-2019. Genomic sequences of viruses from five cases are shown to be almost identical to one another, with the homology of 79.5% to severe acute respiratory syndrome (SARS)-CoV and 85% to 96% to a bat SARS-like coronavirus (bat-SL-CoVZC45) at the whole genome level.[4] The outbreak thus represents a newly emerging viral disease due to species jumping of a virus to humans.

Initially, it was thought that the infection showed no or limited human-to-human transmission. However, soon after it became evident that transmission among humans constitutes the main mechanism of infection spread, in community, health care facilities, and at home. The diagnoses were made purely based on clinical and radiographical grounds, lack of response to antibiotic therapy, and exclusion of other common respiratory infections for the season.[1-3] With the newly identified genetic sequence information, diagnostic tests based on detection of the viral sequence by reverse-transcriptase polymerase chain reaction (RT-PCR) or next-generation sequencing platforms soon became available. This allowed for diagnosis confirmation and better estimates of infection activity, which was found to be increasing at alarming speeds. The municipal government issued a public warning and implemented a total shutdown of transportation on 23 January 2020, advising individual isolation.

On 30 January 2020, a World Health Organization panel declared this outbreak a public health emergency of international concern. As of 31 January 2020, the confirmed cases reached 9811 nationwide, and 15 238 suspected cases (Figure 2).

1 | CLINICAL ASPECTS AND DIAGNOSIS

Clinically, patients with novel coronaviral pneumonia are characterized by fever with or without chills, dry cough, chest tightness, and shortness of breath.[1-3] Chest computerized tomography (CT) scan shows patchy to diffuse interstitial infiltrates or thickening (Figure 1), some with the characteristic ground-glass-like density. Very severe cases can develop radiographic changes of the so-called "white lungs." Laboratory tests show lowered lymphocytes and white blood cell counts in most patients, among other abnormalities.[2] The China National Health Commission communicated guidelines[6] for initial diagnosis and disease severity triage into common (mild), severe, and critical categories. Diagnosis is based on epidemic exposure, plus two of the following clinical findings: fever, radiographic features as described above, normal or lowered white blood cells, or reduced lymphocyte count.

The common (mild) cases are those with fever, respiratory symptoms, and pneumonia on chest radiography. Severe cases need to meet one of the following criteria: (a) respiratory distress, RR ≥ 30/minutes; (b) resting blood oxygen saturation ≤ 93%; or (c) arterial blood oxygen partial pressure (PaO2)/FiO2 ≤ 300 mm Hg. Critical cases meet one of the following: (a) respiratory failure needing mechanical oxygenation; (b) shock; or (c) development of other organ failures, requiring intensive care unit care. Around 70% to 80% of patients are mild, and 20% to 30% are severe or critical (Peng ZY, personal communication).
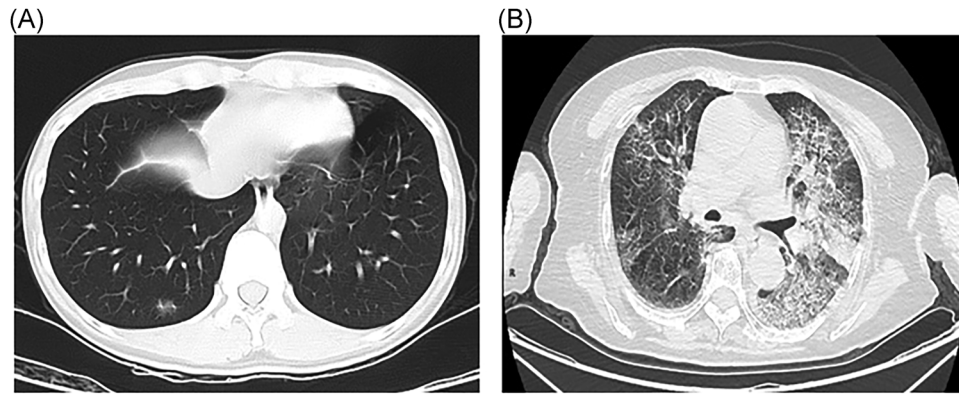
FIGURE 1   CT images of patients with novel coronavirus pneumonia. A, Patchy ground-glass-like density change from a 33-year-old woman who developed a mild illness after exposure at work. B, Bilateral diffuse thickening of interlobular septa with network-like densities, bronchiolar thickening, and consolidation of the left lower lobe from an 83-year-old woman. She presented with fever, chills, and sore throat; she had dry cough, chest tightness, and shortness of breath for a week. Her body temperature at presentation was 38.8°C, CBC showed WBC 4.6×10^9/L, neutrophilic differential 65.8%, and lymphocytic differential 19.9% (reduced). (Courtesy of Prof Haibo Xu). CBC, complete blood count; CT, computed tomography; WBC, white blood cells

The diagnosis needs to be differentiated from influenza, parainfluenza, adenovirus, respiratory syncytial virus, rhinovirus, SARS coronavirus, mycoplasma, chlamydia, and bacterial pneumonia, as well as noninfectious diseases such as vasculitis.[2,6]

2 | DIAGNOSIS USING THE RNA-BASED ASSAYS

Currently, the diagnosis of suspected cases is confirmed by RNA tests with real-time RT-PCR or next-generation sequencing. It had been shown that viral RNA can be detected from the nasal and pharyngeal swab, bronchoalveolar lavage, and blood plasma using RT-PCR targeting the NP gene of the virus.[2,4,6] Before the Wuhan city-wide shutdown, specimens for confirmatory tests had to be sent to China CDC, with a long turnaround time. Subsequently, several main tertiary care hospitals in Wuhan were authorized to perform the tests. Therefore, in the earlier stages of the outbreak, a very limited number of patients were tested and confirmed for the diagnosis.

Since 25 January tests have become increasingly available for clinically suspected patients, with a history of exposure, fever, and positive findings on chest CT. However, since only a limited number of tests can be offered each day due to limited supplies and lab facilities, only a portion of the targeted population received tests. For example, during the month of January 2020, only 1700 specimens were tested (media report by the hospital on WeChat, 1/29/2020), compared with the vast number of clinic patients during that period (between 22 January and 30 January alone, there were over 7000 visits to one of the designated fever clinic). This created a significant backlog, as many patients had to wait for days to receive a confirmation or exclusion of diagnosis. It also led to problems in real-time reporting, causing artificial fluctuation in daily updates of new cases (Figure 2B). For example, as shown in Table 1, using data collected from the National Health Commission's daily updates,[7] on some dates, the change in the number of newly confirmed cases varies drastically. This cannot be explained by realistic changes in the speed of infection spread. Needless to say, the recorded figures of daily confirmed cases are likely underestimated.
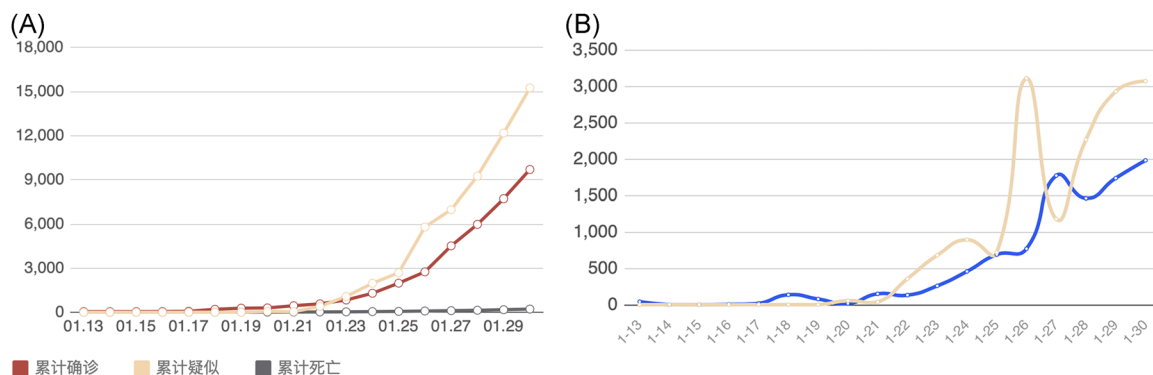

累计确诊   累计疑似   累计死亡

FIGURE 2   A, Daily update of accumulated suspected (yellow curve) and confirmed (red curve) nCov-2019 infections in China as of 31 January 2020. B, Daily update of newly confirmed (blue curve) and suspected cases (from China National Health Commission Bulletin)

TABLE 1 Daily update of newly confirmed and suspected cases in Hubei

	Hubei				Wuhan		
	Newly confirmed	Accumulated	Change from previous day	% Change	Newly confirmed	Change from previous day	% Change
20 Jan	72	270			60		
21 Jan	105	375	33	46	105	45	75
22 Jan	69	444	−36	−34	62	−43	−41
23 Jan	105	549	36	52	70	8	13
24 Jan	180	729	75	71	77	7	10
25 Jan	323	1052	143	79	46	−31	−40
26 Jan	371	1423	48	15	80	34	74
27 Jan	1291	2714	920	248	892	812	1015
28 Jan	840	3554	−451	−35	315	−577	−65
29 Jan	1932	4586	1092	130	356	41	13
30 Jan	1220	5906	−712	−37	378	22	6
31 Jan	1347	7153	127	10	576	198	52

Another concern related to the nucleic acid tests is that there has not been sufficient time to assess their sensitivity and specificity. Based on personal communications with colleagues, a significant portion of patients who otherwise fit the diagnosis based on clinical and chest CT findings, including many hospitalized patients, have tested negative for viral RNA. Other common respiratory etiologies, such as influenza, were excluded. These remain "suspected" cases and may be reflective of false negativity in sampling. In some patients, the virus may be present in the lower respiratory secretion but absent in the upper respiratory tracts.

With the current tests, it is therefore difficult to achieve a meaningful assessment of what proportion of symptomatic patients are infected.

3 | SEROLOGICAL ASSAYS ARE NEEDED

Data from the SARS epidemic show that serological responses, including viral-specific immunoglobulin M (IgM) and IgG, can allow for serologic diagnosis.[8,9] Most recently, it was shown that patients with 2019-nCov pneumonia also possessed similar acute serological responses.[4] Using the bat SARSr-111 CoV Rp3 nucleocapsid protein (NP) as an antigen, both IgG and IgM antibodies could be detected with enzyme-linked immunoassay (ELISA) in these patients. The dynamic pattern is consistent with an acute viral infection, with the IgG concentration beginning to rise as IgM levels start to drop.[4]

As we know, the production of host antibodies to a specific virus during an acute phase infection is consistent in most patients, except for those with immunodeficiency. The IgM antibody can be detected as early as day 3 in many infections. The requirement for specimen quality is less stringent than for RNA-based assays. Whether viruses themselves are present in respiratory specimens, the presence of a specific antibody can be uniformly detected, avoiding false-negative

results due to sampling. Most importantly, with the regular 96-well microplate, and automatic ELISA devices, the test capacity is greatly increased compared to RNA-based molecular tests and can handle a large number of febrile patients such as in the current epidemic, with a quick turnaround time (2-3 hours). For example, hemorrhagic fever with renal syndrome caused by hantavirus was endemic in Hubei Province, with a high annual incidence in the 1980s and 1990s. The rapid and specific etiologic diagnosis was critical for patient management and epidemic controls. An IgM-capture ELISA was used to quickly screen all febrile patients effectively during those epidemic seasons.[10,11] Similarly, for the novel coronavirus pneumonia (NCVP) situation, an IgM-capture ELISA can offer earlier and more efficient confirmation or exclusion of the nCoV-2019 infection in patients with fever, thus adding to the accuracy of epidemiologic monitoring and facilitating proper isolation of patients.

From a technical point of view, although the NP can serve as a sensitive antigen, other 2019-nCoV-specific epitopes or antigens should be explored for use in the serology assay. It has been reported that the N protein of SARS CoV antigenically cross-reacted with antisera of antigenic group-I animal coronaviruses such as human coronavirus 229E, feline infectious peritonitis virus, and porcine transmissible gastroenteritis virus (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC404591/). Therefore, the use of the whole N protein as the antigen for serological assay could lead to specificity and sensitivity issues potentially.

During this current outbreak, one of the authors volunteered in one of the designated fever clinics. The scene was astonishing, with over a thousand of anxious patients with illnesses ranging from mild to severe, all lined up and waiting over 4 hours to be seen. The vast majority could be individuals without infection by nCoV-2019, yet all the protection they had was facial masks. One cannot imagine how much risk for cross infection can occur in such over-crowded situations. If some rapid viral diagnostic tests were available, the situation would be completely different. Patients could quickly have blood

drawn when they first arrive and wait elsewhere to receive the results. Or they could have blood drawn in their own communities (common residential areas called "Xiao Qu" in Wuhan, as in most other cities) to avoid a trip to the clinics and high potential for nCoV-2019 exposure. Coincident with the 2019-nCoV outbreak is the season for several other common respiratory etiologies, such as influenza and the common cold. After the city-wide shutdown and heightened public warning, there was a surge of clinic patients. Many patients would visit several clinics or the same clinic daily, trying to be selected for RNA tests. A significant number of them had symptoms or lab findings that are not consistent with NCVP, such as elevated lymphocytes and lack of chest CT findings. With a serological test, these patients could avoid the clinic visits.

Currently, RNA-based molecular tests require upscale lab facilities with restrictive biosafety levels and technical sophistication, and expensive. The screening patient population is in both large medical centers or smaller community-level hospitals. Serologic tests can be easily implemented in the clinical laboratory of any hospital, thus with a much wider application than molecular tests.

Another issue for consideration is reporting of asymptomatically infected cases, or very mild cases of infection who are a large group of patients but not tested for viral RNA (which is impractical), therefore making the true rate of infection in the population unknown. With the development of a specific IgG antibody test, a large-scale sero-epidemiological study can be conducted after the end of the current outbreak, so that we can understand the true scale of human-to-human transmission of the novel coronavirus of 2019.

A fast-performing serologic assay is acutely needed for the current and future outbreaks.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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