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Review Article

Laboratory Diagnosis of COVID-19

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ABSTRACT

The outbreak of COVID-19 caused by severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has threatened health globally. Rapid and early laboratory diagnoses of COVID-19 is the main focus of treatment and control. Molecular tests are the basis of confirmatory tests of COVID-19, but serological tests are largely available and play an important role in understanding the epidemiology of the virus and identifying populations at higher risk for infection.

Laboratory diagnostic tests for COVID-19 should be readily available, accurate and fast highly sensitive and specific methods. This write up reviews the current laboratory methods available for testing coronaviruses by focusing on the coronavirus disease 2019 (COVID-19) outbreak going on in Wuhan. A nasopharyngeal swab is usually collected to obtain a specimen.

Keywords: COVID-19, Serology, POCT, Real-time PCR.

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INTRODUCTION

A novel corona virus caused a outbreak of pneumonia began from Wuhan, Hubei province, China. It was identified as the seventh corona virus, becoming the third zoonotic human corona viruses.

Coronaviruses belong to the family *Corona viridae* and were characterized as HCoV-229E and HCoV-OC43.[1] The latest coronavirus to emerge in humans appeared in Wuhan City, Hubei Province, China in December 2019[5,6] and has been designated SARS-CoV-2.[7]

CLINICAL AND PUBLIC HEALTH SIGNIFICANCE

Respiratory symptoms including cough and dyspnea usually develop from several days to a week after illness onset. Atypical pneumonia and respiratory deterioration occur in 20-30% of cases. The incubation period is 5.2 days.[8] Fever and cough are frequently reported early in the course of illness.[9,10] Infections are also characterized by dyspnea, respiratory distress and positive chest X-ray.10 Lower respiratory symptoms often develop about 1 week from onset of initial symptoms.[11]

MORBIDITY AND MORTALITY

As of September 23rd 2020, 23,084,982 cases and approximately. 981,217 deaths from COVID-19 have been recorded worldwide. Many countries have adopted drastic measures such as physical distancing and lockdowns in an attempt to mitigate the COVID-19 pandemic. Of countries and continents outside of China, United States, India, Brazil, Italy, Spain, South Korea and Iran have experienced a high number of COVID-19 cases. Mortality is highest in older persons, with median age of 59-75 years.[12,13] All pediatric cases with laboratory- confirmed SARS-CoV-2 infection were mild cases with no deaths reported.[14]

The Chinese Centers for Disease Control and Prevention team analyzed more than 72,000 patient records, of which 44,672 were laboratory-confirmed cases, 16,186 suspected cases, 10,567 clinically diagnosed cases, and 889 asymptomatic cases. Of the confirmed cases, about 14% of the illnesses were severe, which included pneumonia and shortness of breath, and about 5% have critical disease, marked by respiratory failure, septic shock, and multi-organ failure. The overall case fatality rate was 2.3%, and of 1,023 deaths included in the study, the majority were in people age 60 and older or those with underlying medical conditions (www.cidrap.umn.edu/news-perspective/2020/02/more-outbreak-details-emerge-COVID-19-cases-top-70000.Accessed18-02-2020)

Table 1: Advantages and disadvantages of the laboratory diagnostic methods for SARS-CoV-2

Diagnostic approach	Method	Testing scenarios	Advantages	Disadvantages
Neutralization tests	VNT and PVNT	BSL-2 or BSL-3 laboratories, pathogen laboratories	Authoritative, simple, low cost, reliable, high sensitivity	Time-consuming, long-period, laborious, perform in BSL-3 or BSL-2 lab
PCR	qRT-PCR	BSL-2 laboratories, public health institutes, quarantine depots	High specificity, not require expensive equipment, timesaving	Complex pretreatment steps, requires skillful, false negative
	Nested RT-PCR	BSL-2 laboratories, prefectural and municipal public health institutes, quarantine depots	High sensitivity, specificity was higher than that of RT-PCR, suitable for detect low-copy-number viruses, time-saving	Complex pretreatment steps, requires skillful, manpower, the second PCR amplification may cause cross-contamination
	ddPCR	BSL-2 laboratories, public health institutes, quarantine depots	Quantitative, sensitive, suitable for detect samples with low viral load, independent of a traditional standard curve	Susceptible to exogenous contamination, expensive than qRT-PCR, calibrant materials need to be defined
	Nanoparticles - based amplification	BSL-2 laboratories, environmental testing institutions	High sensitivity, adopted in fully automated RNA extraction systems, excellent RNA binding performances	Complex pretreatment steps, requires skillful, expensive than qRT-PCR, with the risk of photobleaching
	RT-LAMP	Basic laboratories, community nursing sites	Timesaving, thermostatic, sensitive, user-friendly, sophisticated equipment-free	Easy to be contaminated and cause false-positive, nonspecific amplification cannot be easily identified, requires skillful
	Portable benchtop-sized analyzers	Clinical laboratories, physicians' office, emergency departments	Automatic, portable, rapid, not requires trained staff	Inconsistent performance, may lack sensitivity in weakly positive samples
Immunological diagnostic	ELISA	Clinical laboratories, public health institutes	Quantitative detection, simple, a low risk of infection, convenient, stable reagent	Time-consuming, low sensitivity, cross-reactivity, expensive monoclonal antibody, low-throughput
	IFA	Clinical laboratories, pathogen laboratories, public health institutes	Avoid the interference of endogenous biotin and contamination of antigens in the blood	Non-specific fluorescence, subjective, low-throughput, time-consuming
	CLIA	Clinical laboratories, public health institutes	Automatic, rapid, quantitative, high sensitivity, broad linear range, stable results	Sophisticated instruments, high requirements for equipment and environment, not suitable for detect whole blood samples,
	LFA	Clinical laboratories, physicians' offices, emergency departments, community service stations	Rapid, convenient, on-site screening, inexpensive, small sample volume	Low sensitivity, cross-reactivity, inconsistent performance, not suitable for early diagnosis, low-throughput
	Microarray and microfluidic chip	Clinical laboratories, emergency departments, community service stations	Small size, high sensitivity, automatic, high-throughput, portable	Core technologies lack norms and standards, high cost, nonspecific binding of proteins
Genome sequencing	Metatranscriptomic sequencing	BSL-2 laboratories, genetic testing centres, research laboratories	Simple, reduce the cost, does not claim a reference sequence	Increase cost, sophisticated instruments, insufficient coverage and depth
	Nanopore targeted sequencing	BSL-2 laboratories, genetic testing centres, research laboratories	Broad detection range, rapid turnaround time, long-read, high-accuracy, monitor the variation	Increase cost, sophisticated instruments, requires skillful
	Amplicon se quencing	BSL-2 laboratories, genetic testing centres, research laboratories	Convenient, high sensitivity, suitable for detect samples with low viral load, economical	Sophisticated instruments, not be used to sequence highly diverse or recombinant viruses
	Hybrid capture - based sequencing	BSL-2 laboratories, genetic testing centres, research laboratories	High sensitivity, suitable for detect intra-individual variations	Sophisticated instruments, not be used to sequence highly diverse or recombinant viruses

Table 2: RT-PCR tests/primers for SARS-CoV-2 in different institutions

Institute	Gene target	Probe (5'-3')	Former primer (5'-3')	Reverse primer (5'-3')
China CDC	ORF1ab gene	FAM-CCGTCTGCGGTATGT GGA AAGGTTATGG-BHQ1	CCCTGTGGGTTTTACACTTA A	ACGATTGTGCATCAGCTGA
	N gene	FAM-TTGCTGCTGCTTGACAGA TT-TAMRA	GGGGAACTTCTCCTGCTAG AAT	CAGACATTTTGCTCTCAAGCTG
US CDC	N1 target	FAM-ACCCCGCATTAC GTT TGGTGGACC-BHQ1	GAC CCC AAA ATC AGC GAA AT	TCT GGT TAC TGC CAG TTG AAT CTG
	N2 target	FAM-ACAATTTGCCCCCAGCGC TTCAG-BHQ1	TTA CAA ACA TTG GCC GCA AA	GCG CGA CAT TCC GAA GAA'
	N3 target	FAM-AYCACATTGGCACCCGCA ATCCTG-BHQ1	GGG AGC CTT GAA TAC ACC AAA A	TGT AGC ACG ATT GCA GCA TTG
France Pasteur Institute	RdRP1 target	HEX-AGATGTCTTGTGCTGCCG GTA-BHQ1	ATGAGCTTAGTCCTGTTG	CTCCCTTTGTTGTGTTGT
	RdRP2 target	FAM-TCATACAAACCACGCCAG G-BHQ1	GGTAACTGGTATGATTTCG	CTGGTCAAGGTTAATATAGG
Japan National Institute of Infectious Disease	N gene	FAM-ATGTCGCGCATTGGCATG GA-BHQ	AAATTTTGGGGACCAGGAAC	TGGCAGCTGTGTAGGTCAAC
Germany Charité	RdRP gene	FAM-CAGGTGGAACCTCATCAG GAGATGC-BBQ	GTGARATGGTCATGTGTGG CGG	CARATGTTAAASACACTATTAGC ATA
	E gene	FAM-ACACTAGCCATCCTTACTGCGC TTCG-BBQ	ACAGGTACGTTAATAGTTAA TAGCGT	ATATTGCAGCAGTACGCACACA
Thailand National Institute of Health	N gene	FAM-CAACTGGCAGTAACCA-BQH1	CGTTTGGTGGACCCTCAGAT -	CCCCACTGCGTTCTCCATT
Hong Kong University	ORF1b-nsp14 gene	FAM-TAGTTGTGATGCWATCATGACT AG-TAMRA	TGGGGYTTTACRGGTAACCT	AACRCGCTTAACAAAGCACTC
	N gene	FAM-GCAAATTGTGCAATTTGCGG-TAMRA	TAATCAGACAAGGAACTGAT TA	CGAAGGTGTGACTTCCATG

Table 3: Current statistics for COVID-19 globally as of 23rd September 2020

Country	Total Cases	New Cases	Total Deaths	New Deaths	Total Recovered	Active Cases	Serious, Critical	Tot Cases/ 1M pop	Deaths/ 1M pop	Total Tests	Tests/ 1M pop
World	**32,082,948**	**312,877**	**981,217**	**6,262**	**23,657,313**	**7,444,418**	**62,402**	**4,116**	**126**		
USA	7,138,708	40,771	206,558	1,077	4,387,230	2,544,920	14,103	21,538	623	100,548,988	303,359
India	**5,730,184**	**89,688**	**91,173**	**1,152**	**4,671,850**	**967,161**	**8,944**	**4,143**	**66**	**66,279,462**	**47,920**
Brazil	4,627,780	32,445	139,065	906	3,992,886	495,829	8,318	21,736	653	17,900,000	84,074
Russia	1,122,241	6,431	19,799	150	923,699	178,743	2,300	7,689	136	43,600,000	298,734
Colombia	784,268	6,731	24,746	176	662,277	97,245	863	15,376	485	3,499,136	68,601
Peru	782,695	6,149	31,870	98	636,489	114,336	1,381	23,663	964	3,751,583	113,421
Mexico	705,263	4,683	74,348	651	506,732	124,183	2,672	5,457	575	1,604,845	12,417
Spain	693,556	11,289	31,034	130	N/A	N/A	1,436	14,833	664	11,820,505	252,796
South Africa	665,188	1,906	16,206	88	594,229	54,753	539	11,183	272	4,083,757	68,658
Argentina	664,799	12,625	14,376	424	525,486	124,937	3,511	14,678	317	1,815,738	40,090

Table 4: Current statistics for COVID-19 in India

Total Cases	5,730,184
Cases per Total Population	0.41%
Total Deaths	91,173
% Deaths per Total Cases	0.01%
Total Recovered	4,671,850
Recovery Rate	81.53%
Active Cases	967,161
Tot Cases/ 1M pop	4,143
Deaths/ 1M pop	66
Total Tests	66,279,462
Tests/ 1M pop	47,920

LABORATORY DIAGNOSIS

Specimen Collection: Samples for HCoVs are taken from. upper and lower respiratory sources including throat, nasal nasopharyngeal, sputum, and bronchial fluid.[15,16] The collection and testing of both upper and lower respiratory samples [sputum, bronchoalveolar lavage fluid (BAL)] is recommended.[17] Specimens collected for laboratory testing of HCoVs should be maintained at refrigerated temperature for up to 72 hours, or frozen at -70C or below.

Rapid Antigen Tests: In a pre-peer reviewed article, Diao et al. reported that a fluorescence immunochromatographic assay is an accurate, rapid, early and simple method for detecting

nucleocapsid protein of SARS-CoV-2 in nasopharyngeal swab for diagnosis of COVID-19 (www.medrxiv.org/content/10.1101/2020.03.07.20032524v2. Accessed 15 March 2020).

Serology: Serological assays, are important for understanding the epidemiology of emerging HCoVs, including the of asymptomatic infections.

Molecular Methods: Several RT-PCR protocols for detection of SARS-CoV-2 RNA have been posted by the World Health Organization at https://www.who.int/emergencies/diseases/novel-coronavirus- 2019/technical-guidance/laboratory-guidance.

CONCLUSIONS

The ID NOWTM (previously Alere i) Influenza A & B assay (Abbott, San Diego, CA) was cleared by the US Food and Drug Administration (FDA) for direct use on NPS. Similarly, the Xpert® Xpress Flu/RSV (Cepheid, Sunnyvale, CA) and cobas® Liat® Flu A/B & RSV (Roche Molecular Systems, Pleasanton, CA) assays are integrated nucleic acid extraction-independent devices that have received FDA clearance recently and CLIA-waiver for simultaneous detection and identification of FluA, FluB, and RSV in nasopharyngeal swabs.[18] The FilmArray® Respiratory EZ Panel (BioFire, Salt Lake City, UT) so far is only CLIA-waived syndromic panel that covers a set of 14 respiratory viral and bacterial pathogens including classical coronavirus species.[19]

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