CoVID-19 & Clinical Laboratory Tests

(Molecular, Serological & Routine) With emphasis on safety, specimen collection & interpretation

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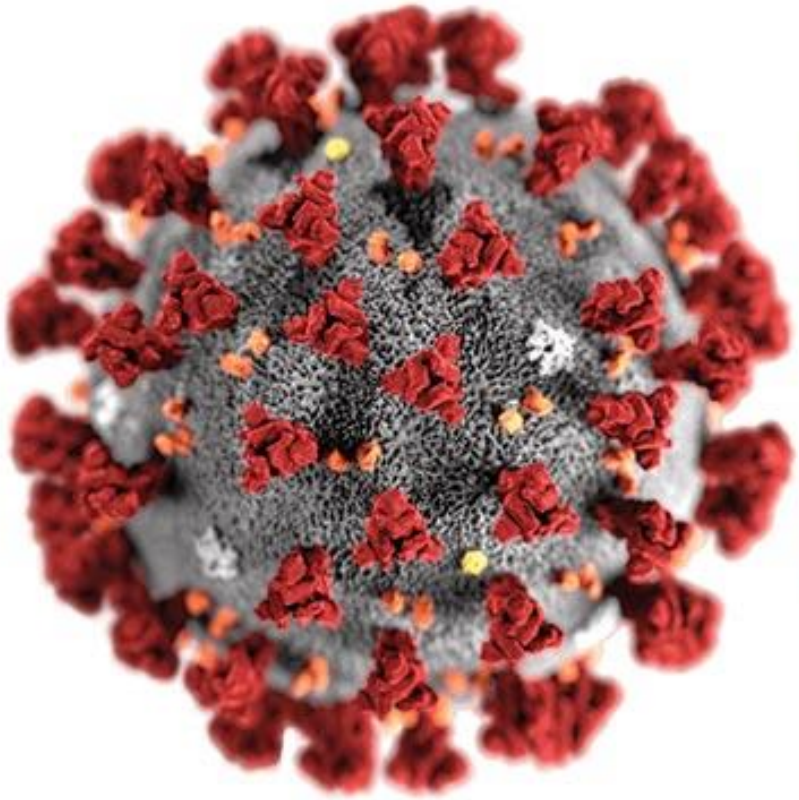


SARS-CoV-2

According to WHO

The disease caused by
Novel Coronavirus, SARS-CoV-2

is now officially called

COVID-19

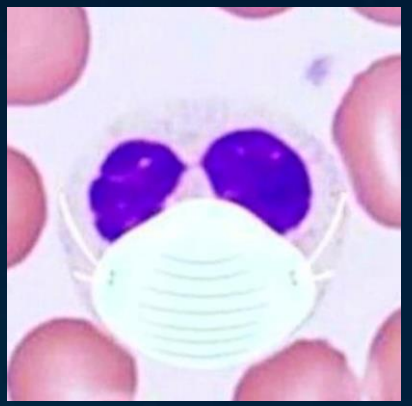
CO - Corona
VI - Virus
D - Disease

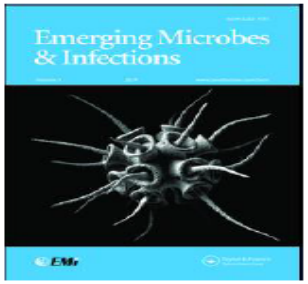
www.microbenotes.com

World Health Organization

Coronavirus Cases: 2,638,477
Deaths:184,248
Recovered:721,997

Coronavirus Cases: 85,996
Deaths: 5,391
Recovered: 63,113





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Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

Laboratory diagnosis of emerging human coronavirus infections – the state of the art

Michael J. Loeffelholz & Yi-Wei Tang

To cite this article: Michael J. Loeffelholz & Yi-Wei Tang (2020) Laboratory diagnosis of emerging human coronavirus infections – the state of the art, Emerging Microbes & Infections, 9:1, 747-756, DOI: 10.1080/22221751.2020.1745095

To link to this article: https://doi.org/10.1080/22221751.2020.1745095

DE GRUYTER

Clin Chem Lab Med 2020; aop

Opinion Paper

Giuseppe Lippi*, Ana-Maria Simundic[a] and Mario Plebani[a]

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

Opinion Paper

Giuseppe Lippi* and Mario Plebani

The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks

https://doi.org/10.1515/cclm-2020-0240
Received March 3, 2020; accepted March 4, 2020

Keywords: coronavirus; COVID-19; laboratory medicine; laboratory tests.

DE GRUYTER Clin Chem Lab Med 2020; aop

Opinion Paper

Giuseppe Lippi*, Ana-Maria Simundic[a] and Mario Plebani[a]

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

Recommendations for Minimal Laboratory Testing Panels in Patients with COVID-19: Potential for Prognostic Monitoring

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Clinical Chemistry 0:0
1–3 (2020)

Opinion

Emergence of a Novel Coronavirus Disease (COVID-19) and the Importance of Diagnostic Testing: Why Partnership between Clinical Laboratories, Public Health Agencies, and Industry Is Essential to Control the Outbreak

Matthew J. Binnicker

Introduction- History

Table 1. Human coronaviruses.

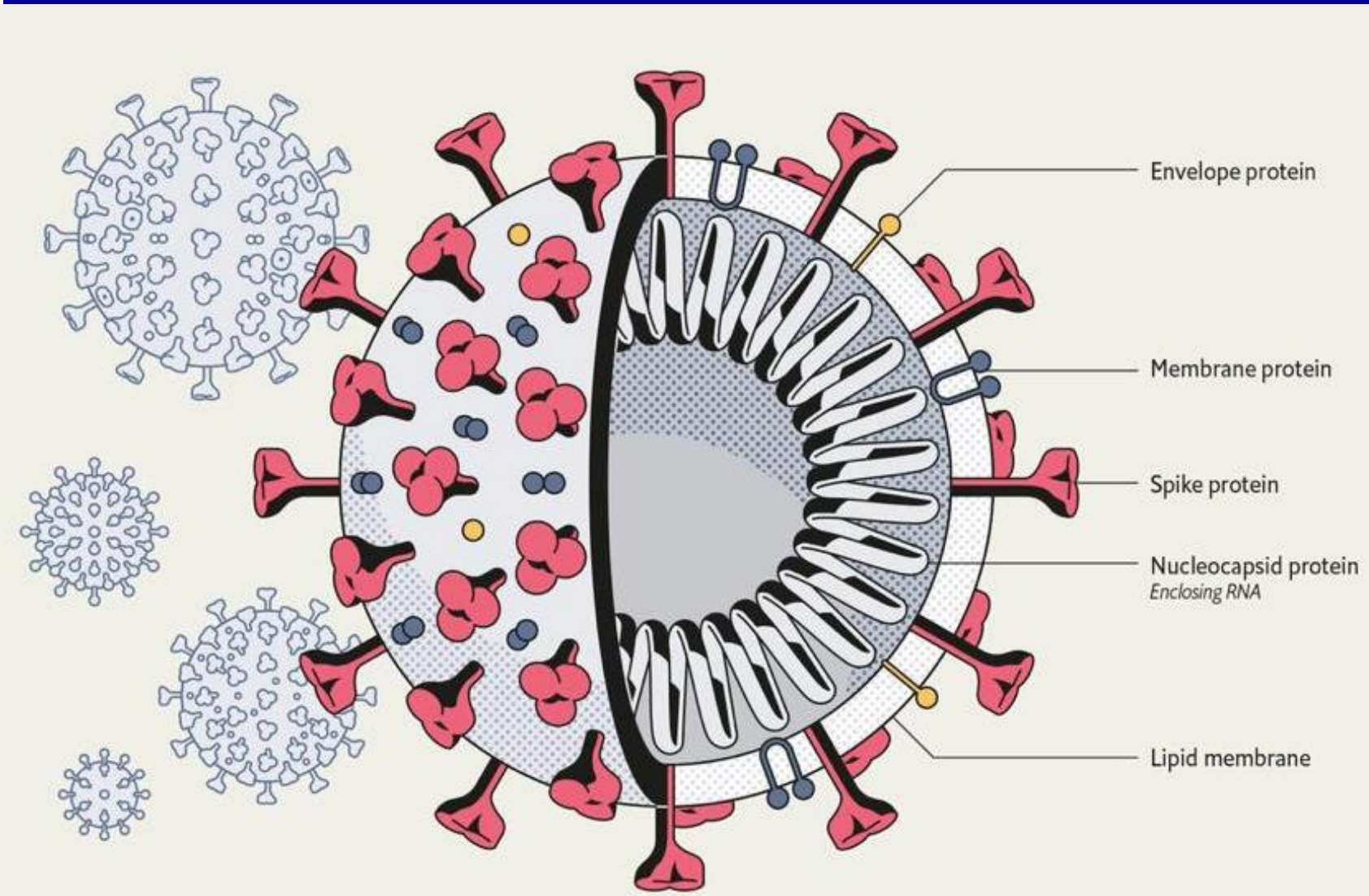
Virus	Genus	Disease	Discovered
CoV-229E	Alpha	Mild respiratory tract infection	1967
CoV-NL-63	Alpha	Mild respiratory tract infection	1965
CoV-HKU-1	Beta	Mild respiratory tract infection; pneumonia	2005
CoV-OC43	Beta	Mild respiratory tract infection	2004
SARS-CoV	Beta	Human severe acute respiratory syndrome, 10% mortality rate	2003
MERS-CoV	Beta	Human severe acute respiratory syndrome, 37% mortality rate	2012
SARS-CoV-2	Beta	Severe acute respiratory infections, <2% mortality rate	2019

88% sequence identity with bats, but distinct from SARS-CoV (79% sequence identify)

- HCoVs (HCoV-229E and HCoV-OC43) were first isolated in cell culture in the **1960**s from persons with upper respiratory infections.

Emerging Microbes & Infections, 2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1745095

Introduction-Virology



Clinical & Public Health Significance Epidemiology

- HCoVs are usually display a **winter seasonality**, although HCoV-229E has been detected sporadically throughout the year.

Endemic (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-

- HKU1), Endemic HCoVs are globally distributed & ***Human*** population restricted.

Epidemic (SARS- CoV, MERS-CoV and SARS-CoV-2). SARS-CoV and MERS-CoV are maintained in ***Zoonotic*** reservoirs.

Emerging Microbes & Infections, 2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1745095

Clinical & Public Health Significance Epidemiology

- The **effective reproductive number** (R; i.e. the average number of secondary cases per infectious case) has been estimated at 2.6 for SARS-CoV-2 compared to 1.1 for SARS-CoV,
- **Doubling time** of the epidemic has also been calculated as **3.6 days** (comprised between 1.0 and 7.7 days) compared to approx. 16 days for SARS-CoV.
- Future mortality projection of the WHO,**2016-2060**, whereby the number of deaths for lower respiratory infections is expected to increase by **over 50%** during the next 40 years (i.e. from 2.96 to 4.62 million deaths per year).

Clin Chem Lab Med 2020; aop
Lippi et al.: Laboratory diagnostics in COVID-2019 infection

Symptoms

- **Endemic HCoVs**, incubation period: **2–5 days** & **mild upper respiratory symptoms** (the "common cold"), among the most frequent cause of upper respiratory tract infections. Lower respiratory tract infections (bronchiolitis, pneumonia) are rare.
- **SARS-CoV**, incubation period: usually **4– 5 days**, often present with symptoms of fever, headache & myalgias. 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.

Emerging Microbes & Infections, 2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1745095

Symptoms

Communicable period:

- expressed as first time of SARS-CoV-2 positive to date of virus clearance was:
- **6 days** (IQR, 2–12 days) in subjects without symptoms
- **12 days** (IQR, 12–14) in those who became instead symptomatic
- It is also worth mentioning here that virus shedding in some patients may continue for some days after symptom relief and recovery

Emerging Microbes & Infections, 2020, VOL. 9
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Symptoms

The largest cohort of >44,000 persons with COVID-19 from China :

- **Mild to moderate** (mild symptoms up to mild pneumonia): **81%**
- **Severe** (dyspnea, hypoxia, or >50% lung involvement on imaging): **14%**
- **Critical** (respiratory failure, shock, or multiorgan system dysfunction): **5%**

- Fever (83–99%)
- Cough (59–82%)
- Fatigue (44–70%)
- Anorexia (40–84%)
- Shortness of breath (31–40%)
- Sputum production (28–33%)
- Myalgias (11–35%)

CDC

Symptoms

When to Seek Medical Attention

If you develop any of these **emergency warning signs*** for COVID-19 get **medical attention immediately:**

- Trouble breathing
- Persistent pain or pressure in the chest
- New confusion or inability to arouse
- Bluish lips or face

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

Diagnosis

1. Clinical Diagnosis

a) History & Physical examination

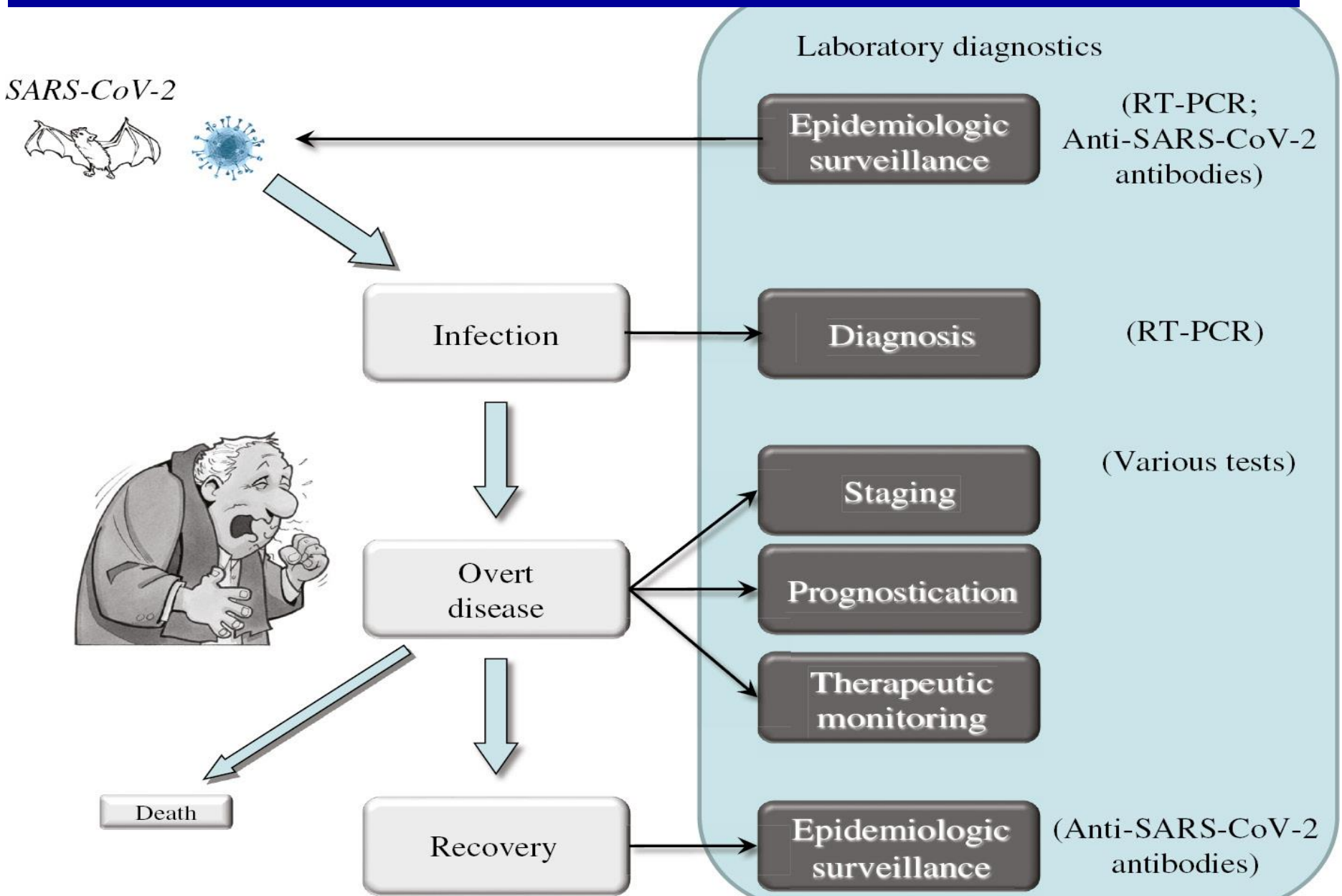
b) CT imaging examination,

The imaging finding vary with the patient`s age, immunity status, disease stage at the time of scanning, underlying diseases, & drug interventions.

2. Laboratory Diagnosis, Should be distinguished from:

- Other known viral virus of pneumonia, such as influenza A, B viruses, parainfluenza, adenovirus, RSV, rhinovirus, SARS-CoV,…
- Mycoplasma pneumonia, chlamydia pneumonia, & bacterial pneumonia.
- Non-infectious diseases, such as vasculitis, dermatomyositis, …

The essential role of laboratory diagnostics in SARS-CoV-2 infection



The essential role of Clinical Laboratory

1. Etiological diagnosis,

the first and most obvious setting where laboratory diagnostics plays an essential role.

2. Patient monitoring,

Staging, Prognostication & Therapeutic monitoring.

RT-PCR tests & many other laboratory tests may help assessing **disease severity** and ***predicting the risk of evolution toward ARDS***, **DIC** and/ or **MOF**(Multi Organ Failure).

3. Surveillance,

Identification of anti-SARS-CoV-2 antibodies, both IgG & IgM may hence enable to gain valuable epidemiological data in the fight against this viral epidemic.

Etiological diagnosis

- Real-time Reverse Transcriptase PCR (**rRT-PCR**) is ***the most common*** method to diagnose COVID-19, mainly targeting various combinations of following genes:
  - **Open reading frame** (**Orf**),
  - **Envelope** (**E**),
  - **Nucleocapsid** (**N**),
  - **RNA-dependent RNA polymerase** (**RdRp**)

genes

Priorities for Testing Patients with suspected CoVID-19 Infection

COVID-19 Symptoms: Fever, Cough, and Shortness of Breath

PRIORITY 1

Ensures optimal care options for all hospitalized patients, lessen the risk of healthcare-associated infections, and maintain the integrity of the healthcare system

- Hospitalized patients
- Healthcare facility workers with symptoms

Priorities for Testing Patients with suspected CoVID-19 Infection

PRIORITY 2

Ensures those at highest risk of complication of infection are rapidly identified and appropriately triaged

- Patients in long-term care facilities with symptoms
- Patients 65 years of age and older with symptoms
- Patients with underlying conditions with symptoms
- First responders with symptoms

Priorities for Testing Patients with suspected CoVID-19 Infection

PRIORITY 3

- **As resources allow, test individuals in the surrounding community of rapidly increasing hospital cases to decrease community spread, and ensure health of essential workers**
- Critical infrastructure workers with symptoms
- Individuals who do not meet any of the above categories with symptoms
- Healthcare facility workers and first responders
- Individuals with mild symptoms in communities experiencing high numbers of COVID-19 hospitalizations

NON-PRIORITY

- Individuals without symptoms

Molecular Methods: rRT-PCR

1. China CDC Method for detection 2019-nCoV (posted on 24 January 2020)

- Target 1 （*ORF1ab*） Target 2 （*N*）

2. Institute Pasteur Method, Paris

- Two RdRp targets (IP2 & IP4)

As a confirmatory assay, the E gene assay from the Charité protocol, Originally proposed by the Charité-Universitätsmedizin Berlin Institute of Virology

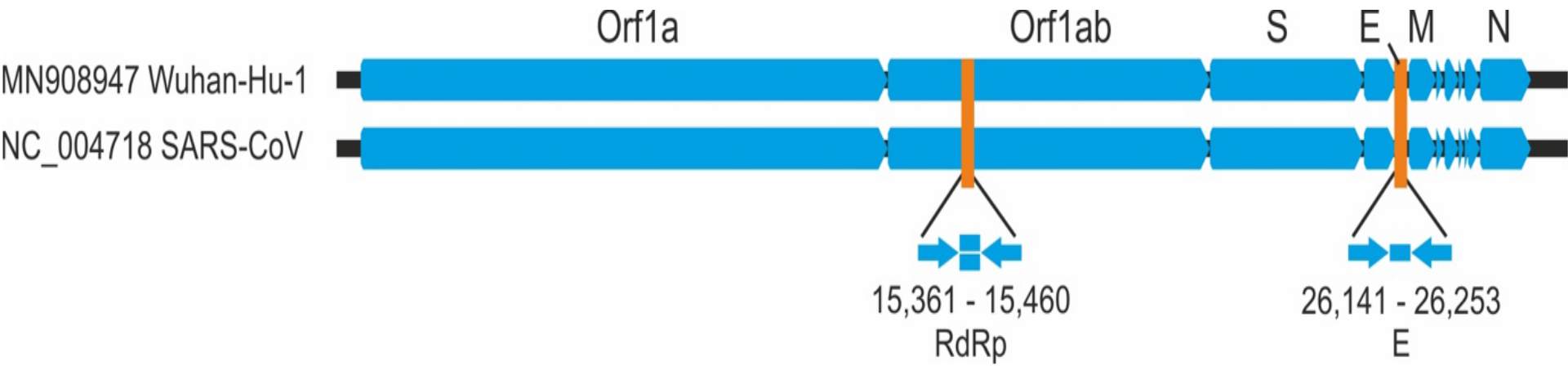
3. CDC rRT-PCR Diagnostic Panel

- 2 targets: *N1* & *N2*, IC: *RNase P*

4. rRT-PCR method- HKU Med.

- Target: ORF1b, Target: N

Relative Positions of Amplicon Targets on SARS-CoV an 2019-nCoV Genome



- **ORF:** Open reading frame;
- **RdRp:** RNA-dependent RNA polymerase
- **E:** Envelope
- **N:** Nucleocapsid

Comparison of the rRT-PCR assay the WHO & the CDC

Test	Molecular targets	Scope	Limit of blank	Reference specimens	Storage conditions
WHO					
	E gene RdRp gene N gene	First-line screening Confirmatory testing Additional confirmatory testing	3.9 copies×reaction 3.6 copies×reaction N/A	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)
CDC					
	N1/2/3 gene RNase P gene	Combined assay Control assay	1.0–3.2 copies/μL N/A	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

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

Tests for SARS-CoV-2/COVID-19 and Potential Uses

Type of Test	Measure	Value	Beneficiary
Nucleic acid amplification test for viral RNA *(nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others)*	Current infection with SARS-CoV-2	• Inform individual of infection status so they can anticipate course of illness and take action to prevent transmission • Inform patient management and actions needed to prevent transmission • Inform actions needed to prevent transmission	• Individual • Healthcare or long-term care facility • Public health
Antibody detection	Past exposure to SARS-CoV-2	• Detect susceptible individuals (antibody negative) and those previously infected • Identify individuals with neutralizing antibodies • Facilitate contact tracing and surveillance	• Identify those potentially immune to SARS-CoV-2 (if tests can detect protective immunity, individuals could be returned to work) • Healthcare facilities: Experimental therapy • Public health

Robin Patel et al. mBio 2020; doi:10.1128/mBio.00722-20

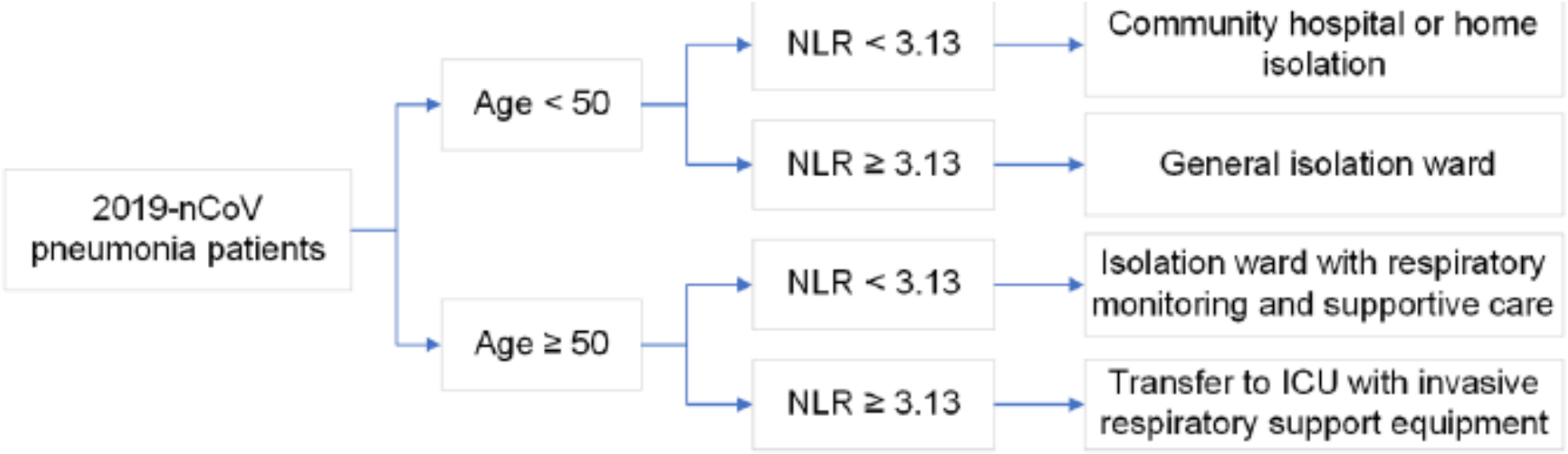
Patient Monitoring
the most important abnormalities

- ***Lymphopenia,*** Increased values of CRP, LDH, ESR & D-dimer, along with diminished concentration of serum albumin.
- ***Parameters predict progression toward severe or critical forms of COVID-19:*** **leukocytosis**, **neutrophilia** & **lymphopenia**.
- Pooled data of 1099 patients with laboratory confirmed SARS-CoV-2 infection from 552 hospitals in 30 Chinese territories demonstrating that COVID-19 patients have:

  - Lymphopenia (83.2%), - Thrombocytopenia(36.2%),

  - Increased values of CRP (60.7%), LDH (41.0%), AST (22.2%), ALT (21.3%) and D-dimer (43.2%).

Patient Monitoring
the most important abnormalities



- Study[6] in Beijing showed that cut-off value of **NLR is 3.13**, sensitivity is 0.875 and specificity is 0.717.
- Patients should be transferred to **ICU** with **age >50** and **NLR>3.13**. If NLR<3.13 and age<50, the patients could isolate at home or community hospital.

Patient Monitoring
the most important abnormalities

- **PT** & **D-dimer** are significant predictors of **disease severity** & confirms that **DIC**
- Elevated D-dimers as one of the predictors of mortality
- Huang and colleagues showed:

D-dimer levels on admission were higher in ***patients needing critical care support*** (median [range] D-dimer level **2400 ng/mL[600–14,400])** than those patients who did not require it (median [range] D-dimer level **500 ng/mL** [300–800], p=0·0042).

4 potential mechanisms leading to lymphocyte deficiency:

(1) The virus might ***directly infect lymphocytes***. Lymph. express the coronavirus receptor ACE2 and may be a direct target of viruses.

(2) The virus might ***directly destroy lymphatic organs*** (thymus & spleen).

(3) ***Inflammatory cytokines*** continued to be disordered, perhaps leading to lymphocyte apoptosis. Basic researches confirmed that **TNFα**, **IL-6** and other pro-inflammatory cytokines could induce lymphocyte deficiency.

(4)Inhibition of lymphocytes by **metabolic molecules** produced by metabolic disorders, such as **hyperlactic acidemia**. The severe type of COVID-19 patients had elevated blood lactic acid levels, which might suppress the proliferation of lymphocytes

Signal Transduction and Targeted Therapy **volume 5**, Article number: 33 (2020)

Laboratory Tests in Patients with COVID-19

Test	Abbreviation	Rationale for inclusion
Hematology (including hemostasis/coagulation)		
Complete/full blood count	CBC/FBC	Identification of lymphopenia, neutrophilia, and thrombocytopenia
Prothrombin Time	PT	Identification of ongoing coagulopathy
Activated partial thromboplastin time	APTT	
Fibrinogen	Fbg or Fib	Identification of ongoing (consumption) coagulopathy
D-dimer		Identification of ongoing (consumption or thrombotic) coagulopathy
Biochemistry and other tests		
Electrolytes		Identification of metabolic derangement
Glucose		
C-reactive protein	CRP	Monitoring of infection/ inflammatory response
Lactate dehydrogenase	LDH	Identification of lung injury and/or multiple organ failure
Aspartate aminotransferase	AST	Identification of liver injury
Alanine aminotransferase	ALT	
Bilirubin		
Albumin		Identification of liver failure

Laboratory Tests in Patients with COVID-19

Test	Abbreviation	Rationale for inclusion
Hematology (including hemostasis/coagulation)		
Creatine kinase (also known as creatine phosphokinase or phosphocreatine kinase)	CK	Identification of muscle injury
Lipase		Identification of pancreatic injury
blood urea nitrogen	BUN	Identification of kidney injury and/or failure
Creatinine		
Cardiac biomarkers (troponin I or T)		Identification of cardiac injury
Brain natriuretic peptide	BNP	Identification of cardiac failure
Ferritin		Monitoring of infection/inflammatory response
Procalcitonin	PCT	Identification of bacterial coinfections
Presepsin		Monitoring of severity of viral infection

DOI https://doi.org/ 10.1055/s-0040-1709498, April 9, 2020.
ISSN 0094-6176.

Hematology Issues during COVID-19
Massachusetts General Hospital
Version 7.0, 4/14/2020

- **Recommendations**

a. Diagnostics: For all patients presenting to MGH for COVID-19:

- Obtain baseline: **D-dimer, PT**, **PTT**, **fibrinogen, ferritin, LDH, troponin, CPK** and **CBC with differential**

b. Monitoring

- **Trend D-dimer daily** (or whenever labs are being drawn if less frequent) if baseline or subsequent **>1000 ng/mL**.

- For patients in the ICU, trend CBC, PT, PTT & fibrinogen daily (or whenever labs are being drawn if less frequent)

c. Management

Advice on the use of point-of-care immunodiagnostic tests for COVID-19

- At present, based on current evidence, WHO recommends the use of these new point-of-care immunodiagnostic tests ***only in research settings***.
- They **should not be used** in any other setting, including for **clinical decision-making**, until evidence supporting use for specific indications is available.

https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19

Rapid diagnostic tests, RDT, Antigen Detection

- Based on this information, ***half or more of COVID-19 infected patients might be missed by such tests***, ***Poor Sensitivity***

- **WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for patient care, although research into their performance and potential diagnostic utility is highly encouraged**.

https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19

Rapid Diagnostic Tests based on Host Antibody Detection

- Based on current data, **WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research**.
- **Paired samples** are necessary for confirmation with the initial sample collected in the **1st week of illness** & the **2nd** ideally collected **2-4 weeks later** (optimal timing for convalescent sample needs to be established).

WHO/COVID-19/laboratory/2020.5

of-point-of-care-immunodiagnostic-tests-for-covid-19

Specimen Collection

1 **Accurate** & **fast** lab. testing depends largely on **correct specimen collection** from the patient **at the right time**.

b. Single swab used for **Throat then Nose** OR

c. **Individual Nose & Throat** swabs in separate collection tubes OR

d. **Nasopharyngeal wash/aspirate** or **nasal aspirate** (NA)

e. **Nasal mid-turbinate** (NMT) swab, also called Deep Nasal Swab (about 2 cm)

f. **Anterior nares specimen** (NS) insert the swab at least 1 cm

https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/laboratory-investigations-and-sample-requirements-for-diagnosing-and-monitoring-wn-cov-infection

Specimen Collection

2. Lower respiratory tract

- Bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy, 2-3 mL

Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/or fatal cases.

- Sputum

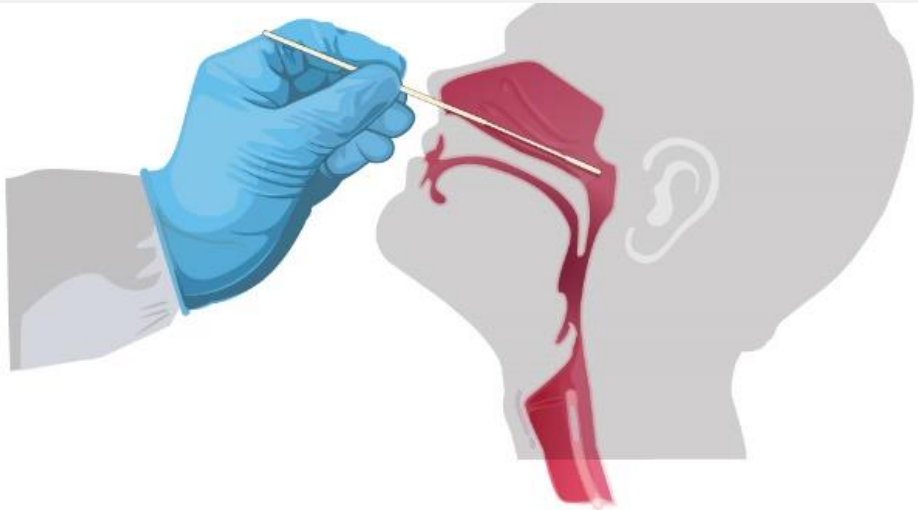
Educate the patient about the difference between sputum and oral secretions (saliva). Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

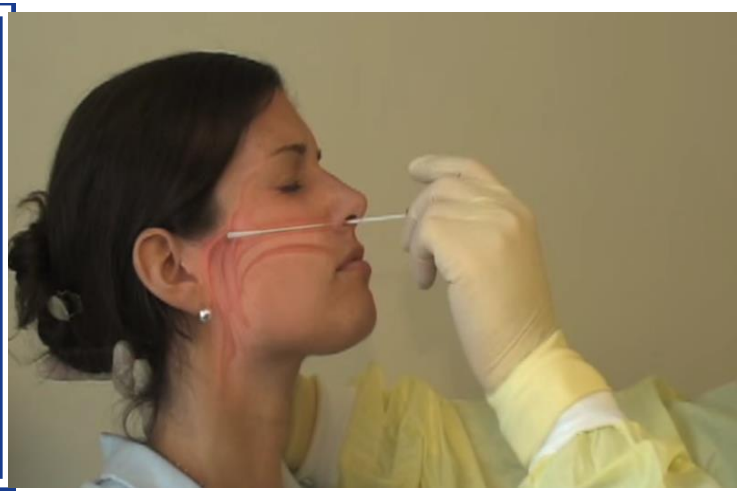
Specimen Collection

Nasopharyngeal swab

IMPORTANT NOTES:

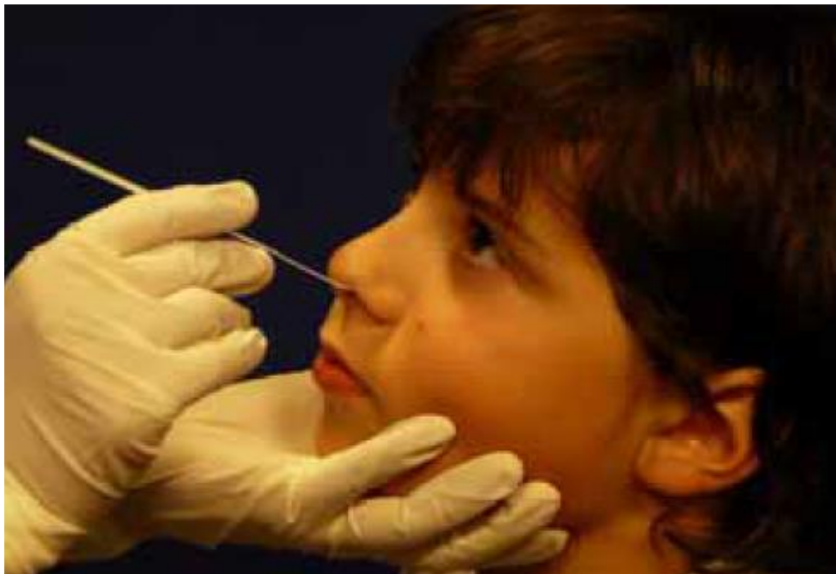
- Appropriate transport medium must be used, 2–3 mL of viral transport media
- **Do not send swabs dry.**
- **If collecting both nasopharyngeal & oropharyngeal swabs, both swabs must be placed in a single collection tube.**





Swab held correctly

Swab held incorrectly



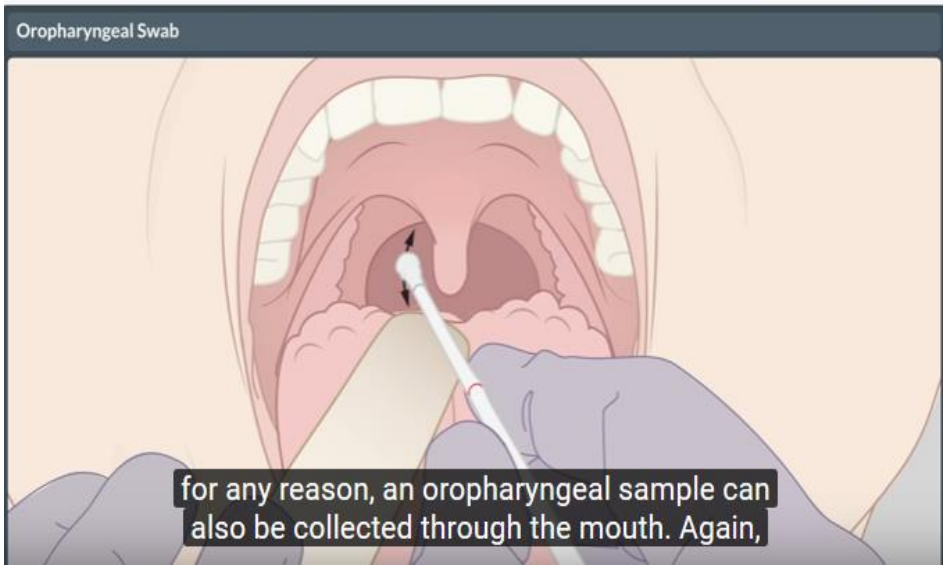
Restraining a small child

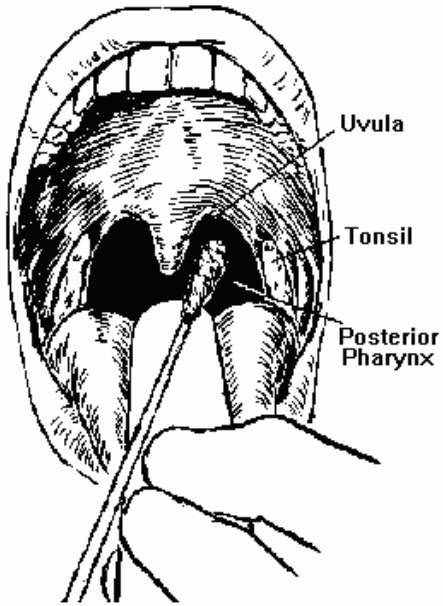
restraining an older child

Specimen Collection
Throat swab

IMPORTANT NOTES:

- Appropriate transport medium must be used.
- **Do not send swabs dry.**
- **If collecting both nasopharyngeal & oropharyngeal swabs, both swabs must be placed in a single collection tube.**





Specimen Types & Sensitivity

- Bronchoalveolar lavage, BAL fluid specimens, the highest positive rates, **93%**
- Sputum, **72%**
- Nasal swabs, **63%**
- Fibrobronchoscope brush biopsy, **46%**
- Pharyngeal swabs, **32%**
- Feces, 29%
- Blood 1% , only 15% of patients hospitalized with pneumonia had detectable RNA in serum
- None of the 72 urine specimens tested positive

High viral loads, ***more in the nose than in the throat***

Emerging Microbes & Infections, 2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1745095

JAMA Published online March 11, 2020

Specimen type	Collection materials	Storage temp. until testing	Recommended temperature for shipment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C	2-8 °C if ≤ 5 days –70 °C (dry ice) if > 5 days
Bronchoalveolar lavage	Sterile container *	2-8 °C	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days
(Endo)tracheal aspirate,nasopharyngeal or nasal wash/aspirate	Sterile container *	2-8 °C	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days
Sputum	Sterile container *	2-8 °C	2-8 °C if ≤ 2 days –70 °C (dry ice) if > 2 days
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood)	2-8 °C	2-8 °C if ≤ 5 days –70 °C (dry ice) if > 5 days
Whole Blood	Collection tube	2-8 °C	2-8 °C if ≤ 5 days –70 °C (dry ice) if > 5 days
Stool	Stool container	2-8 °C	2-8 °C if ≤ 5 days –70 °C (dry ice) if > 5 days
Urine	Urine collection	2-8 °C	2-8 °C if ≤ 5 days

Biosafety considerations

- The U.S. CDC biosafety guidelines state that routine diagnostic testing of specimens from suspected or confirmed SARS-CoV-2 patients, can be handled in a BSL-2 laboratory using standard precautions (https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab biosafetyguidelines.html. Accessed 21 March 2020).

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SARS-CoV-2 Real-Time RT-PCR
Interpretation of Results

- **No Template Control (NTC),** should not exhibit fluorescence growth curves that cross the threshold line If any of the NTC reactions sample contamination may have occurred. **Invalidate** the run and repeat the assay with strict adherence to the guidelines.
- **Positive Control (nCoVPC),** N, E, RdRp, Orf Positive Controls
- **Human Specimen Control (HSC) (Extraction Control),** successful recovery of RNA as well as extraction reagent integrity,
- **RNase P (Extraction Control),**

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves, **< 40.00 Ct**, the presence of the human RNase P gene.
- ***Failure to detect RNase P in any clinical specimens may indicate:***

1. Improper extraction of nucleic acid from clinical
2. Materials resulting in loss of RNA and/or RNA degradation.
3. Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
4. Improper assay set up and execution.
5. Reagent or equipment malfunction.

RNase P (Extraction Control) Interpretation of Negative RNase P

- If the SARS-CoV-2 specific markers (N,E,RdRP) are positive ***even in the absence of a positive RP***, the result should be considered **valid**, some samples may have low cell numbers
- A ***negative RP signal does*** not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
- If all 2019-nCoV markers & RNase P are negative for the specimen, the result should be considered **invalid** for the specimen.
- If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

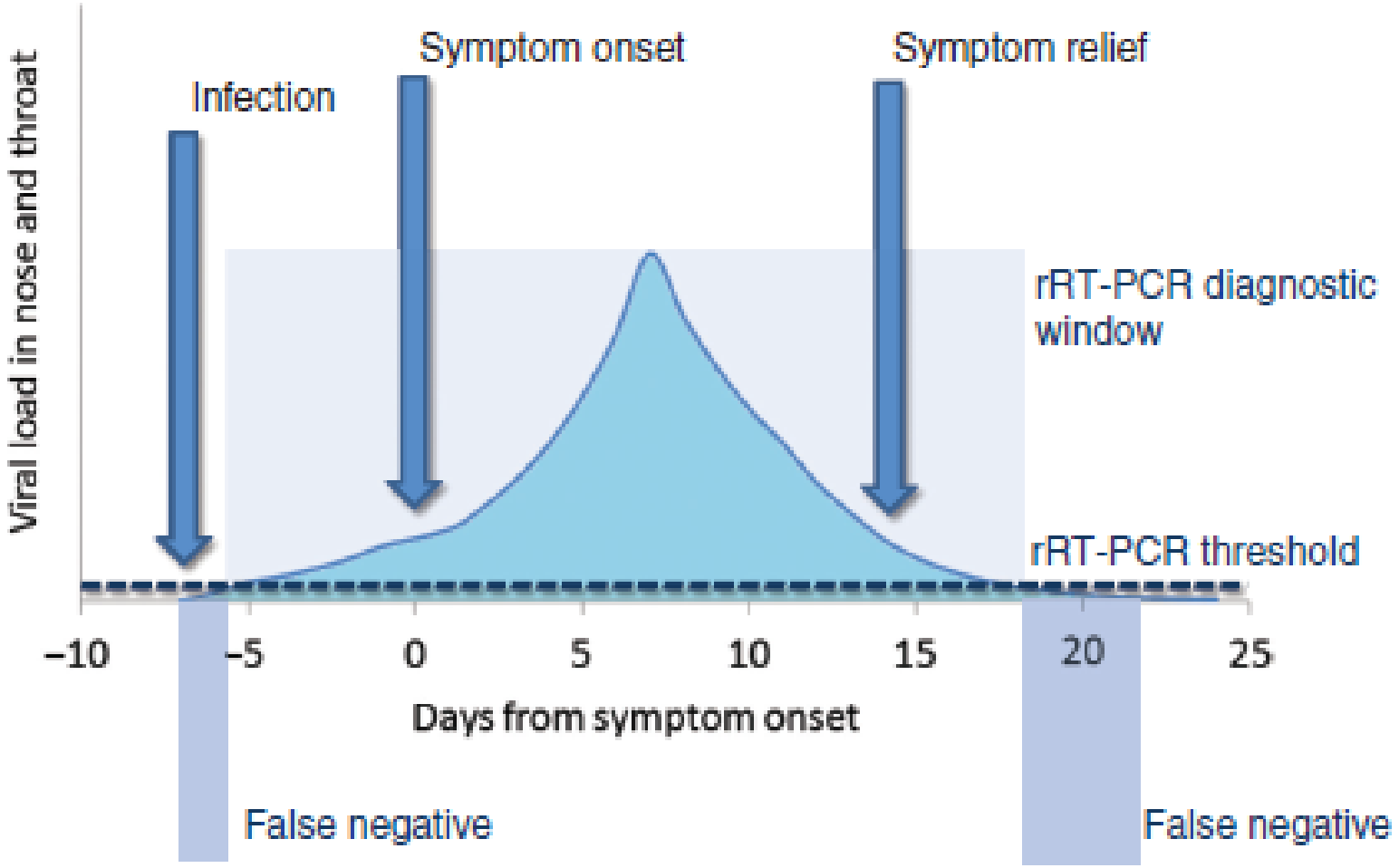
Interpretation of Controls

Control Type	External Control Name	Used to Monitor	E- Gene	N-Gene	RP	Expected Ct Values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	+	+	< 40.00 Ct

SARS-CoV-2 rRT-PCR Diagnostic Panel Results Interpretation Guide

E-Gene	N-Gene	RP	Result Interpretation	Report	Actions
+	+	+	**SARS-CoV-2 detected**	Positive or **Detected**	Report results to Pasteur Institute, Health administration and sender.
If only one of the two targets is positive		**±**	**Inconclusive Result**	**Inconclusive**	Repeat test and/or re-extract and repeat test If result remains inconclusive, contact your …. for transfer of the specimen or further guidance.
-	-	+	**SARS-CoV-2 not detected**	**Not Detected**	Report results. Consider testing for other viruses.
-	-	-	**Invalid Result**	**Invalid**	Repeat extraction & test. If remains invalid, consider collecting a new specimen from the patient.

Correspondence between development of viral load during SARSCoV-2 infection, clinical course & positivity of rRT-PCR assays.



Limitations

- All users, analysts, and any person reporting diagnostic results should be **trained** by a competent instructor.
- **Negative results** do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- Optimum specimen types & timing for peak viral levels have not been determined (late or very early in the infection).
- ***Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.***
- ***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***.

Limitations, cont.

- A false negative result may occur if a specimen is **improperly collected**, **transported** or **handled**.
- **False negative** results may also occur if **amplification inhibitors** are present in the specimen or if **inadequate numbers of organisms** are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence.
- False negative test results are more likely when prevalence of disease is high.
- False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.

Limitations, cont.

- If the ***virus mutates*** in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably.
- Detection of viral RNA ***may not indicate*** the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Potential Pre-analytical Vulnerabilities of SARS-CoV-2 rRT-PCR

- **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

Potential Analytical Vulnerabilities of SARS-CoV-2 rRT-PCR

- Testing carried out outside of the diagnostic window
- Active viral recombination, Shen et al. recently found ***a remarkable level of viral diversity*** in some infected patients, a median number of **4 intra-individual viral variants,**
- 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

Thank you, any question?