Testing in the time of COVID-19

Information for Laboratories

April, 2020
COVID-19 a growing pandemic

Almost one million confirmed positives cases worldwide at the end of March 2020

Observations from the front lines: rapid, often unpredictable, respiratory failure and cardiac arrest in some patients

- Dyspnea is a crucial phase – after may have rapid deterioration of respiratory systems
- The decline is very abrupt. It is respiratory arrest in its true form, requiring emergency intubation.
- Some patients look like they would soon recover, but within 1 or 2 days, they deteriorate and die.

Observations from the front lines: rapid, often unpredictable, respiratory failure and cardiac arrest in some patients

<table>
<thead>
<tr>
<th>Observation</th>
<th>Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with initially mild symptoms can develop hypoxemia (oxygen saturation &lt; 93%)</td>
<td>For hospitals where mild patients are discharged home, who might require hospital admission/ closer monitoring?</td>
</tr>
<tr>
<td>Patients apparently well managed in hospital can develop sudden, abrupt respiratory failure requiring emergency intubation.</td>
<td>Which patients are at higher risk and may benefit from early intubation or more aggressive management?</td>
</tr>
<tr>
<td>Patients apparently well managed in hospital can develop sudden cardiac arrest</td>
<td>Which patients are at higher risk and may need close monitoring?</td>
</tr>
<tr>
<td>Patients on mechanical ventilation who improve, pass all weaning parameters and are extubated, can rapidly relapse and require re-intubation.</td>
<td>Who can be safely extubated?</td>
</tr>
</tbody>
</table>

Current clinical symptoms alone may be insufficient in the assessment of patients with COVID-19. How can we better triage and monitor these patients?
The novel coronavirus: Defining terms

SARS-CoV-2 ("Severe Acute Respiratory Syndrome Coronavirus 2")

SARS-CoV-2 is the virus that causes COVID-19

COVID-19 ("CoronaVirus Disease 2019")

COVID-19 is the disease resulting from infection with the novel coronavirus

The novel coronavirus: Defining terms

SARS-CoV-2:

• Family: Coronavirus (CoV); large family of single-stranded RNA viruses that have been isolated in different animal species

• Closely related to SARS and MERS

• High rate of human-to-human transmission. 1 person may infect >2 others on average.

• Viral receptor appears to be angiotensin converting enzyme 2 (ACE2)

• May be multiple routes of transmission, as virus found in blood and fecal swabs. Respiratory most common

COVID-19:

Existing data suggests:

• COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose with coughing (or sneezing) but may also be airborne

• Significant environmental stability (remains infectious) on surfaces/aerosols

• Incubation period: 2-14 days (assumed based on what is known for MERS-CoV)

• Treatment is supportive – no drug or vaccine with proven efficacy

https://www.who.int/health-topics/coronavirus#tab=tab_1. Accessed March 26, 2020


Guo et al. Military Medical Research (2020) 7:11

What is known about Coronaviruses?

- SARS-CoV-2: ssRNA, betacoronavirus, 60-140 nm diameter
- Studies from SARS and MERS: Inactivated by UV-C, 75°C heat, >70% ethanol, 0.2% sodium hypochlorite, 0.5% H2O2, 0.25% povidone iodine, etc
- Chlorhexidine did not significantly inactivate CoV
- Viral entry via ACE2. TMPRSS2 protease primed the viral S protein for ACE2 binding
- Microarray analysis: high ACE2+TMPRSS2 expression in lung alveolar type 2, esophageal upper epithelium, ileum absorptive enterocytes, colonic enterocytes
- AT2 cells responsible for surfactant production, immune regulation, regeneration/repair

Clinical classification and transmission of COVID-19

Clinical classification
- Incubation period: median 5 days, range 1–7 days
- Patients with mild symptoms in week 1... may progress in week 2. Progression may be rapid and sudden.
- Possible transmission during asymptomatic phase 4–6 days before the onset of symptoms.
- Highest risk of transmission during symptomatic phase
- Possible transmission after remission of the symptoms

Disease spectrum

<table>
<thead>
<tr>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>Mild to Moderate 80%</td>
<td>Severe 10–15%</td>
</tr>
<tr>
<td>Infectious virus can be shed, viral loads may be comparable to symptomatic patients</td>
<td>No signs (mild) or some signs (moderate) of pneumonia on imaging</td>
<td>Respiratory distress of ≥30 breath per minute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxygen saturation ≤93% at rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PaO2/FiO2 ≤300 mmHg</td>
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<tr>
<td></td>
<td></td>
<td>Lung lesion progression &gt;50% in 24–48h</td>
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</tbody>
</table>

Content source:
- National Centre for Immunization and Respiratory Diseases (NCIRD) Division of Viral Diseases (JAMA.2020 Feb7. doi:10.1001/jama.20201585)
COVID-19 presents challenges for healthcare works

- Shortage/lack of PPE (personal protective equipment) such as masks, gloves, gowns, face-shields
  - Especially shortage of N95 masks
- Shortage of hospital beds and other critical equipment, in particular ICU monitored beds and ventilators
- Lack/limited numbers of COVID-19 test kits
- Loss of clinical staff to illness/burnout or insufficient staffing to meet demand
COVID-19 patient pathway

Diagnosis

COVID-19 infection?
- Molecular lab test

Prognosis

How severe is it?
- Immuno/chemistry lab tests
- Hematology lab tests
- Blood gas tests
- CT
- X-ray
- Ultrasound

Therapy

How to treat?
- Immuno/chemistry lab tests
- Hematology lab tests
- Blood gas tests
- CT
- X-ray
- Ultrasound

Follow-up

When recovered?
- CT
- X-ray
- Ultrasound
- Immuno/chemistry lab tests
- Molecular lab test

Staff protection and capacity management

Status: 27.03.2020
Example of Clinical Setup with an Isolated Area for COVID-19 Cases

symptomatic patient with high COVID-19 suspicion

no contact with infectious COVID-19 patients

Radiology department (non-isolated)

Isolated area

Sampling for PCR

Other modalities

Stationary X-ray

Mobile X-ray

CT

Sampling for PCR

if no CT is available inside the isolated area

inpatient / ICU patient with COVID-19

mismatch symptoms/PCR result – or – severe symptoms

CT


Testing for COVID-19

Molecular: Detection of viral RNA
• Confirms infection
• Lab-based (rt-PCR) and Point-of-care (POC)

Serology: Detection of antibodies
• Lab-based and POC
  
  Early or acute  Older or resolved
  
  IgM Antibody  IgG Antibody

• IgG indicates prior/resolving infection
• IgG can aid in prevalence assessment in the community
• It is yet to be proven that IgG seroconverted individuals may be immune to subsequent infection
Diagnosis: RT-PCR is more sensitive during early disease, serology is more sensitive 2-3 weeks after symptom onset

Table: Sensitivity of different methods by time from symptom onset

<table>
<thead>
<tr>
<th>Time from symptom onset</th>
<th>RT-PCR sensitivity</th>
<th>Total Ab sensitivity</th>
<th>IgM sensitivity</th>
<th>IgG sensitivity</th>
<th>PCR+Ab sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 1-7 (n=94)</td>
<td>66.7%</td>
<td>38.3%</td>
<td>28.7%</td>
<td>19.1%</td>
<td>78.7%</td>
</tr>
<tr>
<td>day 8-14 (n=135)</td>
<td>54.0%</td>
<td>89.6%</td>
<td>73.3%</td>
<td>54.1%</td>
<td>97.0%</td>
</tr>
<tr>
<td>day 15-39 (n=90)</td>
<td>45.5%</td>
<td>100%</td>
<td>94.3%</td>
<td>79.8%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Cumulative seroconversion by day from onset
CDC recommends collecting and testing an upper respiratory specimen for COVID-19

Nasopharyngeal specimen is the preferred choice for swab-based SARS-CoV-2 testing.

When collection of a nasopharyngeal swab is not possible, the following are acceptable alternatives:

• An oropharyngeal (OP) specimen collected by a healthcare professional
• A nasal mid-turbinate (NMT) swab collected by a healthcare professional or by onsite self-collection (using a flocked tapered swab)
• An anterior nares specimen collected by a healthcare professional or by onsite self-collection (using a round foam swab)

Samples should be stored at 2-8 degrees Celsius. If over 72 hours freeze at -70 degrees Celsius prior to testing.

Identification of high risk patients

- Age >55 years
- Any age with severe obesity (BMI >40)
- Pre-existing pulmonary disease (chronic lung disease or moderate to severe asthma, COPD, lung cancer, pulmonary hypertension, emphysema (smoking, A1AT deficiency))
- Chronic kidney disease
- Diabetes
- History of:
  - hypertension (treated and untreated)
  - cardiovascular disease
  - liver disease
  - transplants or other immunosuppression (ex. cancer treatment)
- All patients with HIV
- Patients with endocrine pathologies
- Use of biologic drugs

Patient triage

Epidemiologic surveillance
Laboratory testing:
- RT-PCR
- Anti-SARS-CoV-2 antibodies

Recovery

Epidemiologic surveillance
Laboratory testing:
- Anti-SARS-CoV-2 antibodies (IgG, IgM)
Confirm immunization

Therapeutic monitoring

Laboratory testing:
- Arterial blood gas
- Complete blood count
- Acute phase proteins, inflammation & coagulation
- Liver, kidney, cardiac function biomarkers
- Additional testing related to comorbidities

Imaging:
- Chest CT (ground glass opacification)

The essential role of laboratory diagnostics in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

### Current recommended lab testing (WHO, ESICM)

| Recommended daily labs | CBC with differential (trend total lymphocyte count) Comprehensive metabolic panel:  
|                        | Electrolytes: Na, K, Total CO₂, Chloride  
|                        | Total protein and Albumin  
|                        | Creatinine  
|                        | Bilirubin, ALT, AST  
|                        | CPK (total creatine kinase)  
|                        | Lactate |
| Risk stratification    | D-dimer, Ferritin, CRP, ESR, LDH, Cardiac troponin |
| Viral serologies       | HBV, HCV, HIV 1/2/O |

### Frequent laboratory abnormalities in patients with COVID-19 *

<table>
<thead>
<tr>
<th>Blood lymphocyte count (35–75%)</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (50–98%)</td>
<td>Increased</td>
</tr>
<tr>
<td>Hemoglobin (41–50%)</td>
<td></td>
</tr>
<tr>
<td>Neutrophil count</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (ESR, up to 85%)</td>
<td></td>
</tr>
<tr>
<td>Creatine protein (CRP, 75–93%)</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH, 27–92%)</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td></td>
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<tr>
<td>Total bilirubin</td>
<td></td>
</tr>
<tr>
<td>Cardiac troponin</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin (6–25%)</td>
<td></td>
</tr>
<tr>
<td>Prothrombin time (in sec)</td>
<td></td>
</tr>
<tr>
<td>D-dimer (36–43%)</td>
<td></td>
</tr>
</tbody>
</table>

*Approximate percentage of patients

### Additional essential lab testing

<table>
<thead>
<tr>
<th>Arterial blood gas</th>
<th>pH, PaCO₂, PaO₂, and aHCO₃</th>
</tr>
</thead>
</table>
| Hematology         | Lymphopenia with atypical lymphocytes  
|                    | Leukocytosis, Neutrophilia, low eosinophils  
|                    | Thrombocytopenia: Platelet count |
| Hemostasis         | Prothrombin time, D-Dimer |
| Inflammation/infection | CRP, Ferritin, IL6, TNFα, SAA  
|                     | Procalcitonin |
| Cardiac            | High-sensitivity troponin, CK-MB, BNP/NT-proBNP |
| Liver              | Albumin, ALT, AST, bilirubin |
| Renal              | Creatinine, Cystatin C |

### Potential clinical significance

- For ventilator adjustments
- Decreased immunological response to the virus
- Bacterial (super) infection
- Consumption (disseminated) coagulopathy
- Activation of blood coagulation and/or disseminated coagulopathy
- PT and D-dimer are significant predictors of disease severity
- Severe viral infection/necrosis
- Bacterial (super) infection
- Increased levels may be associated with higher mortality
- Impairment of liver function, Liver injury
- Kidney injury

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Information reflects a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.
Patients with comorbidities that are infected by COVID-19 have a worse prognosis, expanding the role of the laboratory

Patients with comorbidities (like diabetes mellitus, hypertension, cardiovascular, chronic lung and chronic kidney disease) are particularly susceptible to COVID-19 infection and are likely to have more severe illness

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Additional testing</th>
<th>Impact of COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>Troponin, Natriuretic peptide, CKMB</td>
<td>Precipitates cardiac complications like: acute heart failure, myocardial infarction, myocardial injury, cardiac arrest.</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>Blood: Creatinine, Cystatin C, eGFR Urine: Albumin</td>
<td>Challenges for patients on dialysis, in particular, in-center hemodialysis. Uremic patients are particularly vulnerable to infection and may exhibit greater variations in clinical symptoms and infectivity.</td>
</tr>
<tr>
<td>Heart/liver/kidney transplant</td>
<td>Immunosuppressant Drugs: Mycophenolate, Cyclosporine, Tacrolimus, Sirolimus, Everolimus</td>
<td>Patients may be more vulnerable due to immunocompromised status.</td>
</tr>
<tr>
<td>Viral co-infection</td>
<td>Hepatitis B serology (anti-Hbe, anti-HBc, and HBeAg) Hepatitis C serology (anti-HCV), unless positive in past 12/2/19, CD4 count</td>
<td>Viral serologies exist in interpretation of ALT elevations, present in ~25% of COVID-19 patients. HIV patients may get severe side effects when taking Tocilizumab (drug being used for COVID-19 pneumonia). Hepatitis patients are at higher risk for liver complications.</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Blood glucose</td>
<td>Patients with diabetes who are infected with COVID-19 may see their glycemic control deteriorate during the illness.</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>LDH</td>
<td>Patients may be more vulnerable due to lung function insufficiency.</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>Albumin, AST, ALT, Total Protein, Bilirubin, PT INR</td>
<td>Patients may be more vulnerable due to liver function insufficiency.</td>
</tr>
</tbody>
</table>


†PDR Tocilizumab

https://www.pdr.net/drug-summary/Actemra-tocilizumab-2359#1

Hematologic parameters in patients with COVID-19 infection 10.1002/ajh.25774

Acute Respiratory Distress Syndrome (ARDS) and COVID-19
Role of Arterial Blood Gas (ABG)

Healthy Lungs:
Oxygen is passed from alveolus (air sacs) to the capillaries to the red blood cells.

COVID-19 Patients:
Coronavirus damages walls of air sacs that help pass oxygen, causing them to thicken and limit the amount of oxygen that gets passed

ABG test provides a status of the patient’s oxygenation levels, enabling caregivers to determine if adjustments to ventilator settings or other treatments are required

Observations from China Study:
Review of 191 patients examined for risk factors found:
• 50 out of 54 died of ARDS
• 9 out of 137 survived ARDS

ARDS:
• Defined by the amount of oxygen in arterial blood to the fraction of oxygen in inspired air

ABG:
• Measures pH (acidity and alkalinity) and the levels of oxygen (pO_2) and carbon dioxide (pCO_2)

Cytokine response to COVID-19 infection (cytokine storm)

1. Virus detected by macrophages

2. Macrophages release IL-6, SAA and other proinflammatory cytokines to stimulate innate immune response

3. Cytokines recruit T-helper cells which hyper-magnify cytokine response (cytokine storm)

4. Cytokines promote vasodilation and reduced endothelial tone cause vascular leakage

5. Fluid, leukocytes, and cytokines flood tissues, causing inflammation and cellular damage

6. Leaking microvasculature stimulates coagulation cascade

7. Clots cause ischemia in end organs and multiple organ dysfunction (MOD)
Elevated IL-6 levels observed in patients with COVID-19 infection

IL-6 is elevated in COVID-19 patients with severe disease

IL-6 is higher in non-survivors


This information represents a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.
Elevated SAA levels observed in patients with COVID-19 infection

Zhang Y, et al. J Translational Medicine BMC 2020 DOI: 10.21203/rs.3.rs-19724/v1C.

This information represents a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.
PCT may help determine if bacteria are present and pathogenic in patients suspected to have COVID-19

- PCT on admission can aid in early risk assessment
- Bacterial sepsis can occur secondary to viral sepsis or severe viral infection
- In a study of community acquired pneumonia patients, median PCT in patients infected with typical bacteria was substantially higher than PCT in patients with infections of viral origins. The difference between the medians is statistically significant.

PCT remains valuable during hospitalization

Monitor to:
- Evaluate risk of bacterial co-infection
- Assess efficacy of antibiotics

Case report PCT examples courtesy of M. Broyles, Pharm D. Five Rivers Medical Center, Pocahontas, AR.
Prevalence of bacterial co-infection in COVID-19 patients

D-dimer elevations in COVID-19 non-survivors

Study 1:
- 183 consecutive patients with confirmed severe COVID-19 induced pneumonia
  - Significantly elevated D-dimer levels in non-survivors (0.61 µg/L (0.35-1.29) vs. 2.12 µg/L (0.77-5.27); p < 0.001)
  - 71.4% of non-survivors but only 0.6% survivors met the criteria of disseminated intravascular coagulation (DIC)

Laboratory evaluation of DIC:
- PT/PTT (prolonged)
- D-dimer (elevated)
- Platelets (decrease)
- Fibrinogen (decrease)

Study 2:
D-dimer profile of 33 patients with COVID-19

Study 3:
D-Dimer level >1 µg/L was a strong predictor of non-survival in 191 COVID-19 patients with an odds ratio of 18.4

This information represents a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.
Cardiovascular complications: patients with elevated cardiac troponin are at very high risk

Patients with elevated cTn have higher mortality

![Chart showing cardiac troponin levels for died and discharged patients with p-value 0.001]

Non-survivors had increasing cTn over the course of hospitalization

![Chart showing plasma cTn levels over hospitalization stages with p-values indicating significant increases]

Ruan, Q. Intensive Care Med. https://doi.org/10.1007/s00134-020-05991-x
This information represents a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.
Cardiac Troponin elevations in severely ill COVID-19 patients: Potential prognostic factor


This information represents a potential novel clinical utility. Data have not been reviewed by FDA or any other regulatory agency.

Non-survivors

Survivors

<table>
<thead>
<tr>
<th></th>
<th>Survivors (n=137)</th>
<th>Non-survivors (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs-cTnI Median (IQR)</td>
<td>3.0 (1.1 - 5.5)</td>
<td>22.2 (5.6 - 83.1)</td>
</tr>
<tr>
<td>cTnI &gt; 99th p (28ng/L)</td>
<td>1/95 (1%)</td>
<td>23/50 (46%)</td>
</tr>
</tbody>
</table>

Note that increasing cTnI may not be Type I MI:
- Myocardial ischaemia in COVID-19 may be secondary to hypoxemia/tachycardia
- Myocardial damage in COVID-19 may be due to ‘cytokine storm’/catecholamines
Case Report: Fulminant myocarditis secondary to COVID-19, “disguised” as myocardial infarction

- M 37y, chest pain, dyspnea, diarrhea for 3 days
- ECG showed III and AVF ST segment elevation
- cTnT > 10,000ng/L; CK-MB 112.9 ng/L
- Coronary angiography revealed no stenosis

- Echocardiography: Left ventricular EF 27%
- X-ray showed enlargement of the heart
- Chest CT indicated pulmonary infection
- Sputum: SARS-CoV-2 positive

- Diagnosis: COVID-19 infection, fulminant myocarditis, cardiogenic shock
- Treatment: glucocorticoid + immunoglobulin

- 1week later: normal X-ray, LVEF 66%, cTnT 220.5ng/L
We are in this together –
Our response to the COVID-19 pandemic

We are fully committed to supporting our partners throughout the healthcare system to provide the best possible care for patients.

The coronavirus (SARS-CoV-2) pandemic continues to spread, confronting healthcare professionals around the world with unprecedented clinical and operational challenges. As they struggle to deal with this extraordinary situation, at the same time they must also continue to care for other patients.

At Siemens Healthineers, we are aware of the urgency and complexity of the current situation, and we are working hard to provide the best possible support to healthcare providers at each stage of COVID-19 patient care: diagnosis, prognosis, therapy and follow-up.

Our aspiration to drive innovations forward so people live healthier and longer lives is more valid today than ever before. We are stepping up as a partner to support healthcare systems helping them to deliver high-value care to patients and families. Please do not hesitate to contact us in case of questions or suggestions.
Siemens Healthineers Response: Laboratory Diagnostics

FTD SARS-CoV-2 Assay
Chemistry/Immunoassay
Hemostasis/Hematology
Blood Gas Testing

*SARS-CoV-2 serology assay in development†

†In development; not available for sale. Future availability cannot be guaranteed
Computed Tomography, Radiology, Ultrasound and Molecular Imaging Hybrid Devices
COVID-19 Summary

• The SARS-CoV-2 virus is novel, highly infectious and populations lack immunity. Asymptomatic transmission likely.

• Disease severity and clinical presentation varies enormously, from asymptomatic to severe. Risk factors for severity include age and comorbidities such as diabetes, hypertension, and pulmonary disease. Viral sepsis can drive pathology.

• Molecular testing confirms the presence of virus. Serology testing identifies antibodies to the virus indicating current or prior infection.

• Multiple testing modalities are utilized to inform diagnosis and treatment.

• We are fully committed to supporting our partners in the healthcare system to provide the best possible care for patients.

https://www.who.int/health-topics/coronavirus#tab=tab_1. Accessed March 26, 2020
Thank you for your enthusiasm!

Siemens Healthineers
siemens-healthineers.com