BIOSPECIMEN TURNAROUND FOR THE PURPOSE OF COVID-19 RESEARCH PROJECTS

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PRESENTATION PLAN

• 1. Types of samples that biobank may store, sample life cycle.
• 2. Presence of virus in different types of samples.
• 3. Laboratory standards for Sars-CoV-2 suspected samples.
• 4. How biobanks can deal with Sars-CoV-2 samples – Dr. Helmuth Haslacher, Department of Laboratory Medicine MedUni Wien Biobank.
TYPES OF COLLECTED BIOLOGICAL MATERIAL FROM COVID-19 PATIENTS

- Upper respiratory track swabs, BAL, sputum – determination of viral presence and viral load (active pathogen)
- Blood (serum, WB EDTA, heparin plasma, citrate plasma, EDTA plasma, arterial blood for POCT testing, blood culture for sepsis determination)
- Urine – general analysis and microbiology
- Stool – in example for FOB

Which type of sample is suitable for your research?
TYPES OF DATA THAT MIGHT BE IMPORTANT FOR COVID-19 PATIENT CHARACTERIZATION

- Disease severity, duration and outcome
- Antibodies titer (IgM and IgG)
- CT imaging of lungs, alternatively Xray
- Clinical symptoms (fever, dry cough, shortness of breath, muscles)
- Blood count and other lab results especially at the moment of hospital admission,
- Treatment protocol (antivirals, antimalaril, tocilizumab, GCs)
COLLECTION SITES FOR BIOLOGICAL MATERIAL

- A&E clinics – first admission when patients present COVID-19 like symptoms
- Dedicated infectious wards - for mild and moderate symptoms
- ICU – for severe patients usually requiring MV.
- Drive-thru clinics for Sars-Cov-2 testing

A&E - accident and emergency ward, ICU – intensive care unit
SAMPLE CYCLE: RECEPTION, PROCESSING, ANALYSIS AND STORAGE AREAS

Hospital wards

Microbiology Labs (BSL 2/BSL 3)

Virology Labs (most often BSL3)

General pathology labs (BSL 2 or other standard)

Biobanks (BSL???) risk assessment
Patients with COVID-19 had the highest viral load near presentation, which could account for the fast-spreading nature of this epidemic. The high viral load on presentation suggests that SARS-CoV-2 can be transmitted easily, even when symptoms are relatively mild.
DETECTION OF SARS-COV-2 IN DIFFERENT TYPES OF CLINICAL SPECIMENS

Wang et al., JAMA, 11th March 2020

Biodistribution of SARS-CoV-2 among different tissues of inpatients with COVID-19. 1070 specimen samples.

The live virus was detected in feces, implying that SARS-CoV-2 maybe transmitted by the fecal route. A small percentage of blood samples had positive PCR test results, suggesting that infection sometimes may be systemic.

Table. Detection Results of Clinical Specimens by Real-Time Reverse Transcriptase-Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>Specimens and values</th>
<th>Bronchoalveolar lavage fluid (n = 15)</th>
<th>Fibrobronchoscope brush biopsy (n = 13)</th>
<th>Sputum (n = 104)</th>
<th>Nasal swabs (n = 8)</th>
<th>Pharyngeal swabs (n = 398)</th>
<th>Feces (n = 153)</th>
<th>Blood (n = 307)</th>
<th>Urine (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive test result, No. (%)</td>
<td>14 (93)</td>
<td>6 (46)</td>
<td>75 (72)</td>
<td>5 (63)</td>
<td>126 (32)</td>
<td>44 (29)</td>
<td>3 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Cycle threshold, mean (SD)</td>
<td>31.1 (3.0)</td>
<td>33.8 (3.9)</td>
<td>31.1 (5.2)</td>
<td>24.3 (8.6)</td>
<td>32.1 (4.2)</td>
<td>31.4 (5.1)</td>
<td>34.6 (0.7)</td>
<td>ND</td>
</tr>
<tr>
<td>Range</td>
<td>26.4-36.2</td>
<td>26.9-36.8</td>
<td>18.4-38.8</td>
<td>16.9-38.4</td>
<td>20.8-38.6</td>
<td>22.3-38.4</td>
<td>34.1-35.4</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>28.9-33.2</td>
<td>29.8-37.9</td>
<td>29.3-33.0</td>
<td>13.7-35.0</td>
<td>31.2-33.1</td>
<td>29.4-33.5</td>
<td>0.0-36.4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, no data.
Limited data have shown that viral RNA could be detected in plasma or serum from COVID-19 patients. In the first 41 patients in the city of Wuhan, viremia was found in 6/41 (15%) patients. The median PCR cycle threshold value was 35.1 (95% CI: 34.7-35.1), suggesting a very low RNA concentration with no difference found between intensive care unit patients and patients with mild symptoms.

Chang et al., Transfusion medicine reviews, 21th February 2020

<table>
<thead>
<tr>
<th>Methods</th>
<th>Commercial systems</th>
<th>Mechanism of action [56]</th>
<th>SARS-CoV</th>
<th>MERS-CoV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>N/A</td>
<td>Denaturing the secondary structures of proteins</td>
<td>Products without cells. 55°C 20 min in serum</td>
<td>DMEM + 5% FBS 50°C 25 min (reduction of 4 log_{10} TCID_{50/mL}) [53]</td>
</tr>
<tr>
<td>S/D treatments</td>
<td>Octaplas (Octapharma)</td>
<td>Disruption of lipid membranes</td>
<td>Products without cells 2 h for TNBP/Triton X-100 in PBS or 10% BSA</td>
<td>N/A</td>
</tr>
<tr>
<td>Amotosalen + UV-A light</td>
<td>INTERCEPT Blood system for plasma and platelets (Cerus)</td>
<td>Amotosalen (5-59) intercalates into nucleic acid and induces covalent cross-linking upon UV-A exposure</td>
<td>MEM + 10% FBS (reduction of 5.8 log_{10} PFU/mL) [51]</td>
<td>Platelet concentrate (reduction of 4.48 log_{10} PFU/mL) [47]</td>
</tr>
<tr>
<td>Riboflavin + UV-B light</td>
<td>MIRASOL PBT system for plasma and platelets (Terumo)</td>
<td>Riboflavin associates with nucleic acids and mediates an oxygen-independent electron transfer upon UV exposure</td>
<td>N/A</td>
<td>Fresh-frozen plasma (reduction of 4.07 log_{10} PFU/mL) [47]</td>
</tr>
<tr>
<td>UV-C light</td>
<td>THERAFLEX UV-Platelets (Macopharma)</td>
<td>UV-C directly interacts with nucleic acids, causing the formation of nucleotide dimers</td>
<td>Platelet concentrates (reduction of 3.3 log_{10} TCID_{50/mL}) [44]</td>
<td>Platelet concentrates (reduction of 2.37 log_{10} TCID_{50/mL}) [48]</td>
</tr>
<tr>
<td>Methylene blue + Visible light</td>
<td>THERAFLEX MB (Macopharma)</td>
<td>MB intercalates into nucleic acid and mediates the formation of singlet oxygen upon illumination</td>
<td>Plasma (reduction of 3.1 log_{10} TCID_{50/mL}) [45]</td>
<td>Plasma (reduction of 3.3 log_{10} TCID_{50/mL}) [48]</td>
</tr>
</tbody>
</table>

BSA, bovine serum albumin; DMEM, Dulbecco modified Eagle medium; FBS, fetal bovine serum; MB, methylene blue; N/A, not available; PBS, phosphate-buffered saline; PFU, plaque-forming units; TNBP, tri-n-butyl phosphosphate.
WHO AND ECDC CLINICAL LABORATORY GUIDELINES FOR COVID-19 SAMPLES COLLECTION AND FRACTIONATION

ECDC and WHO plus local/national guidelines!

- All specimens collected for laboratory investigation should be regarded as potentially infectious, and healthcare workers who collect or transport clinical specimens should adhere rigorously to Standard Precautions to minimize the possibility of exposure to pathogens. The WHO Aide-memoire on Standard Precautions in Health Care is available from: http://www.who.int/csr/resources/publications/EPR_AM2_E7.pdf. 3.4.2

WHO GUIDELINES FROM 12TH OF FEBRUARY AND THE 19TH OF MARCH

- **Critical:**

  - **risk assessment** before you start working with COVID-19 samples, for each process step, that is from sample collection, sample reception, clinical testing and storage

  - **properly trained and competent personnel** in laboratories capable of meeting additional essential containment requirements and practices, that is, BSL3.

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**Highlights of COVID-19 laboratory biosafety**

- All procedures must be performed based on risk assessment and only by personnel with demonstrated capability, in strict observance of any relevant protocols at all times.

- Initial processing (before inactivation) of all specimens should take place in a validated biological safety cabinet (BSC) or primary containment device.

- Non-propagative diagnostic laboratory work (for example, sequencing, nucleic acid amplification test [NAAT]) should be conducted at a facility using procedures equivalent to Biosafety Level 2 (BSL-2).

- Propagative work (for example, virus culture, isolation or neutralization assays) should be conducted at a containment laboratory with inward directional airflow (BSL-3).

- Appropriate disinfectants with proven activity against enveloped viruses should be used (for example, hypochlorite [bleach], alcohol, hydrogen peroxide, quaternary ammonium compounds and phenolic compounds).

- Patient specimens from suspected or confirmed cases should be transported as UN3373, “Biological Substance Category B”. Viral cultures or isolates should be transported as Category A, UN2814, “infectious substance, affecting humans”.

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**Key points**

- Each laboratory should conduct a local (that is, institutional) risk assessment to ensure it is competent to safely perform the intended testing with appropriate control measures in place.

- When handling and processing specimens, including blood for serological testing, laboratory practices and procedures that are basic to good microbiological practices and procedures (GMP) should be followed.

- The handling and processing of specimens from case with suspected or confirmed COVID-19 infection intended for additional laboratory tests, such as haematology or blood gas analysis, should follow local guidelines for processing potentially infectious material.

- Non-propagative diagnostic laboratory work, including sequencing and NAAT, on clinical specimens from patients who are suspected or confirmed to be infected with COVID-19, should be conducted adopting the practices and procedures of “core requirements”, as detailed in Annex 1, and an appropriate selection of “heightened control measures”, as informed by the 1 risk assessment. In the interim, BSL-2 in the WHO Laboratory biosafety manual, 3rd edition (2) remains appropriate until the 4th edition replaces it.

- Handling of material with high concentrations of live virus (such as when performing virus propagation, virus isolation or neutralization assays) or large volumes of infectious materials should be performed **only by**
CDC - CLINICAL LABORATORY GUIDELINES FOR COVID-19 SAMPLES COLLECTION AND TESTING

- CDC (https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html#testing) updated at 31.03.20

- General guidance including sample collection and labelling

- Guidance for routine diagnostic testing (performed in BSL2)

- Procedures with a High Likelihood to Generate Droplets or Aerosols (perform in BSL2 or provide a barrier between the specimen and personnel)

- Isolation of virus

- Decontamination

- Waste management

SELF PROTECTION WHEN WORKING WITH SARS-COV-2

CEN standards available for free:
• EN 149:2001 + A1:2009 Respiratory protective devices – Filtering half masks to protect against particles – Requirements, testing, marking (commonly referred to as ‘FFP masks’)
• EN 14683:2019 Medical face masks – Requirements and test methods
• EN 166:2001 Personal eye-protection – Specifications
• EN 14126:2003 + AC 2004 Protective clothing – Performance requirements and tests methods for protective clothing against infective agents
• EN 13795-2:2019 Surgical drapes, gowns and clean air suits, used as medical devices for patients, clinical staff and equipment – Part 2: Test methods
• EN 455-1:2000 Medical gloves for single use – Part 1: Requirements and testing for freedom from holes (MDD)
• EN 455-2:2015 Medical gloves for single use – Part 2: Requirements and testing for physical properties (MMD)
• EN 455-3:2015 Medical gloves for single use – Part 3: Requirements and testing for biological evaluation (MDD)
• EN 455-4:2009 Medical gloves for single use – Part 4: Requirements and testing for shelf life determination (MDD)

ISO standards
• EN ISO 13688:2013 Protective clothing – General requirements
• EN ISO 10993-1:2009 + AC 2010 Biological evaluation of medical devices
Fast, portable tests come online to curb coronavirus pandemic

Testing kits delivered by courier and digital tools combine to battle the COVID-19 outbreak.

https://www.nature.com/articles/d41587-020-00010-2
How biobanks can deal with Sars-CoV-2 samples – Dr. Helmut Haslacher, Department of Laboratory Medicine MedUni Wien Biobank.