BIOSPECIMEN TURNAROUND FOR THE PURPOSE OF COVID-19 RESEARCH PROJECTS

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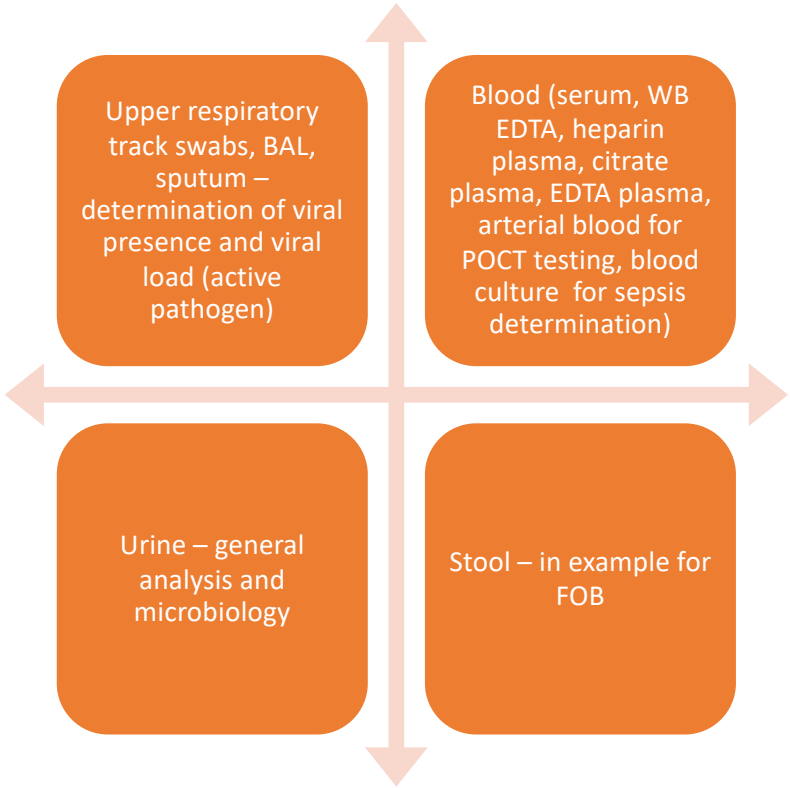
PRESENTATION PLAN

- 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

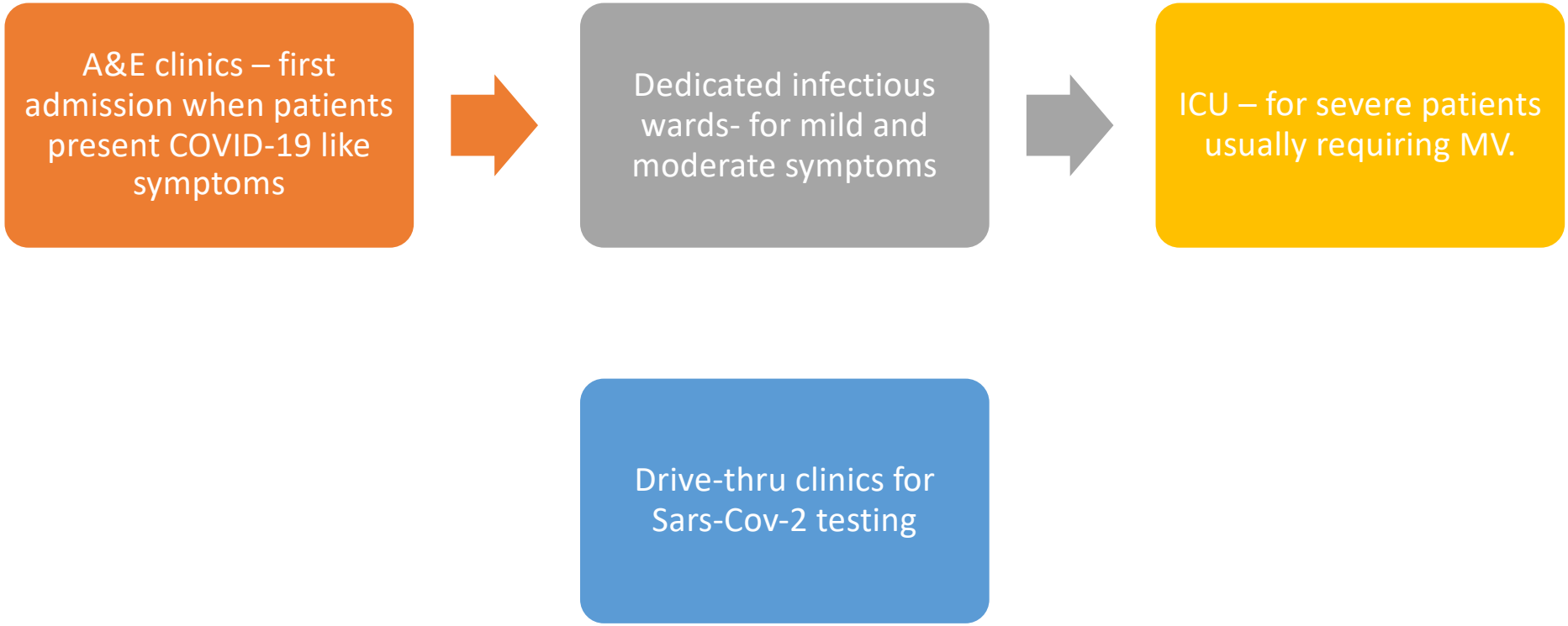


Which type of sample is suitable for your research?

TYPES OF DATA THAT MIGHT BE IMPORTANT FOR COVID-19 PATIENT CHARACTERIZATION

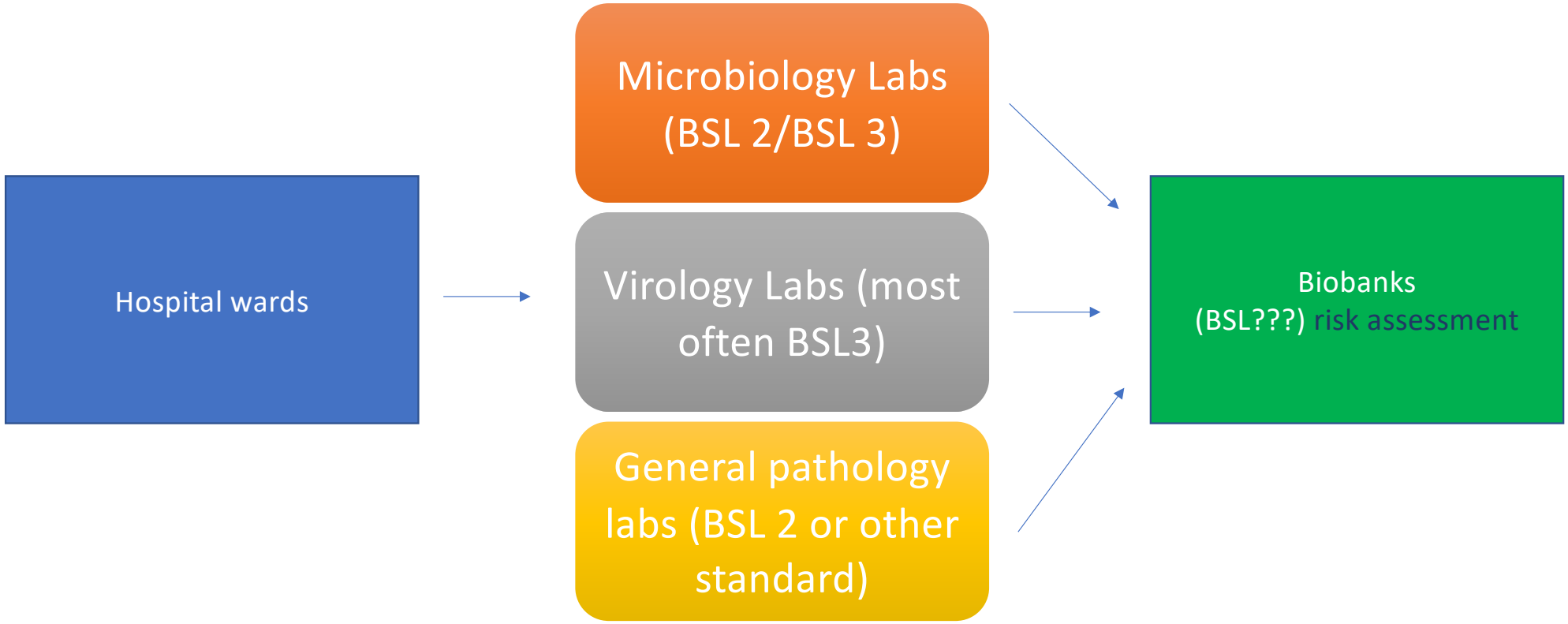
- Disease severity, duration and outcome
- Antibodies titer (IgM and IgG)
- CT imaging of lungs, alternatively Xray
- Clinical symptoms (fever, dry cough, shortness of breath, muscles)
- Blood count and other lab results especially at the moment of hospital admission,
- Treatment protocol (antivirals,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

COLLECTION SITES FOR BIOLOGICAL MATERIAL



A&E- accident and emergency ward , ICU – intensive care unit

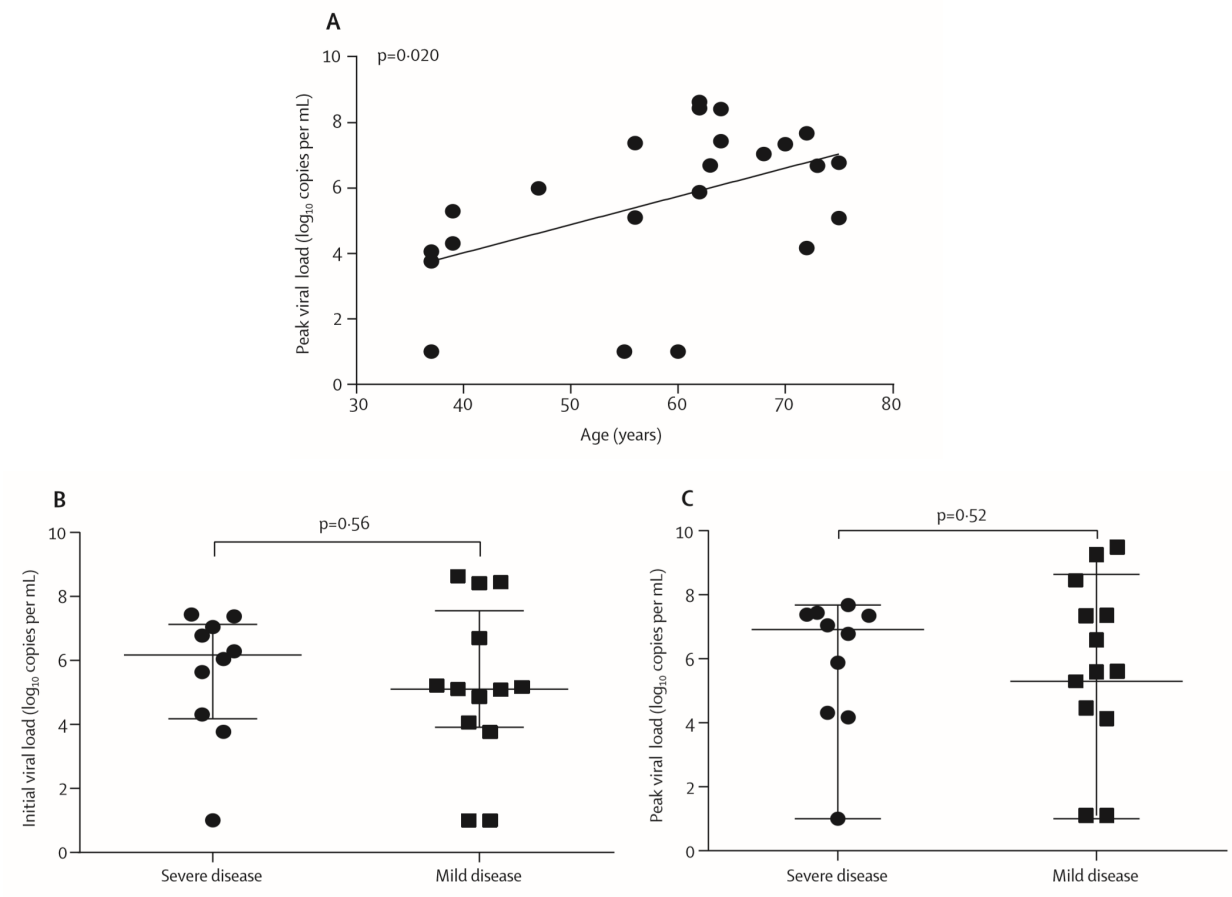
SAMPLE CYCLE: RECEPTION, PROCESSING, ANALYSIS AND STORAGE AREAS



PATIENT VIRAL LOAD IN MILD VS. SEVERE CASES

Wang K-K. et al. Lancet, 23rd March 2020

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.

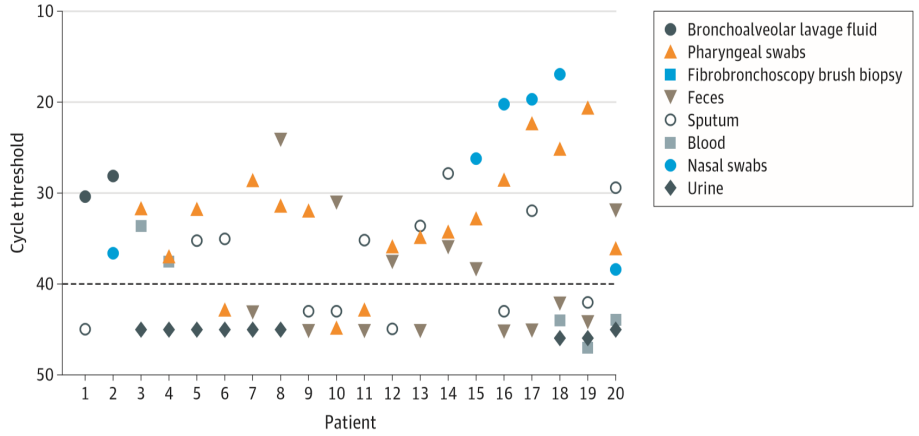


DETECTION OF SARS-COV-2 IN DIFFERENT TYPES OF CLINICAL SPECIMENS

Wang et al., JAMA, 11th March 2020

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

Figure. Severe Acute Respiratory Syndrome Coronavirus 2 Distribution and Shedding Patterns Among 20 Hospitalized Patients



The specimen with a cycle threshold value above the dashed line is interpreted as positive for SARS-CoV-2 RNA; those under, negative.

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

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

Abbreviation: ND, no data.

CORONAVIRUS DISEASE 2019: CORONAVIRUSES AND BLOOD SAFETY

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

"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".

Table 1
Different methods on inactivation of coronavirus in blood products and laboratory tissue culture

Methods	Commercial systems	Mechanism of action [56]	SARS-CoV	MERS-CoV
Heat	N/A	Denaturing the secondary structures of proteins	Products without cells 56°C 20 min in serum 65°C 10 min in serum 60°C 25 min in 25% BSA solution [49] Plasma products 60°C 10 h [52]	DMEM + 5% FBS 56°C 25 min (reduction of 4 $\log_{10}$ $TCID_{50}$/mL) [53]
S/D treatments	Octaplas (Octapharma)	Disruption of lipid membranes	Products without cells 2 h for TNBP/Triton X-100 in PBS or 10% BSA 2 h for TNBP/Tween 80 in PBS or 10% BSA 24 h for sodium cholate in 10% BSA [49] Products without cells 30 min (reduction of >5.75±0.3 $\log_{10}$ $TCID_{50}$/mL) [50]	N/A
Amotosalen + UV-A light	INTERCEPT Blood system for plasma and platelets (Cerus)	Amotosalen (S-59) intercalates into nucleic acid and induces covalent cross-linking upon UV-A exposure	MEM + 10% FBS (reduction of >5.8 $\log_{10}$ PFU/mL) [51]	Platelet concentrate (reduction of 4·48 ± 0.3 $\log_{10}$ PFU/mL) [46] Fresh-frozen plasma (reduction of 4.67 ± 0.25 $\log_{10}$ PFU/mL) [47]
Riboflavin + UV-B light	MIRASOL PRT system for plasma and platelets (Terumo)	Riboflavin associates with nucleic acids and mediates an oxygen-independent electron transfer upon UV exposure	N/A	reduction of >4.07 $\log_{10}$ PFU/mL for pooled plasma reduction of >4.42 $\log_{10}$ PFU/mL for individual donor plasma[54]
UV-C light	THERAFLEX UV-Platelets (Macopharma)	UV-C directly interacts with nucleic acids, causing the formation of nucleotide dimers	Platelet concentrates (reduction of ≥3.4 $\log_{10}$ $TCID_{50}$/mL) [45]	Platelet concentrates (reduction of ≥3.7 $\log_{10}$ $TCID_{50}$/mL) [48]
Methylene blue + Visible light	THERAFEX MB (Macopharma)	MB intercalates into nucleic acid and mediates the formation of singlet oxygen upon illumination	Plasma (reduction of >3.1 $\log_{10}$ $TCID_{50}$/mL [45]	Plasma (reduction of >3.3 $\log_{10}$ $TCID_{50}$/mL) [48]

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 phosphate.

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

- Laboratories should adhere to the guidance in these two documents: The European Committee for Standardisation: CWA15793 Laboratory Biorisk Management, 2011, available from: https://www.uab.cat/doc/CWA15793_2011 and, Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases of January 2020, available from: https://www.who.int/health-topics/coronavirus/laboratory-diagnostics-for-novel-coronavirus

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.

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".

Key points

- Each laboratory should conduct a local (that is, institutional) risk assessment to ensure it is competer 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 (GMPP) should be followe
- The handling and processing of specimens from case with suspected or confirmed COVID-19 infection tha intended for additional laboratory tests, such as haematology or blood gas analysis, should follow loc guidelines for processing potentially infectious mater
- Non-propagative diagnostic laboratory work, includi sequencing and NAAT, on clinical specimens from patients who are suspected or confirmed to be infecte with COVID-19, should be conducted adopting the practices and procedures of "core requirements",[1] as detailed in **Annex 1**, and an appropriate selection of "heightened control measures",[2] as informed by the l risk assessment. In the interim, BSL-2 in the WHO *Laboratory biosafety manual*, 3rd edition *(2)* rema appropriate until the 4th edition replaces it.
- Handling of material with high concentrations of live virus (such as when performing virus propagation, vi isolation or neutralization assays) or large volumes o 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
- Specimen packaging and shipping (follow current guidance of IATA, https://www.iata.org/en/programs/cargo/dgr)

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 14605:2009 + A1:2009 EN 13795-1:2019 Surgical clothing and drapes – Requirements and test methods – Part 1: Surgical drapes and gowns

• 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)

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ISO standards

- EN ISO 374-5:2016 Protective gloves against dangerous chemicals and micro-organisms – Part 5: Terminology and performance requirements for micro-organisms risks
- EN ISO 13688:2013 Protective clothing – General requirements
- EN ISO 10993-1:2009 + AC 2010 Biological evaluation of medical devices

SARS-COV-2 TESTING

- https://www.nature.com/articles/d41587-020-00010-2

NEWS · 23 MARCH 2020 · UPDATE 01 APRIL 2020

Fast, portable tests come online to curb coronavirus pandemic

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

EXPERIENCE SHARING

- How biobanks can deal with Sars-CoV-2 samples – Dr. Helmuth Haslacher, Department of Laboratory Medicine MedUni Wien Biobank.