Preparing to Work with SARS-CoV-2: in supplement to

WHO Laboratory biosafety guidance related to coronavirus disease (COVID-19)

Interim Guidance (28 January 2021)
Overview of Key Points

- Good Microbiological Practice and Procedure
- Biological Risk Assessment
- Clinical testing (non-propagative)
- Culture and isolation of SARS-CoV-2
- Transport and shipping
- Disinfection, inactivation, waste management
Good Microbiological Practice and Procedure
Good Microbiological Practice and Procedure (GMPP)

A series of best biosafe practice and procedure for working with infectious material in the laboratory

• Hand hygiene
• Prevent dispersal
  • Appropriate decontamination and deactivation/disposal
• Avoid injection
  • Safe sharp procedures
• Avoid ingestion and contact with skin and eyes
  • Use personal protective equipment (PPE)
• Avoid inhalation
  • Prevent aerosol formation

GMPP is part of the “Core Requirements” (see Annex I of the WHO Laboratory biosafety guidance related to coronavirus disease (COVID-19)¹ or the fourth edition of the Laboratory Biosafety Manual²)

¹ https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1
² https://www.who.int/publications/i/item/9789240011311
Biological Risk Assessment
Biological Risk Assessment

A systematic process of gathering information and evaluating the likelihood and impact of exposure to or release of workplace hazard(s) and determining the appropriate risk control measures to reduce the risk.

STEP 1. Gather information (hazard identification)
STEP 2. Evaluate the risks
STEP 3. Develop a risk control strategy
STEP 4. Select and implement risk control measures
STEP 5. Review risks and risk control measures

Refer to Annex II of the WHO Laboratory biosafety guidance related to coronavirus disease (COVID-19)¹ or the fourth edition of the Laboratory Biosafety Manual²

¹ https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1
² https://www.who.int/publications/i/item/9789240011311

This process is best carried out by a team of staff that are involved in various processes related to the laboratory work.
STEP 1. Gather information (hazard identification)

Consider the laboratory process to be performed and the following factors that influence risk:

• The biological agent (SARS-CoV-2)
• Laboratory procedures and equipment
• Control measures already in place
• Facility
• Personnel
• Other factors
SARS-CoV-2: the aetiological agent

- Contact and droplet transmission (transmission via aerosols and fomites discussed but not yet proven)
- Vaccination possible but limited availability
- Highly contagious
- Infectious dose unknown
- Surface half-life uncertain
- Non-specific and varied symptoms
- Asymptomatic persons can spread disease
- Severe morbidity among immuno-incompetent and some persons with comorbidities
- Likelihood of mortality increases with age and infirmity
- No preexisting specific immunity in human population but an increasing number of convalescents and vaccinated people. Though the duration of the immunity is not yet reliably determined, and herd immunity could not be assumed at the moment.
- Some antiviral drugs under trial; treatment of symptoms

These factors will influence the consequence of accidental exposure or release!
Procedures and equipment

Aerosol producing procedures:

• Vortexing
• Shaking
• Centrifuging
• Pipetting

Sharps use (glass or needles)

Culture – highly concentrated or large volumes of virus

Laboratory animals - scratches or bites

These procedures increase the likelihood of an accidental exposure or release
Control measures in place

Bioccontainment
• Biosafety cabinet (BSC)
• Glovebox (possible alternative)

Personal Protective Equipment
• Disposable gloves
• Full-length laboratory coats/gowns
• Eye protection
• Face shields
• Masks/respirators

Administrative Controls
• Training
• Good Microbiological Practice and Procedure (GMPP)
• Standard operating procedures (SOPs)
• Biosafety manual

These control measures reduce the likelihood of an accidental release or exposure.
Asset

Integrity
• Ample space with a hand-washing basin
• Intact (no gaps or breaches in structure)
  • Easy to clean and decontaminate
• Designed or refitted for safe, efficient and ergonomic operations

Safety and Security
• Restricted access to labs/corridors
• Doors labelled with biohazard sign
• Workflow – tidy and uncluttered

Ventilation
• Sufficient ventilation
• Directional airflow into the lab (virus isolation)

Facilities with these features reduce the likelihood of an accidental release or exposure
Personnel

Competence

**Trained to perform the work**
- Methods and equipment
- Biosafe practices and correct use of PPE
- Continual learning

**Understanding of risks**
- Mitigation and remediation

**Experience**
- Trained and knowledgeable in relevant lab techniques

**Attitude**
- Professional
- Focused

Well-trained, experienced laboratory personnel reduce the likelihood of an accidental release or exposure
STEP 2. Evaluate the risks

• What situations could lead to potential exposure or release?
  • Spills, aerosols, injury?
• How likely are these situations to happen?

**What are the consequences of exposure or release?**

<table>
<thead>
<tr>
<th>Consequences of exposure/releases</th>
<th>Severe</th>
<th>Medium</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td></td>
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<tr>
<td>Negligible</td>
<td>Very low</td>
<td>Low</td>
<td>Medium</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Likelihood of exposure/release</th>
<th>Unlikely</th>
<th>Possible</th>
<th>Likely</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>Procedures</th>
<th>Hazards</th>
<th>How likely is this ?**</th>
<th>Consequence</th>
<th>Inherent Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample accessioning</td>
<td>• Container leaks</td>
<td>Unlikely to Possible</td>
<td></td>
<td>Low to Medium</td>
</tr>
<tr>
<td></td>
<td>• Container breakage (sharps)</td>
<td></td>
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<tr>
<td></td>
<td>• Infectious material spill</td>
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<tr>
<td>Viral Culture*</td>
<td></td>
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<tr>
<td>Sample collection*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>• Aerosol exposure during sample processing</td>
<td>Possible to Likely</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Eye splash during sample processing</td>
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<tr>
<td></td>
<td>• Infectious material spill</td>
<td></td>
<td></td>
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<tr>
<td>RT-PCR</td>
<td></td>
<td></td>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>ELISA (serology)</td>
<td></td>
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</tr>
<tr>
<td>Near Point-of-Care (PoC)</td>
<td></td>
<td></td>
<td></td>
<td>Low to Medium</td>
</tr>
<tr>
<td></td>
<td>• Aerosol exposure during sample processing</td>
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<td></td>
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<td></td>
<td>• Infectious material spill</td>
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<tr>
<td>PoC</td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Whole Genome Sequencing</td>
<td>None (if sample is already inactivated)</td>
<td>Unlikely</td>
<td>Negligible</td>
<td>Very low</td>
</tr>
</tbody>
</table>

* These are the procedures that involve the greatest risk

**The likelihood will depend on control measures that are already in place
STEP 3. Develop a risk control strategy

Considerations:

- Are resources sufficient to secure and maintain potential risk control measures?
- Will any conditions identified limit the ability to reduce risk?
- Can the work be done without additional risk control measures?
### STEP 4. Select and implement risk control measures

Add control measures (PPE, BSC, others as appropriate)

**Risk should be reduced to a level that is acceptable!**

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Hazards</th>
<th>How likely is this?**</th>
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<td>• Infectious material spill</td>
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</tr>
</tbody>
</table>

**Additional Control Measures**

- BSC, respiratory protection, eye protection, ventilation

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Hazards</th>
<th>How likely is this?**</th>
<th>Consequence</th>
<th>Residual Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample accessioning</td>
<td>• Container leaks</td>
<td>Unlikely</td>
<td>Moderate</td>
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</table>
## Adding control measures to reduce risk

<table>
<thead>
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<th>Hazards</th>
<th>Inherent Risk</th>
<th>Additional Control Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample accessioning</td>
<td>• Container leaks</td>
<td>Low to Medium</td>
<td>BSC, respiratory protection, eye protection, ventilation</td>
</tr>
<tr>
<td></td>
<td>• Container breakage (sharps)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Infectious material spill</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral Culture*</td>
<td>• Aerosol exposure during sample processing</td>
<td>Medium to High</td>
<td>Heightened control measures/BSL3, inward air flow, BSC, enhanced respiratory protection</td>
</tr>
<tr>
<td>Sample collection*</td>
<td>• Eye splash during sample processing</td>
<td></td>
<td>Face shield, respiratory protection</td>
</tr>
<tr>
<td></td>
<td>• Infectious material spill</td>
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<td></td>
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<tr>
<td>RT-PCR ELISA (serology)</td>
<td>• Aerosol exposure during sample processing</td>
<td>Medium</td>
<td>BSC, respiratory protection, eye protection, ventilation</td>
</tr>
<tr>
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<td></td>
<td>• Infectious material spill</td>
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<tr>
<td>Near POC</td>
<td>• Aerosol exposure during sample processing</td>
<td>Low to Medium</td>
<td>Respiratory protection, eye protection or face shield, ventilation</td>
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<tr>
<td>POC</td>
<td>• Aerosol exposure during sample processing</td>
<td>Low</td>
<td>Respiratory protection, eye protection or face shield, ventilation</td>
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<tr>
<td>Whole Genome Sequencing</td>
<td>None</td>
<td>Very low</td>
<td>None needed</td>
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Note: BSC = Biosafety Cabinet
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<td>POC</td>
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<td>Low</td>
<td>Very low</td>
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<tr>
<td>Whole genome sequencing</td>
<td>None</td>
<td>Unlikely</td>
<td>Negligible</td>
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* These are the procedures that involve the greatest risk

** The likelihood will depend on control measures that can be added to reduce risk
STEP 5. Review risks and risk control measures

- Risk assessment should be a continuous process
- Should be performed whenever changes take place:
  - Personnel
  - Facility
  - Equipment
  - Methods
  - Regulations
Clinical testing (non-propagative)
Real Time Reverse Transcriptase PCR (Nucleic Acid Amplification Test)\(^3\)

- Good Microbiological Practice and Procedure (GMPP)
  - (See “Core Requirements”, Annex I\(^1\) or the LBM4\(^2\))
- Appropriate PPE
- Staff Competence
- Biosafety Level 2 (BSL-2) or heightened control measures suitable for diagnostic services in the WHO Laboratory biosafety manual: fourth edition\(^2\)
- BSC or primary containment device should be utilized

\(^1\)https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1
\(^2\)https://www.who.int/publications/i/item/9789240011311
\(^3\)https://www.fda.gov/media/134922/download
Point of Care (PoC) and near-POC Assays
including antigen-detecting RDTs (Ag-RDT)
(No nucleic acid extraction)

• Good Microbiological Practice and Procedure (GMPP)
• Appropriate PPE
• Staff Competence

• May be performed on bench (outside a lab)
  • Well-ventilated area (see the following slides)
  • On absorbent towel or diaper
  • Free of clutter

• Optional
  • Biosafety cabinet/glove box
  • Use primary containment if readily available
SARS CoV-2 Antigen Tests
Detect only active COVID-19 infection
Simple, rapid, easy to perform

WHO interim Guidance
Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays

Suggested Use Cases during Outbreaks
• PCR is unavailable/long turnaround times
  • Remote settings, within institutions
• Screening of at-risk individuals (before NAAT)
• Monitor trends in disease incidence
• Early detection and isolation
  • Widespread transmission
  • Asymptomatic contacts

Ag RDT should meet diagnostic criteria of ≥ 80% sensitivity and ≥ 97% specificity

4 https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays
Antigen Test (POC)

From: [https://doi.org/10.1016/j.nmni.2020.100713](https://doi.org/10.1016/j.nmni.2020.100713)
Ventilation

The movement of fresh air around a closed space, or the system that does this

Types

• Natural:
  Purpose-built, building openings (windows, doors, whirlybirds, chimneys, etc.)

• Assisted (mixed mode):
  Relies on natural driving forces to provide the desired (design) flow rate.

• Mechanical- Fans drive mechanical ventilation.
  Installed in windows, walls, air ducts

The risk assessment decides the type of lab ventilation based on suitability and availability

<table>
<thead>
<tr>
<th></th>
<th>Natural ventilation</th>
<th>Assisted ventilation</th>
<th>Mechanical ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Climate</strong></td>
<td>Cannot be used in extreme hot or cold environments</td>
<td>In extreme climates, must be used with HVAC and heating systems.</td>
<td>Suitable for all weather climates</td>
</tr>
<tr>
<td><strong>Equipment Cost</strong></td>
<td>Inexpensive</td>
<td>Installation costs low to medium</td>
<td>Expensive to install and maintain</td>
</tr>
<tr>
<td><strong>User control</strong></td>
<td>High but binary (all or nothing)</td>
<td>Greater control by user</td>
<td>Greatest control by user</td>
</tr>
<tr>
<td><strong>Air exchange/ventilation rate</strong></td>
<td>Least control. Cannot establish negative pressure</td>
<td>Greater control by user</td>
<td>Greatest control by user, but can fail to maintain air exchange</td>
</tr>
<tr>
<td><strong>Energy cost</strong></td>
<td>Low</td>
<td>Medium</td>
<td>High to very high. May need filter, HEPA</td>
</tr>
<tr>
<td><strong>Comfort</strong></td>
<td>Potential for noise intrusion</td>
<td>Potential for equipment noise</td>
<td>Potential for equipment noise</td>
</tr>
<tr>
<td><strong>Product protection</strong></td>
<td>Highest potential for contamination of the specimens</td>
<td>Potential for contamination of the specimens without containment</td>
<td>Lowest potential for contamination of the specimens without containment</td>
</tr>
</tbody>
</table>
Culture and isolation of SARS-CoV-2
Requirements for culture and isolation of SARS-CoV-2

• Special training
• Detailed risk assessment
• Heightened Control measures or Biosafety Level 3 (BSL-3)
• Appropriate PPE
• Facility with inward directional airflow into the laboratory (negative pressure)

• Not suitable for most laboratories
  • → Outside the main scope of this supplementary guidance
Transport and shipping
Intra-facility transfer\textsuperscript{7,8}

- From clinic to laboratory
- Between buildings
- Between non-adjoining laboratories
- Use a cart if many samples are being moved
- Spill kit available and staff trained
- Pneumatic tube system
  - Detailed risk assessment required if necessary to use
  - Tightly sealable bag system recommended

\textbf{Disinfect} external surfaces of carrier and cart before moving between laboratories

\textsuperscript{7}https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/

\textsuperscript{8}https://www.cdc.gov/csels/dls/locs/2020/transport_recommendations_for_covid-19_specimens.html
Inter-facility (between facilities) transportation

1. Human specimens that may contain SARS-CoV2
   - Ground Transport
     - Follow local and applicable international regulations for ground transport
     - Ideally triple-packaged
     - If using commercial carrier, Category B Regulations apply (UN3373)
   - Air transport
     - Category B UN3373 regulations

2. Live viral cultures
   Must be shipped according to Category A UN2814 regulations

Follow WHO Guidance on regulations for the transport of infectious substances 2019–2020

Follow WHO Guidance on regulations for the transport of infectious substances 2019–2020

Disinfection, inactivation, waste management
Disinfection

1. Sodium hypochlorite (bleach)\(^2\)
   - 1000 parts per million [ppm] (0.1%) for general surface disinfection
   - 10 000 ppm (1%) for disinfection of sample spills
   - Prepare new dilution every 24 hours
   - Contact time ≥ 10 min
2. Ethanol (EtOH) 62–71% (Contact time ≥ 10 min)
3. Hydrogen peroxide (H\(_2\)O\(_2\)) 0.5%
4. Quaternary ammonium compounds and phenolic compounds, if used according to the manufacturer’s recommendations
5. Other compounds according to manufacturer’s directions\(^2\)

\(^2\) [https://www.who.int/publications/i/item/9789240011311]
\(^10\) [https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2-covid-19]
Inactivation
Inactivate SARS CoV-2 whenever possible BEFORE manipulation to prevent accidental exposure or release

1. Chemical
   • Some viral RNA extractions buffers
   • Formalin for tissue samples
2. Gamma Irradiation (≥1 Mrad)
3. Heat
   • 30 min at 65°C (conservative)
   • *Serology – may be affected (Read manufacturer’s instructions)

External lysis buffer of the common RNA extraction kits is effective in inactivating the COVID-19 virus without heat or other additional means

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10 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7354533/pdf/viruses-12-00624.pdf
3 https://www.fda.gov/media/134922/download
11 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7354523/pdf/viruses-12-00622.pdf
Decontamination and waste management principles

1. Control biological risks
   • Any surfaces or materials known to be or potentially contaminated
   • Benchtops, interior surfaces of BSC, equipment and devices

2. Identify and Segregate contaminated materials
   • Sharps
   • Contaminated waste
   • Chemical waste
   • General (non-hazardous) waste

3. For all contaminated materials or liquids
   • Decontaminate onsite to allow further safe handling or package and transport safely to another treatment site
Waste Management

• Autoclave or incinerate infectious waste
• Waste is Category B for transportation purposes
  • Regulated Medical Waste UN 3291
• Disposal of POC spent test cartridges
  • Read manufacturers specific instructions
  • Read Material Safety Data Sheets
  • Follow national, local regulations for disposal

2 https://www.who.int/publications/i/item/9789240011311
Remember…
Use caution when working with products containing guanidinium iso/thiocyanate (GTC/GITC)\textsuperscript{12,13}.

GTC/GITC lyses cells and denatures nucleases (RNase/DNase).

Products containing GTC/GITC:
- Most DNA/RNA extraction kits
- GeneXpert cartridges
- TRIzol™ and similar products
- Some viral transport media (e.g. PrimeStore® MTM, Zymo DNA/RNA Shield)

Read and follow manufacturer’s instructions and Safety Data Sheets (SDS/MSDS).

**Do not use bleach** in the presence of GTC/GITC
- Reaction produces cyanide and chlorine gases
- GTC/GITC inactivates organisms, so bleach not required

**GTC/GITC waste is Hazardous Waste**
- Toxic to marine and aquatic life
- **Do not** dispose of in wastewater stream
- Segregate GTC/GITC waste
  - Dispose of according to federal, state and local guidelines

\textsuperscript{12} Paik SY, Wu X. 2005 Chemical Health and Safety 12(4):33-38
\textsuperscript{13} https://www.ehs.harvard.edu/sites/default/files/lab_safety_guideline_qiagen_kits_0.pdf
## Waste Management at a glance

<table>
<thead>
<tr>
<th></th>
<th>Human test samples</th>
<th>Nucleic Acid POC Cartridges; PCR extraction buffers</th>
<th>Antigen RDT Cartridges; Antibody test buffers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazard</strong></td>
<td>SARS-CoV2 (potential)</td>
<td>GTC (Guanidinium iso/thiocyanate)</td>
<td>Sodium azide (Na$<em>3$)$</em>{15}$ buffers</td>
</tr>
<tr>
<td><strong>Precautions</strong></td>
<td>Handle with appropriate PPE Prevent aerosolization.</td>
<td>Releases toxic gases in the presence of Sodium hypochlorite (bleach)</td>
<td>Toxic to aquatic life and acute toxic for humans.</td>
</tr>
<tr>
<td><strong>Cleaning and disinfection</strong></td>
<td>0.1-1.0% Sodium hypochlorite (bleach) or other recommended disinfectant</td>
<td>GCT inactivates SARS-CoV-2 RNA. Do not use bleach in the presence of GTC. Use a 70% solution of ethanol or isopropyl alcohol.</td>
<td>Sodium azide inactivates SARS-CoV-2, use appropriate disinfectant such as 70% ethanol or isopropyl alcohol. Do not autoclave.</td>
</tr>
<tr>
<td><strong>Disposal</strong></td>
<td>Category B Waste Autoclave or incinerate</td>
<td>Follow manufacturer’s instructions. Segregate PCR extraction buffers as hazardous waste for professional disposal.</td>
<td>Read Safety Data Sheet and follow manufacturer’s instructions for disposal or dispose of with hazardous waste. Do not pour sodium azide down the drain.</td>
</tr>
</tbody>
</table>

Summary
Before beginning laboratory work...

- Understand and practice GMPP
- Use appropriate containment and control measures as per Core Requirements for clinical testing
- Understand and practice Biological Risk Assessment (BRA)
- Understand and practice safe transport and shipping of samples
- Heightened control measures (or BSL3) for work with live cultures
- Understand and practice appropriate viral inactivation, disinfection and waste inactivation procedures
Reference, Acknowledgements, Thanks

WHO Laboratory biosafety guidance related to coronavirus disease (COVID-19) Interim guidance 28 January 2021
https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1

• Christina Scheel (Centers for Disease Control and Prevention, United States of America)
• Stuart Blacksell (Mahidol Oxford Tropical Medicine Research Unit, Thailand)
• Kathrin Summermatter (Institute for Infectious Diseases, University of Bern, Switzerland)

• WHO Health Emergencies Programme and COVID-19 laboratory team: Kazunobu Kojima, Rica Zinsky, Céline Barnadas, Matthew Lim, Mick Mulders, Karin von Eije, Mark Perkins, Maria Van Kerkhove
# Highlights of Guidance

<table>
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<tr>
<th>Focus Topic</th>
<th>Change</th>
<th>Slide #s</th>
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<tr>
<td>POC – Antigen test</td>
<td>Suggested use cases and diagnostic window</td>
<td>23-24</td>
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<td>Ventilation</td>
<td>Types of ventilation and advantages/disadvantages of each according to climate and facility resources</td>
<td>25-26</td>
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<td>Inactivation</td>
<td>Most nucleic acid extraction buffers and some transport media contain detergents and chemicals that deactivate SARS-CoV-2. Sodium azide inactivates SARS-CoV-2.</td>
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<td>Waste</td>
<td>Disposal of POC cartridges; sodium azide cannot be poured down the drain.</td>
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<tr>
<td>GTC: cleaning and disposal</td>
<td>Guanadinium iso/thiocyanate: segregate for professional disposal. Do NOT use bleach in the presence of GTC/GITC. Disinfection is not needed for GTC/GITC waste since it kills SARS-CoV-2.</td>
<td>35-37</td>
</tr>
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