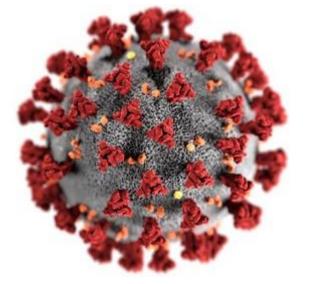
Preparing to Work with SARS-CoV-2: in supplement to

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

Interim Guidance (28 January 2021)









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



This process is best carried out by a team of staff that are involved in various processes related to the laboratory work

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

² https://www.who.int/publications/i/item/9789240011311



STEP 1. Gather information (hazard identification)

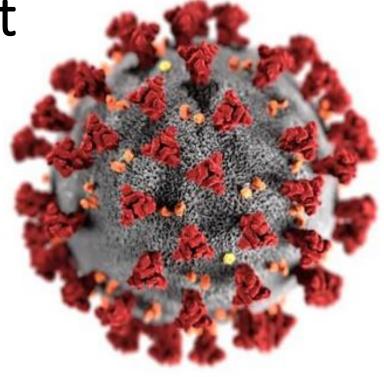
Consider the laboratory process to be performed and the following factors that influence risk:

- 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



Procedures and equipment

Aerosol producing procedures:

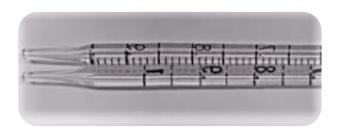
- Vortexing
- Shaking
- Centrifuging
- Pipetting

Sharps use (glass or needles)

<u>Culture</u> – highly concentrated or large volumes of

virus

<u>Laboratory animals</u> - scratches or bites











Control measures in place

Biocontainment

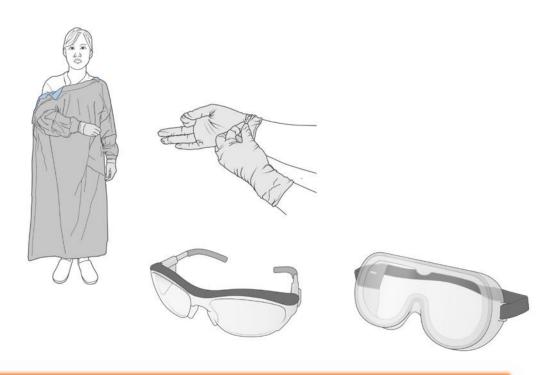
- Biosafety cabinet (BSC)
- Glovebox (possible alternative)

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

Facility

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)





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





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?

	Severe	Medium	High	Very high
of exposure/ release	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium
		Unlikely	Possible	Likely
		Likeliho	od of exposure/	'release

Inherent Risk working with SARS-CoV-2 in the Laboratory

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

^{*} 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)

Procedures	Hazards	How likely is this ?**	Consequence	Inherent Risk
Sample accessioning	Container leaksContainer breakage (sharps)Infectious material spill	Unlikely to Possible	Moderate	Low to Medium



Additional Control Measures

BSC, respiratory protection, eye protection, ventilation



	Severe	Medium	High	Very high
of exposure/ release	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium
		Unlikely	Possible	Likely
		Likeliho	od of exposure/	release

Procedures	Hazards	How likely is this ?**	Consequence	Residual Risk
Sample accessioning	Container leaksContainer breakage (sharps)Infectious material spill	Unlikely	Moderate	Low

Risk should be reduced to a level that is acceptable!

Adding control measures to reduce risk

Procedures	Hazards	Inherent Risk	Additional Control Measures
Sample accessioning	Container leaksContainer breakage (sharps)Infectious material spill	Low to Medium	BSC, respiratory protection, eye protection, ventilation
Viral Culture*			Heightened control measures/BSL3, inward air flow, BSC, enhanced respiratory protection
Sample collection*		Medium to High	Face shield, respiratory protection
RT-PCR ELISA (serology)	 Aerosol exposure during sample processing Eye splash during sample processing 	Medium	BSC, respiratory protection, eye protection, ventilation
Near POC	Infectious material spill	Low to Medium	Respiratory protection, eye protection or face shield, ventilation
POC		Low	Respiratory protection, eye protection or face shield, ventilation
Whole Genome Sequencing	None	Very low	None needed

Residual Risk: Risk remaining after adding controls (previous slide)

Procedures	Hazards	How likely is this ?**	Consequence	Residual Risk
Sample accessioning	Container leaksContainer breakage (sharps)Infectious material spill	Unlikely		Low
Viral Culture* Sample collection*		Unlikely to Possible	Moderate	Low to Medium
RT-PCR ELISA (serology)	 Aerosol exposure during sample processing Eye splash during sample processing Infectious material spill 	Unlikely	iviouerate	Low
Near POC		Unlikely		Low
POC		Unlikely		Very low
Whole genome sequencing	None	Unlikely	Negligible	Very low

^{*} 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)



- Good Microbiological Practice and Procedure (GMPP)
 - (See "Core Requirements", Annex I ¹ or the LBM4²)
- Appropriate PPE
- Staff Competence
- Biosafety Level 2 (BSL-2) or heightened control measures suitable for diagnostic services in the WHO Laboratory biosafety manual: fourth edition²
- 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 <u>active</u> COVID-19 infection Simple, rapid, easy to perform

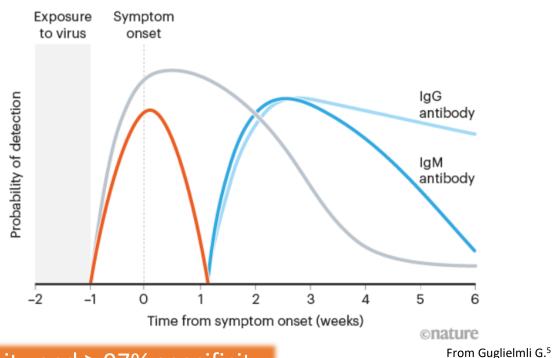
WHO interim Guidance Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays⁴

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

Pre-symptomatic and within 5-7 days after symptom onset

- PCR-based tests can detect small amounts of viral genetic material, so a test can be positive long after a person stops being infectious.
- Rapid antigen tests detect the presence of viral proteins and can return positive results when a person is most infectious.
- Antibody tests detect the body's immune response to the virus and are not effective at the earliest phase of infection.



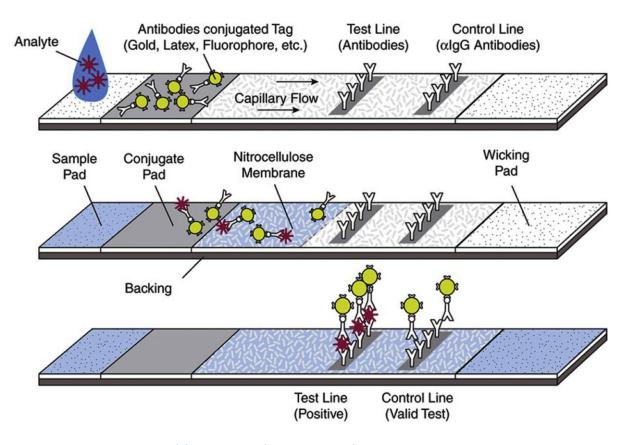
Ag RDT should meet diagnostic criteria of ≥ 80% sensitivity and ≥ 97% specificity

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

⁵ Guglielmli G. 2020. Nature Vol 585. pp 496-98.

Antigen Test (POC)

Lateral Flow Assay Architecture



From: 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

Types⁶

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

⁶ https://medicalguidelines.msf.org/viewport/TUB/latest/appendix-18-advantages-and-disadvantages-of-ventilation-techniques-20324472.html

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



Culture and isolation of SARS-CoV-2

Requirements for culture and isolation of SARS-CoV-2

- Special training
- Detailed risk assessment
- Heightened Control meaures 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

Primary receptacle

Watertight, leak-proof or siftproof receptical wrapped in absorbent material

Intra-facility transfer^{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



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

8https://www.cdc.gov/csels/dls/locs/2020/transport recommendations for covid-19 specimens.html





Pneumatic tube system





Sealable bag

Inter-facility (between facilities) transportation

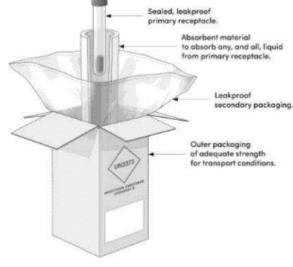
- 1. Human specimens that may contain SARS-CoV2
 - Ground Transport
 - · Follow local and applicable international regulations for ground transport
 - Ideally triple-packaged
 - If using commercial carrier, Category B Regulations apply (UN3373)
 - Air transport
 - Category B UN3373 regulations
- 2. Live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

Must be shipped according to Category A UN2814 regulations

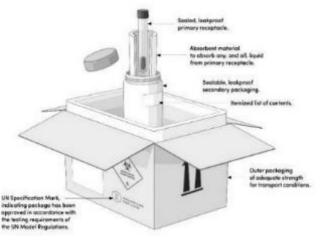
Follow WHO Guidance on regulations for the transport of ple packaging infectious substances 2019–20209

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





Category B





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 (H₂O₂) 0.5%
- 4. Quaternary ammonium compounds and phenolic compounds, if used according to the manufacturer's recommendations
- 5. Other compounds according to manufacturer's directions²

- Use with caution in well-ventilated areas
- Allow appropriate contact time
- Do not use expired chemicals





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^{9,3,10}
- Formalin for tissue samples¹⁰
- 2. Gamma Irradiation (≥1 Mrad)¹¹
- 3. Heat
 - 30 min at 65°C³ (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 means³

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

¹² https://absa.org/wp-content/uploads/2020/04/ABSA2020-InVitroInactivation-ofSARS-CoV-2-UsingGammaRadiation.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 <u>all</u> 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 waste²
- 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)^{12,13} GTC/GITC lyses cells and denatures nucleases (RNase/DNase)

Products containing /GTCGITC

- 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)

<u>Do not use bleach</u> 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

¹³Paik SY, Wu X. 2005 Chemical Health and Safety 12(4):33-38

¹⁴ https://www.ehs.harvard.edu/sites/default/files/lab_safety_guideline_giagen_kits_0.pdf

Waste Management at a glance

	Human test samples	Nucleic Acid POC Cartridges; PCR extraction buffers	Antigen RDT Cartridges; Antibody test buffers
Hazard	SARS-CoV2 (potential)	GTC (Guanidinium iso/thiocyanate)	Sodium azide (NaN ₃) ¹⁵ buffers
Precautions	Handle with appropriate PPE Prevent aerosolization.	Releases toxic gases in the presence of Sodium hypochlorite (bleach)	Toxic to aquatic life and acute toxic for humans.
Cleaning and disinfection	0.1-1.0% Sodium hypochlorite (bleach) or other recommended disinfectant	GCT inactivates SARS-CoV-2 RNA. Do not use bleach in the presence of GTC. Use a 70% solution of ethanol or isopropyl alcohol.	Sodium azide inactivates SARS-CoV-2, use appropriate disinfectant such as 70% ethanol or isopropyl alcohol. Do not autoclave.
Disposal	Category B Waste Autoclave or incinerate	Follow manufacturer's instructions. Segregate PCR extraction buffers as hazardous waste for professional disposal.	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.

^{15 &}lt;a href="https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-azide">https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-azide

Summary

Before beginning laboratory work...



Understand and practice GMPP



Heightened control measures (or BSL3) for work with live cultures



Understand and practice
Biological Risk Assessment (BRA)



Understand and practice safe transport and shipping of samples



Use appropriate containment and control measures as per Core Requirements for clinical testing



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

Focus Topic	Change	Slide #s
POC – Antigen test	Suggested use cases and diagnostic window	23-24
Ventilation	Types of ventilation and advantages/disadvantages of each according to climate and facility resources	25-26
Inactivation	Most nucleic acid extraction buffers and some transport media contain detergents and chemicals that deactivate SARS-CoV-2. Sodium azide inactivates SARS-CoV-2.	34
Waste	Disposal of POC cartridges; sodium azide cannot be poured down the drain.	36
GTC: cleaning and disposal	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.	35-37