RNA EXTRACTION USING QIAamp VIRAL RNA MINI KIT (Qiagen)

Learning Objectives

- At the end of this training, the trainee will be able to:
  - Understand biosafety practices associated with RNA extraction from clinical specimens
  - Explain the conditions and factors affecting extraction
  - Understand sample handling and storage
  - Understand the principle of RNA extraction
  - Perform viral RNA extraction using the Qiagen QIAamp Viral RNA Mini kit

Biosafety Considerations

Clinical specimens are potentially infectious and need to be handled and processed in a way that minimizes the risk to the laboratorian.

N95 respirator

1. Don proper personal protective equipment (PPE) *as recommended by risk assessment*


Safety glass


Gloves


Face shield


Tyvek coveralls


Hood + PAPR


Shoe Covers

Biosafety Considerations

Clinical specimens are potentially infectious and need to be handled and processed in a way that minimizes the risk to the laboratorian.

2. Process clinical specimens in a Biological Safety Cabinet (BSC) that has been certified as operating within the manufacturer's specifications within the last year.

Biosafety Considerations

Clinical specimens are potentially infectious and need to be handled and processed in a way that minimizes the risk to the laboratorian.

3. Use an appropriate decontamination solution at the correct concentration, making sure to expose the potentially contaminated surface and/or material to the solution for the appropriate contact time
    - Sodium hypochlorite (bleach)
    - Alcohol (ethanol, isopropanol)
    - Hydrogen peroxide
    - Phenolic compounds
    - Quaternary ammonium compounds

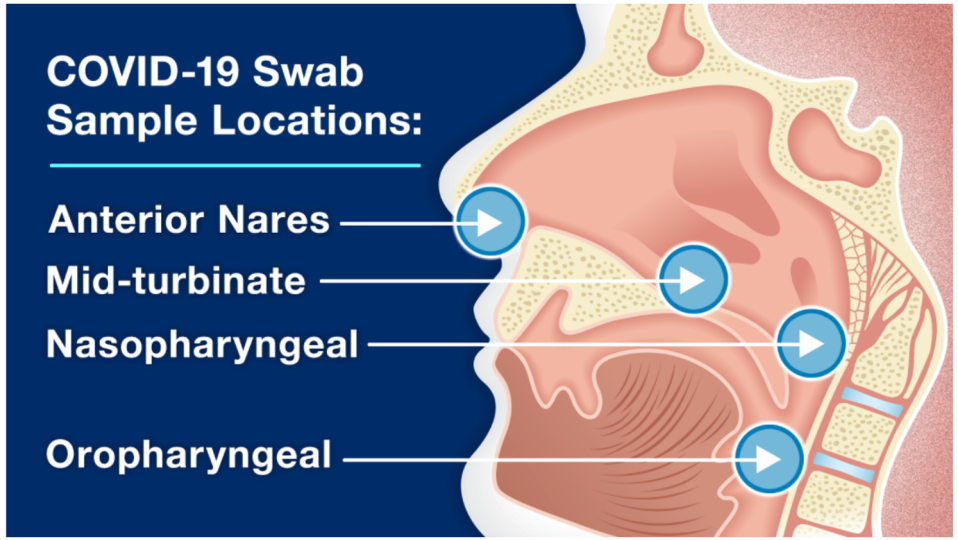
Summary of Biosafety Considerations

- Appropriate personal protective equipment (PPE), based on a risk assessment, should be worn through the whole process
- Handling and processing of clinical samples should be done in a certified class II biological safety cabinet (BSC) with HEPA filters to protect the sample from environmental contamination, the laboratory worker from the sample, and the environment from the sample
- The biological safety cabinet, micropipettes and other equipment, and other working areas should be decontaminated using appropriate decontaminants

Clinical Specimen Types and Handling

- Clinical specimens are:
  - Swabs in viral transport medium:
    - Nasopharyngeal
    - Oropharyngeal
    - Anterior nares (nasal)
    - Nasal mid-turbinate
  - Nasopharyngeal wash/aspirate
  - Nasal wash/aspirate
  - Sputum
  - Endotracheal aspirate or broncho-alveolar lavage fluid (BAL)

- Storage
  - 2-8 ºC for up to 72 hours (short-term storage)
  - -20 ºC or colder (long-term storage)



Precautions when Working with RNA

- RNA stands for RiboNucleic Acid
- Working with RNA is more demanding than working with DNA
  - RNA is very unstable, and is easily degraded by RNAses
- RNases are enzymes that degrade RNA
  - Difficult to inactivate, even by prolonged boiling or autoclaving
  - Present in dust, skin secretions, bodily fluids, and ungloved hands
  - Active at room temperature, 4°C, and colder, although freezing reduces the rate of degradation
  - Small amounts can destroy RNA in a sample, leading to a false negative test result
- Solutions such as RNAse Away can remove RNAses from surfaces
- Wear gloves at all times when preparing and working with RNA, and change them if you touch bare skin or undecontaminated equipment/surfaces

Precautions when Working with RNA

- Use filtered, RNAse-free pipette tips and RNAse-free tubes
- Change out pipette tips if there is a chance the tip has touched a surface
- Decontaminate working surfaces, equipment, tubes, with bleach, RNase AWAY, and alcohol.
- Clean and decontaminate the working area and laboratory frequently
- All reagents and consumables used should be RNase-free
  - Prepare single-use aliquots of reagents in a genome-free space (such as where master mix is prepared) to minimize the risk of contaminating them through reuse
- Store extracted RNA in small single-use aliquots at -80 ºC (long-term storage) or -20 ºC (short-term storage). Avoid repeated freeze/thaw cycles

Overview of RNA Extraction

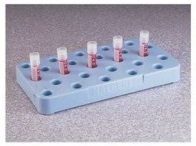
Clinical specimen

Lysis of samples using a lysis buffer

Lysates transferred to spin column for binding

Columns are washed 2X

Nucleic acids are eluted (RT-PCR, sequencing, …)



Samples

Lys
e

Bind

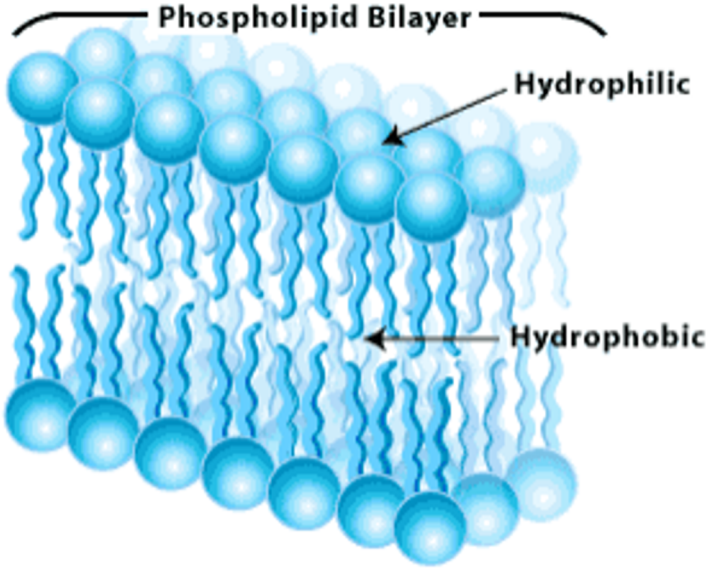
Wash (2X)

Elute

Pure RNA

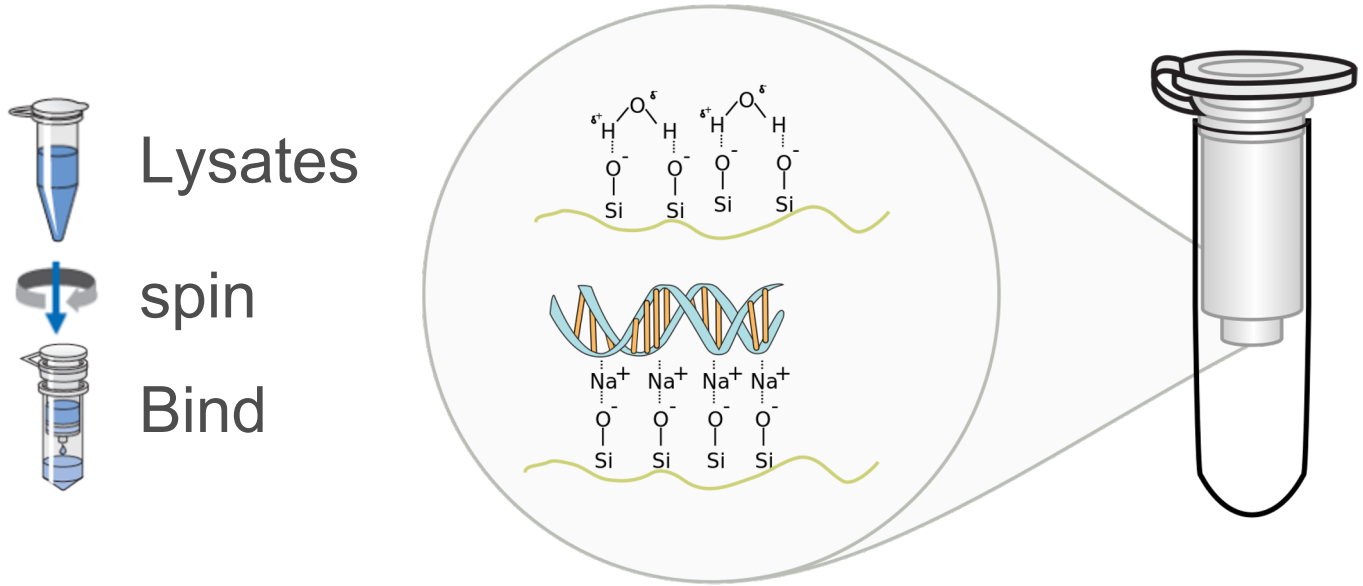
Lysis and Nucleic Acid Precipitation

- Lysis is done with lysis buffer (buffer AVL) containing chaotropic salt that:
  - Breaks down cell membrane, viral envelope and capsid and free nucleic acids
  - Solubilizes hydrophobic regions in lipid bilayers
  - Denatures proteins by disrupting the tertiary structure
  - Inactivate enzymatic proteins activity (this will inactivate RNases and prevent RNA from degradation)
  - Disrupts weak bonds between molecules
- Addition of absolute ethanol
  - Ethanol brings nucleic acid out of aqueous solution (salting out)



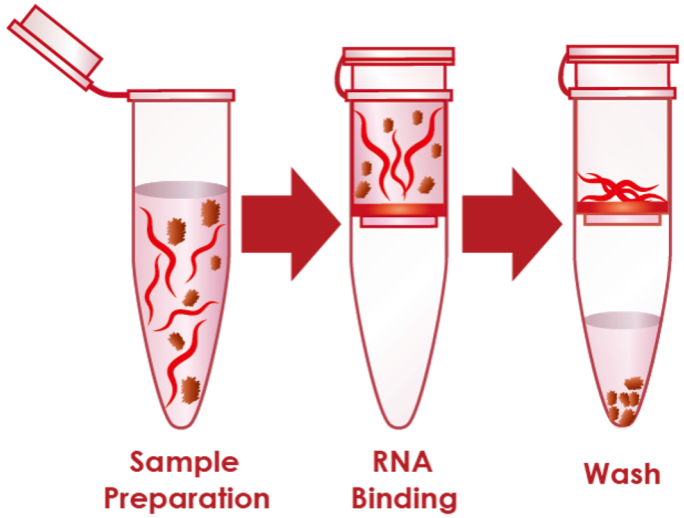
RNA Binding to QIAamp Spin Column Membrane

- Nucleic acids bind to silica-based membrane in the spin column under:
  - Optimal pH and salt conditions



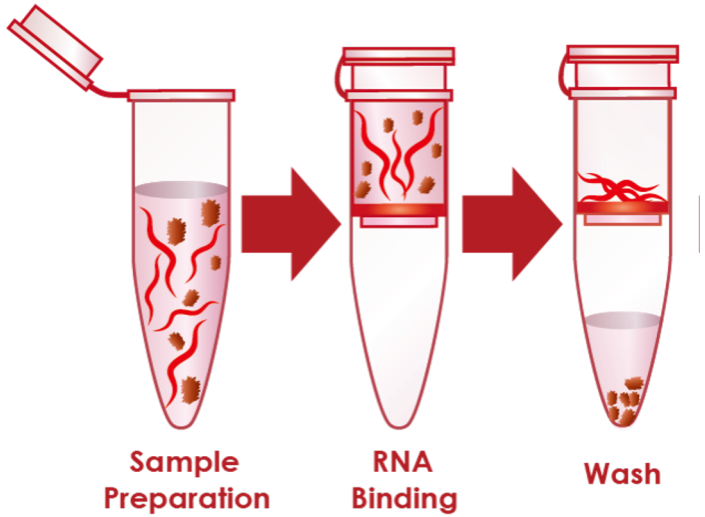
Washing the QIAamp Spin Column

- Column is washed with Wash Buffer AW1 that:
  - Leaves nucleic acids bound to filter membrane
  - Remove contaminants from the filter membrane that flow through



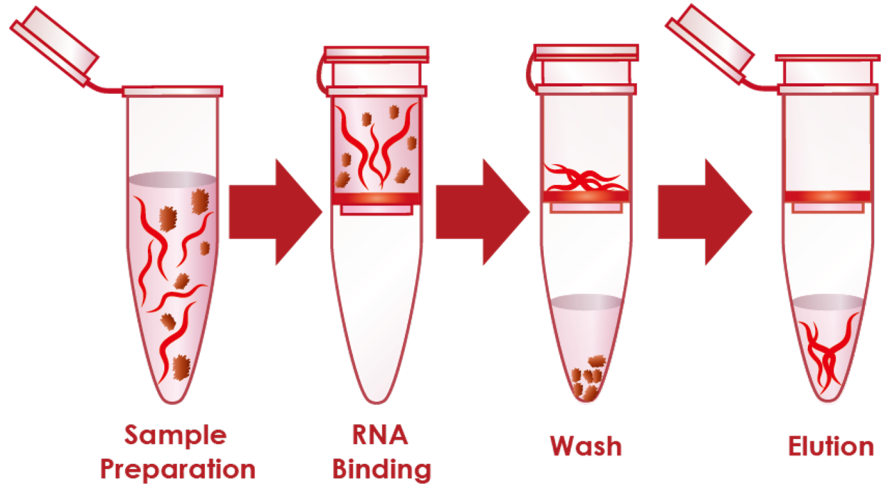
Washing the QIAamp Spin Column

- Column is washed with Wash Buffer AW2 that:
  - Leaves nucleic acids bound to filter membrane
  - Remove residual contaminants from filter membrane that flow through



Elution of Bound RNA from QIAamp Spin Column

- Nucleic acid is eluted with Buffer AVE that:
  - Releases bound nucleic acids from the membrane
  - Allows nucleic acids to flow through the membrane
- Purified RNA is collected in clean tube and ready for downstream application (RT-PCR, sequencing, …)

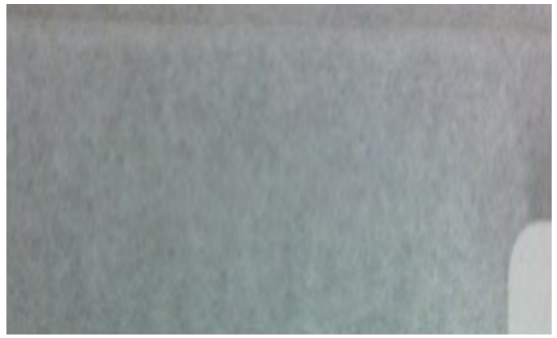


Extraction of SARS-CoV-2 Viral RNA from Naso/Oropharyngeal Samples

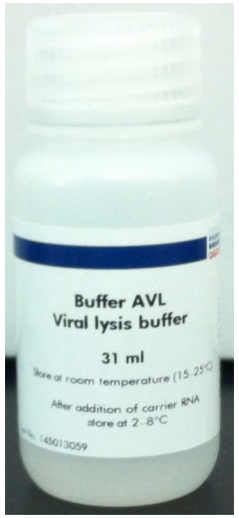
(QIAamp Viral RNA Mini Kit,
Cat#52904 or 52906)

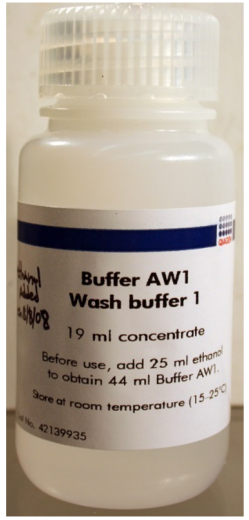
Kit Contents and Required Reagents

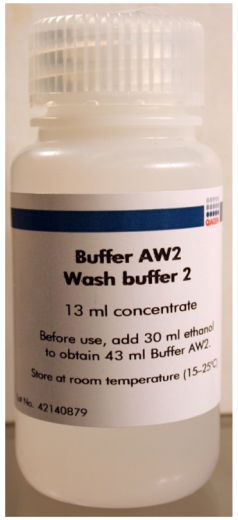
- Kit contents
  1. Mini Spin Columns
  2. Collection Tubes (2 mL)
  3. Lysis buffer (AVL)
  4. Carrier RNA (will not be used)
  5. Wash buffer AW1 (Concentrate)
  6. Wash buffer AW2 (Concentrate)
  7. Elution buffer (AVE)

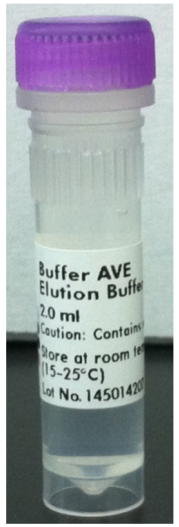


- Reagents Required but not provided
  1. Ethanol (96-100%)
  2. RNase-free microcentrifuge tubes
  3. RNase-free pipettes and pipette tips
  4. Microcentrifuge









Things to do Before Starting

1. Equilibrate samples to room temperature (15–25°C)

1. Equilibrate Buffer AVE to room temperature for elution step

1. Add the required volume of ethanol to Buffer AW1 and Buffer AW2 (check the box and write date and your initials)

1. If any buffers are cloudy or contain crystals, warm the bottles until they are clear and the crystals are gone.

NB: Carrier RNA will not be used : So no carrier RNA should be added to Buffer AVL

Preparing for the Extraction

1. Don appropriate PPE based on risk assessment

2. Clean Biological Safety Cabinet (BSC) with 10% Bleach Solution

3. Clean BSC with RNase AWAY decontamination solution

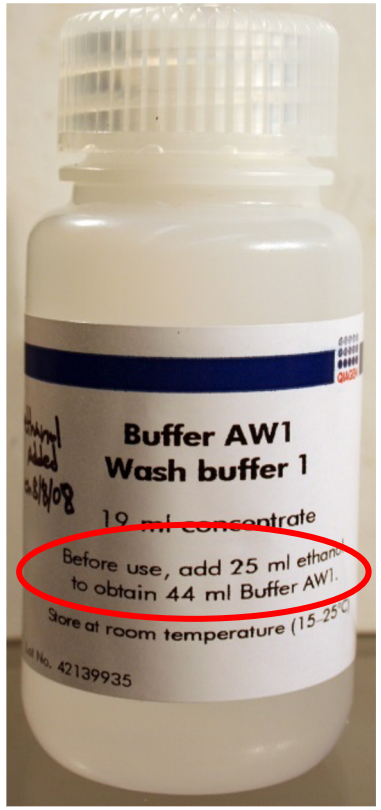
4. Clean BSC with 70% Alcohol solution

5. Wipe down all reagents and consumables (e.g. tip boxes) with RNase AWAY solution

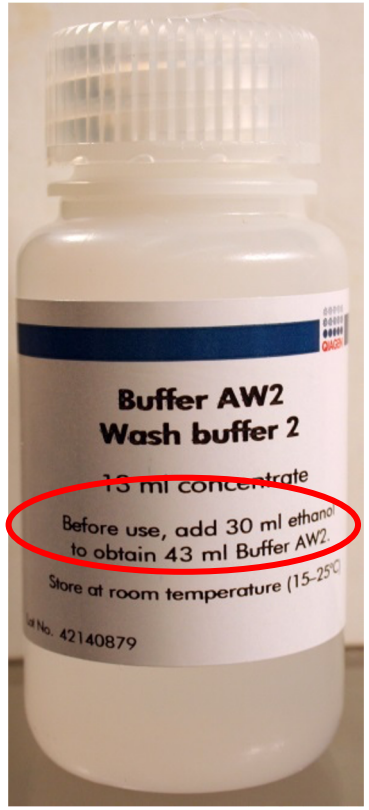
6. Load reagents and consumables in the BSC

Preparation of Reagents

Add 25 mL ethanol



Add 30 mL ethanol



Lysis of Samples

1. Pipet **560 µL Buffer AVL** into 1.5 mL Eppendorf tube

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2. Mix clinical sample by vortexing for 15 sec and add **140 µL sample** to buffer AVL in the microcentrifuge tube

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3. Mix well by pulse-vortexing for 15 sec and incubate at room temperature (15-25 ºC) for 10 minutes

↓

4. Briefly centrifuge the tube for 2 sec to remove drops from inside the lid

↓

5. Add **560 µL of ethanol** (96-100%) to the sample and mix by pulse vortexing for 15 sec, then briefly centrifuge for 2 sec

Binding of Lysates to Mini Column Membrane

6. Carefully transfer **630 µL of lysates** (step 5) to the QIAamp Mini Column

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7. Close the cap and centrifuge @ 6,000 x *g* for 1 minute

↓

8. Place the QIAamp Mini Column into a clean 2 mL collection tube and discard the tube containing the filtrate

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9. Carefully open the QIAamp Mini Column and transfer remaining lysates to the column

↓

10. Close the cap and centrifuge @ 6,000 x *g* for 1 minute

↓

11. Place the QIAamp Mini Column into a clean 2 mL collection tube and discard the tube containing the filtrate

Washing the Mini Column Membrane

12. Carefully open the QIAamp Mini column and add **500 µL Buffer AW1**. Close the cap and centrifuge @ 6,000 x *g* for 1 minute

↓

13. Place the QIAamp Mini Column into a clean 2 mL collection tube and discard the tube containing the filtrate

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14. Carefully open the QIAamp Mini column and add **500 µL Buffer AW2**. Close the cap and centrifuge @ 20,000 x *g* for 3 minutes

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15. Place the QIAamp Mini Column into a clean 2 mL collection tube and discard the tube containing the filtrate

↓

1. Close the cap and centrifuge @ 20,000 x *g* for 1 minute

Elution of Bound RNA from Mini Column Membrane

17. Place the QIAamp Mini Column into a clean 1.5 mL microcentrifuge tube and discard the tube containing the filtrate

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18. Carefully open the QIAamp Mini column and add **60 µL Buffer AVE**. Close the cap and incubate at room temperature for 1 minute

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19. Centrifuge @ 6,000 x *g* for 1 minute

↓

20. Collect eluate (RNA) and discard the QIAamp Mini Column

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21. Use purified RNA in downstream application (RT-PCR, sequencing, …)

Reference

1. QIAamp Viral RNA Mini Kit Handbook (Qiagen)
2. Laboratory Biosafety Manual. 2004. World Health Organization (WHO) Geneva. *Third edition*