DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30329-4027

Automated RNA Extraction using QIAcube HT

Influenza Division
Virology, Surveillance and Diagnosis Branch
Genomics and Diagnostics Team

NOTE: This procedure is provided for research use only.
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1.0 Purpose

1.1 This protocol provides instruction for high throughput isolation of RNA from influenza samples using the Qiagen Custom QIAamp 96 DNA Kit and the automated liquid handler QIAcube HT.

2.0 Responsibility

Job Function	Responsibility
Lab Technicians	Responsible for preforming the protocol as written.
Laboratory Supervisor or designee	Responsible for: • Training Lab Technicians in the protocol as written. • Review of documentation and/or data to support the effective completion of activities associated with this procedure.

3.0 Definitions

Term	Definition
EtOH	Ethanol
BSC	Biological Safety Cabinet
PPE	Personal Protective Equipment

4.0 Critical Equipment

Equipment Name	Description
Qiagen QIAcube HT	N/A
Vortex	N/A
Certified BSC	N/A

5.0 Materials

Material Name	Material Description
Custom QIAamp DNA Kit with Treated Plates for QIAcube HT	• Qiagen catalog No. 1102511 (480 preps) • Kit components include: ○ QIAamp 96 Filter Plate, treated ○ Buffer AVL ○ Buffer AW1 treated filtered ○ Buffer AW2 treated filtered ○ Buffer AVE ○ Carrier RNA ○ Nuclease Free Water
Ethanol	96-100% (EtOH)
Pipettes	20uL, 200uL, 1000uL (Rainin or Eppendorf)
Aerosol resistant pipette tips	Rainin Cat# RT-L10F, RT-L200F, RT-L1000F or equivalent
Gloves	Latex or nitrile

Material Name	Material Description
Reagent Trough with Lid (70ml)	Qiagen Part # 990554
Reagent Trough with Lid (170ml)	Qiagen Part # 990556
QIAcube HT Plasticware	• Qiagen catalog #950067 • Kit components include: o S-block o 200µl Filter-Tips OnCor C o Elution microtubes RS o Tape pad o Caps for elution microtubes
Tip Disposal Box	Qiagen catalog# 990550
DECONQUAT® 100	Veltek Associates catalog #DQ100-06-167-01

6.0 Reagent Storage

Reagent	Storage
QIAamp 96 Plate, treated	Stored at 15°C to 25°C.
Unmixed Buffers and Reagents	Stored at 15°C to 25°C.
Buffer AVL-Carrier RNA solution	• Can be prepared in advance and stored at room temperature for up to 60 days. • Occasionally develops a precipitate when stored at 15°C to 25°C that must be re-dissolved by warming (at 80°C) with light vortexing before use. • Unused portions of carrier RNA dissolved in Buffer AVE should be frozen in aliquots at -20°C. Do not freeze-thaw more than three (3) times.

7.0 Safety Precautions

Precaution Type	Precaution Description
Isolate handling	Isolates are opened and handled only in BSC with proper PPE until AVL is added. Once AVL buffer is added and sample incubated for 10min, isolate can be handled outside BSC.
QIAcube Waste Disposal	Waste Liquid from vacuum control station should be disposed in a sealable container and marked for chemical waste collection.

8.0 Procedure

8.1 When opening a new Qiagen kit, follow the steps below for Wash Buffer Preparation and AVL Preparation.

8.2 **Wash Buffer Preparation**

8.2.1 When first opening a Viral RNA Kit, add EtOH to Qiagen wash buffers according to manufacturer's instructions.

8.2.1.1 Add 2134~~0~~mL EtOH to Buffer AW1 (Wash Buffer 1)

8.2.1.2 Add 290mL EtOH to Buffer AW2 (Wash Buffer 2)

8.3 **AVL Preparation**

8.3.1 Add 1350uL (1uL of solvent per 1ug of RNA) of Buffer AVE (in purple-capped tube) to Carrier RNA (red-capped tube) and vortex to mix.

8.3.2 For 96 samples, add 310uL of the re-suspended carrier RNA to 31mL of AVL buffer (2.8uL of carrier RNA per 280uL of AVL needed for each sample). Vortex to mix.

8.3.3 Pipette 280uL of AVL + carrier into the well of a 2.2mL S-Block (deep well plate).

8.3.3.1 QIAcube HT processes samples one column at a time.

8.3.3.2 Do not leave unused wells empty in columns being used. Add water or leftover AVL.

8.3.3.3 **Note:** Leftover carrier RNA/AVE should be aliquoted and stored in -20°C freezer.

8.3.3.4 **Note:** Leftover AVL + resuspended Carrier RNA can be stored at room temperature for up to 60 days.

8.4 **Sample Preparation**

8.4.1 Thaw, vortex, and briefly spin down all samples to be extracted.

8.4.2 In a BSC, transfer 70uL of each viral sample into 280uL of Buffer AVL (with Carrier RNA) in the S-Block well and vortex.

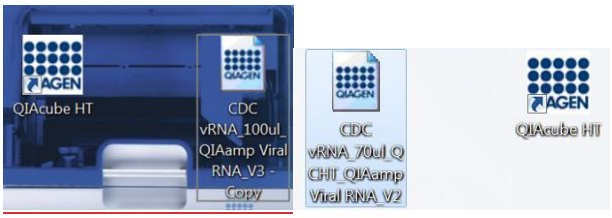
8.4.2.1 If samples are known to have low titer, volume of samples used can be increased beyond 70uL.

8.4.3 Repeat for all samples to be extracted.

8.4.4 Incubate all samples at room temperature (15-25°C) for 10 minutes.

8.5 **QIAcube Setup**

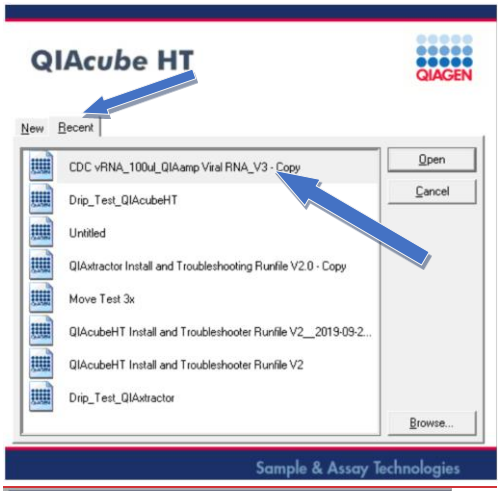
8.5.1 Using the QIAcube laptop, click on the QIAcube HT link on the desktop. Alternatively, the link for the protocol used can be selected, which leads straight to step 8.5.3 below.

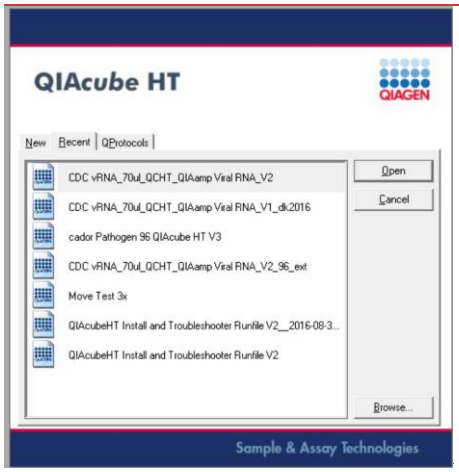


8.5.2 Select the **Recent** tab in the dialog box (see below).

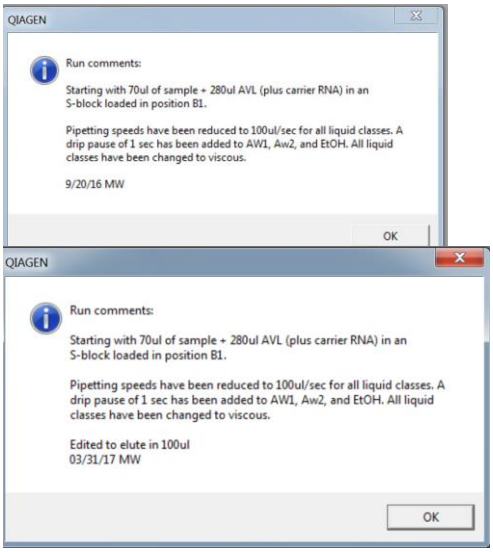
8.5.2.1 Select **CDC vRNA_70ul_QCHT_QIAamp Viral RNA_V2**

8.5.2.2 Click on **Open**.





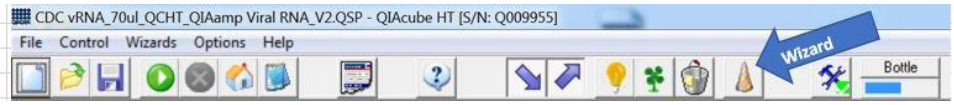
8.5.3 A dialog box with run comments will appear with modifications made to the protocol.



8.5.4 Software will open with the layout of the deck according to the protocol selected.

8.5.4.1 Deck layout image does not show location of elution plate, which is to the right of A1 on top of the tip box B2.

8.5.5 Go to the **Wizard** hat icon.



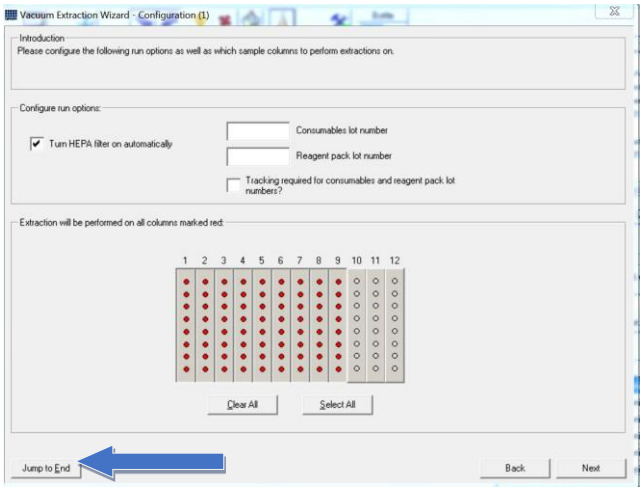
8.5.5.1 Select the columns where samples are located.

- Columns selected for extraction will be marked red. Unused columns have wells in white.

8.5.5.2 By selecting **Next**, a detailed step-by-step layout of the whole protocol will be shown, which also allows modifications to the protocol.

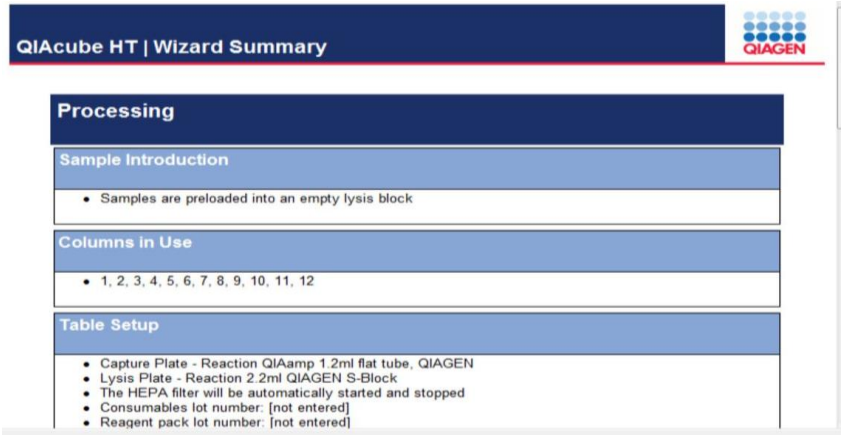
Note: **To avoid unwanted changes, do not use the Next link, use the Jump to End link instead.**

8.5.5.3 Click on **Jump to End** link.

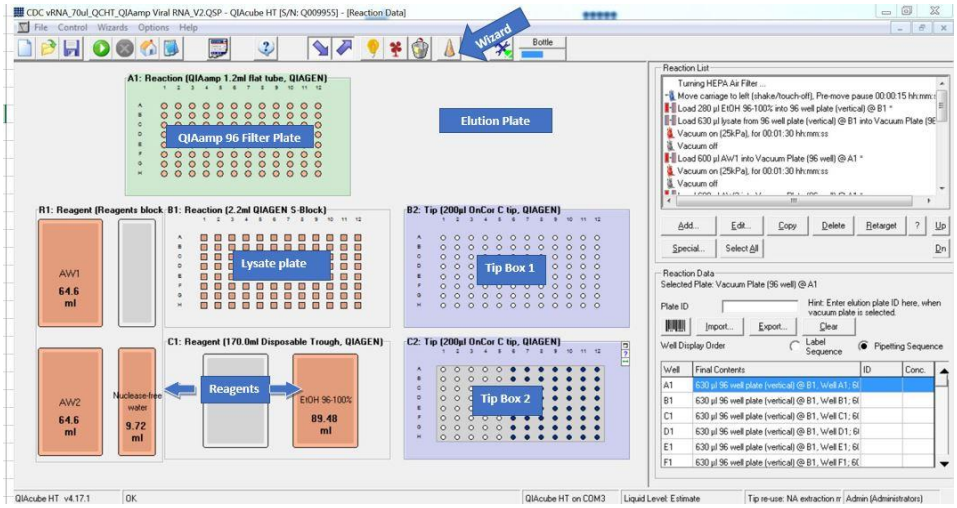


8.5.5.4 The **Wizard Summary** will be displayed.

- Summary can be saved and used as documentation for the run.
- Click **Finish** to go back to deck layout.



8.5.6 **Deck Layout**



8.5.6.1 **R1 and C1: Reagents (Reagents Block)**

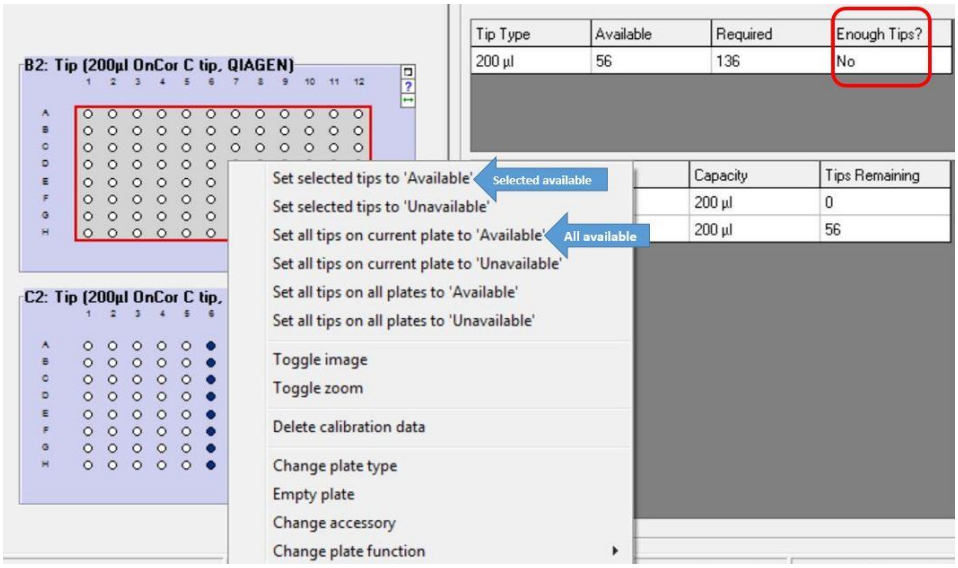
- Shows location and volume of reagents according to the number of columns selected in step 8.5.5.1 above.
- Information regarding reagent type, liquid quality, and extra volume can be found by clicking on each colored reagent box show above. Detailed information will be displayed to the left of the screen.
- Type of reagent through used are listed below:

Reagent	Through Type	Location
AW1	170ml Trough with Lid	R1 reagent block
AW2	170ml Trough with Lid	R1 reagent block
Nuclease Free Water	70ml Trough with Lid	R1 reagent block
96-100% Ethanol	170ml Trough with Lid	C1 reagent block

8.5.6.2 **B1 Reaction (2.2mL QIAGEN S-Block): Lysate Plate**

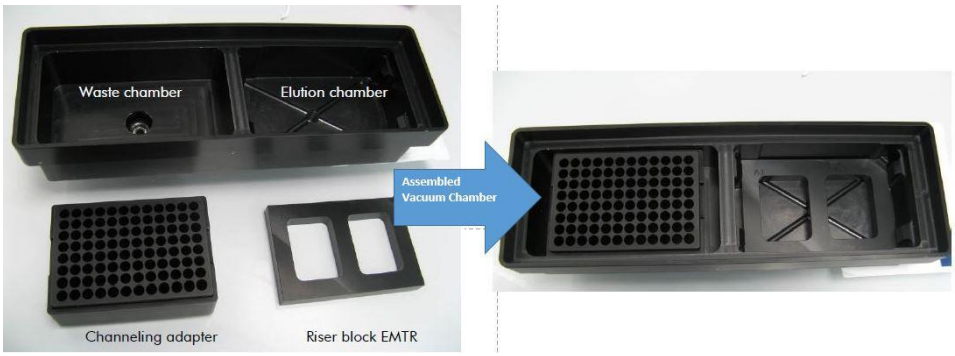
- S-Block containing sample lysate from section 9.4.
- Shows columns selected for extraction in red.

8.5.6.3 **B2 and C2: Tips (200uL OnCor C Tip, Qiagen)**



- Leftover tips from the previous instrument run will be taken as the currently available tip count and location by software.
- If tips leftover are not enough for the number of samples being processed, software will indicate so (as shown above) and more tips need to be added or the run will not start.
- To add tips to plate or a new tip plate, right click on the tip box that needs to be changed on the deck and select either one of the options shown in the figure above.

8.5.6.4 **A1 Reaction (QIAamp 1.2mL Flat Tube Plate) and Elution Chamber: Vacuum Chamber**



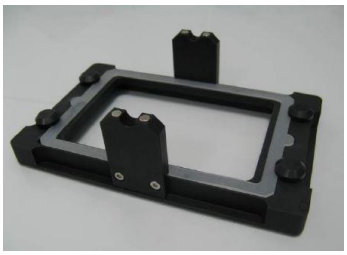
- **Vacuum chamber** must be assembled as shown above

- Channeling adapter (block and block holder) goes over the waste chamber and riser block over the elution chamber.
- Channeling adapter gasket should be greased before every run.

8.5.6.4.1 **Elution plate (Elution microtubes RS)** must be placed to the leftmost position on top of the riser block.

8.5.6.4.2 **Transfer carriage** (shown below) should be positioned over the channeling adapter.

Note: It is very important to place the carriage to the leftmost position on the vacuum chamber.



8.5.6.4.3 **The QIAamp filter plate** must go on top of the transfer carriage so it can be moved from the waste chamber to the elution plate.

- **Note:** any empty columns must be covered using the plastic seal included in the kit.
- Place as far to the left as possible.

8.5.7 **Vacuum Control Station and Waste Bottle: l**ocated below the QIAcube HT instrument .

8.5.7.1 Volume of waste bottle is controlled by the QIAcube HT software based on the number of runs and samples processed.

8.5.7.2 An icon in the toolbar will indicate volume of waste bottle and when full (about half of the volume of the actual bottle), the container must be emptied before starting a run.

8.5.7.3 After emptying the waste bottle, software volume can be reset by right-clicking on the icon and selecting to empty the bottle.

8.5.7.4 If volume reaches the full mark during a run, the software will stop the run and request that the bottle be emptied before resuming processing.

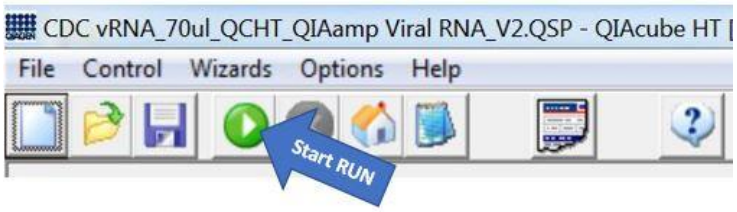
8.5.8 **Tip Disposal**

8.5.8.1 Ensure the tip disposal box is present and uncovered ~~toby~~ catch tips.

8.5.8.2 Tip disposal box should be emptied after each run and tips disposed into chemical bins.

8.5.9 **Start Run**

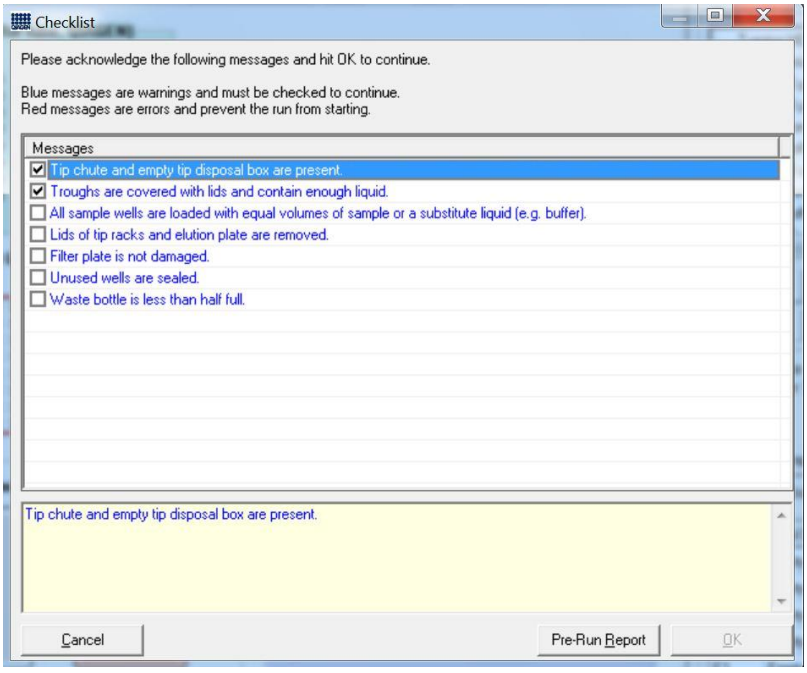
8.5.9.1 When all items listed in steps 8.5.6 through 8.5.8 have been properly added and assembled, click on the green button on toolbar to start the run.



8.5.9.2 The program will request acknowledgment that all the parameters have been met before starting the run.

8.5.9.3 Please take the time to read all points and review the deck before checking the boxes.

8.5.9.4 Click **OK** to start the run.



8.5.10 After completion of RNA extraction, the RNA should be transferred from the elution plate into a PCR plate for proper storage at -20°C to -90°C for up to one year.

8.6 **QIAcube Maintenance**

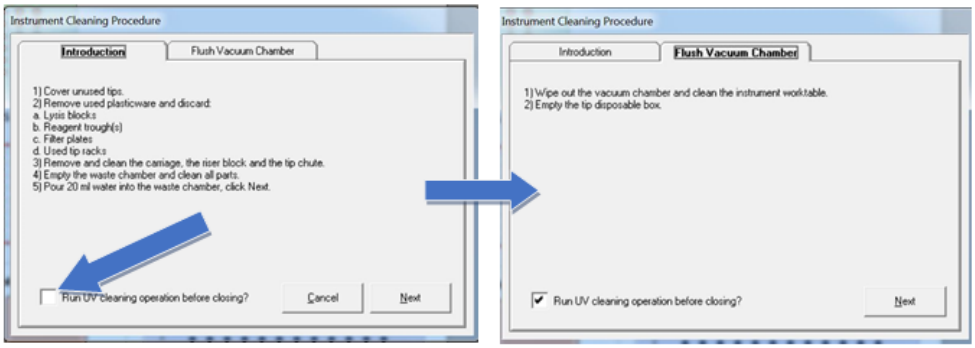
8.6.1 **Flushing Vacuum Chamber**

8.6.1.1 Vacuum chamber should be flushed after every run by pouring 20mL of water in the waste chamber by closing the QIAcube software or clicking on the  **Clean vacuum** station icon on the menu.

8.6.1.2 A message box will appear with instructions (shown below).

8.6.1.3 There is an option to perform UV decontamination of the working deck before closing the software if the check box is checked. See step 8.6.2 for details.

8.6.1.4 Click **Next** for additional set of instructions.

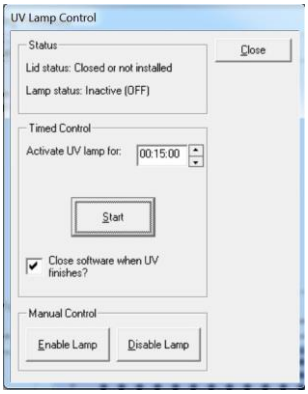


8.6.2 **Running the UV Cleaning Operation**

8.6.2.1 The operation of the UV lamp can be initiated together with the flushing of the vacuum chamber or by selecting the icon in the toolbar.

8.6.2.2 It Is recommended to run the UV lamp for at least 15 minutes after running the instrument.

8.6.2.3 A text box will appear, and user needs to acknowledge that security measures have been taken before turning on the UV lamp.



8.6.3 **Cleaning Instrument Accessories**

8.6.3.1 Rinse with room temperature tap water.

8.6.3.2 Soak in DECONQUAT® 100 or other approved solution for 15-30 minutes.

8.6.3.3 Rinse with room temperature deionized water and allow to dry.

8.6.4 **Cleaning the Worktable and Lid**

8.6.4.1 Lid must be cleaned with water only and a clean, soft cloth. **DO NOT** wipe with alcohol or any other solution.

8.6.4.2 The instrument's worktable should be wiped with 70% ethanol solution or DECONQUAT® 100. For other approved cleaning solutions or for more information, refer to the QIAcube HT Manual.

8.6.5 **Troubleshooting**

8.6.5.1 Refer to the QIAcube HT User Manual for help with troubleshooting any errors or for any additional information.

9.0 Related Procedures – None

10.0 References

10.1 QIAcube HT User Manual, First Edition, July 2013

10.2 Custom QIAamp DNA

11.0 Attachments ~~- None~~

~~**11.0**~~11.1 Appendix A: QIAcube HT Wizard Summary

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