### Front View of the BioRobot 8000


- Technical tower side panel for access to the dilutors
- Technical tower front door for access to the reagent carousel
- Safety shield
- High-speed dispensing system
- Automated vacuum system
- High-speed shaker system
- Cooling and heating system
- Calibration pyramid
- Wash station
- Tip-disposal unit
- Probes/tip adapters and robotic handling system
- Robotic arm
- Power switch

**Note**: The components installed on your BioRobot 8000 depend on your application requirements.

**BioRobot 8000 Installed with Worktable Hood (Open)**

**BioRobot 8000 Installed with Worktable Hood (Closed)**

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## 3.1 Modular design concept

The BioRobot 8000 is based on a modular design concept. Each major function of the BioRobot 8000 is performed by an independent component of the instrument. The modular design maximizes the flexibility of the instrument and simplifies maintenance and servicing procedures.

The major components of the BioRobot 8000 are:

- The worktable
- The technical tower
- The robotic arm
- The robotic handling system
- The dilutor system
- The high-speed dispensing system
- The wash station
- The automated vacuum system
- The cooling and heating system
- The labware tracking system
- The high-speed shaker system
- The QIAsoft Operating System

**Note**: Depending on your application requirements, only a selection of the above listed components may be available on your instrument.

## 3.2 Principles of operation

The BioRobot 8000 is a system of components controlled by the QIAsoft Operating System stored on the hard disk of a personal computer. Molecular biology applications, such as purification of DNA, RNA, and recombinant proteins, are automated by running QIAsoft protocols. Protocols are either supplied by QIAGEN or can be developed by the user. A protocol can be customized in order to optimize each stage of the molecular biology application, from pipetting of samples and reagents to final reporting and data analysis (note that the availability of some of these features depends on which hardware components are installed on your BioRobot 8000).

### 3.2.1 The worktable

The BioRobot 8000 worktable accommodates in the correct position various hardware components (e.g., cooling and heating system) and various accessories (e.g., adapters, reagent holders, microplate stations, and plate stackers) that are required for processing of samples.

The worktable is equipped with an arrangement of locating pins that allow repositioning of microplate stations and plate stackers whenever necessary.

The microplate stations hold adapters, reagent holders, and any labware of 96-well plate format. Some adapters are held by the hardware components (e.g., the heat transfer adapter fits into the cooling and heating system).

### 3.2.2 The technical tower

The technical tower is the housing on the right-hand side of the BioRobot 8000 (viewed from the front). It accommodates electronics, the pumps of the liquid-handling systems, and the reagent carousel and the buffer tracking system.

### 3.2.3 The robotic arm

The X, Y, Z robotic arm with the variable spacing system (V) provides accurate and precise positioning of the probes/tip adapters and the robotic handling system on the worktable.

All X, Y, Z, and V movements are driven by DC motors with encoders.

The robotic arm is moved in the X direction (left and right) by the X-motor inside the instrument housing.

The probes/tip adapters are mounted on the Y-slide, which is located inside the robotic arm and moves in the Y direction (front to rear). Each probe/tip adapter can be moved independently in the Z direction (up and down).

The BioRobot 8000 has the variable spacing system, which varies the spacing evenly between probes/tip adapters. The variable spacing system allows creation of protocols that use a variety of containers with different spacing between

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neighboring pipetting positions. The spacing between the probes/tip adapters is adjusted symmetrically using the variable spacing system motor (V-motor).

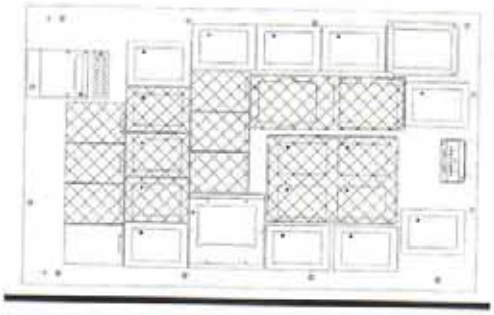
The robotic handling system motor (G-motor) controls the gripping function of the robotic handling system. The HZ-motor controls movement in the Z direction (there are separate Z-motors for the probes/tip adapters and an HZ-motor for the robotic handling system). The X-motor and Y-slide control movement in the X and Y direction respectively.

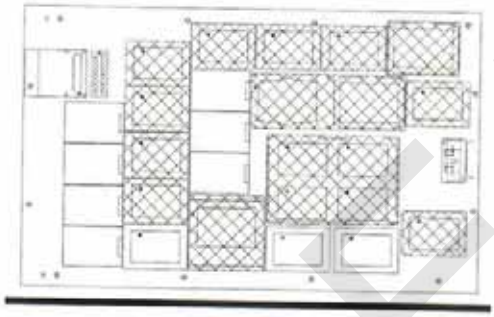
Pipetting positions can be specified with a resolution of less than 1 mm in X, Y, and Z directions.

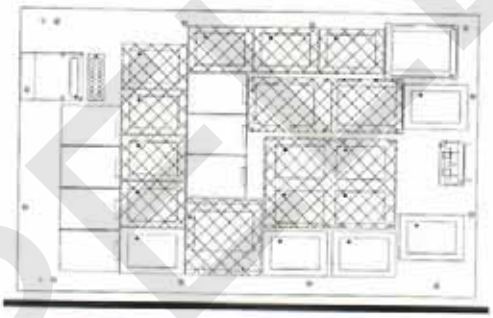
**Note**: Access by probes/tip adapters and the robotic handling system to locations on the worktable is limited to the corresponding working areas.

The accessible working areas and worktable layout of the BioRobot Universal System are shown in the figures on pages 3-7 and 3-8. The accessible working areas on your worktable depend on the components installed on your BioRobot 8000.

## The Worktable Layout of the BioRobot Universal System







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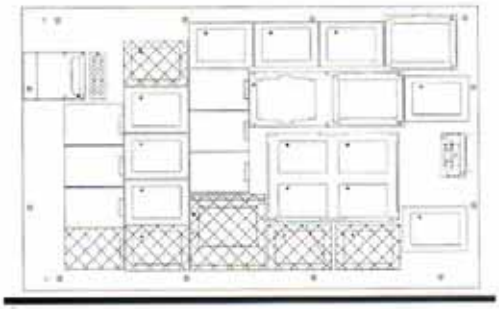
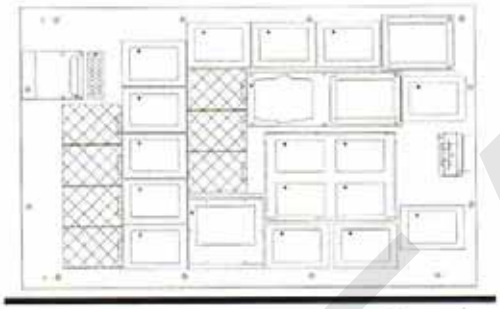




The hatched areas indicate ■ Full probe working area; ■ Robotic handling system working area; ■ Dispenser head working area; ■ Area accessible by certain probes only; ■ Positions of Tip Racks.

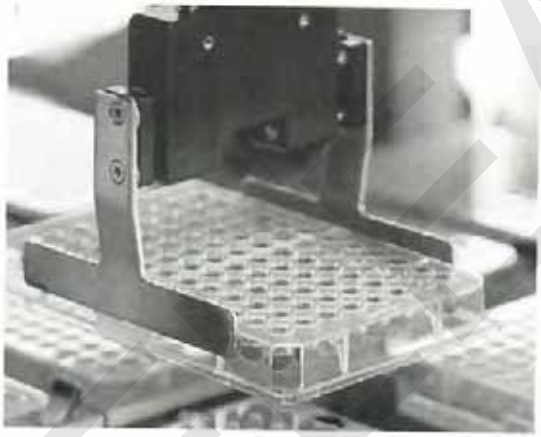
### 3.2.4 The robotic handling system

The robotic handling system is used to:

- Transport labware between different locations on the worktable
- Load and unload the vacuum manifold of the automated vacuum system
- Manipulate the dispenser head of the high-speed dispensing system

The robotic handling system can also be used to manipulate certain accessories (e.g., channeling adapter). The robotic handling system is installed on the robotic arm.

**Robotic Handling System Gripping a Microplate**



**Note**: Items can only be maneuvered within the worktable area accessible by the robotic handling system (see the figures on page 3-7 and 3-8). The robotic handling system and the probes/tip adapters can only be used asynchronously.

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### 3.2.5 The dilutor system

The BioRobot 8000 is equipped with 8 pipetting channels which can be fitted with washable probes, tip adapters, or a combination of both.

**Four Probes (left) and Four Tip Adapters (right)**

Each probe/tip adapter can be moved independently in the Z direction, and the spacing between the probes/tip adapters (Y direction) is variable (from 9 mm to 20 mm). This feature is termed the variable spacing system.

The pipetting channels are connected to precision syringe pumps and can be programmed to aspirate and dispense liquids at any position within the worktable area accessible by the probes/tip adapters (see the figures on pages 3-7 and 3-8).

**Note:** The dilutor system can only be used to transfer liquids from a source position, where liquid is aspirated, to a destination position, where the liquid is dispensed. Continuous dispensing of liquids from a single bottle to multiple destination positions is performed using the high-speed dispensing system.

### Syringe pumps

The syringe pumps are computer-controlled syringes that are connected via tubing to the probes/tip adapters and a membrane pump.

Each probe/tip adapter has a dedicated syringe pump controlling its aspiration and dispensing function.

All parts that come into contact with liquid are made of inert materials such as stainless steel, polytetrafluoroethene (PTFE), and fluorinated ethene propene (FEP).

The membrane pump supplies system liquid from the system liquid container (located below the BioRobot 8000) to the syringe pumps, and is used to flush the dilutor system and to rinse and clean the probes.

### Probe formats

QIAGEN offers a choice of probe formats for liquid handling. Each pipetting channel can be equipped with either a washable probe or a tip adapter:

- Probes are hollow pipet tubes usually made of ceramic coated stainless steel; for available probe sizes, see page B-1. The probes are washed at a dedicated wash station with clean system liquid delivered by the membrane pump.

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- Cross-contamination–free pipetting is provided by using disposable tips that attach to tip adapters.
  - Disposable tips with a total capacity of 10 µl, 50 µl, 300 µl, and 1100 µl are available.
  - Disposable tips containing filter barriers (10 µl, 50 µl, 300 µl, and 1100 µl tips) are available to prevent aerosols, an essential safety feature when processing potentially infectious specimens.

### Liquid detectors

Each pipetting channel is equipped with a liquid detector that enables it to detect the surface of ionic solutions and other conductive surfaces upon contact. The liquid detectors operate by monitoring the changes in capacitance between the probes/tip adapters and the liquid.

**Note**: QIAGEN cannot guarantee the function of the liquid detectors if labware (e.g., bottles, tubes) that are not approved by QIAGEN are used to hold samples and reagents.

The liquid detectors are used to detect sample and reagent levels. If there is insufficient sample or reagent, the protocol run stops to alert the user, if specified in the protocol.

**Note**: Since the liquid detectors cannot determine the materials that cause changes in capacitance, it is imperative that the probes/disposable tips do not touch any surface other than the liquid to be detected. An error may be encountered if the probes/disposable tips touch the side of a vessel, foam, a hand, and so on, even if the contact is insufficient to cause resistance.

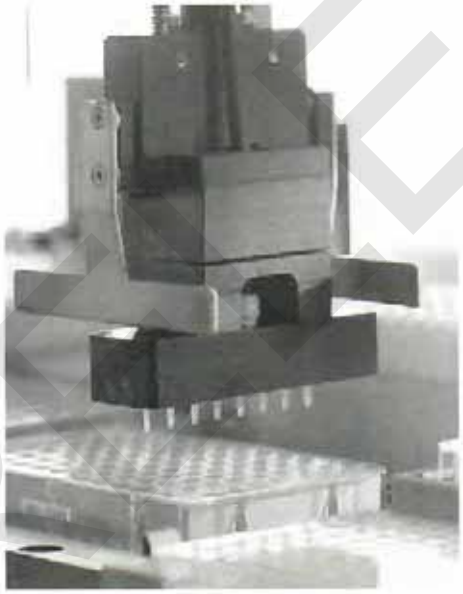
### Liquid level sensor system

The system liquid container, the waste container, and the vacuum trap are each equipped with a liquid level sensor. The sensors operate by monitoring changes in capacitance. They detect when there is insufficient liquid in the system liquid container, and when the maximum level in the waste container and the vacuum trap are reached. The sensors prevent working without system liquid, and prevent overflow of the waste container and vacuum trap.

## 3.2.6 The high-speed dispensing system

The high-speed dispensing system provides high-speed dispensing of liquids using an 8-channel dispenser head that is manipulated by the robotic handling system.

**Dispenser Head Being Manipulated by the Robotic Handling System**



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The dispenser head is connected by tubing to a membrane pump and can dispense liquid to any position within the worktable area accessible by the dispenser head (see the figures on page 3-7 and 3-8).

**Note**: During operation of the high-speed dispensing system, the 8 dispensing channels are always used simultaneously.

Liquid is aspirated via a probe from bottles placed into the reagent carousel.

Protocol run times are significantly shortened due to a continuous flow of liquid and reduced arm movements. 250 µl of liquid can be dispensed into 96 wells in less than 20 seconds.

All parts that come into contact with liquid are made of inert materials such as stainless steel, PTFE, and FEP.

### Dispenser head

The dispenser head is made of Delrin® acetal resin and is equipped with 8 dispensing channels fitted with precision outlets.

Manipulation and precise positioning of the dispenser head is performed by the robotic handling system. When the high-speed dispensing system is not in use, the dispenser head is placed in a dedicated wash station where it is held in position magnetically.

After dispensing reagents or buffers, the complete system is washed at the dedicated wash station with clean system liquid delivered by the membrane pump.

The dispenser head is connected via tubing to the membrane pump. This tubing is mounted using a roll-up mechanism to prevent any loose tubing from interfering with robotic arm movements.

### Membrane pump

The computer-controlled membrane pump is located in the technical tower and provides a continuous flow of liquid for faster sample processing. The pump is connected via tubing to the dispenser head and to a probe that aspirates liquid from bottles placed into the reagent carousel.

The membrane pump ensures that accurate and consistent liquid volumes are dispensed by all 8 dispensing channels. When dispensing water, 200–1000 µl are delivered with a coefficient of variation (CV) of less than 7%. The accuracy of the membrane pump is maintained by performing a calibration protocol installed with the QIAsoft software. For details, see "Calibration and System Diagnostics" (Section 8).

### Reagent carousel

The reagent carousel is located inside the technical tower and is accessed from the door at the front of the technical tower.

**Reagent Carousel**

The carousel accommodates up to four 250 ml bottles (60 mm diameter), three 500 ml bottles (72 mm diameter), and a special system liquid bottle with a capacity of 1000 ml (provided by QIAGEN).

Liquid is aspirated from these containers by a stainless steel probe that is connected to the membrane pump. The probe moves up and the carousel turns until the required reagent bottle is positioned under the probe.

Movement of the probe in the Z direction is controlled by the P-motor and movement of the reagent carousel is driven by the R-motor.

Bar-code–labeled reagent bottles placed in the reagent carousel can be automatically identified using a bar-code reader camera (the buffer tracking system). The bar-code reader camera is installed inside the technical tower.

Bar-code reading is software controlled. Identified bar codes can be stored (e.g., to document reagent lots used for sample processing).

### 3.2.7 The wash station

The specially designed wash station protects the worktable from splashes while the dilutor system is being flushed with system liquid. The wash station also allows the inside and outside of the washable probes to be rinsed.

The wash station contains a cup for each probe. When the tip of the probe is positioned in the cup, system liquid (usually distilled or deionized water from the system liquid container) is dispensed under software control via the membrane pump (wash pump) through the probe. The system liquid swirls upward, caused by the shape of the cup, and washes the outside of the probe before spilling into the moat and down the drain. Both the inside and outside of the probe are thoroughly cleaned in one step. Tubing delivers the waste system liquid from the drain to the waste container.

You can adjust the volume of system liquid for flushing the dilutor system and washing the probes.

### 3.2.8 The automated vacuum system

The automated vacuum system consists of a vacuum manifold on the BioRobot 8000 worktable and a vacuum trap and pump below the instrument. This system allows rapid and thorough filtration of wash and elution buffers through QIAGEN 96-well plates (e.g., QIAprep® 96 plates).

The BioRobot 8000 uses the robotic handling system for automated and accurate assembly of the vacuum manifold and the accessories and labware that fit inside it (e.g., QIAGEN 96 well plate, channeling adapter, elution microtubes).

The vacuum manifold consists of 3 parts:

- Vacuum manifold base
- Vacuum manifold top
- Vacuum manifold lid

The vacuum manifold base is always located on the worktable, where vacuum is applied. At the start of a protocol, the user places first the vacuum manifold lid and then the vacuum manifold top onto the slot to the right of the vacuum manifold base. During the protocol, the robotic handling system transfers both parts to and from the vacuum manifold base.

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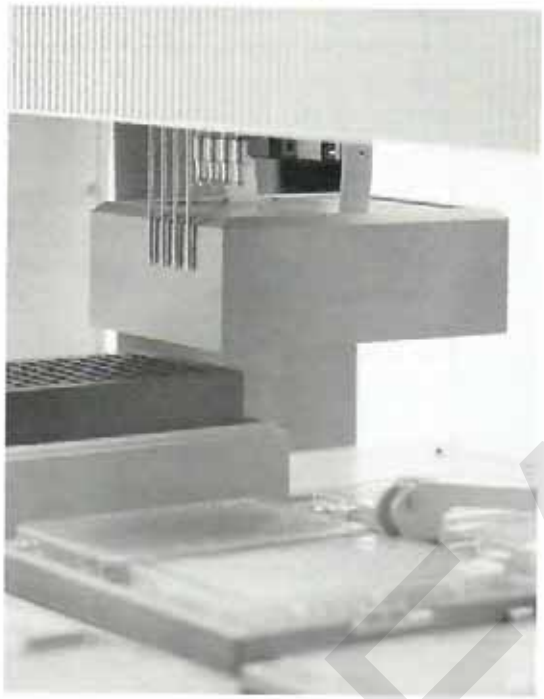
### Assembly of the Vacuum Manifold by the Robotic Handling System



During washing of the samples in the QIAGEN 96-well plate, wash buffers pass from the plate, through a channeling adapter or a filtration adapter, and into the bottom of the vacuum manifold.

After the samples in the QIAGEN 96-well plate are washed, they are eluted and collected in an item of labware (e.g., microplate or elution microtubes) that is placed into the vacuum manifold base.

### 3.2.9 The cooling and heating system

The cooling and heating system is used for fully automated cooling and heating of samples and reagents.

**Cooling and Heating System Receiving a Microplate from the Robotic Handling System**

A specially developed "boost" technology provides rapid and accurate temperature changes.

Heating of samples using the cooling and heating system is performed twice as fast compared to conventional heating systems due to extremely short ramp times. Thus, protocol run times are significantly shortened.

Operation of the cooling and heating system is controlled via the QIAsoft Operating System. Temperatures can be set between 4°C and 80°C. The temperature set represents the actual liquid temperature to be achieved. Based on the volume and type of liquid, the software automatically adjusts the heating temperature for the cooling and heating system accordingly.

The cooling and heating system accommodates one item of labware in microplate format (e.g., S-Block, microplate, or PCR plate). This type of labware can be placed in or removed from the cooling and heating system using the robotic handling system. The heat transfer adapter for microplate RB is used to ensure tight contact between the labware and the cooling and heating system and to allow excellent heat transfer. Special adapters for PCR plates, 12 ml tubes, 2 ml tubes, 1.5 ml tubes, and 20 ml disposable troughs are also available.

## 3.2.10 The labware tracking system

The labware tracking system is a hand-held bar-code reader and is for identifying bar-code–labeled labware used on the BioRobot 8000 (e.g., S-Blocks, microplates).

The user holds the bar-code reader for a short time over the bar code on the item of labware until the QIAsoft software identifies the code. A beep tone indicates that the bar code is read.

### Bar code types

The labware tracking system can read the following types of bar codes:

- EAN 13
- EAN 8
- UPC-A
- UPC-E
- CODE39
- CODE93
- CODE128
- CODABAR (NW-7)
- EAN with supplemental codes 2 of 5 Interleaved, 2 of 5 Standard
- UPC with supplemental codes 2 of 5 Interleaved, 2 of 5 Standard, Code 2 of 5 Striche Industrie, Code 2 of 5 Striche IATA, Code 2 of 5 Striche Matrix
- Code CIP
- MSI

- Plessey
- Code 11
- IBM Delta Distance
- Telepen

### 3.2.11 The high-speed shaker system

The shaker system is used for resuspension of bacterial pellets and mixing of liquids. QIAGEN supplies shaker systems for 4 plates.

### 3.2.12 The QIAsoft Operating System

The QIAsoft Operating System is the controlling software for the BioRobot 8000.

The QIAsoft 4.2 software operates under the Microsoft Windows XP Professional operating system or Windows 2000 operating system and the QIAsoft 5 software operates under the Windows XP Professional operating system. The QIAsoft software is stored in a subdirectory on a computer hard disk. The software gives the user complete control over sample processing and data handling. Clear on-screen prompts and point-and-click menus make it simple to create customized protocols. Complete documentation and sample tracking are provided in standard file formats for easy data exchange with other laboratory instruments and laboratory information management systems (LIMS).

It is strongly recommended that you do not install additional software onto the computer, since it may interfere with proper operation of QIAsoft software.

QIAsoft software is written in Microsoft Visual C++. For more information on QIAsoft software, refer to the separate software user manual.

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# 4. Installation Procedures

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# 4 Installation Procedures

The unpacking and installation of your BioRobot 8000 is carried out by a QIAGEN Instrument Service Specialist. Someone familiar with laboratory and computer equipment should be present during the installation.

## 4.1 Requirements

### Site

The BioRobot 8000 must be located out of direct sunlight and away from heat sources. Refer to Appendix A for the operating conditions (temperature and humidity).

Use a level workbench that is large enough and strong enough to accommodate the BioRobot 8000 and the computer hardware. Alternatively, QIAGEN offers 2 specially designed cabinets (see next page). Refer to Appendix A for the weight and dimensions of the BioRobot 8000.

Make sure that there is enough space for placing the system liquid container, the waste container, and the vacuum pump and trap within 1.5 m (5 ft.) of the BioRobot 8000.

The BioRobot 8000 and the computer hardware must be placed near to properly grounded (earthed) AC power outlets. The power lines to the equipment should be voltage regulated and surge protected.

### Computer

The operation of the BioRobot 8000 requires a separate personal computer. The computer is supplied by QIAGEN. The minimum hardware and software specifications are described in Appendix A.

### Additional requirements

The system liquid container, the waste container, and the vacuum trap are provided with the BioRobot 8000. These containers can be placed in a special holder that is also supplied with the BioRobot 8000. This holder should be placed near the workbench with the instrument.

The system liquid container must be filled with distilled or deionized water.

### Optional requirements

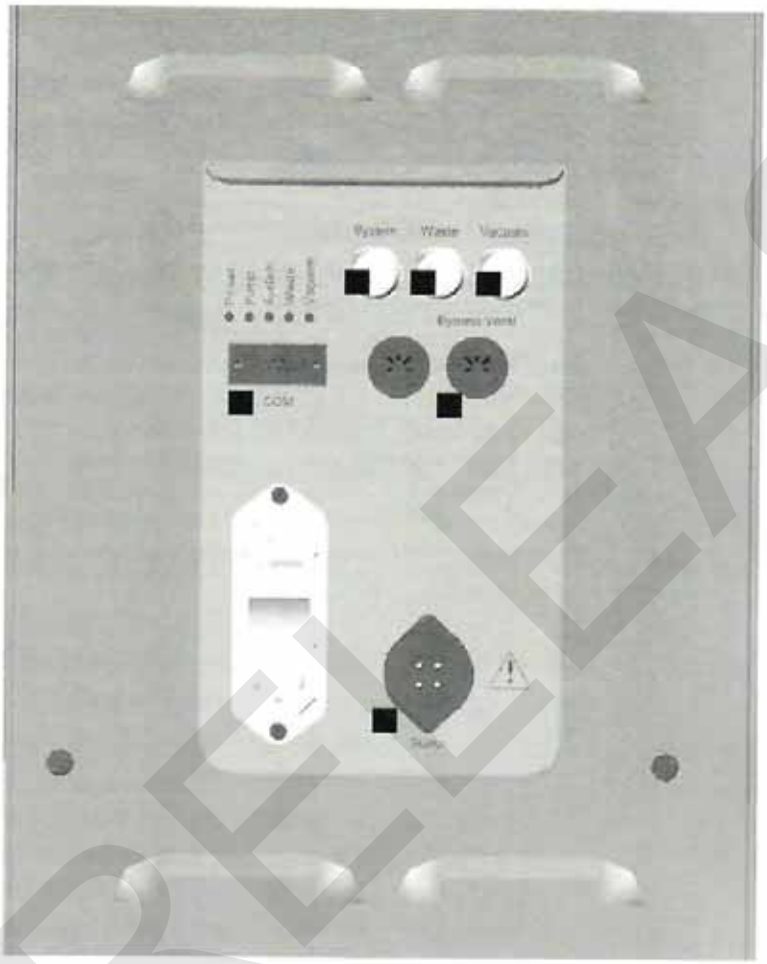
As an alternative to using your own workbench for accommodating your BioRobot 8000, QIAGEN offers 2 specially designed cabinets:

- **Laboratory Cabinet 8000** — supports the BioRobot 8000 on top and stores the system liquid container, waste container, vacuum trap, vacuum pump, and accessories in the cupboard and drawer underneath
- **Accessory Cabinet 8000** — supports the computer and monitor on top and stores accessories in the cupboard underneath

## 4.2 AC power connection

### Connector panel

The rear of the BioRobot 8000 contains a connector panel, as shown below.



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- System
- Waste
- Vacuum
- COM
- Bypass Ventilation
- Pump

Above the COM port are 5 LED lights (from left to right): "Power", "Pump", "System", "Waste", and "Vacuum".

Below the COM port is the socket for the power cord (the power connector), and the compartment for the fuses.

**Note**: The connector to the left of that for "Bypass Ventilation" is not used.

### Power requirements

The power requirements for the BioRobot 8000 are stated on the label on the rear of the instrument (see page 1-3).

BioRobot 8000 workstations sold in Australia and the UK operate at one of the following:

- 240 V AC, 50/60 Hz, 800 VA
- 240 V AC, 50/60 Hz, 1000 VA

BioRobot 8000 workstations sold in all other countries have self-regulating power units and operate at one of the following:

- 100–115/220–230 V AC, 50/60 Hz, 800 VA
- 100–115/220–230 V AC, 50/60 Hz, 1000 VA

Make sure that the voltage rating of the BioRobot 8000 and the computer hardware are compatible with the AC voltage available at the installation site.

### Grounding requirements

To protect operating personnel, the National Electrical Manufacturers' Association (NEMA) recommends that the BioRobot 8000 and the computer hardware be correctly grounded (earthed). The equipment are equipped with 3-conductor AC power cords that, when connected to appropriate AC power outlets, ground (earth) the equipment. To preserve this protection feature, do not operate the equipment from AC power outlets that have no ground (earth) connection.

### Installation of AC power cord

Connect one end of the AC power cord to the power connector located on the rear of the BioRobot 8000, and the other end to the AC power outlet. Repeat this procedure for the computer hardware.

| WARNING | **Risk of electric shock** [W10]<br>Disconnect the AC power cord before removing or installing a fuse to avoid the possibility of serious injury from electrical shock. |
|---|---|

The AC line fuse (slow blow) compartment is located near the power connector on the rear of the BioRobot 8000. Fuses for the power supplies are specified on the label situated on the rear of the instrument. If a fuse needs to be changed, contact QIAGEN Technical Services.

The following BioRobot 8000 components have fuses:

- Power inlet
- Shaker system
- Cooling and heating system
- X-assembly main board
- Automated vacuum system
- Syringe pump units
- YZ CPU units

## 4.3 Hardware installation

Installation of the BioRobot 8000 is performed by a QIAGEN Instrument Service Specialist upon delivery.

## 4.4 Software installation

The QIAsoft Operating System is installed on the computer before the BioRobot 8000 is delivered. The program, including all subdirectories and files, is stored under the directory **C:\Program Files\QIAsoft**.

A CD-ROM containing the installation file for the QIAsoft Operating System is supplied. Contact QIAGEN Technical Services if you need to reinstall the software.

# 5. Preventive Maintenance

# 5 Preventive Maintenance

The following preventive maintenance procedures must be carried out to ensure reliable operation of the BioRobot 8000:

- **Regular maintenance** — performed between protocol runs
- **Daily maintenance** — every day
- **Weekly maintenance** — every week
- **Monthly maintenance** — every month
- **Biannual maintenance** — every 6 months (only for the BioRobot Universal System)
- **Preventive maintenance** — every year

The regular, daily, weekly, monthly, and biannual maintenance procedures are performed by the user. These procedures involve the following:

- Cleaning and disinfection of the workstation
- Calibration of the high-speed dispensing system
- Checking for malfunctioning of the dilutor system

| WARNING | **Risk of electric shock** [W11]<br>Do not open any panels on the BioRobot 8000.<br>**Risk of personal injury and material damage**<br>Only perform maintenance which is specifically described in this section. |
|---|---|

## Record keeping

A record should be kept of each preventive maintenance procedure carried out on the BioRobot 8000. It is important to maintain this record, as repairs necessitated by misuse, abuse, or negligence are not covered under the Warranty or the Service Support Agreement.

If your BioRobot system is installed with QIAsoft 5 Operating System, the "Maintenance" environment of the QIAsoft 5 software keeps a record in the "Maintenance" tab of daily, weekly, monthly, biannual, and preventive maintenance procedures that have been performed.

The environment also provides an up-to-date list in the "New Maintenance" tab of maintenance procedures that should be performed. For each procedure, you read a brief description of what needs to be done, carry out the procedure (refer to the instructions in this section), and then give confirmation in the software. For details about using the "Maintenance" environment, see the *"QIAsoft 5 Operating System User Manual"*.

### Cleaning agents

The following types of disinfectants and detergents are recommended for the cleaning of the BioRobot 8000:

- Ethanol-based disinfectants, such as Mikrozid® Liquid (Schülke & Mayr GmbH), for wiping surfaces (consists of 25 g ethanol and 35 g 1-propanol per 100 g Mikrozid Liquid)
- Glyoxal and quaternary ammonium salt based disinfectant, such as Antifect® Liquid (Schülke & Mayr GmbH), for submerging worktable items (consists of 10 g glyoxal, 12 g lauryldimethylbenzylammonium chloride, 12 g myristyldimethylbenzylammonium chloride, and 5–15% nonionic detergent per 100 g Antifect Liquid)
- Anionic and nonionic surfactant based detergent, such as RBS®-35 Detergent Concentrate (Pierce Chemical Co.) for cleaning the system liquid container
- 0.1 M NaOH, 1 mM EDTA solution for cleaning the tubing of the high-speed dispensing system and inactivating RNases (see Section 5.4, page 5-9)

**Note**: If you want to use disinfectants different from those recommended, ensure that their compositions are similar to those described above. A suitable alternative to Antifect Liquid is DECON-QUAT® 100 (Veltek Associates, Inc.).

| | |
|---|---|
| **WARNING**  | **Toxic fumes** [W7]<br>Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |

## 5.1 Regular maintenance procedure

The regular maintenance procedure needs to be carried out by the user between each molecular biology application. When you run a QIAGEN protocol, the wizard will provide you with the appropriate instructions at the start and/or end of the protocol.

1. Typically, you need to perform the following tasks for the regular maintenance procedure:
   Remove the samples, reagents, and disposable labware (including tip disposal bag) from the worktable and discard them.
2. Remove the channeling adapter, the multiwell-plate holders, and the elution microtube adapter from the worktable, and soak them in disinfectant according to the manufacturer's instructions.
   **Note**: Ensure that these items are completely submerged in disinfectant and that there are no air pockets. After disinfection, rinse the items with deionized water and dry them with lint-free paper towels.
3. Soak the heating plate of the automated vacuum system with the remaining system liquid from the bottle in the reagent carousel. Start the vacuum pump by selecting "Tools/Start Vacuum Pump". Ensure that you empty the bottle completely.

| WARNING | **Hazardous chemicals and infectious agents** [W12] |
|---|---|
|  | The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |

For further details of the regular maintenance procedure, refer to the handbook of the QIAGEN kit you are using.

## 5.2 Daily maintenance procedure

The daily maintenance procedure involves the following tasks. For BioRobot systems installed with QIAsoft 5 Operating System, these tasks are listed in the "New Maintenance" tab of the "Maintenance" environment, and require confirmation from the user that they have been carried out. For details, see the *"QIAsoft 5 Operating System User Manual"*.

### Liquid containers

1. Empty the system liquid container (from underneath the workstation) and the system liquid bottle (from the reagent carousel).
   **Note**: When opening the system liquid container, first disconnect the tubing, then remove the lid.
   Rinse the container and bottle thoroughly with fresh deionized water and refill them with fresh deionized water.
   **Note**: After refilling the system liquid container with deionized water, first close the lid, then connect the tubing.
2. Flush the dilutor system with 30 ml deionized water by selecting "Tools/Flush System".
3. Empty the vacuum trap and the waste container.
   **Note**: When opening the vacuum trap, first disconnect the tubing, then remove the lid. After emptying the vacuum trap, first close the lid, then connect the tubing.
   The trap and container, and their lids and tubing, may be cleaned by washing with detergent and then rinsing with water.

| WARNING | **Hazardous chemicals and infectious agents** [W12] |
|---|---|
|  | The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |

| WARNING | **Toxic fumes** [W7]<br>Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |
|---|---|

### Tip-disposal station

1. Select "Tools/Move Arm to..." and select *"Left"*, *"Middle"*, or *"Right"* to move the robotic arm to the appropriate position on the worktable.
2. Thoroughly wipe all parts of the tip-disposal station with a soft lint-free cloth moistened with ethanol-based disinfectant (e.g., Mikrozid Liquid). Incubate as appropriate and wipe dry with paper towels.

| WARNING | **Hazardous chemicals and infectious agents** [W12]<br>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

### Worktable

1. Select "Tools/Move Arm to..." and select *"Left"*, *"Middle"*, or *"Right"* to move the robotic arm to the appropriate position on the worktable.
2. Remove all removable objects from the worktable. Examples include the tube holders and the reagent holders.
   **Note**: The vacuum manifold base remains on the worktable.
3. Wipe the worktable and the removed objects with a soft lint-free cloth moistened with ethanol-based disinfectant (e.g., Mikrozid Liquid) and incubate as appropriate. Then wipe with a soft cloth moistened with water and dry with paper towels. Return the objects to the worktable.
4. **Option**: Clean the removed objects by soaking them in a glyoxal and quaternary ammonium salt based disinfectant (e.g., Antifect Liquid).

| WARNING | **Hazardous chemicals and infectious agents** [W12]<br>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

| WARNING | **Toxic fumes** [W7]<br>Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |
|---|---|

## 5.3 Weekly maintenance procedure

The weekly maintenance procedure involves the following tasks. For BioRobot systems installed with QIAsoft 5 Operating System, these tasks are listed in the "New Maintenance" tab of the "Maintenance" environment, and require confirmation from the user that they have been carried out. For details, see the *"QIAsoft 5 Operating System User Manual"*.

If possible, perform the daily maintenance procedure before performing the weekly maintenance procedure.

### Bar-code reader windows

1. Inspect the bar-code reader window of the:
   - Labware tracking system (both hand-held and internal)
   - Buffer tracking system
2. If the window is dirty, wipe the surface with a soft lint-free cloth moistened with a little deionized water. Then dry immediately with a dry soft lint-free cloth or paper towel.

| CAUTION | **Damage to the bar code readers** [C4]<br>Do not use solvents to clean the bar code reader. Solvents can damage the bar code reader window. |
|---|---|

### Reagent carousel

Open the door of the technical tower and remove all bottles from the reagent carousel. Inspect the interior surface for splashed liquid. If splashes are present:

1. Remove the reagent carousel and wipe off the splashes.
2. Carefully wipe the interior and exterior surfaces of the reagent carousel with a soft lint-free cloth moistened with ethanol-based disinfectant (e.g., Mikrozid Liquid). Ensure that the disinfectant reaches all parts of the reagent carousel.
3. Wipe off the disinfectant and rinse with deionized water.
4. Wipe off any excess water and dry with lint-free paper towels.
5. Return the reagent carousel to the technical tower.

| WARNING | Toxic fumes [W7] |
|---|---|
| ⚠ | Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |

## 5.4 Monthly maintenance procedure

The monthly maintenance procedure involves the following tasks. For BioRobot systems installed with QIAsoft 5 Operating System, these tasks are listed in the "New Maintenance" tab of the "Maintenance" environment, and require confirmation from the user that they have been carried out. For details, see the *"QIAsoft 5 Operating System User Manual"*.

If possible, perform the weekly maintenance procedure before performing the monthly maintenance procedure.

### System liquid container

1. Remove the tubing attached to the cap of the system liquid container. Then unscrew and remove the cap. Remove the system liquid bottle from the reagent carousel.

2. Empty the system liquid container and the system liquid bottle.
3. Clean the container and bottle with strong detergent (e.g., RBS-35 detergent) according to the manufacturer's instructions.
4. Rinse the container and bottle 3 times with tap water to remove all traces of detergent. Repeat using deionized water.
5. Wipe the interior surface of the cap and tubing with a lint-free paper towel moistened with dilute detergent (e.g., RBS-35 detergent).
6. Rinse the cap and tubing 3 times with tap water to remove all traces of detergent. Repeat using deionized water.
7. Refill the system liquid container and the system liquid bottle with deionized water.
8. Close the cap of the system liquid container, and reconnect the tubing to the cap.
   Return the system liquid bottle to the reagent carousel.
9. Flush the dilutor system with 30 ml deionized water by selecting "Tools/Flush System".

| WARNING | **Hazardous chemicals and infectious agents** [W12]<br>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

| WARNING | **Toxic fumes** [W7]<br>Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |
|---|---|

### High-speed dispensing system and liquid detectors

1. Clean the surface of the dispenser head with a soft lint-free cloth moistened with ethanol-based disinfectant (e.g., Mikrozid Liquid).
   Wipe away the disinfectant with a soft lint-free cloth moistened with a little deionized water.
2. Check the dispenser head nozzles for damage.
   If the glass capillaries are broken, the nozzles need to be replaced. Contact QIAGEN Technical Services.
3. Check the high-speed dispensing system and calibrate if necessary. Check the liquid detectors.
   For the BioRobot Universal System:
   - Enter the "Execute" environment
   - Select "Maintenance Protocol UNIV" from the protocol selection box and click "RUN"
   - A wizard appears and provides you with the necessary instructions

   For other BioRobot systems, contact QIAGEN Technical Services.

The calibration results are saved in a report file.

**Note**: The calibration of the high-speed dispensing system depends on the liquid detectors functioning properly. The report file will indicate whether calibration failed due to malfunctioning liquid detectors.

If the BioRobot system is for RNA purification, the tubing of the high-speed dispensing system should be flushed with 0.1 M NaOH, 1 mM EDTA solution to inactivate RNases.

For the BioRobot Universal System:

1. Enter the "Execute" environment.
2. Select "Dispenser RNase-free UNIV" from the protocol selection box and click "RUN".
   A wizard appears and provides you with the necessary instructions.

For other BioRobot systems, we recommend running the RNeasy Maintenance Protocol once a month.

### Condensate trap

**Note:** The condensate trap is the small glass bottle connected to the vacuum pump.

1. Disconnect the condensate trap from the vacuum pump and the vacuum filter.
2. Empty the condensate trap and clean it using a glyoxal and quaternary ammonium salt based disinfectant (e.g., Antifect Liquid).
3. Rinse the condensate trap 3 times with deionized water.
4. Reconnect the condensate trap to the vacuum filter and the vacuum pump.

| WARNING | **Hazardous chemicals and infectious agents** [W12]<br>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

| WARNING | **Toxic fumes** [W7]<br>Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes. |
|---|---|

### Worktable

1. Select "Tools/Move Arm to..." and select *"Left"*, *"Middle"*, or *"Right"* to move the robotic arm to the appropriate position on the worktable.
2. Remove all removable objects from the worktable. In addition, remove the tip-disposal station:
   - Unscrew the knurled nut holding the tip-disposal station
   - Lift off the tip-disposal station, including the tip slide

   Do not remove the vacuum manifold base from the worktable.

3. Disinfect the removed objects by soaking them in a glyoxal and quaternary ammonium salt based disinfectant (e.g., Antifect Liquid).
4. Rinse the removed objects 3 times with tap water to remove all traces of disinfectant. Repeat using deionized water.
5. Wipe off any excess liquid and dry with lint-free paper towels.
6. Return the objects to the worktable.

| WARNING | **Hazardous chemicals and infectious agents** [W12]<br>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

### Robotic handling system

1. Carefully wipe the 2 grips of the robotic handling system with a lint-free cloth or tissue moistened with ethanol-based disinfectant (e.g., Mikrozid Liquid).
2. Wipe the grips with a soft cloth moistened with warm water. Then wipe off any excess liquid and dry with paper towels.

## 5.5 Biannual maintenance

Only for the BioRobot Universal System.

### Dilutor system

Check the dilutor system of the BioRobot Universal System as follows:

1. Enter the "Execute" environment.
2. Select "Syringe Calibration Check UNIV" from the protocol selection box and click "RUN".
   A wizard appears and provides you with the necessary instructions.

3. If the dilutor system needs to be calibrated, select "Syringe Calibration Adjust UNIV" from the protocol selection box and click "RUN".
   A wizard appears and provides you with the necessary instructions.

The results are saved in a report file.

## 5.6 Preventive maintenance

Each BioRobot workstation is supplied with a 1-year Warranty that includes all repairs due to mechanical breakdown. Worldwide, the maximum time for response to a breakdown is 5 days. Application development, software upgrades, worktable accessories, disposable items, and replacement of spare parts such as syringes, tubing, and pipet tips are not included in the Warranty.

QIAGEN offers comprehensive Service Support Agreements, including Warranty Extensions, Full Cover Support Agreements, and Preventive Maintenance Agreements. Service Support Agreements minimize downtime and ensure high performance from your workstation. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Instrument Service representative, or your local distributor for more information about flexible Service Support Agreements from QIAGEN.

# 6. Minor Corrective Maintenance

# 6 Minor Corrective Maintenance

This section provides instructions for removing and replacing minor parts and for carrying out occasional maintenance.

Removal and replacement of minor parts should only be attempted by experienced users or specially trained personnel.

| WARNING | **Personal injury or equipment damage hazard** [W14]<br>Do not attempt to remove any parts which are not specifically mentioned in this section. Doing so may invalidate your Warranty and could cause personal injury or equipment damage. If you think any part of the BioRobot 8000 not listed in this section requires removal or replacement, contact QIAGEN. |
|---|---|

## 6.1 Switching off the BioRobot 8000

| WARNING | **Risk of electric shock** [W15]<br>Switch off the power and disconnect the power cable before attempting any maintenance. |
|---|---|

The BioRobot 8000 is normally left switched on. If you need to switch off the workstation, switch off at the power switch at the front right of the workstation.

**Note**: If you want to shut down the BioRobot system for more than a week, contact QIAGEN Technical Services.

**Note**: If you need to move the workstation, contact QIAGEN Technical Services.

## 6.2 Removing and replacing a probe/tip adapter

If a probe or tip adapter is damaged and needs to be replaced, contact QIAGEN Technical Services.

## 6.3 Removing the tip-disposal unit and tip disposal bag

Clean the tip-disposal unit regularly to prevent buildup of contaminants. The unit can be removed for cleaning.

### Removing the tip-disposal unit

Remove the tip-disposal unit as follows:

1. Switch off the BioRobot 8000 (see page 6-1).
2. Carefully move the robotic arm to the middle of the worktable.
3. Remove the tip disposal bag from the tip-disposal unit (see below).
4. Unscrew the knurled nuts holding the wash station.
5. Remove the wash station.
6. Unscrew the knurled nut holding the tip-disposal unit.
7. Lift off the tip-disposal unit.

Reverse the procedure to replace the tip-disposal unit.

### Removing the tip disposal bag

1. Gently pull out the hinged tip disposal container from under the left side of the BioRobot 8000.
   The hinged tip disposal container is held in place by a magnetic catch.
2. Gently pull the tip disposal bag out of the tip disposal container.
3. Dispose of the tip disposal bag according to your local safety regulations.

| WARNING | **Hazardous chemicals and infectious agents** [W13]<br>The used tips may contain remnants of samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly.<br>Refer to your local safety regulations for proper disposal procedures. |
|---|---|

**Note**: The tip disposal bag and its tips can be autoclaved.

Reverse the procedure to fit a new bag.

## 6.4 Removing and cleaning a tip-tray holder

The tip-tray holders may be removed for cleaning.

### Removing a tip-tray holder

1. Carefully grip the holder and push it upward.
2. Lift the holder off the worktable.

### Cleaning a tip-tray holder

1. Rinse thoroughly with distilled or deionized water.
2. Wipe off any excess liquid and dry with paper towels.

## 6.5 Loading a tip tray

Take a tip tray filled with disposable tips and place it in the tip tray holder.

| CAUTION | **Damage to the instrument** [C2]<br>Only use the tips supplied by QIAGEN. Tips from other suppliers may cause serious damage to the instrument. |
|---|---|

Make sure the tip tray is correctly oriented in the tip-tray holder. The tip tray has a notch that allows the tip tray to be placed in only one direction on the tip-tray holder.

**Note**: Always load one more tip tray than is required by the protocol; the tray does not have to be full. During protocol execution, if a tip adapter unexpectedly fails to pick up a tip, it will try to pick up another tip instead. Thus, the extra tip tray acts as a safeguard to prevent disruption of the protocol due to lack of tips.

## 6.6 Major corrective maintenance

If the BioRobot 8000 malfunctions even after you have performed the maintenance described in this section and followed the advice in "Troubleshooting" (Section 7), contact QIAGEN Technical Services.

Do not access any electronic modules via the back panel or the robotic arm. Any major corrective maintenance attempted by unauthorized personnel will void your Warranty.

June 2005

# 7. Troubleshooting

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# 7 Troubleshooting

## 7.1 Hardware

### 7.1.1 Initialization errors

**Note**: If you need to contact QIAGEN Technical Services about an error, record the events leading to the error and the information from any dialog boxes that appear. This record will help the QIAGEN Instrument Service Specialist to resolve the error.

Initialization errors occur when the initialization procedure cannot be completed. The beeper sounds and an "Initialization Error" dialog box appears.

The "Initialization Error" dialog box displays the module (e.g., *Module Z Motor 1*) that caused the error, the error (e.g., *Communication Error*), and the error code (e.g., *8001*).

If an initialization error occurs, follow these steps:

1. Ensure that the BioRobot 8000 is switched on.
2. Ensure that all connections between the computer and the BioRobot 8000 are secure.
3. Click "Retry Initialization".
4. If the BioRobot 8000 does not initialize correctly, a "Robot Message" dialog box appears. Match the module and error code displayed in this dialog box with the module and error code listed in "Module and error code key" on page 7-3.
5. Follow the recommendations in "Module and error code key".
6. If the BioRobot 8000 still cannot be initialized, contact QIAGEN Technical Services.

### 7.1.2 Operating errors

**Note**: If you need to contact QIAGEN Technical Services about an error, record the events leading to the error and the information from any dialog boxes that appear. This record will help the QIAGEN Service Engineer to resolve the error.

Operating errors may occur during protocol execution. The beeper sounds and an "Error Message" dialog box appears.

The "Error Message" dialog box displays the module that caused the error (e.g., *Module Vacuum Pump*), the error (e.g., *"Pressure could not be reached."*), and the error code (e.g., *02*).

If an operating error occurs, follow these steps:

1. Match the module and error code displayed in the "Error Message" dialog box with the module and error code listed in "Module and error code key" on page 7-3.
2. Follow the recommendations in "Module and error code key".
3. Click the "Retry failed action" button.
4. If the error persists, contact QIAGEN Technical Services.

#### "Retry failed action"

If the "Retry failed action" button is clicked, the BioRobot 8000 will attempt to carry out the action that originally caused the error.

Use this button if the error resulted from a situation that is now corrected (e.g., the probe attempted to enter a capped bottle and the cap has now been removed).

**Note:** If the system is unable to carry out the action after "Retry failed action" is clicked, a dialog box may appear and ask you to switch off the system, wait for a few seconds, and switch on the system again. Follow the on-screen instructions and then click either:

- "Done" to continue with the interrupted protocol, or
- "Stop Protocol" to terminate the protocol run.

### 7.1.3 Module and error code key

**Module: Shaker Motor**

| Error code | Error text | Comments and suggestions |
|---|---|---|
| 8001 | Initialization error | Module is not initialized. Retry initialization using "Tools/Reinitialize Robot".<br>If the BioRobot 8000 still cannot be initialized, contact QIAGEN Technical Services. |
| 8002 | Overload | Shaker system is overloaded. Reduce the weight on the shaker system.<br>Movement is blocked due to friction. Contact QIAGEN Technical Services. |
| 8004 | Range error | Values are out of range. Contact QIAGEN Technical Services. |

### Module: Motor Valve, or Syringe Drive *n*

(*n* is the number of the module)

| Error code | Error text | Comments and suggestions |
|---|---|---|
| 8001 | Initialization error | Module is not initialized. Retry initialization using "Tools/Reinitialize Robot".<br>If the BioRobot 8000 still cannot be initialized, contact QIAGEN Technical Services. |
| 8002 | Overload | Syringe movement blocked. Contact QIAGEN Technical Services. |
| 8004 | Range error | Syringe stroke too large. Contact QIAGEN Technical Services. |
| 8008* | Syringe stroke deviation from home | Fault due to program error. Contact QIAGEN Technical Services.<br>Dilutor is defective. Contact QIAGEN Technical Services. |
| 8100* | No valve drive | Defective valve drive. Contact QIAGEN Technical Services. |
| 8200 | Hardware error | Dilutor valve is blocked. Contact QIAGEN Technical Services. |
| 8400* | Valve movement | Dilutor valve is worn or defective. Contact QIAGEN Technical Services.<br>Defective valve drive. Contact QIAGEN Technical Services. |
| 8080 | Syntax error | Invalid command sent to the BioRobot 8000. Contact QIAGEN Technical Services. |

* Does not apply to the BioRobot Universal System.

## Module: Vacuum Pump

| Error code | Error text | Comments and suggestions |
|---|---|---|
| 8001 | Initialization error | Module is not initialized. Retry initialization using "Tools/Reinitialize Robot".<br>If the BioRobot 8000 still cannot be initialized, contact QIAGEN Technical Services. |
| 8002 | Pressure could not be reached | Ensure that:<br>■ The vacuum manifold top and base are assembled correctly<br>■ The quick-disconnect couplings are connected correctly to the vacuum trap<br>■ The cap of the vacuum trap is tightly closed<br>■ Vacuum tubing is not kinked, bent, or damaged<br>■ The "Pressure" and the "Rise Time" dialog fields in the "VACUUM PUMP: Parameters" dialog box are given reasonable values |
| 8004 | Parameter out of range | The parameters specified in the "VACUUM PUMP: Parameters" dialog box are out of range. Check these parameters. |
| 8008 | Vacuum trap full | Liquid level in vacuum trap is too high. Empty the vacuum trap.<br>Vacuum trap is not connected. Connect the vacuum trap. |
| 8010 | Measured pressure not reliable | Damaged electronics. Contact QIAGEN Technical Services. |

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| | | |
|---|---|---|
| 8032 | Fatal error | Damaged electronics. Contact QIAGEN Technical Services. |

**Module: Motor *n***

| Error code | Error text | Comments and suggestions |
|---|---|---|
| 8001 | Initialization error | Module is not initialized. Retry initialization using "Tools/Reinitialize Robot".<br>If the BioRobot 8000 still cannot be initialized, contact QIAGEN Technical Services. |
| 8002 | Movement blocked | Z drive may be distorted. Contact QIAGEN Technical Services. |
| 8004 | Movement out of range | Instrument setup is not correct. Contact QIAGEN Technical Services. |
| 8008 | Movement blocked | Movement is obstructed (e.g., when the cap of a buffer bottle has not been removed and the probe cannot enter the bottle). Ensure movement is unobstructed. |
| 8100 | | The door in front of the reagent carousel is open. Close the door.<br>If the door is closed and this error appears, then the light barrier for the door is defective. Contact QIAGEN Technical Services. |

### 7.1.4 General errors that do not have error codes

| Error | Comments and suggestions |
|---|---|
| Cannot access message file *xxxxx* | Close any other applications accessing message file *xxxxx* (e.g., a text editor).<br>Ensure that the directory for message file *xxxxx* exists. |
| Unable to open file *xxxxx* | Exit the QIAsoft software, exit the Windows operating system, and switch off the computer.<br>Switch on the computer and try to open file *xxxxx*.<br>If this file still cannot be opened, contact QIAGEN Technical Services. |
| Version *xxxxx* of command *xxxxx* not supported | The protocol to be loaded was created on a newer version of the QIAsoft software. Contact QIAGEN Technical Services. |
| Loading file: Sharing violation | Close any application that may be using the file.<br>Exit the QIAsoft software, exit the Windows operating system, and switch off the computer.<br>Switch on the computer and try to open the file.<br>If this file still cannot be opened, contact QIAGEN Technical Services. |
| Error solving expression *xxxxx* | Check the syntax of the displayed expression for errors. |
| Unable to reach position *xxxxx* on rack *xxxxx* | The position cannot be accessed with the selected probe. Use a different slot for the rack. |
| Unknown probe set selected for this command | Check the settings for the probe in the command's parameters dialog box. |
| Failed to start application *xxxxx* | Software components are missing. Reinstall the QIAsoft software. |

| Error | Comments and suggestions |
|---|---|
| Module *xxxxx* not found | Use the Robot Configuration menu in the "Setup" environment to load the backup robot configuration file (*.rcf) from the "Backup" folder. If this is not possible or the problem persists, contact QIAGEN Technical Services. |
| Empty expression: {} found! | Check the parameters specified in the command's parameters dialog box. |
| Source slot and destination slot must not be the same! | Ensure that 2 different slots are selected for this command. |
| File *xxxxx* cannot be accessed! | Close any other applications accessing file *xxxxx*.<br>Exit the QIAsoft software, exit the Windows operating system, and switch off the computer.<br>Switch on the computer and try to open file *xxxxx*.<br>If this file still cannot be opened, contact QIAGEN Technical Services. |
| X-/Y-/Z-Position *xxxxx* not reachable with probe *xxxxx* | In the "Setup" environment, check how the rack that is being accessed is taught. Otherwise, consider using a different slot for this pipetting command. |
| No detection! Probe is probably too heavily bent, or pyramid is not mounted! | Check the probe and, if necessary, wipe off any droplets.<br>Check the pyramid and, if necessary, wipe off any droplets.<br>If the problem persists, contact QIAGEN Technical Services. |
| Rack *xxxxx* does not contain disposable tips. Use a different rack in this context! | Ensure that you are using a tip tray with disposable tips for the intended action. |

| Error | Comments and suggestions |
|---|---|
| There is no rack for disposable tips *xxxxx* placed in any tip slot! | Assign a tip tray to one of the tip-tray holders in the layout configuration you are using in the "Layout" tab of the "Develop" environment. |
| Module *xxxxx* not initialized! | Reinitialize the BioRobot 8000.<br>If the problem persists, contact QIAGEN Technical Services. |
| Out of disposable tips | Refill the tip-tray holders and click "OK". |
| No Wash Station rack found! | Reinstall the QIAsoft software.<br>If the problem persists, contact QIAGEN Technical Services. |
| Robot device *xxxxx* not defined! | Contact QIAGEN Technical Services. |
| External position *xxxxx* is empty, or no content name has been assigned! | In the "Setup" environment, check and edit the parameters of the position in the external rack.<br>Enter a name for the content of this position. |

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## 7.2 General troubleshooting

| | Comments and suggestions |
|---|---|
| **Poor pipetting performance** | |
| a) System liquid container is empty | Refill the system liquid container. |
| b) Air gaps in system liquid tubing or syringe | Flush the tubing. |
| c) System liquid tubing not correctly connected | Tighten the screws and connecting tubing. |
| d) Syringe and/or valve may be leaking | Contact QIAGEN Technical Services. |
| e) Probe is not adjusted correctly to the Z drive | Contact QIAGEN Technical Services. |
| **Wrong delivery destination** | |
| a) Incorrect arrangement of buffers on worktable | Ensure that the arrangement of the tubes corresponds to the numbering on the tube holder. |
| b) Probe may be distorted | Contact QIAGEN Technical Services. |
| c) Malfunctioning of X, Y, Z movement | Contact QIAGEN Technical Services. |
| **Power failure** | |
| a) Power cord not connected | Ensure that the power cord is connected. |

| | Comments and suggestions |
|---|---|
| b) Power outlet not functioning | Check the power outlet by using it to operate another electrical instrument. |

**Robotic handling system drops plate**

| | |
|---|---|
| Problem with grips of robotic handling system | Check the grips of the robotic handling system:<br>■ If the rubber surface on the inside of the grips is damaged and needs replacement, contact QIAGEN Technical Services<br>■ If necessary, clean the grips (see page 5-11)<br>If the problem persists, contact QIAGEN Technical Services. |

**Robotic handling system misses plate or destination**

| | |
|---|---|
| a) Plate positioned incorrectly in slot | Ensure that the plate is positioned correctly in its slot. |
| b) The plate and slot were not taught properly in the "Setup" environment | Check that they were taught properly. |
| c) Faulty Z drive | Contact QIAGEN Technical Services. |

**Camera fails to read bar code**

| | |
|---|---|
| a) Bar codes are faulty, incorrectly positioned, or dirty | ■ Check the bar codes for faults.<br>■ Ensure that the bar codes are correctly positioned.<br>■ Ensure that the bar codes are clean; errors can also occur if the bar codes are shiny. |
| b) Problem with the CCD camera window | Check the window for dirt. The window should be transparent, red in color, and free from scratches. If necessary, carefully clean the window.<br>If the problem persists, contact QIAGEN Technical Services. |

| | Comments and suggestions |
|---|---|
| **Vacuum error: Pressure could not be reached** | |
| a) Lid on vacuum trap is not tightly closed | Close the lid tightly. |
| b) Plate or lid is not positioned properly in the top of the vacuum manifold | Place the plate or lid properly in the top of the vacuum manifold.<br>If the plate or lid was positioned automatically by the robotic handling system, check that it was taught properly in the "Setup" environment. |
| c) Unused positions are not sealed (if processing fewer than 96 samples) | Use tape to seal the unused positions. |
| d) Seal defect on the connectors for the vacuum trap | Change the connector; contact QIAGEN Technical Services. |
| e) Poor pump performance | See below. |
| **No vacuum on the vacuum pump** | |
| Vacuum pump defect, or liquid in the vacuum pump (vacuum trap overflowed) | Contact QIAGEN Technical Services. |
| **Tips do not attach properly** | |
| Tip-tray holder was not taught properly in the "Setup" environment | Check that the tip-tray holder was taught properly.<br>If the problem persists, contact QIAGEN Technical Services. |
| **Tips do not detach properly** | |
| Tip-disposal unit is not installed correctly | Install the tip-disposal unit correctly. |

| Comments and suggestions | | |
|---|---|---|
| **Tip touches when pipetting into a 96-well microplate** | | |
| The slot and rack were not taught properly in the "Setup" environment. | Check that they were taught properly. If the problem persists, contact QIAGEN Technical Services. | |
| **Liquid detection problem** | | |
| a) Small droplets or foam around the edge of small tubes are leading to premature detection | Ensure that there are no droplets or foam around the edges of small tubes. | |
| b) System liquid tubing is not filled with system liquid | Fill the tubing with system liquid by selecting "Tools/Flush System". | 8 |
| c) System liquid container is not filled with deionized water | Empty the container, and fill it with deionized water. | |
| d) The system liquid in the system liquid container is contaminated | Empty and clean the container, and replace the system liquid. | 9 |
| **Tubing system running empty** | | |
| a) Tubing is incorrectly placed in the system liquid container | Place the tubing correctly in the system liquid container. | |
| b) System liquid container is empty | Fill the container with deionized or distilled water. | A |
| c) Sensor for the system liquid container is faulty | Check that the cable for the system liquid container sensor is connected properly. If the problem persists, contact QIAGEN Technical Services. | |

| Comments and suggestions | |
|---|---|
| **Buildup of droplets on the tip adapters** | |
| a) Air bubble in the system liquid tubing | Flush the tubing to remove air bubbles by selecting "Tools/Flush System". |
| b) Precipitate buildup on the tip adapter | Clean the tip adapter. If the problem persists, contact QIAGEN Technical Services. |
| c) Leak in the system liquid tubing | Tighten all tubing connections. If the problem persists, contact QIAGEN Technical Services. |

# 8. Calibration and System Diagnostics

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# 8 Calibration and System Diagnostics

## 8.1 Pipetting system calibration

The dilutor system and the high-speed dispensing system of the BioRobot 8000 can be calibrated.

The frequency of calibration depends on the type of applications performed and on how often they are performed. For advice, ask the QIAGEN Instrument Service Specialist or contact QIAGEN Technical Services.

### 8.1.1 Calibrating the dilutor system

See the *"Precision Pipetting BioRobot 8000 Handbook"* for more information.

### 8.1.2 Calibrating the high-speed dispensing system

See the *"Precision Pipetting BioRobot 8000 Handbook"* for more information.

## 8.2 Diagnostics

The BioRobot 8000 has diagnostic routines which are used to verify the correct operation of the instrument after maintenance and service procedures, and to investigate suspected malfunction of the instrument.

Relevant diagnostic tests should be conducted after completing installation of all hardware and software for the system. These tests are to verify correct operation of the system and should be performed before placing the instrument into service. The diagnostic tests should also be performed after transporting the unit to another location, or after certain corrective maintenance procedures.

| CAUTION | **Damage to the instrument** [C3]<br>Diagnostic tests should only be performed by QIAGEN Instrument Service Specialists.<br>Unauthorized personnel should not attempt any diagnostic test especially if the instrument is under Warranty, or if a maintenance contract is in effect. |
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# 9. Glossary

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| Word | Description |
|---|---|
| Dilutor unit | A module consisting of a syringe pump (syringe, dilutor valve, motor), tubing, and a probe/tip adapter. The dilutor unit is for high-precision aspiration and dispensing of liquid. |
| Encoder | Optical device used to determine the position of the motor axle. |
| Error codes | Numeric codes that represent various software and hardware errors. |
| "Execute" environment | The "Execute" environment of the QIAsoft Operating System is for running protocols. |
| Exit | An option in the File menu of the QIAsoft Operating System that is for closing the software. |
| Flush | An operation to clean the probes/tip adapters and connected tubing and syringes by flushing system liquid through them.<br>A flush also removes air bubbles from the tubing. |
| Initialization | An operation performed automatically before each protocol run to check the electronic and mechanical operation of the BioRobot 8000. |
| Layout configuration | The arrangement of slots (e.g., microplate stations and tip-tray holders) and racks (e.g., microplates and tip trays) on the worktable for a particular protocol run. Layout configurations are defined in the "Layout" tab of the "Develop" environment. |
| LED | Light Emitting Diode. Used as indicators on the CCD camera and power indicator. |
| Liquid detector | A system that detects ionic liquids or conductive surfaces by monitoring the change in capacitance between the probe/disposable tip and worktable as the probe/disposable tip enters a liquid. |
| Locator pins | The pins arranged on the worktable surface which hold the slots (e.g., microplate stations, tip-tray holders) in their correct positions. |

| Word | Description |
|---|---|
| Luer lock | A screw mechanism that connects a syringe to a dilutor valve. |
| Menu bar | A bar located at the top of each environment of the QIAsoft Operating System. It contains menus with various options for the user to choose from. |
| Mixing | A liquid-handling operation in which the liquid contained within a vessel (e.g., tube) is aspirated and dispensed in order to mix the contents of the vessel. |
| Moat | The outer reservoir of the wash station. Waste liquid collects in the moat before draining to the waste container. |
| Position | An area of a rack that can contain something. Examples of positions include the wells of a microplate or the holes of a tip tray for holding disposable tips. |
| Power switch | A button located at the front of the BioRobot 8000 in the bottom-right corner. It allows the user to switch the workstation on and off. |
| Precision | The reproducibility of an assay or pipetting action determined by its standard deviation. |
| Probe | A probe is a specially designed steel tube for aspirating and dispensing liquids.<br>A tip adapter is a special probe designed for use with disposable tips. |
| Protocol | A protocol runs on the QIAsoft Operating System and contains instructions for the BioRobot 8000 to perform a molecular biology application. A protocol contains commands that are executed sequentially, and information about the layout of labware, samples, and reagents on the instrument.<br>The BioRobot 8000 is installed with several QIAGEN protocols.<br>In addition, customized protocols can be created, edited, and deleted by the user. These protocols can be protected to prevent them from being inadvertently changed. |

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| Word | Description |
|---|---|
| Tip adapter | A tip adapter is a special probe that allows aspiration and dispensing of liquid through an attached disposable tip. |
| Tool bar | A bar located below the menu bar. It contains buttons that, when clicked, allow the BioRobot 8000 or QIAsoft software to perform an operation. |
| Valve | A computer-controlled 3-way valve located at the top of each syringe. |
| Variable spacing system | A computer-controlled system that varies the spacing between the probes/tip adapters. |
| Waste | The outlet of the wash station or liquid that is discarded. |
| Waste container | The bulk container that collects waste liquid from the wash station. It is located below the BioRobot 8000. |
| Waste outlet | The junction between the wash station and the waste tubing. Situated on the underside of the wash station. |
| Waste tubing | Tubing that delivers waste liquid from the waste outlet to the waste container. |
| Worktable | The surface of the BioRobot 8000 where samples are loaded and processed. It contains an array of slots and racks and is accessible by the probes/tip adapters, the dispenser head, and the robotic handling system. |
| X, Y, and Z movement | Describes the movement of the probes/tip adapters and the robotic handling system: left to right (X), back to front (Y), and up and down (Z). |
| Z drive | A drive unit that moves a probe/tip adapter in the Z direction (up and down). |
| Z-max | The lowest point reached by a probe/tip adapter. |

| ord | Description |
|---|---|
| ravel | The height at which the probes/disposable tips travel above the surface of a rack. This height is defined individually for each rack. If several different racks are used together in one protocol, the QIAsoft software automatically calculates the optimum travel height. Z-travel should be set above the highest point of the rack. |

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June 2005

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# Appendices

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# Appendix A

## Technical data

### Environmental conditions

| | |
|---|---|
| Power requirements | One of 4 configurations is supplied:<br>■ 240 V AC, 50/60 Hz, 800 VA<br>■ 240 V AC, 50/60 Hz, 1000 VA<br>■ 100–115/220–230 V AC, 50/60 Hz, 800 VA<br>■ 100–115/220–230 V AC, 50/60 Hz, 1000 VA<br>Check the label on the back of the BioRobot 8000 (see page 1-3). |
| Fuses | T10L250V; 2 fuses for the main power supply unit |

| WARNING | **Electrical hazard** [W16]<br>Never install a fuse different from that specified on the label on the rear of the BioRobot 8000. If a fuse needs to be changed, contact QIAGEN Technical Services. |
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|---|---|
| Ambient operating temperature | 20–26°C (68–79°F) |
| Humidity range | 15–75% relative humidity at 30°C (85°F) or below without condensation |
| Altitude | Up to 2000 m (6500 ft.) |
| Place of operation | For indoor use only |
| Storage temperature | –10°C to 40°C (14–104°F) |
| Pollution level | 2 |
| Overvoltage categeory | II |

## Mechanical data and hardware features

| | |
|---|---|
| Dimensions (approximate) | Width: 1.45 m (58 in.)<br>Height: 0.79 m (31 in.)<br>Depth: 0.81 m (32 in.) |
| Mass | Approximately 135 kg (298 lb.)<br>**Note**: The figure given is for the basic instrument. |
| Computer | ■ 500 MHz Pentium® III processor or higher<br>■ 128 MB RAM<br>■ CD-ROM drive<br>■ Graphics card, 1024 x 768 pixels, true color<br>**Note**: Specifications are subject to change as technology improves |
| Software | QIAsoft 4.2 Operating System running on Microsoft Windows XP Professional operating system or Windows 2000 operating system.<br>QIAsoft 5 Operating System running on Microsoft Windows XP Professional operating system. |
| Interface | RS 232 C/CCITT V.24 asynchronous serial ASCII |
| Liquid detection | The probe, or tip adapter with conducting tip, functions as a liquid detector and measures the change in capacitance when entering or leaving a liquid.<br>The minimum detectable volume is 200 µl of 0.009 g/ml sodium chloride solution in a tube (16 mm diameter) or a microtube placed in a metal block. |
| Probe | The probe is made of ceramic-coated stainless steel and is designed to eliminate droplet formation.<br>The inner diameter is 0.4 mm or 0.9 mm depending on the probe set used. |
| Tip adapter | The tip adapter is made of stainless steel and is designed to eliminate droplet formation.<br>The tip adapter can pick up conducting disposable tips of various sizes. |

| | |
|---|---|
| Disposable tips | The conducting disposable tips are specially molded for use with the tip adapters. The following tips are available:<br>■ Disposable tips, 10 µl, 50 µl, 300 µl, and 1100 µl<br>■ Disposable filter-tips, 10 µl, 50 µl, 300 µl, and 1100 µl |
| Pipetting configuration | Probes and tip adapters can move independently in the Z direction as required.<br>Probes and tip adapters can be combined as required. |
| Wash station | Here, the inner and outer surfaces of the probes can be thoroughly washed using distilled or deionized water, or a non-conductive disinfectant solution. |
| Precision pipetting system | Each pipetting channel has a high-precision syringe pump for small-volume liquid handling. The syringes can be operated independently or simultaneously.<br>For the BioRobot Universal System, the full volume can be dispensed in 12,000 steps. The syringe size is 1000 µl and the dilutor syringe delivers 1–1000 µl of liquid.<br>For other BioRobot systems, the full volume can be dispensed in 2000 steps. There are 4 syringe sizes available, covering the range 1 µl to 2500 µl:<br>■ The 100 µl dilutor syringe delivers 1–100 µl of liquid<br>■ The 500 µl dilutor syringe delivers 5–500 µl of liquid<br>■ The 1000 µl dilutor syringe delivers 10–1000 µl of liquid<br>■ The 2500 µl dilutor syringe delivers 25–2500 µl of liquid |
| High-speed shaker system | ■ 4-plate shaker system — operates at speeds of 200–1100 rpm<br>The shaking direction can be automatically reversed.<br>The 4-plate shaker system accommodates up to 4 microplates (or other labware with the same base dimensions). |
| Robotic handling system | A T-grip model is available for moving plates on the worktable.<br>The system can move a plate from one location to another in less than 10 seconds, depending on the worktable settings. |

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|---|---|
| Probe spacing | The spacing between probes/tip adapters can be varied evenly from 9 mm to 20 mm using the variable spacing system. |
| Cooling and heating system | Computer-controlled cooling and heating system with adapters for plates, blocks, and tubes.<br>Working temperature range for cooling: room temperature (approximately 20–26°C) to 4°C.<br>Working temperature range for heating: room temperature to 80°C. |
| Labware tracking system | The labware tracking system is a hand-held CCD camera bar-code reader for identifying bar-code labeled labware. The reading field is 65 mm x 10 mm. |
| Buffer tracking system | The buffer tracking system is a CCD camera bar-code reader integrated into the technical tower. The reader identifies bar-code labeled reagent and buffer bottles placed in the reagent carousel. The reading field is 65 mm x 10 mm. |
| Compatible bar codes | The following are compatible with the labware tracking system:<br>EAN 13; EAN 8; UPC-A; UPC-E; CODE39; CODE93; CODE128; CODABAR (NW-7); EAN with supplemental codes 2 of 5 Interleaved, 2 of 5 Standard; UPC with supplemental codes 2 of 5 Interleaved, 2 of 5 Standard, Code 2 of 5 Striche Industrie, Code 2 of 5 Striche IATA, Code 2 of 5 Striche Matrix; Code CIP; MSI; Plessey; Code 11; IBM Delta Distance; Telepen. |
| Safety features | The BioRobot 8000 is installed with one of the following:<br>■ Safety shield — helps to protect the user from the movements of the robotic arm<br>■ Worktable hood — protects the user from all movements of the BioRobot 8000 |
| Vacuum manifold | The vacuum manifold accommodates one QIAGEN 96-well plate (e.g., QIAprep 96 plate).<br>Samples are eluted into labware such as 96-well microplates or deep-well blocks. |

| | |
|---|---|
| Vacuum pump | The vacuum pump pressure is computer-regulated from atmospheric pressure (approximately 1000 mbar) down to 20 mbar. |

# Appendix B

## BioRobot 8000 system component list

**Note**: Some component combinations may exclude the use of other components. If you have questions about combinations of components, call QIAGEN Technical Services.

| Product | Contents |
|---|---|
| BioRobot 8000 | System includes: robotic workstation comprised of 8 dilutor units and selected system components; variable spacing system; QIAsoft Operating System; installation and training; 1-year warranty on parts and labor |
| **Probe/Tip sets** | |
| Probe set 8000, small | Pipetting probe (0.4 mm) and precision syringe (0.5 ml) for aspirating and dispensing volumes from 1 to 500 µl |
| Probe set 8000, medium | Pipetting probe (0.4 mm) and precision syringe (1.0 ml) for aspirating and dispensing volumes from 10 to 1000 µl |
| Probe set 8000, large | Pipetting probe (0.9 mm) and precision syringe (2.5 ml) for aspirating and dispensing volumes from 25 to 2500 µl |
| Tip adapter set 8000, small | Tip adapter and precision syringe (0.5 ml) for use with 10 µl or 300 µl disposable tips |
| Tip adapter set 8000, medium | Tip adapter and precision syringe (1.0 ml) for use with 300 µl and 1100 µl disposable tips |
| Tip adapter set 8000, large | Tip adapter and precision syringe (2.5 ml) for use with 1100 µl disposable tips |
| Tip adapter set 8000, universal | Tip adapter and precision syringe (1.0 ml) for use with 10 µl, 50 µl, 300 µl, and 1100 µl disposable tips. |

| Product | Contents |
|---|---|
| **Modules** | |
| High-speed dispensing system | Computer-controlled, high-precision multiple-channel dispensing device; reagent carousel equipped with capacitance-based liquid-level sensing system; tubing and bottles; buffer tracking system must be purchased separately |
| High-speed shaker system | Computer-controlled, high-speed shaker for mixing samples in up to four 96-well plates (or other labware with the same base dimensions) |
| Labware tracking system, hand-held | Hand-held bar-code reader for identification of labware |
| Buffer tracking system | Bar-code reader fitted to the reagent carousel compartment for identifying buffers and reagents |
| Robotic handling system | Robotic handling system (T-grip) for moving labware, module components, and accessories on the worktable |
| Cooling and heating system | Computer-controlled cooling and heating system; adapters for accommodating various labware must be purchased separately |
| Automated vacuum system | Vacuum manifold and computer-controlled pressure system for walk-away processing of a QIAGEN 96-well plate |
| Tip-disposal unit 8000 | Unit for detaching and collecting used disposable tips |
| **Accessories** | |
| Reagent holder, tubes and bottles | Holder for accommodating 4 microcentrifuge tubes (1.5 ml), 4 tubes (13 ml), and 2 bottles (125 ml and 60 ml) on the worktable |
| Reagent holder, 3-trough, 20 ml | Holder for 3 disposable troughs (20 ml) for use with multiple-channel pipetting systems |

| Product | Contents |
|---|---|
| Reagent holder, 8-tube, 1.5 ml | Holder for accommodating 8 microcentrifuge tubes (1.5 ml) on the worktable; must be used with the reagent-holder tray |
| Reagent holder, 4-tube, 13 ml | Holder for accommodating 4 tubes (13 ml) on the worktable; must be used with the reagent-holder tray |
| Reagent holder, 1-trough, 20 ml | Holder for accommodating a single disposable trough (20 ml) on the worktable; must be used with the reagent-holder tray |
| Reagent-holder tray | Tray for accommodating up to 3 of the following reagent holders: 8-tube, 4-tube, 1-trough |
| Cooling block, plate, 0.2 ml | Holder for a 96-well amplification plate (0.2 ml per well) on the worktable |
| Cooling block, 24-tube, 1.5 ml | Holder for accommodating 24 tubes (1.5 ml) on the worktable |
| Cooling block, 1-tube, 13 ml | Holder for accommodating a single tube (13 ml) on the worktable |
| Microplate station 8000, short | Station for accommodating a 96-well plate (or other labware with the same base dimensions) on the worktable |
| Microplate station 8000, tall | Station for accommodating a 96-well plate (or other labware with the same base dimensions) on the worktable |
| Plate stacker 8000, detachable | Unit for stacking up to eight 96-well plates; for use with the robotic handling system |
| Laboratory cabinet 8000 | Cabinet for accommodating the BioRobot 8000, accessory items, vacuum trap, vacuum pump, waste and system liquid containers |
| Accessory cabinet 8000 | Cabinet for accommodating computer, monitor, keyboard, and additional accessories |

| Product | Contents |
|---|---|
| Computer system, Windows XP Professional | For operating BioRobot systems: computer, monitor, keyboard, Microsoft Windows XP Professional operating system |
| Tip-tray holder | Holder for accommodating a tip tray containing 96 disposable tips |
| Disposable tips, 10 µl (24 x 96) | Conducting disposable tips; 24 packs of 96 each |
| Disposable tips, 50 µl (960) | Conducting disposable tips; pack of 960 |
| Disposable tips, 300 µl (960) | Conducting disposable tips; pack of 960 |
| Disposable tips, 1100 µl (960) | Conducting disposable tips; pack of 960 |
| Disposable filter-tips, 10 µl (24 x 96) | Conducting disposable filter-tips; 24 packs of 96 each |
| Disposable filter-tips, 50 µl (960) | Conducting disposable filter-tips; pack of 960 |
| Disposable filter-tips, 300 µl (960) | Conducting disposable filter-tips; pack of 960 |
| Disposable filter-tips, 1100 µl (960) | Conducting disposable filter-tips; pack of 960 |
| Tip disposal bags (20) | Autoclavable bags for collection and disposal of used disposable tips; pack of 20 |

# Appendix C

## Warranty statement

Thank you for your purchase of QIAGEN instrumentation. Your instrument has been carefully tested to ensure optimum operating efficiency and reproducibility of results. QIAGEN warrants that all new instrumentation manufactured by QIAGEN will correspond to the product specifications and be free from defects in workmanship and materials for a period of twelve (12) months from the original date of shipment. Repair or replacement of defective parts will be provided to the purchaser during this time period provided the QIAGEN instrumentation is operated under conditions of normal and proper use, but not for damage caused by the customer. If any part or subassembly proves to be defective, it will be repaired or replaced at QIAGEN's sole option, subsequent to inspection at the factory, or in the field by an authorized factory representative, provided that such defect manifested under normal and proper use. The shipper will pay all transport fees.

**Limitation of warranties and remedies**

THE FOREGOING WARRANTY IS QIAGEN'S SOLE AND EXCLUSIVE WARRANTY, AND REPAIR OR REPLACEMENT OF DEFECTIVE PARTS IS THE SOLE AND EXCLUSIVE REMEDY. THERE ARE NO OTHER WARRANTIES OR GUARANTEES, EXPRESS OR IMPLIED. THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE EXPRESSLY EXCLUDED, TO THE FULLEST EXTENT PERMITTED BY LAW. (NOTE: SOME STATES DO NOT PERMIT DISCLAIMERS OF IMPLIED WARRANTIES SO THIS LIMITATION MAY NOT APPLY TO YOU). WITH THE EXCEPTION OF THE ABOVE-REFERENCED REPAIR OR REPLACEMENT REMEDY, QIAGEN SHALL HAVE NO OBLIGATION OR LIABILITY OF ANY NATURE WHATSOEVER WITH RESPECT TO THE QIAGEN INSTRUMENTATION, WHETHER ARISING IN CONTRACT, TORT, STRICT LIABILITY, OR OTHERWISE, INCLUDING BUT NOT LIMITED TO, LIABILITY FOR INDIRECT, CONSEQUENTIAL, INCIDENTAL AND/OR SPECIAL, PUNITIVE, MULTIPLE AND/OR EXEMPLARY DAMAGES AND/OR OTHER LOSSES (INCLUDING LOSS OF USE, LOST REVENUES, LOST PROFITS AND DAMAGE TO REPUTATION), EVEN IF SUCH DAMAGES WERE FORESEEN OR FORSEEABLE, OR WERE BROUGHT TO QIAGEN'S ATTENTION. IN NO EVENT SHALL QIAGEN'S LIABILITY TO YOU EXCEED THE PURCHASE PRICE OF THE PRODUCT.

## Liability clause

QIAGEN shall be released from all obligations under its Warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.

All materials replaced under this Warranty will be warranted only for the duration of the original Warranty period, and in no case beyond the original expiration date of original Warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this Warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

# Index

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## W

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Please see back cover for contact information for your local QIAGEN office.

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# Public Health Virology Validation Report for *Sarbecovirus "Wuhan" E gene TaqMan 2020* Nucleic Acid Testing

## 1 Purpose and Scope

This document describes the results of validating an *in vitro* molecular test used by Public Health Virology. It is used for any molecular assays that require validation.

## 2 Principle

Public Health Virology is a NATA Accredited laboratory and to maintain accreditation is required to validate all assays. This is performed in accordance with the NPAAC guidelines. Due to availability issues regarding positive material and volume of patient samples, a 3 Tier Validation system has been developed. When changes to an oligonucleotide primer or probe sequence, amplification kit brand, cycling condition or synthetic control are made to a validated test that will impact the result outcome, verification of the change must be performed.

### Tier 1

Full validation with a minimum of 50 target-positive samples and 100 target-negative (some containing other related viruses, some from a relevant sample matrix, some from clinically similar presentation/request) and the following must be completed:

☐ Limit of detection

☒ Sensitivity

☒ Specificity

☒ Precision

### Tier 2

Partial validation with fewer than 50 positive samples and the following must be completed:

☐ Limit of detection

☐ Sensitivity

☐ Specificity

☐ Precision

### Tier 3

No positive samples available – validate on synthetic controls only. The following must be completed:

☐ Limit of detection

☐ Precision

## 3 Associated Documentation

- NPAAC Guidelines
- NATA Standard

## 4 Amendment History

| Version | Date | Updated By | Amendments |
|---|---|---|---|
| 1 | 10/05/2016 | Ian Mackay | New document |
| 2 | 09/08/2016 | Ian Mackay | Added measurement uncertainty (MU), Appendices updated, edits of precision and numbering |
| 3 | 10/05/2017 | Ian Mackay | Edits to description, limit of detection, authorisation, precision and MU |
| 4-5 | 30/08/2017 | Ian Mackay | Edits to MU, numbering, precision |
| 6 | 11/04/2019 | Ian Mackay | Annual review minor edits |
| 7 | 08/10/2019 | Ian Mackay | Edits to amendment table, checkboxes and cycle number |

## 5 Appendices

Appendix 1 –

- *Data index file*

Appendix 2 –

- *Cover sheet file*

# 6 Validation Report

## 1 Recommendations

- Referred to in the laboratory as the Wuhan E TaqMan 2020 (WuhanE-TM2020) assay, it is suitable for use as a screening assay for members of the *Sarbecovirus* subgenus of *Betacoronavirus*. This includes the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

## 2 Description of Assay

- A real-time RT-PCR (RT-rtPCR) TaqMan assay targeting the E gene of *Sarbecovirus*.
- The assay is based on a published probe and primer sequences published by Corman[1] et al. The assay has been optimised using a different concentration of reverse primer and validated using both the SuperScript™ III Platinum® One-Step Quantitative RT-PCR (Invitrogen) and the SensiFast™ Probe Lo-ROX One-Step (Bioline) kits. The preferred method is the SuperScript III kits for clinical samples extracted using the EZ1 Virus Mini Kit v2.0 or the QIAamp One-For-All nucleic acid kit. Precision, sensitivity and specificity data is calculated using the SuperScript™ III Platinum® One-Step Quantitative RT-PCR (Invitrogen) kit.
- The assay commences from the receipt of extracted nucleic acids.
- Acceptable requested sample types for test and those which have been used in the validation process:
    - Aspirates
    - Pharynx swabs
    - Nasopharynx swab
    - Throat swabs
    - Nasal swabs
    - Nasopharyngeal swabs
    - Nasopharyngeal oropharyngeal swabs
    - Nasopharyngeal aspirates
    - Sputum
    - Bronchial washing
    - Swabs, site not stated
    - Faeces
    - Cell culture supernatant (TCS)
- Other sample types may produce acceptable results but have not yet been included in the validation process.
- The concentration of the reverse primer varies from the published method, as does the kit used and subsequent cycling conditions.

## 3 Limitations

- The assay may not detect levels of RNA which fall below the limit of detection of the assay.

## 4 Test Method Protocol

A rapid RT-rtPCR employing two oligonucleotide primers and an exonuclease probe (“TaqMan probe”) complementary to *Sarbecovirus* genetic sequences. The validated PCR-based assay amplifies small amounts of virus-specific genetic material through a cyclical process of enzyme-driven copying of the genetic sequence spanned by two primers. The amplification process is monitored via detection of the fluorescence produced by release of a fluorophore during cyclical

destruction of a target sequence-specific probe. This capture occurs via a thermal cycling instrument which also provides the reaction temperatures and timing for the amplification process.

- This is a new assay modified from a previously published assay and employs a newly designed pair of synthetic oligonucleotide primer and probe positive controls.

4.2 Primers and Probes

Sequences are described in the Cover Page.

4.3 Mastermix preparation

- All mastermix must be prepared in the mastermix room in a laminar flow cabinet
- Enzymes should be kept at -20°C in a manual defrost freezer or in a lab top cooler in a frost-free freezer
- All other reagents must be stored and handled according to the manufacturer's instructions

Master mix components are described in the Cover Page.

## 5 Full reaction set-up

1. Add 15µL of required mastermix to sufficient wells
2. Add 5µL of nucleic acid to assigned wells of:
    a. Run Controls RNA/DNA (Probe, Primer, NTC)
    b. Extracted nucleic acids from samples
    c. Positive Extraction Control
    d. Negative Extraction Control

## 6 Cycling Conditions

For the Qiagen/Corbett Rotor-Gene thermal cyclers, the conditions are as follows:

| *1 cycle*<br>*50˚C / 5 min*<br>*95˚C / 2 min* | *50 cycles*<br>*95˚C / 3secs*<br>*60˚C / 30sec* |
|---|---|

## 7 Acceptance Criteria

See:

QIS 27340 7.2 – defining a satisfactory positive real-time PCR signal
QIS 27340 7.10 – use of controls

Controls must give expected results.

| Controls | Expected result |
|---|---|
| NTC | No amplification |
| Primer and Probe Controls | *Amplification within accepted limits* |
| Positive Extraction Control | *Amplification within accepted limits* |
| Negative Extraction Control | No amplification |

## 8 Basic Local Alignment Search Tool (BLAST) nucleotide analysis of oligonucleotides

Both primers and the probe are 100% match with many sequences from the *Sarbecovirus* subgenus, with small mismatches with a few SARS coronaviruses.

For further details see section 2 of the Data index.

| Sequence | Acceptable | Explanation |
|---|---|---|
| Forward primer unique to target | Yes | |
| Reverse primer unique to target | Yes | |
| Probe sequence unique to target (TaqMan test only) | Yes | |

## 9 Evidence of clinical or biological association

Some infectious diseases are defined qualitatively, and some are defined quantitatively. It is often difficult to determine if the detection of the organism is indicative of disease as both viable and non-viable organisms are detected using molecular methods. Test results must be assessed within a clinical and epidemiological context.

## 10 Reagents and consumables

All reagents and consumables must:

- be obtained from approved suppliers
- have their Lot No. and Expiry date recorded
- have passed internal or external quality control
- be stored under appropriate environmental conditions
- have records of purchase, quality control and storage conditions retained

See section 8 of Data index page for manufacturers reagent inserts.

## 11 Equipment

All equipment must:

- Be under calibration controls where appropriate and records kept
- Be under maintenance controls and records kept
- Service records can be found in the following folder: EQUIPMENT

## 12 Optimisation

The latest work is summarised in herein and referred to in the Data index.

## 13 Limit of Detection

An absolute limit of detection has not yet been determined

## 14 Precision

The precision is determined after repeatability and reproducibility analyses. Mean and CV values are rounded to 4 significant digits.

Repeatability analysis amplified 10 replicates of each synthetic control using the same instruments, reagents, aliquots and user

**UBE-WuhanE-synPri**

- Mean of repeatability = *23.60* $C_T$
- CV of repeatability = *0.003154*

**UBE-WuhanE -synPrb**

- Mean of repeatability = *21.60* $C_T$
- CV of repeatability = *0.004761*

See the Repeatability spreadsheet for specific values.

Reproducibility analysis amplified 10* duplicates of each synthetic control on separate days using different aliquots, instruments and users.
(*__NOTE__: the number of data points was reduced due to the restrictive supply of conductive tips)

**UBE-WuhanE-synPri**
- Mean of reproducibility = *23.95* $C_T$
- CV of reproducibility = *0.03163*

**UBE-WuhanE-synPrb**
- Mean of reproducibility = *23.18* $C_T$
- CV of reproducibility = *0.02701*

See the Reproducibility spreadsheet for specific values.

## 15 Sensitivity

Sample extracts or samples that had previously tested positive were tested or re-extracted and tested again using this assay. These included:

- 40 of swabs (sited not stated)
- 9 of nasopharyngeal swabs
- 2 of nasopharyngeal oropharyngeal swab
- 1 of nose swab
- 1 of aspirate
- 1 of pharyngeal swab
- 1 of nasopharynx swab
- 1 of sputum
- 1 of faeces
- 2 of cell culture supernatant (TCS)

*Sensitivity is the ability of the assay to detect true positives in samples of the same type as those listed in section 6.2. These samples must contain organism variants of the type targeted by this assay. The formula below is used to determine the sensitivity. Values are rounded to 3 significant digits.*

Sensitivity = [True Positive / (True Positive + False Negative)] X 100%

The ability of the assay to detect true positives was determined to be: *98.4%*

From *60* previously genotyped positive nucleic acid extracts, *59* were detected.

(It should be noted that the extract not detected was from a previously SARS-CoV-2 diagnosed patient and only one of the 2 extracts from the sample was detected in this assay. Only one of the extracts was available for validation purposes.)

See section 5 of Data index for detail.

## 16 Specificity

Sample extracts or samples that had not previously tested positive for the target virus were tested or re-extracted and tested again using this assay. These included:

- 30 of nasopharyngeal swabs
- 7 of nasopharyngeal aspirates
- 10 of nasal swabs
- 2 of throat swabs
- 51 swabs, site not stated
- 2 sputum
- 1 aspirate
- 1 bronchial washing

*Specificity is the ability of the assay to detect true negatives in samples of the same type as those listed in section 6.2. Some samples should contain organisms with similar taxonomy to, found in the same sample type as, or producing a clinical disease similar to that caused by, the organism this assay targets. Specify which organisms and/or disease states have been selected with rationale. The following formula is used to determine the specificity. Values are rounded to 3 significant digits*.

Specificity = [True Negative / (True Negative + False Positive)] X 100%

*The ability of the assay to detect only the target was determined to be: 100%*

From *104* extracts tested, *0* produced a signal that suggested nonspecific amplification.

- These included extracts previously detected for
  - Alphacoronavirus 229E
  - Influenza A (H1)
  - Influenza A (H3)
  - Influenza B

See section 5 of Data index for detail.

## 17 Measurement uncertainty (MU)

The extended measurement uncertainty (U) is a parameter that characterises the dispersion of values reasonably attributed to the measurand (STO). Values are rounded to 4 significant digits and presented as the expected range around the mean value for a fixed STO concentration.

**UBE-WuhanE-synPri MU**

$Mean_{synPri}$ of reproducibly and repeatability: *23.83* $C_T$

UBE-WuhanE-synPri concentration is described in the Cover Page.

$MU_{synPri}$ was determined to be: *0.03179*

$= [(CV1)^2 + (CV3)^2]^{0.5}$

$= [0.03163^2 + 0.003154^2]^{0.5}$

$U_{synPri}$ was determined to be: *0.06548*
= *2.06* x $MU_{synPri}$

Expected assay $C_T$ range for synPri: *23.76 – 23.90 $C_T$*

**UBE-WuhanE-synPri MU**

$Mean_{synPrb}$ of reproducibly and repeatability: *22.65 $C_T$*

synPrb concentration is described in the Cover Page.

$MU_{synPrb}$ was determined to be: *0.02743*
= $[(CV2)^2 + (CV4)^2]^{0.5}$
= $[0.02701^2 + 0.004761^2]^{0.5}$
$U_{synPrb}$ was determined to be: *0.05650*
= *2.06* x $MU_{synPrb}$

Expected assay $C_T$ range for synPrb: *22.59 – 22.71 $C_T$*

Where CV1 = coefficient of variation (CV) of synPri reproducibility rounded to 4 significant digits; CV2 = CV of synPrb reproducibility rounded to 4 significant digits; CV3 = CV of synPri repeatability rounded to 4 significant digits; CV4 = CV of synPrb repeatability rounded to 4 significant digits;

*See the MU calculations sheet for data and detail.*

## 18 References

1. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3):2000045
2. Northill JA, Mackay IM. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR E gene 2020. Protocols.io dx.doi.org/10.17504/protocols.io.bcv9iw96

## 19 Authorisation

**Authorised by Supervising Scientist:**

**Name**

**Signature**

**Date**

**Authorised by Scientific Manager:**

**Name**

**Signature**

**Date**

**Authorised by Clinical Microbiologist:**

**Name**

**Signature**

**Date**

***Full test name:*** *Sarbecovirus "Wuhan" E gene TaqMan 2020*
***Laboratory test name:*** *WuhanE-TM2020*

| **Section 1:** Previous validation/summary documents |
|---|
| NIL |
| **Published reference:**<br>Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3):2000045<br>Northill JA, Mackay IM. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR E gene 2020. Protocols.io dx.doi.org/10.17504/protocols.io.bcv9iw96 |
| **Previous assays: NIL** |

| **Section 2:** Oligonucleotide structure and specificity |
|---|
| 9/3/2020: Oligo structure and specificity |

| **Section 3:** Preparation of synthetic template oligonucleotide (STO) controls |
|---|
| 3/2/2020: Synthetic control production |
| 4/2/2020: Crude titration of the synthetic RNA controls |
| 4/2/2020: Batch of working controls |
| 24/4/2020: Control summary |

**Section 4:** Oligonucleotide data sheets

| Name | Date | Lot number | Manufacturer |
|---|---|---|---|
| UbcH58-CALFLUOR ORG 560 | 22/3/2019 | WD7307408 | Sigma |
| UBE2D2_01.2 | 18/11/2015 | 1161111 | Geneworks |
| UBE2D2_02.2 | 21/2/2018 | SD540971 | Sigma |
| E_Sarbeco_P1 | 3/2/2020 | 102921593 | IDT |
| E_Sarbeco_F1 | 3/2/2020 | 102921594 | IDT |
| E_Sarbeco_F1 | 1/4/2020 | 102981021 | IDT |
| E_Sarbeco_R2 | 3/2/2020 | 102921595 | IDT |
| E_Sarbeco_R2 | 1/4/2020 | 102981023 | IDT |
| UBET7WuhanEsynPrim | 3/2/2020 | SD753329 | Sigma |
| UBET7WuhanEsynPrb | 3/2/2020 | SD753328 | Sigma |
| | | | |
| | | | |

| **Section 5:** Optimisation and validation documents |
|---|
| 3/2/2020: Initial check of the synthetic RNA controls |
| 4/2/2020: Primer chequerboard with synthetic RNA |
| 4/2/2020: Probe chequerboard with synthetic RNA |
| 5/2/2020: Clinical extract titration |
| 5/2/2020: Primer chequerboard with sample RNA |
| 6/2/2020: Repeatability |
| 6/2/2020: Sensitivity run 1 using SSIII kit |
| 12/2/2020: Check of TCS extract supplied for validation purposes |
| 18/2/2020: Summary of TCS tested by TaqMan |
| 27/2/2020: Kit comparison using TCS extract dilutions |
| 27/2/2020: Kit comparison using synthetic RNA dilutions |
| 28/2/2020: Kit comparison using clinical extracts |
| 3/4/2020: Sensitivity using clinical extracts and Bioline kit |
| 7/4/2020: Sensitivity using clinical extracts and SSIII kit |
| 8/4/2020: Sensitivity using clinical extracts and both SSIII & Bioline kits |
| 29/4/2020: Specificity using Influenza clinical extracts |
| 29/4/2020: Specificity using clinical respiratory sample extracts and alphacoronavirus 229E |

| **Section 6:** Links to raw Rotor-Gene run files |
|---|
| **Date/short description** |
| 20200203 WuhanE-TM2020 RNA check |
| 20200204 WuhanE-TM2020 crude syn RNA titration |
| 20200204 WuhanE-TM2020 primer chequerboard |
| 20200204 WuhanE-TM2020 probe titration |
| 20200205 WuhanE-TM2020 sample RNA titration |
| 20200205 WuhanE-TM2020 primer chequerboard2 |
| 20200206 WuhanE-TM2020 repeatability |
| 20200206 WuhanORF1ab and E sensitivity |
| 2020-02-10-AP TCS check |
| 2020-02-12-AP TCS check |
| 20200212 WuhanE-TM2020 RNA check of TCS supplied for validation |
| 20200227 WuhanE SSIII run 4 kit comparison with synthetic RNA |
| 20200227 WuhanE Bioline run 3 kit comparison with synthetic RNA |
| 20200227 Qiagen mix run 2 kit comparison with synthetic RNA |

| |
|---|
| 20200227 Qiagen mix run 1 kit comparison with TCS RNA |
| 20200227 WuhanE SSIII & Bioline run 1 kit comparison with TCS RNA |
| 20200228 Qiagen mix run 4 kit comparison with clinical RNA |
| 20200228 Wuhan E kit comparison with clinical RNA |
| 20200403 WuhanE & ORF1ab sensitivity |
| 20200407 WuhanE-TM2020 sensitivity |
| 20200408 WuhanE sensitivity |
| 20200429 WuhanE-TM2020 specificity1 |
| 20200429 WuhanE specificity2 |

| **Section 7:** Mastermix documents | |
|---|---|
| **Date** | **Filename** |
| 19/9/2019 | UBE probe control base mix |
| 16/1/2020 | UBE probe control base mix |
| 16/1/2020 | UBE primer control base mix |
| 5/2/2020 | UBE primer control base mix |
| 16/3/2020 | UBE probe control base mix |
| 16/3/2020 | UBE primer control base mix |
| 30/7/2020 | SSIII TaqMan base mix |
| 29/1/2020 | SSIII TaqMan base mix |
| 13/3/2020 | SensiFast RNA TaqMan base mix (Bioline) |
| 16/3/2020 | SensiFast RNA TaqMan base mix (Bioline) |
| 3/2/2020 | WuhanE-TM2020 mix (RNA & DNA mix) |
| 6/2/2020 | WuhanE-TM2020 mix SSIII mix |
| 21/2/2020 | WuhanE-TM2020 kit comparison mix |
| 28/2/2020 | WuhanE-TM2020 Bioline mix |
| 1/4/2020 | Oligo mix |
| 3/4/2020 | WuhanE-TM2020 Bioline mix |
| 7/4/2020 | WuhanE-TM2020 SSIII mix |

| **Section 8:** Reagents used during validation | | |
|---|---|---|
| **Manufacturer** | **Item** | **Part number** |
| Life Technologies | SuperScript™ III Platinum® One-Step Quantitative RT-PCR | 11732088 |
| Bioline | SensiFast™ Probe Lo-ROX kit | BIO-84005 |
| G-Biosciences | Molecular grade water, 1l | 16574 |
| Bioline | SensiFast™ Probe Lo-ROX One-Step Kit | BIO-78005 |

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**APPENDIX 2**

## FULL TEST NAME / LABORATORY TEST NAME

Sarbecovirus "Wuhan" E gene TaqMan 2020 / WuhanE-TM2020

## LEVEL OF VALIDATION ACHIEVED

1

## MIX COMPONENTS (per reaction)

| *Reagent* | *Vol (µL) / reaction* | *Final concentration* |
|---|---|---|
| Nuclease-free water | 4.39 | |
| E_Sarbeco_F1 200pmol/µl | 0.04 | 400nM |
| E_Sarbeco_R2 200pmol/µl | 0.09 | 900nM |
| E_Sarbeco_P1 100pmol/µl | 0.04 | 200nM |
| 2X Reaction Mix [1] | 10.0 | 1X |
| SuperScript® III/Platinum® *Taq* Mix [1] | 0.4 | |
| Rox Reference Dye 25mM | 0.04 | 50nM |
| Template | 5.0 | |
| Final volume | 20µl | |

[1] SuperScript® III Platinum® One-Step qRT-PCR Kit, Cat No. 11732088

## CYCLING CONDITIONS

This assay has been optimised and validated for use with a Rotor-Gene 6000 or Rotor-Gene Q thermal cycler

| *RT-PCR / PCR* | | |
|---|---|---|
| 50°C | 5min | |
| 95°C | 2min | |
| 95°C | 3s | 50X |
| 60°C | 30s* | |

*Fluorescence acquisition step

## OLIGONUCLEOTIDES

E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1: 6FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1

## CONTROLS

RNA is produced from a pair of synthetic oligonucleotide primer and probe positive controls based on UBE2D2 and target genetic sequences.

- UBET7-WuhanE synthetic probe control $10^{-6}$
- UBET7-WuhanE synthetic primer control $10^{-6}$

## NOTES

- This is a summary cover page only. Full details of this PEHV method are available upon request.
- This assay has been optimised using synthetic positive control templates.
- Precision, sensitivity and specificity should be determined if used by another laboratory sites.

## REFERENCES

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3):2000045

Northill JA, Mackay IM. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR E gene 2020. Protocols.io dx.doi.org/10.17504/protocols.io.bcv9iw96