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Rapid Capture® System User Manual

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Sample to Insight

QIAGEN

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APPLICATION DESCRIPTION

The Rapid Capture® System is a general use automated pipetting and dilution system that can be used with the digene® Hybrid Capture® 2 (HC2) DNA Tests* for high-volume sample-throughput testing. This system handles up to 352 specimens in an eight-hour shift, including a 3.5-hour period during which user intervention is not required. Up to 704 specimen results can be generated sequentially in 13 hours. User intervention is limited to specimen preparation, loading of specimen racks onto the deck, deck setup, chemiluminescent signal detection, and result reporting. To accomplish this degree of semi-automation with the assays, the following 6 procedural steps of the manual method are performed by the Rapid Capture System on the instrument deck:

1. Specimen Pipetting
2. Reagent Dispensing
3. Microplate Handling
4. Microplate Mixing
5. Microplate Incubation
6. Microplate Washing

Denaturation of the specimens in preparation for testing with the digene HC2 DNA Tests is performed independently of the Rapid Capture System. In addition, amplified chemiluminescent signal detection and result reporting are performed using a QIAGEN-approved off-line luminometer system utilizing the digene HC2 System Software. Microplate mixing, incubation, and washing are performed by the same type of equipment used as separate bench-top accessories for the manual method of the tests. However, this equipment is integrated on the Rapid Capture System Instrument deck. Each of the digene HC2 procedural steps is performed in the same sequence as the manual test procedure. The Rapid Capture System deck allows for the staggered processing of up to 4 microplates, each plate containing specimens and the required assay Controls and Calibrators. The operator prepares the specimens according to the instructions stated in the current version digene HC2 DNA Test instructions for use (IFU). After loading the racks onto the Rapid Capture System deck, the operator returns at a set time to retrieve the microplate and perform the detection step. The amplified signal generated is detected in a separate chemiluminescent QIAGEN-approved luminometer, and the results are calculated and reported using the digene HC2 System Software. Instructions for the luminometer are available in the corresponding QIAGEN-approved luminometer user manual. Because the required accessories for the digene HC2 DNA Tests and procedural steps remain unchanged, the assay can also be performed manually as instructed in the aforementioned product labeling.

*NOTE: Not all digene HC2 DNA Tests have been approved for use on the Rapid Capture System. Check the IFU for the digene HC2 DNA Test of interest to determine if the desired assay and/or sample type is approved.

INSTRUMENT DESCRIPTION

The Rapid Capture System is a robotic microplate processor comprised of microprocessor-controlled components. The system is controlled using operating software resident on the hard drive of a required PC interfaced with the Rapid Capture System. (Note: Separate software applications reside on this single PC, which controls both the Rapid Capture System and the QIAGEN-approved luminometer.)

Rapid Capture System Specifications (Refer to the Functional System Description section for more details.)

- Dimensions: (W x D x H) 116 x 73 x 66 cm (55 x 31 x 35 inches).
- All Rapid Capture Systems have self-regulating power and operate at 100-240 volts AC with an online frequency of 47 - 63 Hertz; fluctuations not to exceed 10% of nominal voltage.
- Power measurements for the Rapid Capture System, PC, and QIAGEN-approved luminometer show a maximum total power consumption of 355 watts/4.1 A @ 120VAC or less.
- Installation category II, Pollution Degree 2.
- Environmental: 15-30°C; Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 50 percent relative humidity at 40°C. For indoor-use only up to 2000 meters altitude.

Note: These environmental specifications are for the Rapid Capture System; digene HC2 DNA Test conditions may be more restrictive. Please refer to the High-Risk HPV Application Procedure and the CT/GC, CT-ID, and GC-ID Application Procedures of this user manual for additional environmental considerations.

MATERIALS REQUIRED

Rapid Capture System Instrument Includes:

- Rapid Capture System (Robotic Microplate Processor)
- Bottle**s:**
  - System Liquid
  - Wash
  - Waste
- Power cord

Rapid Capture System Required Equipment1

- PC System [includes: CPU, Windowsâ 7, RCS System Software, RCS ScriptSelect Software
- Country Kit [includes: keyboard, mouse]
- Monitor
- Printer[3]
- Printer Cable
- Multi-Specimen Tube (MST) Vortexer 2
- RS232 cables
- QIAGEN-approved luminometer

Reagents[2]

See the appropriate digene HC2 DNA Test Rapid Capture System Application Procedure of this Manual.

Accessories[1]

- digene Specimen Rack (blue) and Lid (For single- and dual-probe testing of STM Specimens)
- Conversion Rack (silver) and Lid (For single and dual probe testing of liquid cytology samples)*
- DuraSeal™ Tube Sealer Dispenser and Cutting Device
- DuraSeal Tube Sealer Film
- Rapid Capture System Reagent Troughs
- Rapid Capture System Reagent Trough Lids
- Rapid Capture System Disposable Tips
- Rapid Capture System Drop-on Caps
- Hybridization Microplate
- Microplate Lids
- Specimen Collection Tube Rack
- Screw Caps
- Rapid Capture System Microplate Well Strips
- Extra-Long Pipette Tips (200 ml) for specimen transfer
- Empty Specimen Collection Tubes

***See appropriate *digene* HC2 DNA Test IFU for sample types approved for use with the Rapid Capture System.**

Equipment and Accessories Required but Not Supplied

- Disposable bench cover
- Powder-free disposable gloves
- Sodium hypochlorite solution, final concentration of 0.5% v/v
- Disposable aerosol-barrier pipette tips for single-channel pipettor (20-200 ml and 200-1,000 ml)
- 15-ml polypropylene conical tubes and caps
- Disposable tips for Eppendorfâ Repeaterâ Pipette (12.5 ml)
- 5-ml and/or 15-ml snap-cap round-bottom polypropylene tubes
- 50-ml polypropylene conical tubes
- Kimtowelsâ Wipers or equivalent low-lint paper towels
- Alcohol wipes
- Labels (water and heat resistant).
- 65 ± 2°C water bath(s) of sufficient size to hold up to 4 specimen tube racks [(33 cm x 18.7 cm or 15 x 8.5 x 6 or 9 inches)]
- Single-channel micropipettor; variable settings for 20-200 µl and 200-1,000 µl volumes
- Repeating positive displacement pipettor such as Eppendorf or equivalent
- Timer
- Vortex mixer with cup attachment

- Uninterruptible Power Supply (UPS), with a capacity of ≥ 1000 VA, Surge suppression, EMI/REI filtering.3

[1]Only the equipment and accessories provided above have been validated for use with the Rapid Capture System and are available from QIAGEN.

[2]The performance characteristics of this system were established only with the reagent test kits and the two specimen collection kits indicated and available from QIAGEN. Use of alternate reagent test kits or specimen collection devices require validation by the user.

[3]Do not plug the printer directly into the UPS.

WARNINGS AND PRECAUTIONS

Instrument Safety

Read this section before operating the Rapid Capture System. Operators of this instrument must be trained in both general laboratory safety practices and the specific safety requirements specific to the Rapid Capture System. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Symbols and Conventions

The following chart is an illustrated glossary of symbols that are used on the Rapid Capture System. Whenever such symbols appear on instruments, please observe appropriate safety procedures.

CAUTION 	This symbol alerts you to consult the manual for further information and to proceed with caution.
CAUTION 	This symbol illustrates a hot surface hazard. Proceed with caution when working around these areas to avoid being burned by hot components.
CAUTION 	This symbol indicates the presence of high voltage and warns the user to proceed with caution.

Precautions for Use

Place the instrument on a sturdy workbench large enough to accommodate the Rapid Capture System (weight 150 lb, 68 kg), System Liquid Bottle, Wash Bottle, and PC. The equipment should not be located near a heat source or exposed to direct sunlight. Make certain that the space for placing the waste container is within 1.5 meters (or 5 feet) of the instrument. The equipment must be close to an AC power outlet. Ensure the equipment power lines are voltage regulated and surge protected.

Bottles for System Liquid, Wash Liquid, and Waste are provided. Place the Wash and System Liquid bottles on or near the workbench on the same level and close to the right-hand side of the instrument. Fill the System Liquid Bottle with deionized or distilled water. Place the Waste Bottle on a visible, secure spot on the floor, behind the instrument, to prevent spilling.

The Hybridization Incubator reaches a set temperature of 65ºC.

Chemical and Biological Hazards

See the appropriate digene HC2 DNA Test IFU for additional Warnings and Precautions related to reagents and specimens. Handle specimen collection tube caps as potentially infectious. Use Universal Precautions when handling specimens as no known test method can offer complete assurance that specimens will not transmit infections.

Use only *digene* HC2 DNA Test components (specific to detection of a particular analyte) from the same kit lot number when running more than one plate. Do not mix different component lot numbers. Refer to the appropriate *digene* HC2 DNA Kit IFU Limitations of the Procedure section for limitations and reagent lot usage restrictions.

Electrical Hazards

The Rapid Capture System does not pose uncommon electrical shock hazards to operators if it is installed and operated without alteration and is connected to a power source of required specifications. Refer to the Instrument Description section for power requirement details.

Note: Do not connect the printer provided with the Rapid Capture System directly into the UPS.

Users must connect the Rapid Capture System to the UPS. This will allow a run to continue for at least 30 minutes in the event of a power interruption and will prevent potential damage to the instrument due to loss of power during a run.

Basic electrical hazard awareness is essential to the safe operation of any system. Elements of electrical safety include, but are not limited to, the following:

- Periodically inspect electrical cables in and on the Rapid Capture System for signs of wear and damage.
- Do not disconnect any electrical connection while the power is ON.
- In the event of a blown fuse, call your local QIAGEN representative for service. Only qualified personnel should perform electrical servicing.
- Keep liquids away from all connectors of electrical components.
- Keep the floor dry and clean under and around the Rapid Capture System.

- Use only approved power cords and electrical accessories, such as those supplied with the instrument, to protect against electric shock. Connect power cords only to properly grounded outlets.
- Do not touch any switch or outlet with wet hands.
- Switch the instrument OFF before disconnecting the AC power cord.
- Unplug the instrument before cleaning any major liquid spills.
- Replace all access covers before operating the instrument.

CAUTION 	To protect operating personnel, the National Electrical Manufacturers' Association (NEMA) recommends that the instrument be correctly grounded. The instrument is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground connection.

CAUTION 	Disconnect the AC power cord before removing or installing a fuse to avoid the possibility of serious injury from electrical shock! Only qualified personnel should perform electrical servicing. Replace all access covers before operating the instrument.

The AC line fuse (slow blow) compartment is located below the main power switch on the rear of the instrument. Information on replacement fuses for the Main Power Supply is specified on the label situated below the main connector. Only qualified and authorized personnel should perform replacement of fuses on internal modules. Call your local QIAGEN representative for service.

See the appropriate user manual for Warnings and Precautions related to operating the QIAGEN-approved luminometer, MST Vortexer 2, or other equipment.

Mechanical Hazards

The robotic arm can exert enough force to be a pinch hazard. DO NOT reach onto the Rapid Capture System deck while the instrument is running unless the system has paused and displayed a dialog box indicating a user-intervention is required. Reaching onto the deck at any other time during a run may result in injury to the user and/or an aborted run.

DO NOT remove the safety shield from the instrument.

The computer keyboard must be placed within reach of the Rapid Capture System to ensure access to the Escape key. The Escape key is considered an emergency stop mechanism.

Do not wear clothing or accessories that could catch on the Rapid Capture System.

In the event of a mechanical jam or other instrument problems, immediately contact your local QIAGEN representative for proper instructions.

The Rapid Capture System weighs over 150 lb (68 kg). Do not attempt to lift or move the Rapid Capture System. Contact your local QIAGEN representative.

System Setup Precautions

Locate the Rapid Capture System such that the user is able to hear the audible alarm, allowing for immediate attention in the event of an error or malfunction.

It is critical that the Rapid Capture System deck is set up and maintained in the exact manner described in this user manual. It is also critical that no extraneous items are placed on the Rapid Capture System deck during operation.

- Strict adherence to reagent usage and limitations is critical for reproducible and consistent assay results. Failure to follow the reagent usage guidelines may result in invalid assays and inaccurate specimen results.

- Ensure that the System Liquid bottle and Wash bottle are adequately filled before the start of each run (see the Reagent Preparation and Storage section in the appropriate digene HC2 DNA Test IFU).

- Empty the Liquid Waste bottle at the end of each run (see Daily/System Cleanup section of the appropriate assay application procedure of this user manual), as there is no liquid level detection on the Waste bottle. If the container is allowed to fill to the level of the tubing that extends inside the bottle, the waste solution will back up and may flood the Tip Rinse station or plate wash station on the deck. This would result in contamination of the instrument with alkaline phosphatase. This may lead to an invalid run.

- Ensure that the tubing extending from the instrument to the Liquid Waste bottle **does not have any kinks and that there are no loops in the tubing path** that would prevent the waste solution from flowing downward. Ensure that the tubing from the System Liquid bottle and the Wash bottle is free of kinks and properly connected. Note especially the points where the tubing attaches to the bottles and the instrument inlet ports.

- Empty the container used to collect disposable tips as often as necessary to ensure that tips fall completely clear of the tip eject station (see Daily/System Cleanup section of the appropriate assay application procedure of this user manual).

- If a plate with fewer than 88 specimens is included in the run, all wells of the capture plate that have been removed for later use must be replaced with Rapid Capture System Microplate Well Strips.

- **It is critical that the correct number of specimens be entered into the Rapid Capture System software. Failure to do so may result in invalid assays, inaccurate specimen results, and instrument failure.**

FUNCTIONAL SYSTEM DESCRIPTION

The Rapid Capture System is a general use automated pipetting and dilution system that can be used with the digene HC2 DNA Tests for high-volume sample-throughput testing. The Rapid Capture System is a computer controlled robotic sample processor. All operations are directed by a host PC that addresses nine embedded microprocessors in the instrument through an RS-232 link. The system is powered by a line voltage sensing switching type power supply and all power is distributed through the system at 240 volts AC or less.

The software-controlled functions and equipment mechanisms include:

- Specimen transfer to microplate
- Reagent addition
- Microplate washing
- Incubation
- Agitation
- A robotic handler transports the microplates between functional stations and moves plate lids and reagent trough covers.
- Motion control of the four pipetting tips and plate transport is accomplished with eight DC servomotors utilizing optical shaft encoders for position and velocity control.
- Fluid handling is accomplished with 4 stepper motor syringe drives, 2 DC diaphragm pumps, and a DC peristaltic pump.
- An orbital 4-plate shaker is stepper motor driven, as are the X-carriage and Z-manifold axis of the plate washer.
- The incubator is firmware controlled and regulates each of 5 chambers to 65°C. Each incubator chamber contains a DC motorized drawer that extends to enable loading/unloading of the microplates.
- Optionally, automatic scanning of plate barcodes and export to the digene HC2 System Software (only available with the RCS barcode upgrade)

HARDWARE COMPONENTS

Base Unit

The base unit of the Rapid Capture System is comprised of:

A) The instrument chassis sub-assembly (the base chassis, deck supports, mechanical deck, side and top panels, safety shield, and tubing telescope), and
B) The electrical sub-assembly (the power supply, printed circuit boards (PCBs), shielding, connectors, and fuses).

X/Y/Z/V Movement Mechanism (Arm)

All X/Y/Z/V (V=VariSpan) movements of the Rapid Capture arm are driven by DC motors with encoders. Each tip can move independently of the others in the Z-direction (up and down). The tips are mounted on the Y-slide, which moves front to rear (Y-

direction) inside the arm of the Rapid Capture System. The arm is mounted on the X-slide located inside the instrument casing and moves left and right (X-direction).

The Rapid Capture System is equipped with VariSpan – the variable spacing of the tips. This is achieved by the VariSpan motor, which is also used to vary the range of the plate gripper.

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

Sample Processor

The Rapid Capture Robotic Microplate Processor features four sampling tips carried by the robotic arm. Each tip is connected to the four-port valve of a precision syringe pump module and can aspirate, dispense, and dilute at most positions on the instrument's work surface.

The Rapid Capture System software controls pipetting sequence, volumes, and pipetting modes.

Syringe Pump and Peristaltic Pump Modules

The syringe pump is a microprocessor-controlled syringe with a four-port valve that connects to the syringe, peristaltic pump, sampling tips, and systems liquid reservoir. Liquid is fed into the syringe from the external reservoir and the tips are flushed via the peristaltic pump. All parts that come into contact with liquid are made of inert materials such as stainless steel, TEFLON®, FEP, Santoprene®, etc.

Each pipette tip of the Rapid Capture System has a dedicated syringe pump, controlling the aspiration and dispensing functions of the sampling tip.

The four-channel peristaltic pump is used to supply system liquid used to flush the tubes at an average flow rate of 2 ml/sec./channel.

Tip Adapters

The Rapid Capture System has four tip adapters. Each tip features independent movement in the Z-direction, while the span movement of tips (Y-direction) is variable. This feature is known as VariSpan.

1. The Rapid Capture System uses 300 µl conductive disposable tips.
2. An automatic procedure checks for the presence of disposable tips. If disposable tips are not detected after four attempts, the system will pause and an audible alert will notify the operator.

Liquid Detector

Each tip in the Rapid Capture System is equipped with a liquid sensor, which enables it to detect ionic solutions upon contact. Liquid detectors monitor changes in capacitance between the disposable pipette tip and the Rapid Capture System deck. When the disposable pipette tip touches the liquid surface, this sudden change in capacitance immediately generates a detection signal. QIAGEN cannot guarantee proper functioning of the liquid level detector if the racks used to contain samples and reagents are not supplied by QIAGEN.

The liquid level detector is used to detect an insufficient amount or total absence of Controls, Calibrators, and reagent liquids*. If this is the case, the system will immediately stop and display a dialog box, allowing the user an opportunity to replenish any liquids.

***Liquid level detection is not activated during specimen transfer.**

NOTE: Because the Level Detector cannot identify what material causes a change in capacitance, it is imperative that the tips do not touch any surface (e.g., foam on top of the meniscus) except the liquid to be detected.

Tip Rinse Station and Drain

The system lines and tip adapters are flushed, through the tip adapters, at the Tip Rinse Station.

When the tip assemblies are positioned in the station, deionized or distilled water from the System Liquid reservoir is aspirated by the Peristaltic Pump and forced through each sampling tip. The flow is dispensed into the Tip Rinse station moat and down the drain. Any air bubbles in the lines or adapters are purged. Tubing takes the waste fluid from the drain to a waste reservoir.

Robotic Plate Handler with Integrated Plate Gripper

The manipulative Plate Gripper, which is an integrated part of the robotic plate handler, is used to transport microplates, capture plates, and microplate lids between positions and modules such as plate stackers, the incubator tower, pipetting positions, the shaker, and the plate washer.

The VariSpan motor is used to vary the spread of the two-gripper tools, and it has an independent Z-motor and drive.

Plates are loaded manually onto the Rapid Capture deck (into detachable stackers and plate shaker positions) and are delivered by the grippers to automatically defined positions when the run is started.

Plate Stackers and Incubators

The fixed Ambient Temperature Plate Stacker houses microplates and microplate lids at a few degrees above room temperature during room temperature incubations.

The five-drawer automatic Hybridization Incubator tower is temperature controllable from ~5°C above ambient air temperature to 65°C in graduations of 0.1°C.

Each Hybridization Incubator consists of five drawers that contain closed shelf units protected from ambient temperature and light by motor-driven, spring-loaded doors. The door is opened and closed by the action of the motor/drawer; the robotic plate handler with grippers delivers and retrieves the plate from the individual drawer.

Pipetting Position and Precision

For pipetting steps, the Robotic Plate Handler with Plate Grippers carries the plate to a pipetting position. This is a permanent plate mounted on the deck surface. The pipetting station is designed for up to two regular dimension microplates and/or microplate lids. Each position is defined in the machine configuration, and the Plate Gripper will always deposit the correct plate in the appropriate position, provided the plates were placed in the proper locations during setup of the Rapid Capture System deck. (Refer to the appropriate digene HC2 DNA Test Application for instructions on proper deck setup.)

All specimen transfer operations and reagent additions are performed using 500 ml syringes operated by pumps. The following specification is based on pipetting normal saline solution (0.9% NaCl with deionized or distilled water): at 10% of full stroke and up to the maximum pipetting volume of the syringe, the % CV is equal or less than 1%. When pipetting low volumes of a viscous solution (i.e. 25 ml of probe mixture), a maximum CV of 5% is expected.

Shaker

The plate shaker is used for mixing after reagent and Probe additions and for shaking during incubation. The shaker accommodates up to four plates. The shaker positions have specially designed clamps that secure the combination of microplate and microplate lid. The orbit has a 1.5-mm diameter and a speed of1100 ± 50 rpm.

Plate Washer

The Rapid Capture System has a modular microplate washer with an eight-channel wash head for flexibility and speed. The washer utilizes aspiration and dispensing pumps, a solenoid valve manifold, and a restriction valve to control liquid pressure.

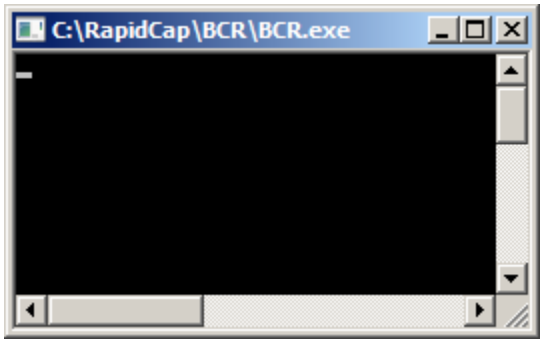
The washer can function independently of other Rapid Capture System functions due to multitasking capabilities of the system. The Wash Bottle supplies the washer.

For the digene HC2 DNA Test Application, the washer dispenses 1.5 ml ± 10% into each well while aspirating from the top of the wells. The flow rate is determined by the dispense pressure of 10 psi and is approximated to be 500 µl/sec. The wells are then aspirated to a maximum mean residual volume of 7 µl/well. The fill/aspirate cycle is repeated six times.

Barcode scanner

If the RCS is equipped with the barcode upgrade, the barcode scanner will scan the hybridization and capture plate barcodes during the run. The plate barcodes will then be available for association in the DML software (see the applicable Suite software user manual for details). The barcode scanner must be connected to the RCS PC.

The barcode upgrade includes an application that saves the scanned barcodes for use by the digene HC2 System Software. While the barcode scanning application is running, a command window will be displayed in the upper left hand corner of the screen. Do not close the command window. The window will close automatically after the barcode is saved. If the command window is closed by the user, then the scanned barcode will not be saved.



The barcode upgrade includes functionality to ensure that the scanned capture plate corresponds to the correct capture plate. However, it is important that users do not switch the sequence of plates on the RCS (for example, during error recovery) to ensure that the association of the capture plate and hybridization plate are correct. Incorrect plate association could lead to incorrect results.

ADDITIONAL EQUIPMENT

Multi-Specimen Tube Vortexer 2 and Racks

The Multi-Specimen Tube Vortexer 2, inclusive of specimens, rack and lid accessory components, are required for sample preparation, processing and denaturation. Two different specimen rack designs are available.

Rack Name	Rack Color	Intended Use
digene Specimen Rack	Blue	Single- and dual-probe testing of STM specimens.
Conversion Rack	Silver	Single- and dual-probe testing of liquid cytology sample.* (This rack accommodates 15-ml conical tubes.)

DML Instrument and digene HC2 System Software

The system is designed for measuring and analyzing light produced by chemiluminescence from digene HC2 DNA Tests.

*See appropriate digene HC2 DNA Test IFU for sample types approved for use with the Rapid Capture System.

Rapid Capture System Software

The Rapid Capture System includes the RCS Software, including the barcode scanning application, and ScriptSelect Software.

Rapid Capture Software controls the Rapid Capture System. Rapid Capture System Software is a flexible, simple-to-use system control package that allows the user to automate microplate-based assay protocols. Rapid Capture System Software is installed on the computer hard disk. Rapid Capture System Software utilizes the Windows® 7 Operating System, which makes the software easy to learn and simple for day-to-day use. The software uses Microsoft Access®, which allows for flexibility, networking, host file transfer, and multitasking.

Rapid Capture System Software Icons and Descriptions

Software	Icon	Software Description	Function
Rapid Capture System Software and Menu Icons		Rapid Capture System Operations Software	Controls the Instrument
		**Run**	Displays the Script List Window
		**Flush System**	Flushes the system.
		**Park**	Moves the robotic arm of the instrument to the park position.

Rapid Capture System Associated Software Icons and Descriptions

The software icons listed below correspond to the following menu items:

Software	Icon	Software Description and Function
Rapid Capture System ScriptSelect Software		**Rapid Capture System ScriptSelect Software** simplifies the user-interface to facilitate selection of the appropriate script for a Rapid Capture System run. See the Rapid Capture System ScriptSelect Software Application Procedure section in this user manual.

Installation of Rapid Capture System Software

The Rapid Capture System Software is preinstalled on the Rapid Capture System computer.

Virus scanners

QIAGEN acknowledges the threat of computer viruses when computers exchange data. The digene HC2 System, including the RCS, is intended for environments where local policies exist to minimize the virus threat and where the system is NOT networked to the internet. Local policies usually require the use of a particular anti-virus tool. While the RCS Software has been tested on computers protected by McAfee Endpoint Protection Essential - For SMB and Windows Defender, QIAGEN has not validated the RCS software for use with any virus scanning software. The selection of an appropriate virus scanning tool is the customer's responsibility.

The system administrator should ensure the following:

- QIAGEN directories are excluded from virus scanning. For the Suite software, these directories are:
  - C:\RapidCap
  - C:\Program Files\Selector
- File access is not intercepted by a virus scanner when the RCS system is in use
- Updates to the virus database are not performed when the RCS system is in use
- File scans are not performed when the RCS system is in use

QIAGEN strongly recommends disabling virus scanner activity during laboratory working hours to prevent interference with the operation of the digene HC2 System, including the RCS. The virus scanner tasks described above can only be safely carried out when the digene HC2 System, including the RCS, is not running; otherwise, there is a risk of adverse impact on the performance of the system.

Powering up the Rapid Capture System

1. Turn on the power to the PC System.
2. Click the icon for the appropriate Windows user account. The RCS computer is set up with two administrative user accounts and one standard user account. QIAGEN recommends users operate the RCS software under the Standard user account. Note that users cannot change Windows users while the RCS is running. Use the following case-sensitive credentials for the Windows operating system:

- Administrative user account:
  - User ID: Administrator
    - Password: digene
    - The system will prompt you to change the password the first time you log in to the Administrator account
- Standard user account:
  - User ID: Welcome
  - Password: welcome
- The Technician user account is intended for use by QIAGEN service personnel.

3. In the welcome screen, type the appropriate password in the password field (password is case sensitive). Press the "Enter" key on the RCS PC keyboard.

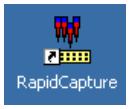
4. After entering the password, the Rapid Capture System Desktop with icons will appear.

5. Check that the pipette adapters and gripper arms are located in the area of the Pipetting Positions or the Specimen Tube Rack (Figure 1). If not, manually lift the adapters and grippers, and move the arm to the appropriate location. Lower the adapters and grippers to their natural stop point. Ensure that there are no miscellaneous items on the deck.

6. Turn the Rapid Capture System's main power switch to the ON position. The power toggle switch is located on the lower right corner of the back panel.

   6a. Position the computer keyboard so that it is adjacent to the Rapid Capture System. In the event that the instrument must be stopped immediately, press the Escape key as an emergency stop mechanism. Refer to Warnings and Precautions section for additional safety instructions.

   6b. Launch the Rapid Capture System software by double clicking the **Rapid Capture System** desktop icon.



   Alternatively, click **Start**, **Programs**, then **Rapid Capture.**

7. Click the **Park** icon from the RCS Tool Menu bar. 

   The pipette adapters and gripper arm will move slowly to the home position, and the system will initialize all components and signal the incubator to reach 65°C.

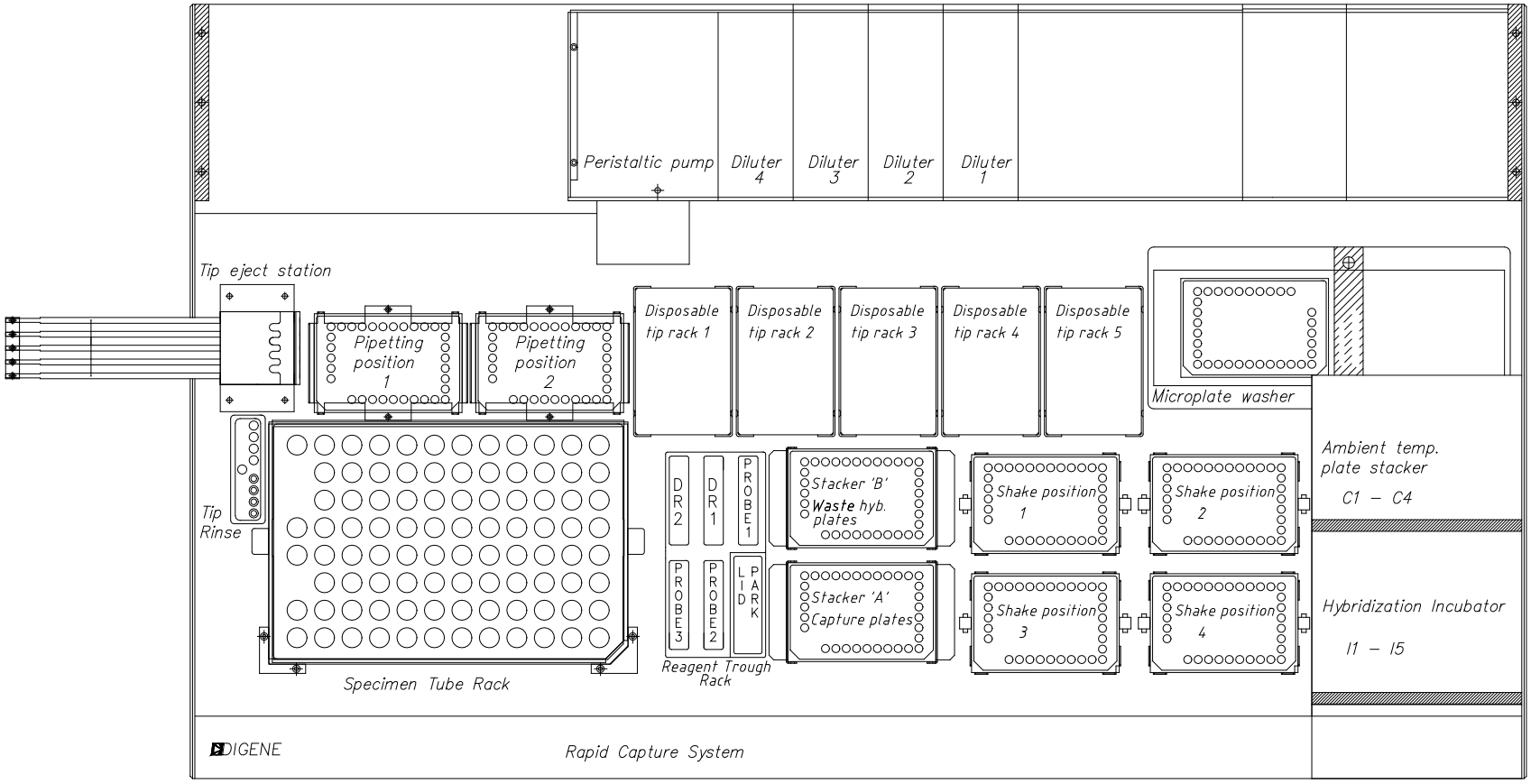


Figure 1: RCS Deck Layout

Routine Maintenance of Rapid Capture System

Daily

See the appropriate digene HC2 DNA Test Application Procedure.

Monthly

a. Replace reagent troughs with new troughs. Label for use with appropriate Probe Mix, Detection Reagent 1, or Detection Reagent 2.
   Note: It is not required to replace the trough lids monthly.

b. For each of the five disposable tip rack holders, pull the center tabs on the front and back ends of the holder toward the center to ensure the clips maintain sufficient tension on the disposable tip racks.

c. Clean the Rapid Capture System Tubing Lines and Bottles with 0.5% v/v Sodium Hypochlorite solution (see procedure below).

Procedure for cleaning the Rapid Capture System Tubing Lines and Bottles

Safety Precautions

The user should never reach onto the deck area when the system is in operation.

Users must wear lab coats, gloves, and safety goggles when performing this procedure.

Procedure

1. To run sodium hypochlorite solution through the system lines, proceed as follows:

   1a. **Verify that the instrument is** ON**, but not running. There should be no** Rapid Capture System Control Program **window open or minimized on the PC screen.**
   1b. Disconnect the quick-release fitting of the System Liquid (deionized or distilled water) Bottle. To prevent alkaline phosphatase contamination, rest the disconnected end of the tubing on a clean Kimtowels Wiper or equivalent low-lint paper towel.
   1c. Remove the lid and empty the bottle into a sink.
   1d. Fill the bottle with 1 liter of freshly prepared 0.5% v/v Sodium Hypochlorite solution.
   1e. Replace the bottle lid. Tighten it securely.
   1f. Cover the air vent in the lid with a Kimtowels Wiper or equivalent low-lint paper towel and shake the bottle vigorously to ensure that the sodium hypochlorite solution rinses all inside surfaces, including the lid.
   1g. Restore tubing connection.

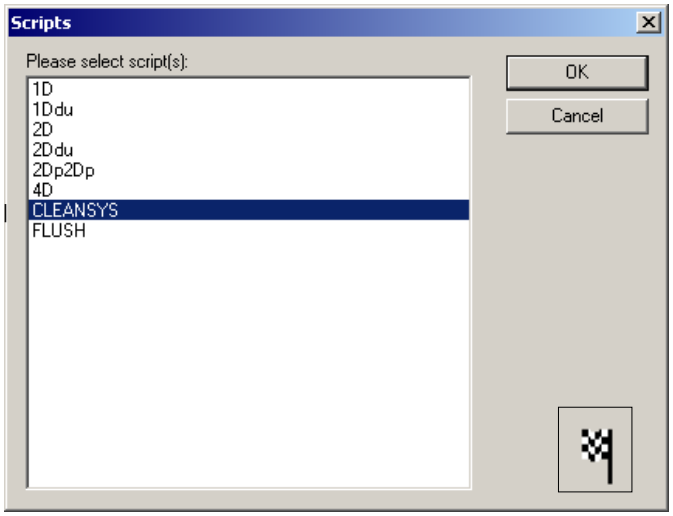
1h. Repeat steps b through g, this time with the Wash Bottle.

1i. Run the script entitled **CLEANSYS**. This will flush all system liquid lines, including the syringes and plate wash cannulas, thoroughly with the sodium hypochlorite solution.

i.1. Launch the Rapid Capture System operating software by double clicking the Rapid Capture System desktop icon.

i.2. Click on the Flag icon from the Rapid Capture main menu.

i.3. Select the CLEANSYS script and click OK.



2. To rinse bottles off-line with deionized or distilled water, proceed as follows:

    a. Disconnect the quick-release fittings of the System Liquid (deionized or distilled water) Bottle and the Wash Bottle. Rest the free ends of the tubing on clean Kimtowels Wipers or equivalent low-lint paper towels to prevent alkaline phosphatase contamination.

    b. Remove the lids and empty the bottles into a sink.

    c. Add 1 liter of deionized or distilled water to the System Liquid Bottle and 2 liters of deionized or distilled water to the Wash Bottle.

    d. Replace the lids securely.

    e. For each bottle, cover the air vent in the lid with a Kimtowels Wiper or equivalent low-lint paper towel and shake vigorously to rinse all interior surfaces with the deionized or distilled water.

    f. Empty each bottle and repeat the deionized or distilled water rinse once more for a total of two deionized or distilled water rinses for each bottle.

3. To rinse and prime Rapid Capture System lines, proceed as follows:

    a. Once both bottles are emptied of their second deionized or distilled water rinse, fill the System Liquid Bottle with deionized or distilled water and the Wash Bottle with Wash Buffer at the working 1X concentration (see Reagent Preparation section in CT/GC, CT-ID, GC-ID Application Procedures section of this user manual).

    b. Reattach the tubing from the instrument to the bottle lids. Be sure that each bottle is connected to the appropriate tubing line. The entrance port of each tubing line into the instrument is labeled. Ensure that the quick-release valves click securely in place.

    c. Run script **CLEANSYS**. This will replace the sodium hypochlorite solution in all lines with the deionized or distilled water or Wash Buffer, as appropriate.

4. To bleach Waste Bottle, proceed as follows:

    a. Disconnect both quick-release fittings to the Waste Bottle. Ensure that the disconnected ends are allowed to rest on a clean Kimtowels Wiper or equivalent low-lint paper towel to prevent contamination of lab surfaces.

    b. Remove the lid and empty the bottle carefully into a sink. Rinse the sink thoroughly, as this waste is a source of alkaline phosphatase.

    c. Add 2 liters of 0.5% v/v freshly prepared sodium hypochlorite solution to the bottle.

    d. Replace the lid securely.

    e. Cover the air vent in the lid with a Kimtowels Wiper or equivalent low-lint paper towel and shake the bottle to rinse all sides with the sodium hypochlorite solution.

    f. Empty the bottle and add 2 liters of deionized or distilled water.

    g. Replace the lid securely.

    h. Cover the air vent with a Kimtowels Wiper or equivalent low-lint paper towel and shake the bottle to rinse all sides with the deionized or distilled water.

    i. Empty the bottle into the sink.

j. Replace the lid securely and reattach both waste tubing lines to the bottle, ensuring that the quick-release valves click securely in place. The system liquid lines and bottles are now clean and ready for use. Be sure to record the date, instrument serial number, and your initials in the maintenance log.

System Shut Down

Note: It is not necessary to turn off the power upon completion of the application procedure.

The Rapid Capture System safely parks the Plate Handler and Pipetting Tip Assemblies at the end of each script. The power switch is located on the lower right side of the back panel. If it becomes necessary to shut down the Rapid Capture System, support the Plate Handler and Pipetting Tip Assemblies by hand from below, and two people should follow these steps:

1. The first person should support the Tip Assemblies (A) by placing one hand under the black plastic at the bottom of each vertical bar. Take care not to push or pull on the bars horizontally as their alignment is sensitive.

2. The first person should support the plate grippers (B) from below with the other hand. (This step is not required following completion of an assay, as the grippers will already be located close to the deck surface.)

3. The second person may now turn OFF the power using the power switch located on the lower right side of the back panel. If there is a plate in the grippers, remove it now.

4. The first person may now move the Plate Handler to the P1 position (See Figure 1: RCS Deck Layout) using the Plate Handler and **not** the Tip Assemblies to pull the arm into position. Tip Assemblies and Plate Handler may now be lowered to the deck.

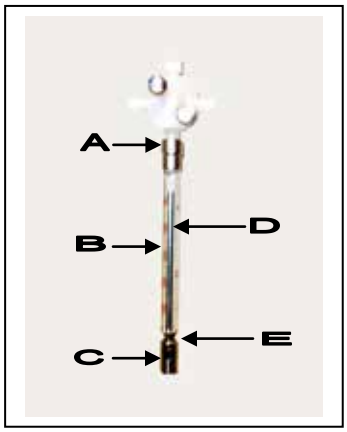
5. If there are disposable tips on the tip adapters, it is best to let the Rapid Capture System unload them by turning the power back ON and running the **Flush** script. If this is not possible due to a malfunction, tips may be removed individually by pulling **straight down** on the tip while supporting the black plastic at the bottom of each vertical bar. It is critical that the Tip Assemblies not be pulled horizontally! Users must follow universal precautions regarding potentially infectious material. Do not place any part of your hand under a disposable tip while pulling down to remove it.

Syringe Cleaning & Replacement

If you need to replace the Syringes because of leaks, bubbles, or internal contamination (i.e., particles, crystals, etc.), turn the instrument off and remove the Syringes from the Syringe Pump modules as described below. Contact your local QIAGEN representative for ordering replacement Syringes.

Removal

Note: Syringes are glass. Use caution when handling.



1. Ensure that the power is switched OFF.
2. Unscrew the Luer-lock connector (A) of the Syringe from the lower port of the Valve.
3. Pull the Syringe barrel (B) slowly down until it is clear of the Valve.
4. Loosen the plunger retainer screw (C), and carefully pull the Syringe away from the plunger drive pin (E).
5. If a Syringe was leaking, clean it or replace it. To clean the Syringe, remove the plunger (D) from the Syringe barrel, wash with a mild detergent, rinse with deionized or distilled water, and follow with 70% isopropanol.

Replacement

1. Place the bottom of the Syringe plunger over the plunger drive pin (E), and tighten the screw on the underside of the plunger (C).
2. Pull the Syringe barrel up until the Luer-lock connector (A) on the Syringe can be inserted into the Luer-lock hub on the lower port of the Valve, and then carefully screw the Syringe clockwise into the Valve. Take care not to cross-thread.

NOTE: Be sure that all the valve screws, the Luer lock connection, all the syringe-tubing connections, and the plunger screw are tight to prevent leakage.

3. Turn the power ON and park the instrument. Check that the Syringe initializes.
4. Run the Flush scripts at least twice to check for leaks. Flush the system until air bubbles in the Syringe or Tubing are removed.

IMPORTANT: **Authorized, trained personnel must perform all maintenance, excluding that discussed in this user manual.**

ScriptSelect

Application Procedure

Intended Use

Scripts define the specific set of Rapid Capture System (RCS) software instructions. The script controls the processing sequence required to run a digene Hybrid Capture 2 (HC2) DNA Test on the Rapid Capture System. There are 43 scripts defined in the Rapid Capture System. Scripts offer the user flexibility in terms of number of specimens, types of specimens, and type of digene HC2 DNA Test for a specific Rapid Capture System run. The scripts are generically named for use with multiple digene HC2 DNA Tests.

Rapid Capture System ScriptSelect Software assists the user in selection of the Script required to perform a digene HC2 DNA Tests on the Rapid Capture System. It functions by generating a series of screen options in which the user makes selections based on the particular digene HC2 DNA Test, the number of Probes, digene Specimen Racks, Conversion Racks, and Probe Configurations. The user must select a script from the RCS ScriptSelect software to add it to the Rapid Capture System Run menu.

Note: Some of the 43 scripts are designated for future applications and are not available for current use. When these scripts become available, QIAGEN will issue a password to unlock them. Disclaimers for non-FDA-approved applications as well as statements regarding FDA-approved applications are both listed in the “Disclaimers:” section of the various windows and the “Disclaimers:” section of printouts.

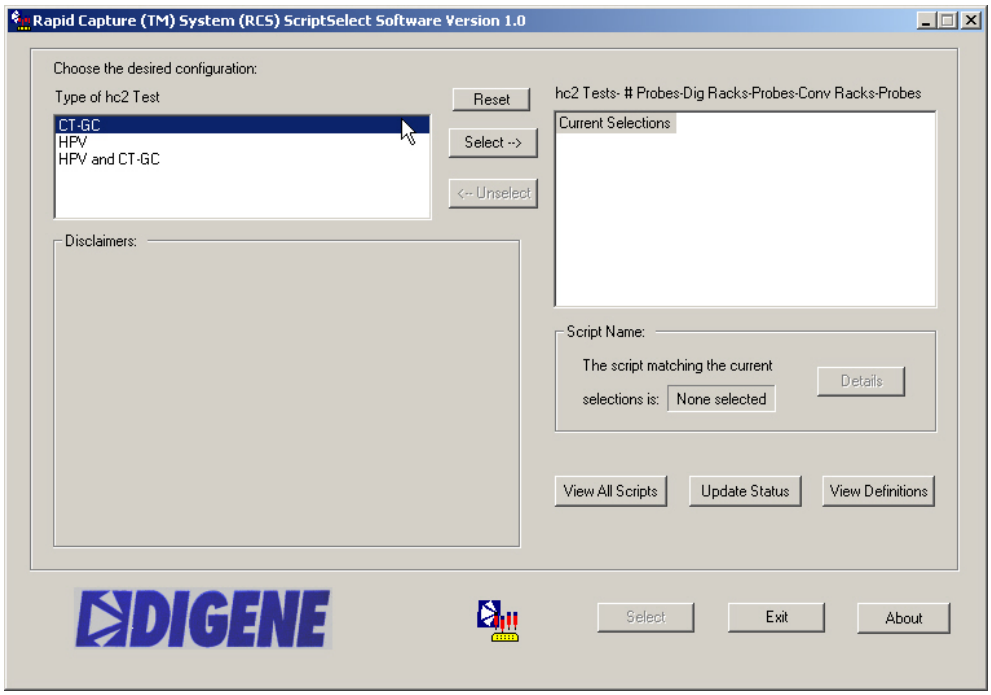
RCS ScriptSelect Software Installation

RCS ScriptSelect Software is installed on the Rapid Capture System computer by QIAGEN service personnel.

Starting the RCS ScriptSelect Software

Double-click on the shortcut icon on the desktop.

The **RCS ScriptSelect Software** main window opens.



Script Selection Procedure

Note: The software is designed to provide the user specific choices based on the preceding selection. Menu option screens are bypassed when there is only one option. The software will default to the only possible configuration based on the user's previous selections.

List of all possible Configuration Screen Menu options

- **Type of Assay to be run:** Select the digene HC2 DNA Test to be performed.
- **Number of Probe(s):** Select the number of Probes to be used.
- **Number of Rack(s) with *digene* Specimens:** Select the number of digene Specimen Racks to be used.
- **Probe Configuration(s) with *digene* Specimens:** Select the types of Probe to be used for the specimens in digene Specimen Rack(s).
- **Number of Conversion Rack(s):** Select the number of Conversion Racks (of converted specimen type) to be used.
- **Probe Configuration(s) with Converted Specimens:** Select the types of Probe to be used for specimens in Conversion Rack(s).

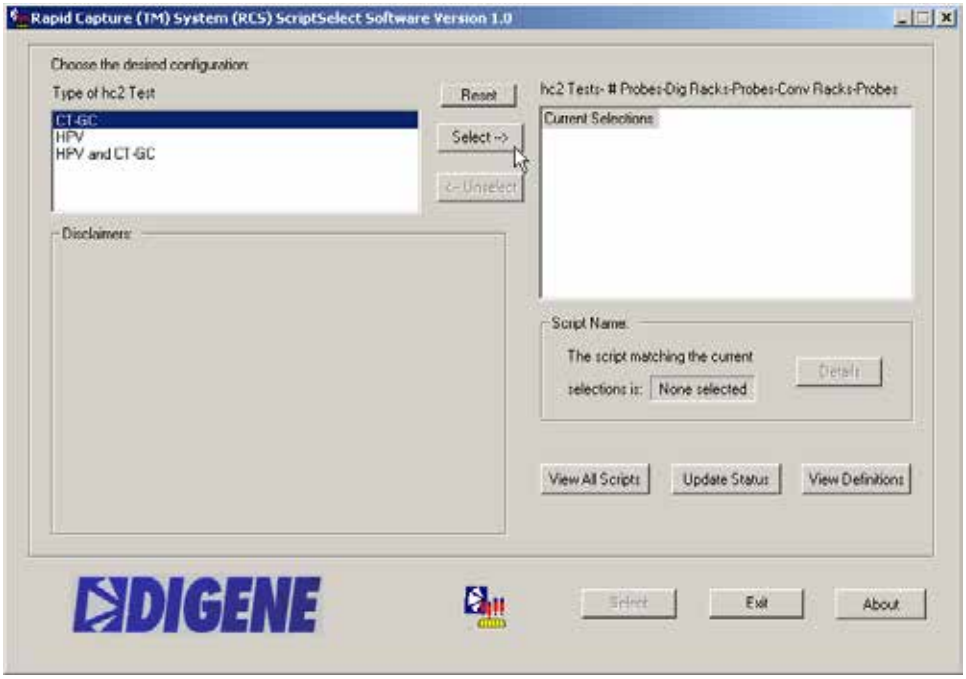
Notes:

- To select an option in the left dialog box of the main window, double-click on the option or highlight the option and click on the **Select -- >** button.
- As configurations are selected, they are transferred to the window on the right. Configurations can be unselected by double clicking on the selection in the window on the right or by highlighting the selection and clicking the **< -- Unselect** button. To unselect more than one option at a time, click on the highest level.

Note: The following RCS ScriptSelect screenshots attempt to show the possible configurations available for selection.

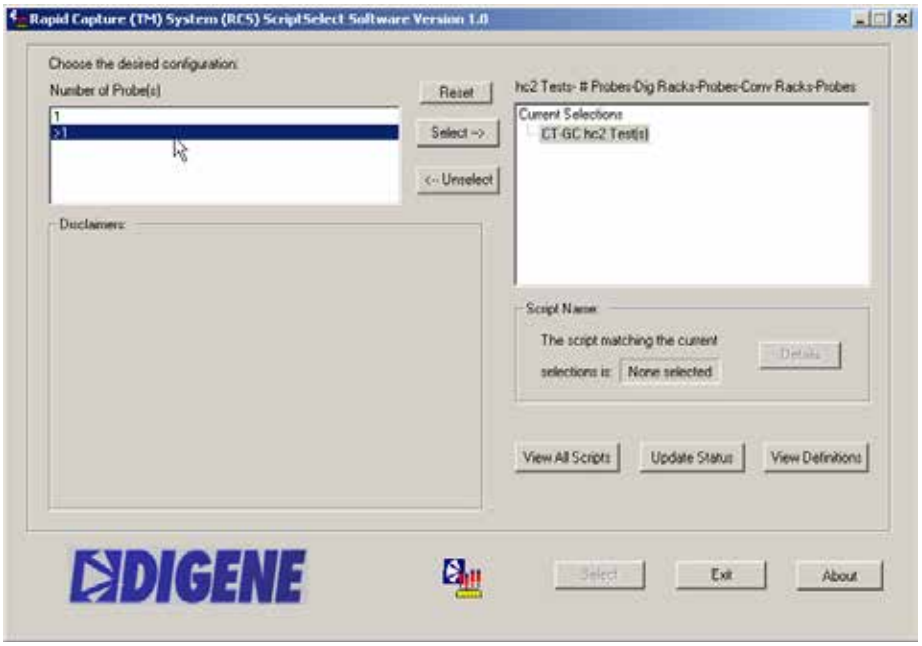
1. Select the type of digene HC2 DNA Test to be run.

   From the **main ScriptSelect** screen, the user must first select the type of digene HC2 DNA Test(s) desired for a Rapid Capture System run. Three options are available: CT-GC, HPV, or HPV and CT-GC.

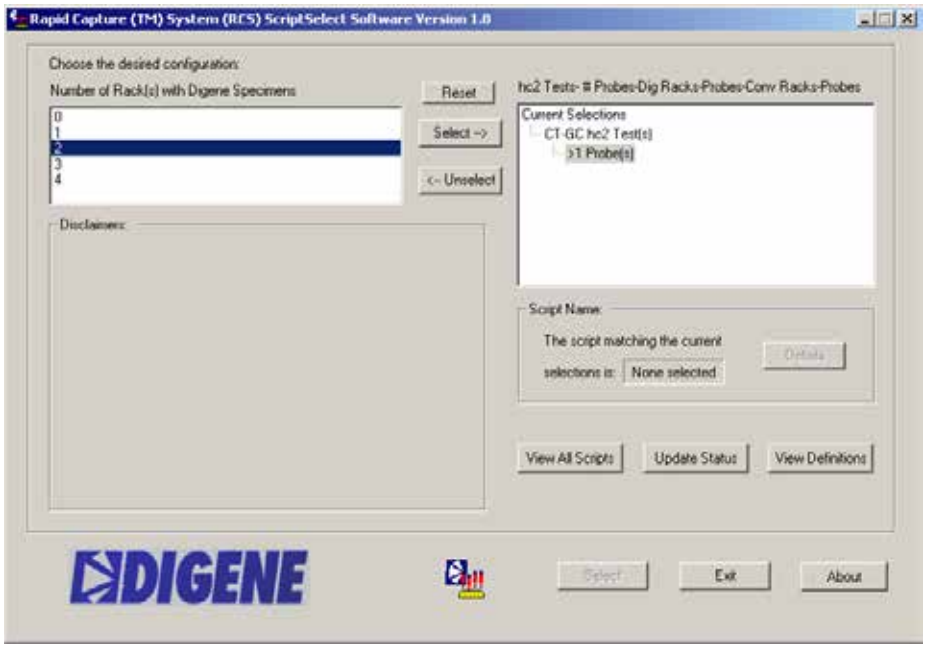


2. The user will then select the number of Probes desired. Two options are available. Select "**>1**" if multiple Probes are to be run.

   The "**1**" option is selected if only one Probe type is required.



3. Select the number of digene Specimen Racks to be used.



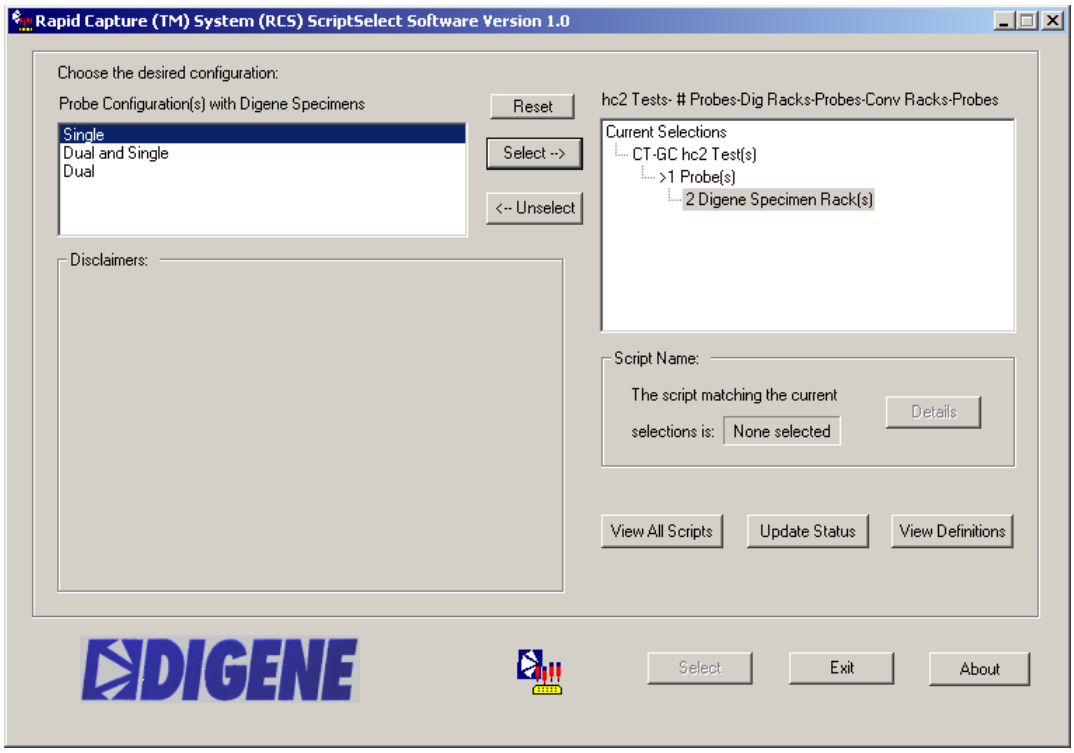
4. Select the Probe Configuration(s) to be use with the digene Specimens:
Single, **Dual and Single**, and **Dual**

Note: The number of Probe types tested with a specimen rack determines the probe configuration to be used.

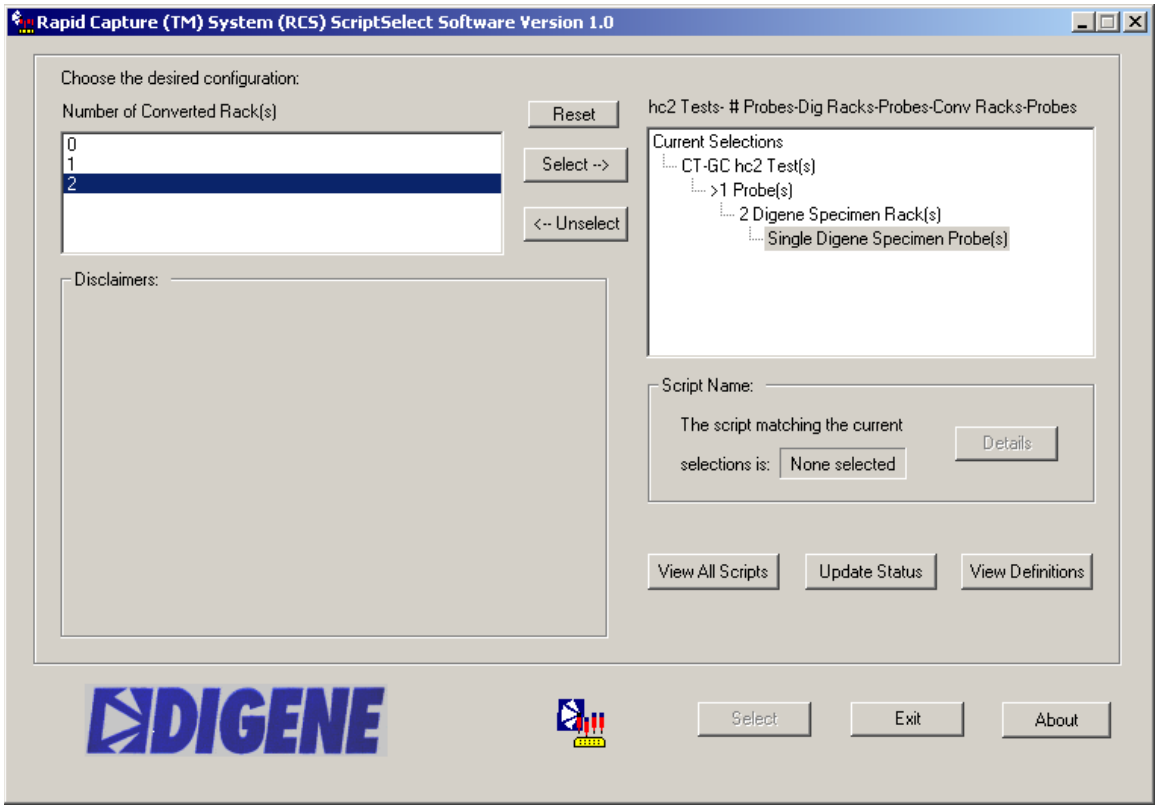
The "**Single**" option indicates that the specimen rack(s) is/are only tested with one Probe type. Selecting **"Single"** does not limit the run to only one probe. More than one probe type can be used; however, each specimen rack(s) is tested with only one probe type.

The **"Dual"** selection indicates that the specimen rack(s) will be tested with two Probe types. For example, one digene Specimen Rack will be tested with the CT Probe and the GC Probe.

The "**Dual and Single"** selection indicates that digene Specimen Rack is tested with two Probes and that the other rack(s) is/are tested with one Probe type.



5. Select the number of Conversion Racks to be used.

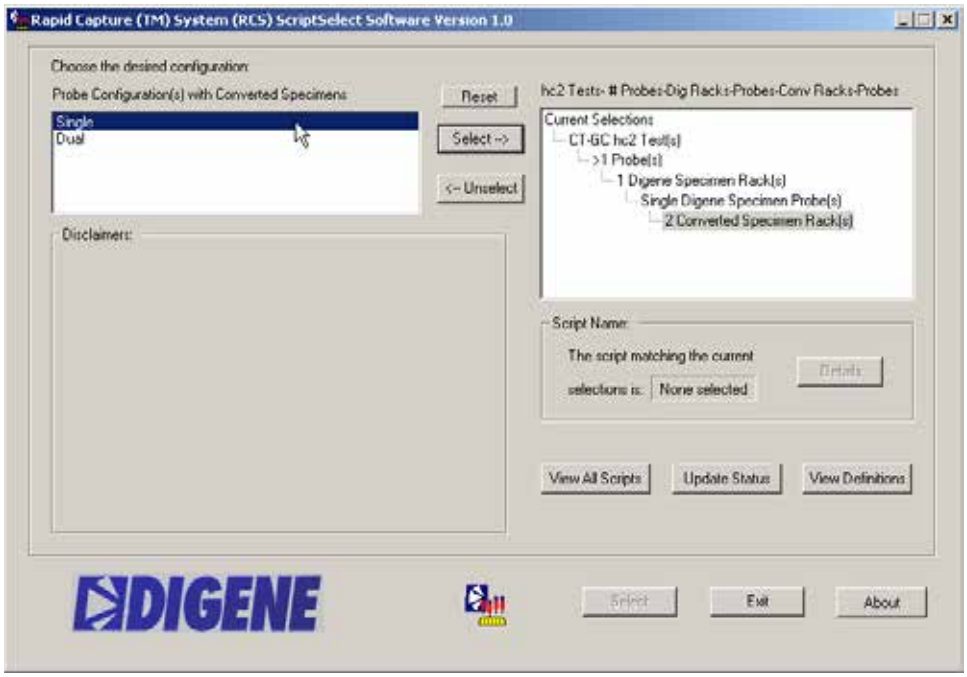


6. Select the Probe Configuration(s) with Converted Specimens.

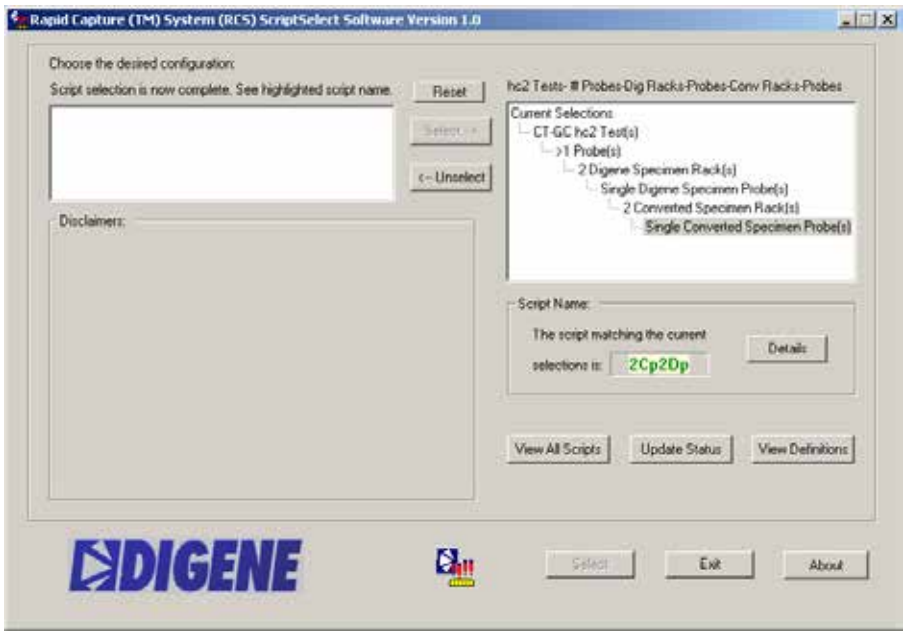
Note: The number of Probe types tested with a Conversion Rack determines the probe configuration to be used.

The "**Single**" option indicates that previously selected Conversion Rack(s) are only tested with one Probe type. Selecting **"Single"** does not limit the run to only one probe. More than one probe type can be used; however, each Conversion Rack is tested with only one Probe type.

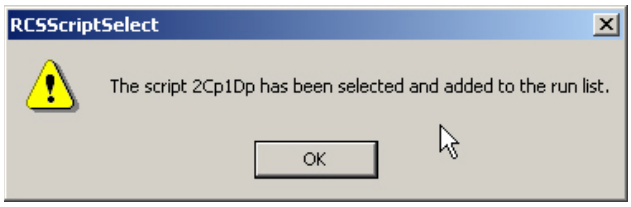
The **"Dual"** selection indicates that specimen rack(s) to be tested will be tested with two Probe types.



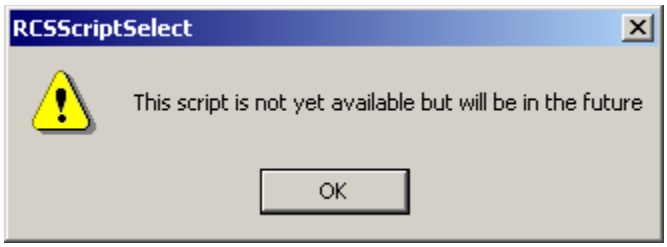
7. The Script Name appears.



8. Click the **Select** button to add the **Script** to the Rapid Capture System Run List. If the Script Application is approved for use on the Rapid Capture System, the following Dialog box will appear:



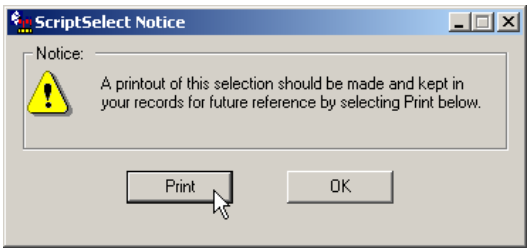
9. If the Script Application is not approved for use on the Rapid Capture System, the following Dialog box will appear:



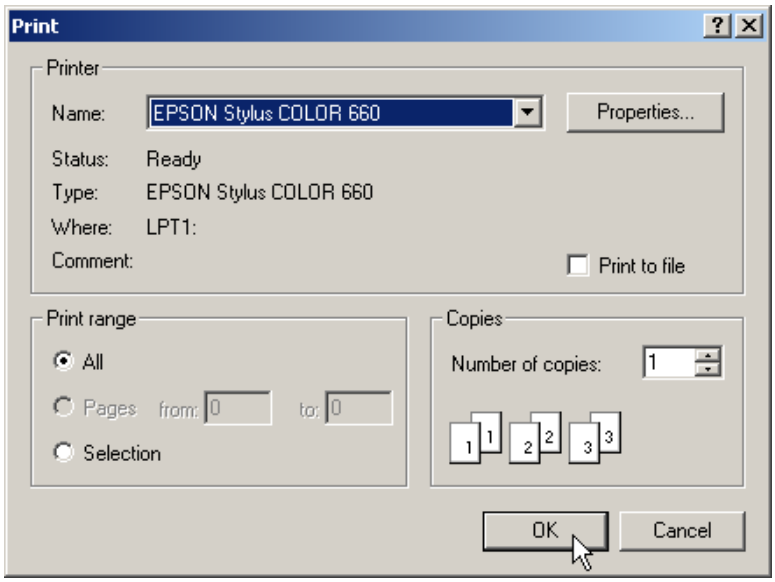
Note: Some of the 43 scripts are designated for future applications and are not available for current use. When these scripts are available for use, QIAGEN will issue a password to un-lock them using the ScriptSelect software.

10. If the script is approved and available for use, the **ScriptSelect Notice** window will appear (see Printing Script Information section of this section for more details).

11.



12. The **Print** window will appear. Select **OK** for printout of script information.



View All Scripts Button

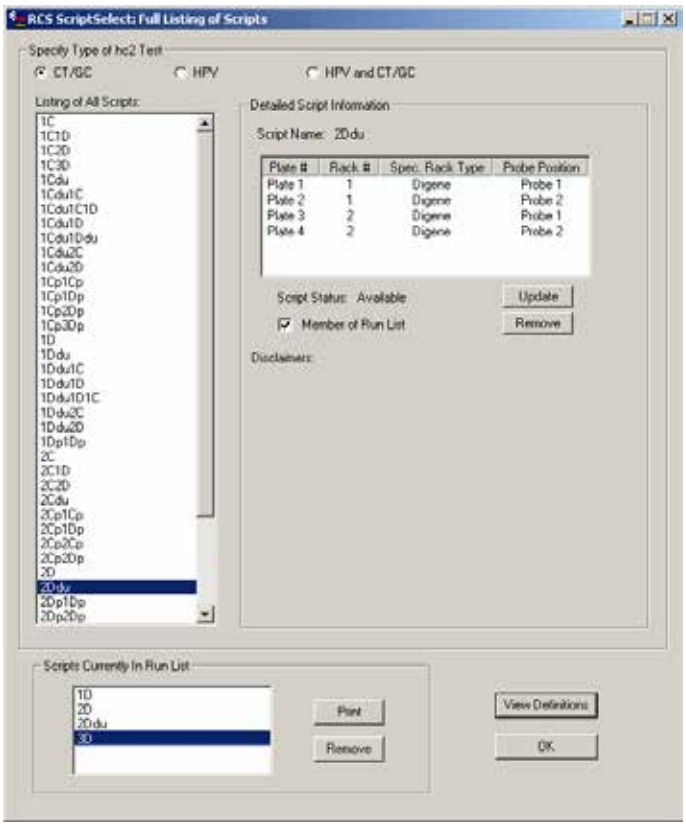
Click the **View All Scripts** button.

The **View Scripts** button displays a complete list of all Scripts installed on the system. However, some scripts are designed for future applications and are not available for current use.

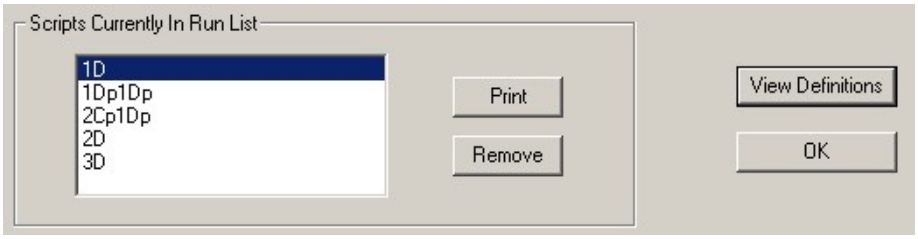
Select the digene HC2 DNA Test type (CT-GC, HPV, or HPV and CT-GC). Highlight a Script name to view the information for a particular Script from the **Listing of All Scripts** box.

Selection activates the **Detailed Script Information** window and lists the Plate/Rack/Probe information for each plate. Double-clicking the script name in **Listing of All Scripts** list box activates the script name and adds the script to the Rapid Capture Run List. Script names may be added or removed from the Run List by clicking the **Select** or **Remove** button in this grouping. Activated scripts may also be removed from the Run List by clicking in the **Remove** button in the **Scripts Currently In Run List** grouping box.

Scripts Currently in Run List Window: Lists all the Scripts that have been added to the Rapid Capture System Run list.



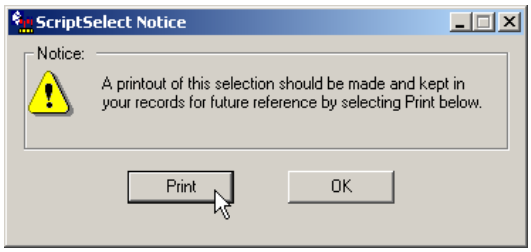
Clicking the button **Remove** will delete the selected script from the Rapid Capture Run List.



Clicking **Print** from this screen displays the **Prints** dialog box. Click **OK** to print out the details of the selected script.

Printing Script Information

Print option 1:



Click the button **Print** in the **ScriptSelect Notice** dialog box to print the selected script. Below is an example of a printout, which contains the name of the selected script, plate/rack/probe information, and applicable regulatory disclaimers.

RCS ScriptSelect Software version 1.0

This script was last selected using the RCS ScriptSelect software on: 01/15/04 3:43 PM

Selected Parameters:

Type of hc2 Test:	CT-GC
Number of Probe(s):	>1
Number of Rack(s) with Digene Specimens:	2
Probe Configuration(s) with Digene Specimens:	Dual and Single
Number of Converted Rack(s):	0

Script Selected : 1Ddu1D

Plate/Rack Configuration:

Plate #	Rack #	Spec. Rack Type	Probe Position
Plate 1	1	Digene	Probe 1
Plate 2	1	Digene	Probe 2
Plate 3	2	Digene	Probe 3

Disclaimers:

Print option 2:

The following printout can be obtained by selecting the **View All Scripts** from the RCS ScriptSelect main window and selecting **Print** from the **Scripts Currently in Run List Window**:

RCS ScriptSelect Software version 1.0

This script was last selected using the RCS ScriptSelect software on: 01/20/04 11:20 AM

Script Selected : 1Ddu1D

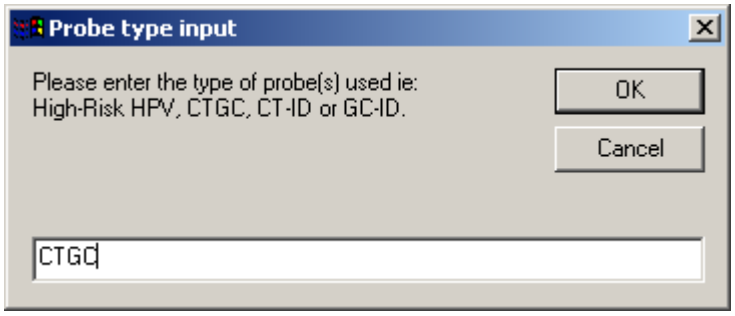
Plate/Rack Configuration:

Plate #	Rack #	Spec. Rack Type	Probe Position
Plate 1	1	Digene	Probe 1
Plate 2	1	Digene	Probe 2
Plate 3	2	Digene	Probe 3

Disclaimers:

Linking ScriptSelect Software to the Rapid Capture System Software

When a script name is selected by clicking the **Select** button, the script is automatically moved to the Rapid Capture Run List in the Rapid Capture Software. From the Rapid Capture Software, the user selects the script and the following dialog box is activated:



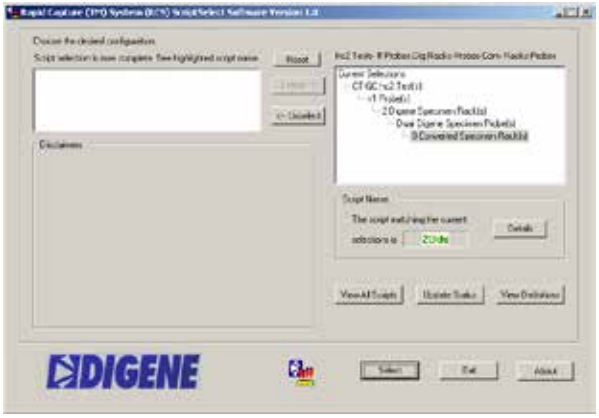
The user is required to enter the Probe(s) used in the run. This information is printed to the default printer along with the script name selected, the date, and the time.

The user can then compare the name of the script file selected in the ScriptSelect software to the name selected in the Rapid Capture Software to verify that the correct script has been selected. The user should also verify the plate specimens, rack types, and Probe locations for the assay being performed.

Script Detail Screen

Script Name Description

A detailed listing of the Plate/Rack/Probe layout can be viewed from the main **ScriptSelect** screen by clicking the **Details** button once a Script has been selected.



The table lists plates by plate number, the rack number for each plate, the specimen rack type (digene Specimen Rack or Conversion Rack) for each plate, and each probe position for each plate.

Member of Run List allows for the activation and customization of the Rapid Capture System Software Script availability menu.

The check box next to **Member of Run List** indicates the script status within the Rapid Capture Software and if the Script is included in the **Script Menu List**. If there is a check in the box, the Script is listed in the Rapid Capture System Script list. If the box is not checked, the Script is unavailable in the Rapid Capture System Run List.

The Script can be added to the Rapid Capture System Script menu by clicking the **Select button.** The Select option is not available if **Script Status** is **Locked.**

Scripts that are listed in the Rapid Capture System software Script menu, and not required by the user, may be removed from the run list by selecting the name of the script and clicking the **Remove** button.

Script Status

Scripts are defined as either “Available for use” on the Rapid Capture System or “Locked.”

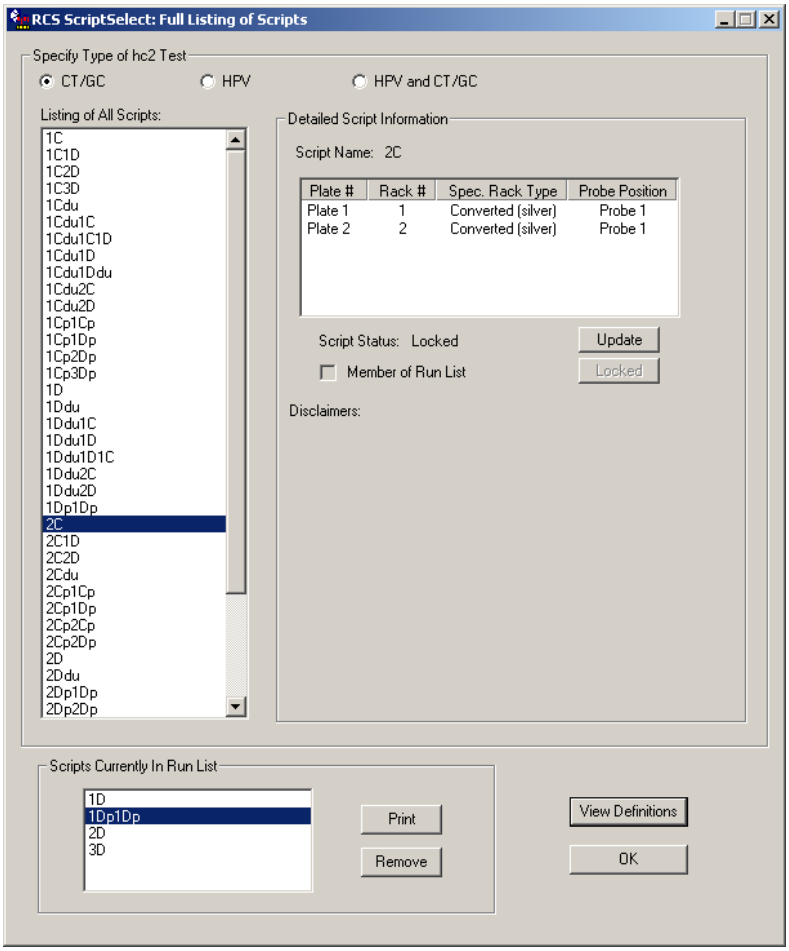
- “Script Status: Available” indicates that the script can be added to the Run List.
- “Script Status: Locked” indicates that the script cannot be added to the Run List and is not available for use.

Locked Scripts/ Unlocking Scripts

Scripts are made available for use on the Rapid Capture System after the validation of the specific script for any digene HC2 DNA Test and specimen type. Disclaimers further define other digene HC2 DNA Tests that may not yet be approved for use on the Rapid Capture System.

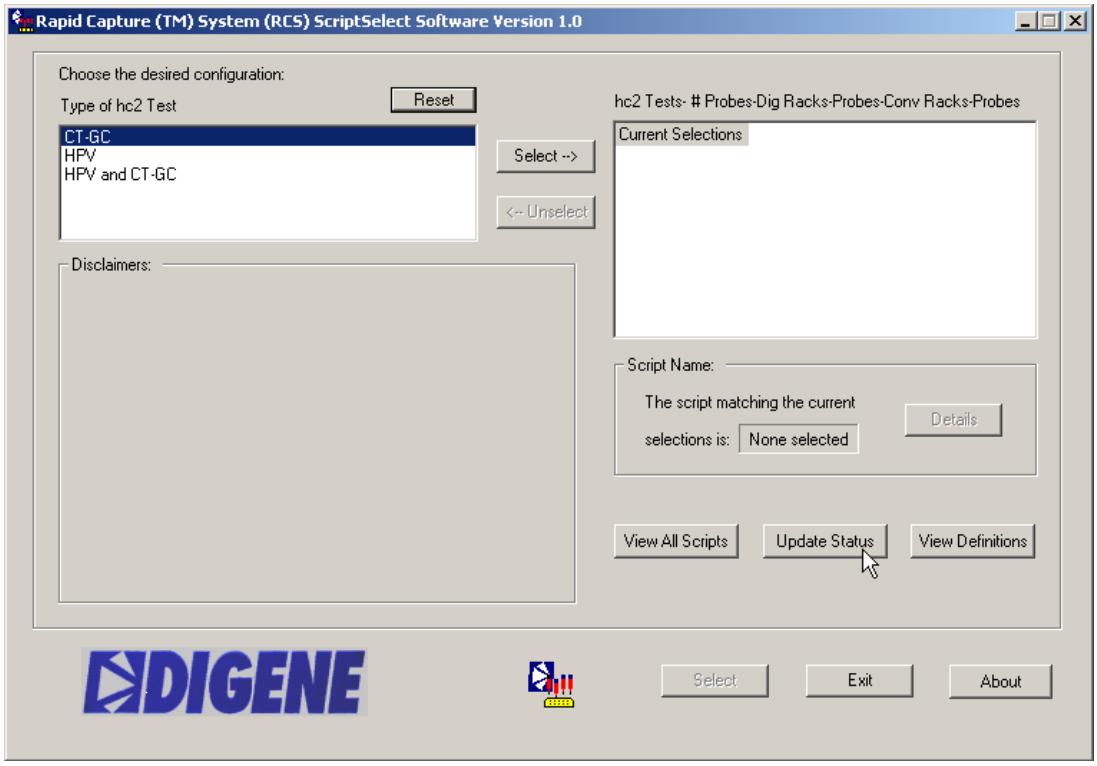
Option 1

If a script is currently not available for activation, the **Select** button is labeled **Locked** and is grayed out and the **Update** button is activated.



Option 2

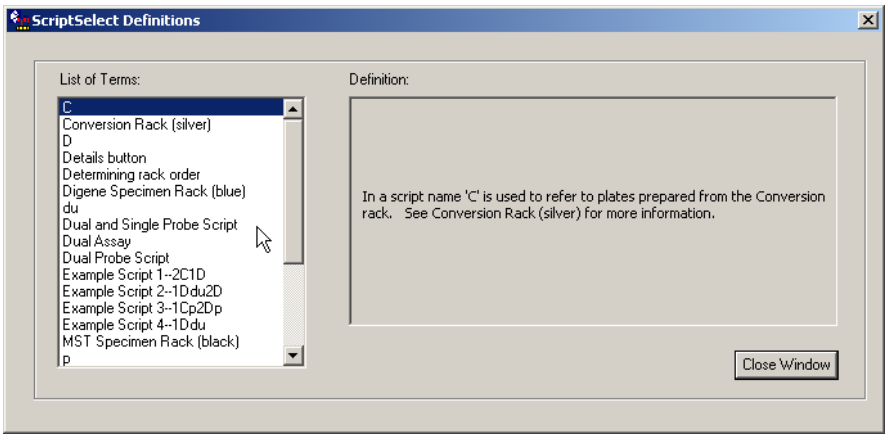
Scripts can also be unlocked from the main window using the **Update Status** button.



Enter the password supplied by QIAGEN and click **OK**.

VIEW DEFINITIONS BUTTON

Clicking the **View Definitions** button activates the **ScriptSelect Definitions** window.



Select the desired term in the left pane to view the definitions on the right. A complete list of the ScriptSelect Definitions is listed below.

Script Definitions

Rapid Capture Script Terms	Definition
digene Specimen Rack (blue)	Refers to the blue specimen rack used for specimens collected in Specimen Transport Medium (STM). This rack can be used for both single- and dual-probe assays.
Conversion Rack (silver)	Refers to the silver specimen rack used for converted Hologic PreservCyt® Solution specimens. These specimens require processing prior to converting them for use with digene HC2 High-Risk HPV DNA Test specimens.
Dual Assay	A dual assay refers to a test where one rack of specimens is distributed to two different plates. Each plate is then tested with a different probe. See Example Script 4 for further clarification.
Single-Probe Script	"Single-Probe Script" indicates that all racks are tested with the same Probe located in trough position Probe 1 of the Rapid Capture System deck. The terminology “Single-Probe Script” is printed on the printout generated at the beginning of an RCS run. See Example Script 1 for further clarification.
Two-Probe Script	"Two-Probe Script" indicates that each rack being tested generates results using a different Probe. Multiple Probes are used for multiple racks, but each rack is only tested with one Probe. The terminology “Two-Probe Script” is found on the printout generated at the beginning of the Rapid Capture System run and defines the Probe and script selection used in a specific Rapid Capture System run. See Example Script 3 for further clarification.
Dual-Probe Script	The Dual-Probe Script indicates that one specimen rack distributed on two plates is tested with two different Probes. The “Dual-Probe Script” terminology is included on the Rapid Capture System confirmation printout generated at the beginning of the Rapid Capture System run. See the definition of Dual Assay and see Example Script 4 for more information.
Dual- and Single-Probe Script	The "Dual- and Single-Probe Script" terminology listed on the printout generated at the beginning of the RCS run indicates the RCS will perform a Dual-Probe assay and Single-Probe assay. The Dual assay is always performed first by the RCS. The Dual assay allows for one rack of specimens to be tested on two plates using Probe from trough positions 1 and 2. The remaining racks are tested using Probe from trough position 3. See Example Script 2 and Dual Assay for additional information.
C	In a script name 'C' is used to refer to plates prepared from the Conversion rack. See Conversion Rack (silver) definition for more information.
D	In a script name, 'D' is used to refer to plates prepared from the digene Specimen Rack. See digene Specimen Rack (blue) or MST Specimen Rack (black) for more information.
du	'du' is used in script names to indicate a Dual assay. See Dual Assay for more information.
p	The “p” is used as a suffix in script names to indicate a different Probe usage. 'p' is used in script names to indicate multiple Single-Probe assays. For example: the script 2Dp1Dp indicates that two digene Specimen Racks are being run using Probe solution from trough position Probe 1 on the Rapid Capture System deck, and a third digene Specimen Rack is tested using Probe 2 from trough position Probe 2 on the Rapid Capture System deck. This is different from a Dual-Probe Script where one specimen rack is tested using 2 Probes. See Example Script 3 for additional information.
Probe 1	Probe 1 refers to the Probe solution located in trough position Probe 1 of the Rapid Capture System deck.
Probe 2	Probe 2 refers to the Probe solution located in trough position Probe 2 of the Rapid Capture System deck.

Rapid Capture Script Terms	Definition
Probe 3	Probe 3 refers to the Probe solution located in trough position Probe 3 of the Rapid Capture System deck.
Run List	The list of scripts currently available in the Rapid Capture System software. Scripts can be added or removed from the Rapid Capture System run list using the ScriptSelect software. Only scripts present in the run list can be used in the Rapid Capture software.
Example Script 1--2C1D	2C1D: Defines a three-rack / three-plate script. This assay utilizes only one Probe located in trough position Probe 1. The 2C defines using two Conversion Racks (silver) for plates 1 and 2. The 1D denotes use of a digene Specimen Rack for plate 3. All specimens are tested using Probe from trough position Probe 1. This is a Single-Probe script.
Example Script 2--1Ddu2D	1Ddu2D: Defines a three-rack/four-plate script. The 1Ddu (1 digene dual) indicates using one digene Specimen Rack (blue) to run two plates from one specimen. The Probe for plate 1 is located in trough position Probe 1 and the Probe for plate 2 is located in trough position Probe 2. The 2D (two digene Specimen Racks tested with a single Probe) refers to two additional digene Specimen Racks tested on plates 3 and 4 using Probe from trough position Probe 3. This is a Dual- and Single-Probe script.
Example Script 3--1Cp2Dp	1Cp2Dp: Defines a three-rack/three-plate script. The 1Cp indicates that one Conversion Rack (silver) is used to run one assay on plate 1 with the Probe in trough position Probe 1. The 2Dp specifies the use of two digene Specimen Racks. The Probe located in trough position Probe 2 is used to test each of the digene Specimen Racks on plates 2 and 3. Refer to the "p" definition. This is a Two-Probe script.
Example Script 4--1Ddu	1Ddu: Defines a one-rack/ two-plate script. The 1 Ddu (1 digene dual) indicates using one digene Specimen Rack (blue) to run two plates from one specimen. The Probe for plate 1 is located in trough position Probe 1 and Probe for plate 2 is located in trough position Probe 2. This is a Dual-Probe script.
Update Button	The Update Button is a utility feature of the ScriptSelect software that will allow for expanded use of the RCS. Upon entering QIAGEN-specified password, new permissions will be granted for extended applications for the RCS. QIAGEN will issue passwords as new approvals are obtained.
Determining rack order	The correct rack order is always indicated by the script name. In general, if there is a dual assay, the rack for the Dual assay is first, followed by any other racks of the same specimen type. If a Dual assay is not required by the script, then Conversion Racks will always be first, followed by digene Specimen Racks.
Script	Set of instructions the Rapid Capture System utilizes to perform an assay or a series of assays.
Details button	Clicking on the Details button generates a window that displays the plate, rack and, Probe configuration for a specific script.
Select Button	Clicking on the Select button automatically adds the script to the Rapid Capture Run List.

CT/GC, CT-ID, and GC-ID

Application Procedures

Required Reagents and Guidelines

Reagents Required:

- Hybrid Capture 2 (digene HC2) CT/GC DNA Test, digene HC2 CT-ID DNA Test, digene HC2 GC-ID DNA Test or digene HC2 CT-GC Dual ID kit
- digene HC2 DNA Collection Device: One cervical brush and one tube containing 1 ml of Specimen Transport Medium (STM)
- Hybrid Capture (HC) Female Swab Specimen Collection Kit (2 swabs and one tube containing 1 ml of STM)

Rapid Capture System Test Reagent Guidelines:

Multiple digene HC2 DNA Tests can be performed on the Rapid Capture System using the multi-digene HC2 DNA Test Guidelines. It is necessary to combine components from multiple kit boxes of the same lot number to provide the required reagent volumes for processing more than one full plate of specimens for a single-probe assay. Use the digene HC2 CT-GC Dual ID kit to perform multi-digene HC2 Tests on the Rapid Capture System. Only the digene HC2 CT-GC Dual ID Kit is qualified for this usage. See multi-digene HC2 testing instructions contained in the digene HC2 CT-GC Dual ID kit IFU supplement. Refer to individual digene HC2 DNA Test IFUs for lot usage restrictions.

Reagent Preparation and Storage

<table>
<tr>
<td>CT/GC, CT-ID, or GC-ID Denaturation Reagent</td>
<td>PREPARE FIRST:

Add five drops of Indicator Dye to each bottle of Denaturation Reagent and mix thoroughly. It is not necessary to combine bottles of Denaturation Reagent. The Denaturation Reagent should be a uniform dark purple color. Once prepared, the Denaturation Reagent is stable for three months when stored at 2-8°C. Label it with the new expiration date. If the color fades, add three additional drops of Indicator Dye and mix thoroughly before using.

Warning: Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, and eye/face protection. Use care when handling.</td>
</tr>
<tr>
<td>CT/GC, CT, or GC Probe Mix (Prepared from CT/GC, CT, or GC Probe and Probe Diluent Reagents)

(Prepare Fresh Daily)</td>
<td>IMPORTANT: SOMETIMES PROBE GETS TRAPPED IN THE VIAL LID.

Note: Take extreme care this step to prevent RNase contamination of Probe and Probe Mix. Use aerosol-barrier pipette tips for pipetting probe. Probe Diluent is viscous. Take care to ensure thorough mixing when preparing Probe Mix. A visible vortex must form in the liquid during the mixing step. Incomplete mixing may result in reduced signal.

· Centrifuge each vial of the CT/GC, CT, or GC Probe briefly to bring liquid to the bottom of the vial. Tap tube gently to mix.
· Determine the amount of Probe Mix required from the table below. Extra Probe Mix is required to account for the void volume required in the Rapid Capture System reagent troughs and is included in the table. The smallest number of wells recommended for each use is 96 or one microplate.
· Transfer the required amount of Probe Diluent to a polypropylene conical tube to accommodate the volume of Probe mixture. Make a 1:25 dilution of Probe in Probe Diluent to prepare Probe Mix using the table below.

<table>
<tr><th>No. of Plates</th><th>Volume of Probe Diluent *</th><th>Volume of Probe *</th></tr>
<tr><td>£ 1**</td><td>5.0 ml</td><td>200 µl</td></tr>
<tr><td>£ 1.5</td><td>6.0 ml</td><td>240 µl</td></tr>
<tr><td>£ 2</td><td>8.0 ml</td><td>320 µl</td></tr>
<tr><td>£ 2.5</td><td>9.0 ml</td><td>360 µl</td></tr>
<tr><td>£ 3</td><td>10.0 ml</td><td>400 µl</td></tr>
<tr><td>£ 3.5</td><td>12.0 ml</td><td>480 µl</td></tr>
<tr><td>£ 4</td><td>13.0 ml</td><td>520 µl</td></tr>
</table>

* These values include the recommended extra volume needed to fill void volume of reagent troughs.
** Processing fewer than 88 specimens requires the same amount of Probe and Probe Diluent as a full plate to ensure a sufficient volume to cover the bottom of the trough.

· Pool Probe from each individual vial of the same kit lot number into one Probe vial and mix by pipetting. Measure Probe and dispense into Probe Diluent by placing a pipette tip against the inner wall of the tube just above the meniscus and expelling the contents. Do not immerse the tip into the Probe Diluent. Do not mix different lot numbers of Probe or Probe Diluent.
· Vortex for at least 5 seconds at maximum speed to mix thoroughly.
Note: A visible vortex must form in the liquid during the mixing step. Incomplete mixing may result in reduced signal.
· Label as CT/GC, CT, or GC Probe Mix and keep in a clean, closed container until ready for use. Discard unused Probe Mix. Do not store probe for future use. Prepare Probe fresh for the day of use.
· For Rapid Capture System run using more than 1 probe type, prepare each probe type in a separate polypropylene conical tube as described above and label accordingly.
· A maximum of three probe types can be utilized on one Rapid Capture System assay.

Caution: The Probe Diluent may cause reversible eye irritation. Wear eye/face protection.</td>
</tr>
</table>

Wash Buffer	For the Rapid Capture System, the Wash Buffer can be prepared as described below and stored in the Wash Bottle at 20-25ºC. See the table below for mixing volumes: No. of plates / Amount of Wash Buffer Concentrate / Amount of Deionized or Distilled Water / Final Volume of 1 X Wash Buffer* £ 2 / 100 ml / 2.9 L / 3 L > 2 / 200 ml / 5.8 L / 6 L ***These values include the recommended extra volume needed to fill the 300 ml void volume of bottle.** **Note:** Prepared Wash Buffer is stable for three months at 2-30ºC. Label with the new expiration date. If Wash Buffer has been refrigerated, equilibrate to 20-25ºC prior to use **Warning: Wash Buffer Concentrate is toxic by ingestion. Wear suitable protective clothing, gloves, and eye/face protection. To minimize exposure, add water to Wash Buffer Concentrate when preparing.**

Volumes for Ready-to-Use Reagents

Detection Reagent 1 & Detection Reagent 2	Determine the volume of Detection Reagent 1 or Detection Reagent 2 required for the run. The minimum run size is one plate. For one plate plus partial plates, use the volumes indicated in the table below. Mix individual reagent bottles thoroughly, then combine the appropriate volume of Detection Reagent 1 or Detection Reagent 2 into a clean disposable 50-ml polypropylene conical tube. Mix thoroughly. Pour entire contents into the designated reagent trough. To avoid contamination, these reagents **must not** be returned to the original bottles. **Discard unused material after use**. No. of Plates / **Minimum** Volume for Detection Reagents 1 and 2 ≤ 1 / 10 ml ≤ 1.5 / 14 ml ≤ 2 / 18 ml ≤ 2.5 / 22 ml ≤ 3 / 26 ml ≤ 3.5 / 30 ml ≤ 4 / 34 ml **Note: Only combine reagents of the same lot number.**

Specimen and Rack Setup

Preparation of Specimens for Transfer on the Rapid Capture System

Multi-Specimen Tube Vortexer 2 and Racks

The Multi-Specimen Tube (MST) Vortexer 2, of the appropriate specimen rack and lid, and the accessory components are required for sample preparation, processing, and denaturation. Two different specimen rack designs are available for the digene HC2 CT/GC, CT-ID, and GC-ID DNA Tests. The specimen racks allow the laboratory to customize their testing. The rack names and usage are listed in the table below. The specimen racks are color coded to differentiate the rack designs.

Name of Specimen Rack	Rack Color Key	Type of Specimen Rack Accommodates	Acceptable Rapid Capture System Scripts
digene Specimen Rack	Blue	Specimens collected in STM including digene HC2 DNA Collection Device and HC Female Swab Specimen Collection Kit.	Single-Probe Assay Dual-Probe Assay Two-Probe Assay

Specimens may contain infectious agents and should be handled accordingly.

Notes

- **The Rapid Capture System Application for *digene* HC2 CT/GC, CT-ID and GC-ID DNA Tests is validated for specimens collected in Specimen Transport Medium.**
- **Denaturation of specimens is performed offline.**
- **Determine the proper rack required for denaturation before placing specimens in the appropriate rack. When testing specimens with two probes, use the *digene* Specimen Rack (blue). This is a Rapid Capture System Dual-Probe Assay.**

1. Remove the specimens and all required reagents from the refrigerator prior to beginning the assay. Allow them to equilibrate to room temperature (20-25oC).

2. Label each digene Specimen Rack (blue) and corresponding lid with a number 1 through 4. Be sure to use a label and marker that will not wash off in the 65°C water bath (see Materials Required section in the Rapid Capture System User Application section of this user manual).

Notes:

- Each digene Specimen Racks (blue) is serialized. The serial number is engraved on both the rack and lid. Serial numbers of each rack and lid must match. Label accordingly.
- Up to four racks of 88 specimens each may be tested per Rapid Capture System run. For Single-Probe assays the digene Specimen Racks are labeled 1 through 4 and must be filled and loaded onto the Rapid Capture System in that order. Remember to include the kit Calibrator and Controls with each rack.
- When a Dual-Probe Script is run on the Rapid Capture System, the samples in the digene Specimen Rack (blue) will be distributed consecutively on two plates. The digene Specimen rack is used to perform a Dual-Probe Script. In this case, the rack is used for two specimen transfers. The rack is recognized as one rack by the Rapid Capture System software. Therefore, when running a Dual-Probe assay, the maximum number of racks the Rapid Capture System can accommodate is three. One rack for two plate transfers (Dual-Probe Script) and two additional racks for single plate transfer.
- Dual-Probe Scripts require two Positive Calibrators to be included on the digene Specimen Rack. Place the Positive Calibrator 1 to be used with the probe in trough position Probe 1 in the D1 position in the rack. Place the Positive Calibrator 2 to be used with the probe in trough position Probe 2 in the position E1 of the rack.

3. Use the digene HC2 System Software to enter specimen IDs and create plate layouts for each specimen rack. (Refer to the digene HC2 System Software User Manual for instructions.) **It is critical that specimen plate layout file names are correlated to the corresponding Specimen Rack.**

Note: Use the digene HC2 System Software to create the Control/Calibrator/specimen template to emulate the order of the specimen rack. See the digene HC2 System Software User Manual for more detailed information.

4. Remove and discard caps from the Negative Control, Positive Calibrator, Quality Controls and specimens to be tested and place tubes in the appropriate specimen Rack as determined below.

Note: Caps removed from the specimen tubes are considered potentially infectious. Dispose of infectious material in accordance with local, State, and Federal regulations.

4a. **The Negative and Positive Calibrators and Quality Controls are required for each rack of specimens to be tested. The Rapid Capture System distributes the Negative Calibrator and Positive Calibrator in triplicate on the first column for each plate of specimens tested.** The Quality Controls and specimens are tested individually.

4b. **For a *digene* Specimen Rack (blue) tested with one Probe**, place the Negative **Calibrator** (NC) in the A1 and Positive Calibrator (PC) in the D1 positions of the rack. Place the Quality Control CT (QC CT) in the G1 and the Quality Control GC (QC GC) in H1 positions of the rack. Controls, Calibrators, and specimens are run in an 8-microwell column configuration. The Rapid Capture System will automatically pipette the Negative **Calibrator** and Positive Calibrator in triplicate from the single tubes in the digene Specimen Rack. (The locations described in the following sentence refer to locations on a Microplate and not a vortexer rack.) The Negative **Calibrator** replicates are in A1, B1, C1; the Positive Calibrator (PC) in D1, E1, F1; QC CT in G1; and QC GC in H1. Place specimens beginning in A2. **See Example 1 rack layout**.

4c. **For a *digene* Specimen Rack (blue) tested with Probe 1 and Probe 2 (Dual-Probe Assay)**, place the Negative **Calibrator** (NC) in the A1, Positive Calibrator 1 (PC1) in the D1, and Positive Calibrator 2 (PC2) in the E1 positions of the digene Specimen Rack. Place the Quality Control CT (QC CT) in the G1 and the Quality Control GC (QC GC) in H1 positions of the digene Specimen Rack. The Rapid Capture System will distribute the Negative Control, Positive Calibrator 1, Quality Controls and specimens tested with probe 1 first. The Rapid Capture System will then distribute the entire rack again using the Negative **Calibrator**, Positive Calibrator 2, Quality Controls and specimens tested with probe 2. (The locations described in the following sentence refer to locations on a Microplate and not a vortexer rack.) The Rapid Capture System will automatically pipette the Negative **Calibrator** and Positive Calibrator in triplicate and Quality Controls once from the single tubes in the digene Specimen Rack, such that the Negative **Calibrator** (NC) replicates are in A1, B1, C1; the Positive Calibrator (PC1 or PC2) in D1, E1, F1; QC CT in G1; and QC GC in H1 and specimens beginning in A2 for each plate to be tested. **See Example 2 rack layout.**

5. Proceed to the Denaturation of the digene HC2 DNA Collection Device specimens, kit Controls and Calibrators section after specimens are placed in the appropriate rack and the plate layouts are created.

> **Note: The *digene HC2* System Software will report Control and Calibrator results based on their location in the plate to verify the assay run. Properly placing Controls and Calibrators in the MST Rack or *digene* Specimen Rack and using the proper assay protocol are essential for valid results.**

EXAMPLE 1: Single-Probe *digene* SPECIMEN RACK (BLUE) LAYOUT FOR THE FIRST 24 MICROWELLS

Row	Column		
	1	2	3
A	NC	Spec. 1	Spec. 9
B		Spec. 2	Spec. 10
C		Spec. 3	Spec. 11
D	PC	Spec. 4	Spec. 12
E	Empty	Spec. 5	Spec. 13
F		Spec. 6	Spec. 14
G	QC CT	Spec. 7	Spec. 15
H	QC GC	Spec. 8	Spec. 16

The digene Specimen Rack is designed to emulate a 96-well plate.

Column 1 of the digene Specimen Rack is designed with five holes for the Negative **Calibrator**, Positive Calibrator, and Quality Controls.

Single-Probe Script can be run using this rack type.

The remaining 11 Columns can accommodate up to 88 specimens.

EXAMPLE 2: Dual-Probe *digene* SPECIMEN RACK (BLUE) LAYOUT FOR THE FIRST 24 MICROWELLS

Row	Column		
	1	2	3
A	NC	Spec. 1	Spec. 9
B		Spec. 2	Spec. 10
C		Spec. 3	Spec. 11
D	PC1	Spec. 4	Spec. 12
E	PC2	Spec. 5	Spec. 13
F		Spec. 6	Spec. 14
G	QC CT	Spec. 7	Spec. 15
H	QC GC	Spec. 8	Spec. 16

The digene Specimen Rack is designed to emulate a 96-well plate.

Column 1 of the digene Specimen Rack is designed with five holes for the Negative **Calibrator**, Positive Calibrator 1 (PC1), Positive Calibrator 2 (PC2) and two Quality Controls.

Dual-Probe Script can be run using this rack type.

The remaining 11 Columns can accommodate up to 88 specimens.

Denaturation of Kit Controls, Calibrators, AND SPECIMENS

Notes:

- **Warning:** Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, and eye/face protection. Use care when handling. Dilute remaining Denaturation Reagent in bottle prior to disposing. Dispose of corrosive material in accordance with local, State, and Federal regulations
- **Important:** Some specimens may contain blood or other biological material that may mask the color changes upon addition of Denaturation Reagent and Probe Mix. Specimens that exhibit a dark color prior to the addition of Denaturation Reagent may not give the proper color changes at these steps. In these cases, failure to exhibit the proper color change will not affect the results of the assay. Proper mixing can be verified by observing the color changes of the Calibrators and Controls
- Do not remove specimen collection device prior to denaturation.
- During the denaturation step, be sure that the water level in the water bath is adequate to immerse the entire volume of specimen in the tube.
- Specimens may be prepared up through the denaturation step and stored at 2-8°C overnight or at -20°C for up to 3 months. A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. Mix well before using.
- To avoid false-positive results, it is critical that all Control, Calibrator, and specimen material come into contact with Denaturation Reagent. Mixing after Denaturation Reagent addition is a critical step. **Make sure the MST Vortexer 2 is set on 100 (maximum speed) setting and that the Pulser Button is OFF.**
- Following denaturation and incubation, the specimens should no longer be considered infectious. However, lab personnel should still adhere to universal precautions.

1. Pipette Denaturation Reagent with Indicator Dye into each Control, Calibrator, or specimen using a repeating or adjustable pipettor. Take care not to touch the sides of the tube, as cross-contamination of specimens could occur. The volume of Denaturation Reagent needed is equivalent to half the sample volume. The exact volume for each type of Control, Calibrator, and specimen is listed in the table below.

Control, Calibrator, or Specimen	Volume of Denaturation Reagent Required
Negative Calibrator, 2 ml	1000 µl
Positive Calibrator and Quality Controls, 1 ml	500 µl
Cervical Specimen, 1 ml	500 µl

Note: Users must prepare Negative **Calibrator**, Positive Calibrator, and Quality Controls fresh for each run. Users may prepare Controls and Calibrators up through the denaturation step and stored at 2-8°C overnight, **but may not be frozen**. The entire volume of the Negative **Calibrator**, Positive Calibrator, and Quality Controls must be denatured for testing with the Rapid Capture System Application.

2. Mix the specimens using the MST Vortexer 2.

   2a. Cover the Control/Calibrator/Specimen tubes with DuraSeal® film by pulling the film over the tubes in the rack.

   2b. Place the rack cover over the film-covered tubes and lock into place with the two side clips. Cut the film with the cutter device.

   2c. Place the rack on the MST Vortexer 2 in the correct orientation and secure the rack with the clamp. Verify speed setting is at 100 (maximum speed), and turn the vortexer power switch to the ON position. Vortex the tubes for 10 seconds. The Controls, Calibrators, and specimens should turn purple.

3. Incubate the tubes in each rack in a 65°C ± 2°C water bath for 45 ± 5 minutes (denatured Controls, Calibrators, and specimens may be tested immediately, or stored as described in Notes above).

4. Prepare the reagents and set up the Rapid Capture System deck during the specimen denaturation incubation.

Setup of the Rapid Capture System Deck

Flush with deionized or distilled water before first use each day by running the "FLUSH" script after initializing the system. Failure to complete a system flush may result in improper aliquot volumes being dispensed.

Notes:

- Wear disposable, powder-free gloves during setup.
- Reference the Rapid Capture ScriptSelect Application Procedure section of this user manual to aid in choosing the correct script for the specific RCS Run. The ScriptSelect Software allows the user to select the proper script and add it to the RCS Run list.
- Use the RCS ScriptSelect printout after script selection to aid in deck setup.

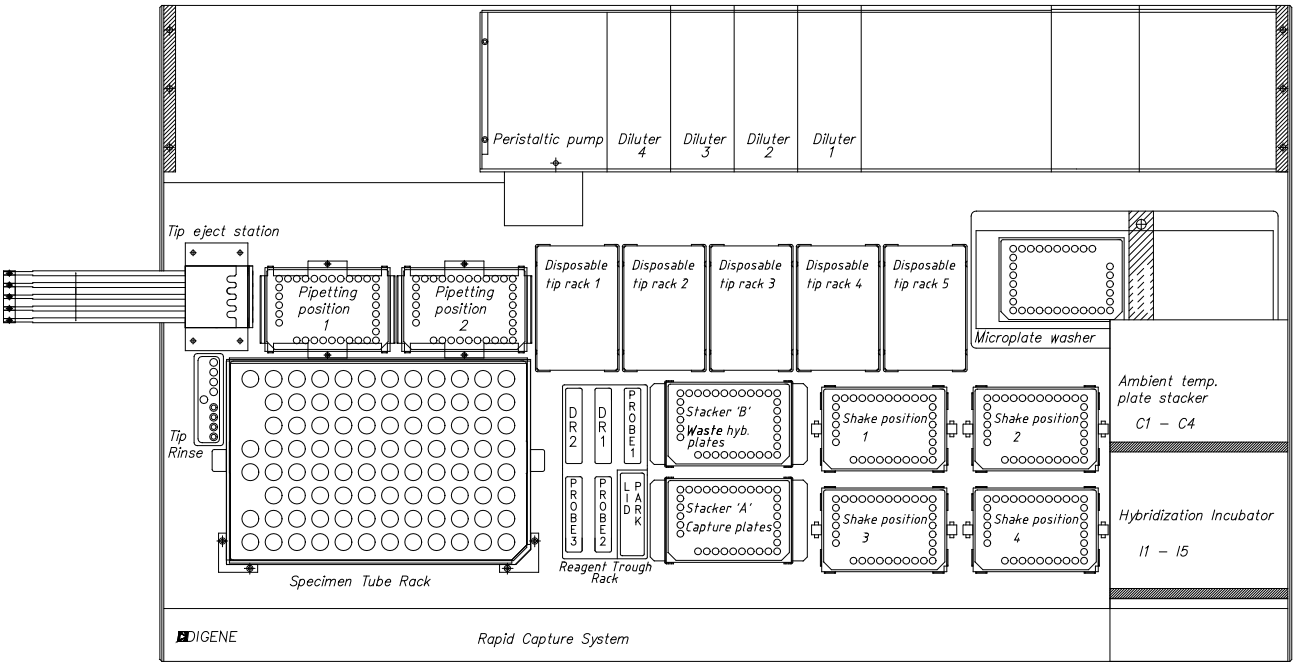
Each rack of 88 specimens to be tested with *digene* HC2 DNA Test will require:

- One digene HC2 DNA Test kit
- One hybridization plate
- One plate lid
- 204 disposable tips (two racks and 12 tips)
- One additional plate lid will be required for each run, regardless of the number of specimen racks to be tested.

Testing a rack of 88 specimen with a Dual-Probe Assay will require:

- Two digene HC2 DNA Test kits
- Two hybridization plates
- Two plate lids
- 408 disposable tips (4 racks and 24 tips)
- One additional plate lid will be required for each run, regardless of the number of specimen racks to be tested.

Figure 1: RCS Deck Layout



Deck Preparation

1. Inspect the deck, including all stackers and incubators, and remove any plates, lids, or other miscellaneous items. If the preceding run was aborted, inspect the 65ºC incubator by manually opening each chamber door using a disposable pipette tip. If plates are present, contact your local QIAGEN Technical Services Representative for instructions. **Failure to remove all such items may result in an instrument crash that can damage the instrument.**

    **Caution**: The Hybridization Incubator reaches a set temperature of 65ºC.

2. Wearing powder-free disposable gloves, fill all 5 disposable tip (DT) rack holders with disposable tip racks. **When loading the tips, the "u-shaped" notch of the rack must be positioned in the front left of the holder**. The rack should snap into place. If it does not, remove the rack of tips and pull the center tabs on the front and back ends of the holder toward the center to increase the tension on the rack. Replace the tip rack. Failure to load disposable tip racks will result in an audible alarm and a dialog box will appear indicating the requirement to load tips.

3. Label the front side of the Hybridization (hyb) Plates as 1 through 4. Place a lid on each plate.

   Note: If using multiple probes for the RCS run, it is recommended to number the Hybridization Plates and Lids and label them with the probe type to be distributed on the plate.

4. Place the Hybridization Plates with lids on the Shakers in the corresponding labeled positions, S1 – S4. Ensure that the plates are correctly oriented and **seated within the guides** (see RCS Deck Layout, Figure 1).

5. Label the front side of the Capture Plates with numbers 1 through 4 to correspond with hybridization plates. If any plate has fewer than 88 specimens, remove the appropriate number of capture strips or wells from the plate, return them to their original Mylar^{Ò} bag, and store at 2-8ºC. Replace **all** missing wells in the capture plate with RCS Microplate Well Strips.

   Note: If using multiple probes for the RCS run, it is recommended to number the Capture Plates and label them with the probe type to be distributed on the plate.

6. Stack the capture plates in numerical order, with plate number 1 on top. Ensure that each plate is correctly oriented with the A1 well position in the back left corner. Place a lid on Plate 1 **only,** and set the plates in Stacker A (see RCS Deck Layout, Figure 1).

   CAUTION: Gripper Arm Crash Hazard - If the correct number of hybridization and capture plates are not loaded on the RCS when the instrument attempts to retrieve them from the Shaker or Stacker A, a system interruption or error may generate. This may require the run to be restarted and/or may damage the instrument.

7. Empty the Liquid Waste bottle, if necessary.

   Note: Ensure that the waste container is empty before starting each run! The waste container may overflow onto the deck causing flooding and alkaline phosphatase contamination. Always change gloves after handling the liquid waste bottle or any possible contact with the waste solution, including contact with the quick-disconnect fittings, to prevent contamination of work areas with the alkaline phosphatase present in the waste solution.

8. If not already labeled from a previous run, label reagent troughs and lids as required for the RCS Script Run: Probe 1, Probe 2, Probe 3, Detection Reagent 1, and Detection Reagent 2 accordingly. It is important to label the troughs and segregate reagents to prevent possible contamination of reagents from run to run. Once labeled, do not use reagent troughs with other reagents. It is recommended to maintain two sets of reagent troughs so that a clean dry set is always available.

Reagent Preparation

1. Fill the Wash Bottle with 1x Wash Buffer with required volume (See Reagent Preparation and Storage section for the appropriate digene HC2 DNA Test run on the Rapid Capture System). Ensure that the quick-release valve clicks securely in place.

> **CAUTION:** Ensure the Wash Bottle is adequately filled before each run with a minimum of 6 L for > 2 plates or 3 L for £ 2 plate.

2. Empty the System Liquid Bottle and refill with fresh deionized or distilled water. Ensure that the quick-release valve clicks securely in place.

> **CAUTION:** Ensure the System Liquid Bottle is adequately filled before each run with a minimum of 1L.

3. Add the required volume of Detection Reagent 2 to the designated reagent trough and place in the back left well of the Reagent Trough Rack. Cover the trough using the corresponding lid (see Reagent Preparation and storage section and RCS Deck Layout, Figure 1).
4. Add the required volume of Detection Reagent 1 to the designated reagent trough and place in the back center well of the Reagent Trough Rack. Cover the trough using the corresponding lid (see Reagent Preparation and Storage section and RCS Deck Layout, Figure 1).
5. Add the prepared Probe Mix to the designated probe reagent trough(s), and place the trough(s) in the appropriate position(s) in the Reagent Trough Rack. Cover the trough(s) using the corresponding lid.

> **Note**:
>
> See Figure 1: RCS Deck Layout or reference the printout from the RCS ScriptSelect Software for the proper positioning of the probes for the specific RCS run.
>
> The following rules apply for the proper placement of the Probe Mixture:
>
> Single-Probe Assay indicates one digene HC2 DNA Test result will be generated for specimens. A Single-Probe RCS run requires the Probe Mixture to be placed in the trough position labeled Probe 1 on the RCS deck.
>
> Dual-Probe assay indicates two digene HC2 DNA Test results will be generated for each specimen; one rack of specimens is distributed to two different plates for testing with two digene HC2 DNA Tests. For a Dual-Probe RCS run, the probe to be tested with the Positive Calibrator 1 is placed in the trough position labeled Probe 1, and the probe to be tested with the Positive Calibrator 2 is placed in the trough position labeled Probe 2. Plates associated with a Dual-Probe assay are always the first to be distributed. Therefore always load the Rack to be used for the Dual-Probe assay first on the RCS deck.
>
> Two-Probe Assay indicates one digene HC2 DNA Test result will be generated for each rack of specimens. For a Two-Probe RCS run, the trough position labeled Probe 1 contains the probe to be distributed on the first plate(s) designated by the script selected by the user. The trough position Probe 2 contains the probe to be distributed on the remaining plate(s).

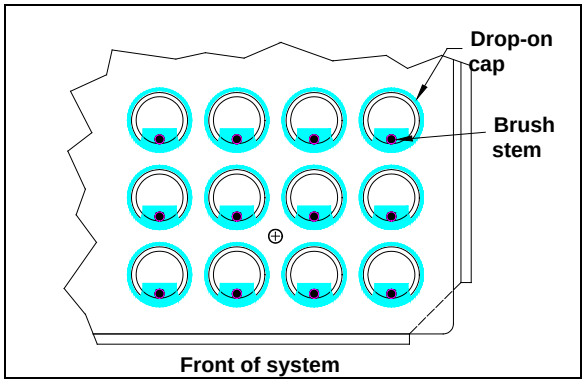
> **Note:** The RCS employs liquid-level sensing when dispensing reagents from the troughs to a plate. In the event of insufficient (or no) volume, the system will pause, display a dialog box indicating the problem, and signal the user with an audible alarm. The user can then place the filled reagent trough on the deck or add additional reagent, as appropriate.

6. When the specimens have completed the 45-minute denaturation incubation, retrieve the racks from the water bath and drain excess water onto paper towels.

> **Note:** **DO NOT** allow specimen racks to cool to room temperature before removing the lid. If cooling occurs, tubes may stick to the lid and subsequently spill.

7. Immediately place the labeled Rack 1 on the MST Vortexer 2 and vortex for a minimum of 10 seconds with motor speed of 100 (maximum speed).
8. Immediately place the rack on the bench top and release the latches. Lift the rack lid ~1 cm and move it gently left and right to release any specimen tubes that may have adhered to the DuraSeal Film. Remove the lid by lifting it straight up until it clears the rack base.
9. Carefully peel the DuraSeal Film from the lid and discard.
10. Repeat steps 7-9 for the remaining specimen racks.
11. Orient the rack so that the Negative **Calibrator** is in the upper left corner. Place a drop-on cap onto each tube containing a brush or swab in Specimen Rack 1. Ensure that the shaft of the collection device is trapped between the tab of the drop-on cap and the side of the specimen tube. The drop-on caps must be oriented so the tab is closest to the user facing the rack (Figure 2).

Figure 2. Orientation of Drop-On Caps



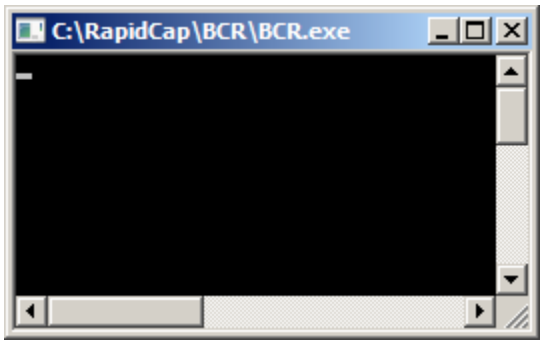
Starting the Rapid Capture System Run

CAUTION: Do not attempt to reach into the instrument while gripper arms are moving. Pause the instrument by pressing the **Esc** key or clicking the **Abort Run** icon and wait for a display dialog box to appear before readjusting or repositioning plates.

Rapid Capture System Run Example 1: 1Ddu Script

Note: The Script 1Ddu is a Dual-Probe assay and is a practical example of testing specimens on two digene HC2 DNA Tests. The Rapid Capture System user selects this Script when testing one rack of cervical specimens with the digene HC2 CT-ID DNA Test and digene HC2 GC-ID DNA Test using the digene HC2 CT-GC Dual ID kit.

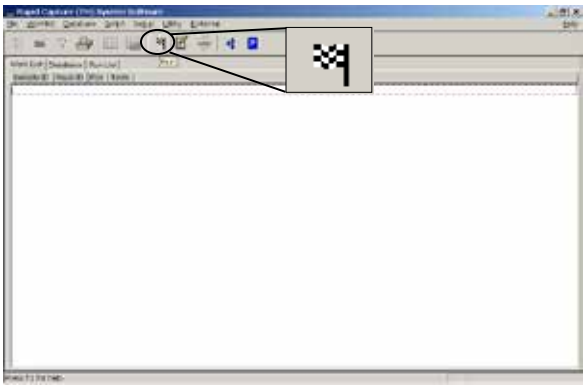
Note: The barcode upgrade includes an application that saves the scanned barcodes for use by the digene HC2 System Software. While the barcode scanning application is running, a command window will be displayed. Do not close the command window. The window will close automatically after the barcode is saved. If the command window is closed by the user, then the scanned barcode will not be saved.



The barcode upgrade includes functionality to ensure that the scanned capture plate corresponds to the correct capture plate. However, it is important that users do not switch the sequence of plates on the RCS (for example, during error recovery) to ensure that the association of the capture plate and hybridization plate are correct. Incorrect plate association could lead to incorrect results

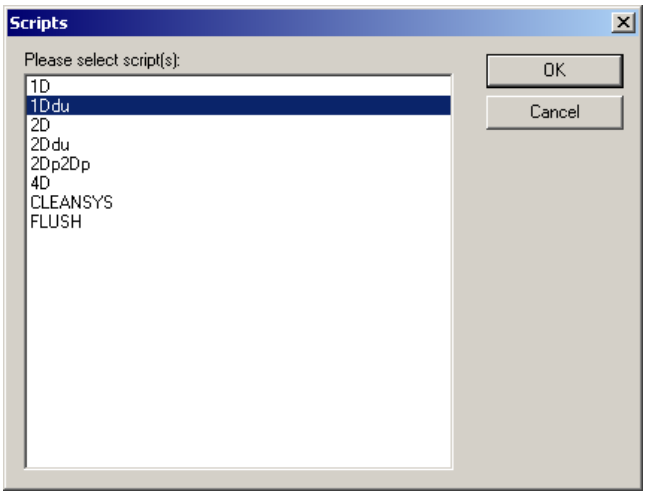
1. Use RCS ScriptSelect to choose appropriate script.

From the Rapid Capture System Software main menu click on the Flag icon.



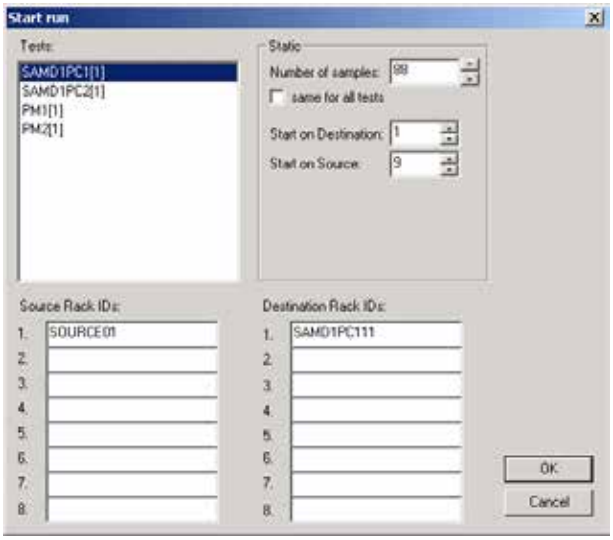
2. The **Script** dialog box appears, listing the scripts added to the Rapid Capture System Run list via the RCS ScriptSelect Software. Select the appropriate script for the Rapid Capture System run. The example given below shows the selection of a **1Ddu** Script.

3. Highlight **1Ddu**. Click **OK.**



> **Note:** The Script **1Ddu** requires 1 digene Specimen Rack (blue) and two Probes placed in the Probe 1 and Probe 2 positions. It cannot be used for a Dual-Probe Script denoted by the abbreviation "**du**." Reference the RCS ScriptSelect Application Procedure section of this user manual for more details of script terminology

4. A window titled **Start run** will appear.



5. The **Start run** window provides an option for entering the number of specimens per plate. In the **Static** box of the **Start Run** window the default specimen number for a full plate is 88 specimens. The **SAMD1PC(1)** test determines the number of specimens to be transferred from the specimen rack to the hybridization plate. The **PM1(1)** determines the number of wells designated to receive reagents and includes the Calibrators and

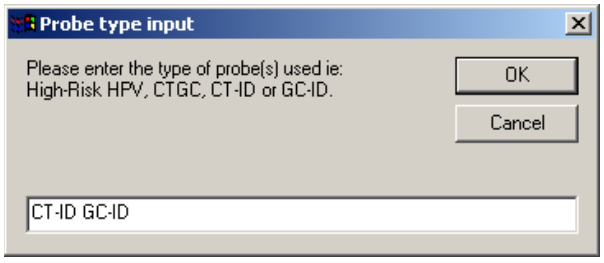
Controls. This is necessary only if a partial plate (fewer than 88 specimens) is included in the run. For this example, 88 specimens are being tested and the default settings apply.

See Rapid Capture System Run Example 2: 3Dp1Dp Script, Steps 6-11 for details.

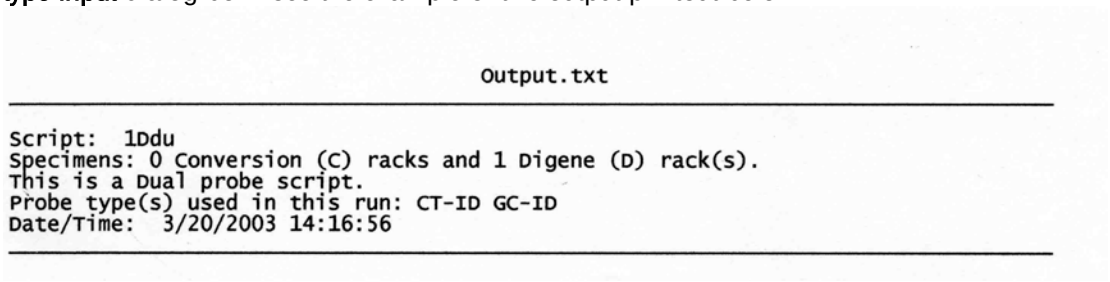
> **Caution:** Under no circumstances should the box "same for all tests" be checked while running any digene HC2 DNA Test. Checking this box can lead to the improper amount of reagent added to some patient specimens.

6. Click **OK** to begin the script.

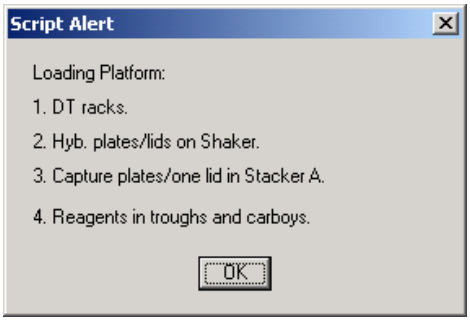
7. The **Probe type input** dialog box appears. Type in the probe types to be utilized on the Rapid Capture System run. For this example the CT and GC Probes are to be utilized on the Rapid Capture System run. Type ***CT-ID GC-ID*** in the dialog box. Click **OK.**



8. The Rapid Capture System software automatically prints the selected script and Probe types entered in the **Probe type input** dialog box. See the example of this output printout below:



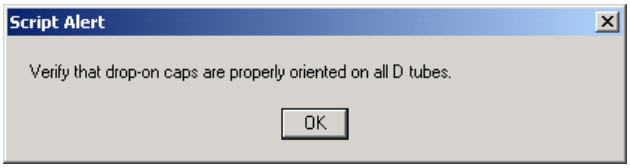
9. All onboard components will initialize, and a window will appear reminding the user of the required deck preparation.



> **Note:** For a 1 Ddu Rapid Capture System Run, the Probe for plate 1 is placed in Trough Probe Position 1 and corresponds to Positive Calibrator 1. The Probe to be distributed on plate 2 is placed in Trough Probe Position 2 and corresponds to Positive Calibrator 2.

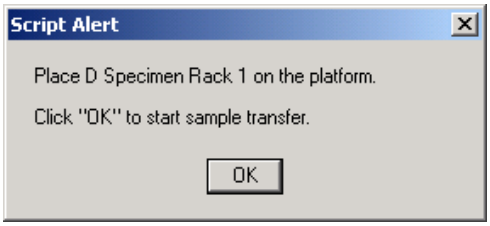
10. Click **OK** after verifying the Rapid Capture System platform is set up properly according to the printout. The Rapid Capture System primes and flushes the lines with system liquid.

1. Another dialog box will then appear reminding the user to verify that drop-on caps have been placed on the digene specimen tube types.



11. Click **OK** following the addition of drop-on caps on all specimens.

12. Place digene (D) Specimen Rack 1 on the deck, placing it so the notched corner of the rack is front and right and the base is positioned within the rack guides on the deck.

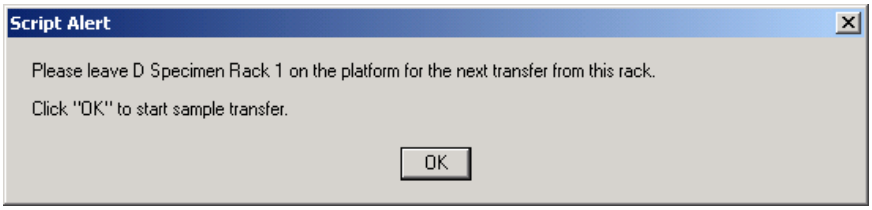


13. Click **OK.**

14. Once rack 1 specimens have been transferred, the screen will display another alert window directing the user to verify that all specimens have been transferred. **After removing the hybridization plate from the deck, visually inspect the hybridization plate for any empty wells that should have received specimen.** Any specimens that failed to transfer must be manually transferred using a single-channel pipettor (20-200 µl) and extra-long pipette tips. The transfer volume is 75 µl. The position of the well in the plate directly corresponds to the position of the specimen tube in the rack. The Specimen Rack may be removed from the deck to facilitate a manual transfer. **However, before continuing the run, it is critical that the plate and Rack be properly situated when returning to the pipetting position.**

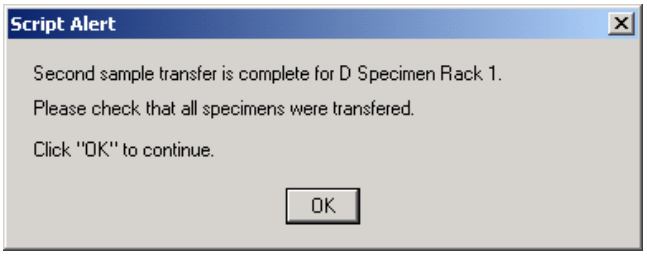
15. Click **OK.**

16. For a Dual-Probe assay the same digene Specimen Rack is used to transfer specimens to plate 2. The digene Specimen Rack is returned to the deck position.



17. Click **OK** to begin sample transfer.

18. An alert box will appear to instruct the user the second sample transfer is complete. Remove the digene Specimen Rack and plate, and verify that all specimens were transferred. If any specimens were not transferred, refer to step 13.



19. After the last rack of specimens has been transferred and been checked, a window will appear reminding the user to refill the DT racks. Replace the plate.

20. 22. At this time, refill all empty and partially empty disposable tip rack holders with full racks of tips. Empty the disposable tip waste container. It is important to follow the instructions in the Script Alert boxes before clicking **OK.** The operating software will control the timing of the RCS once the Probe Mix addition step begins. Any user interruptions after that point will interfere with assay incubation times.

21. Click **OK** and the Rapid Capture System will complete all subsequent steps of the assay through Detection Reagent 2 incubation, providing 3.5 hours of user-free run time. Set a timer for 3 hours and 20 minutes to ensure returning to the instrument in time to read the first plate.

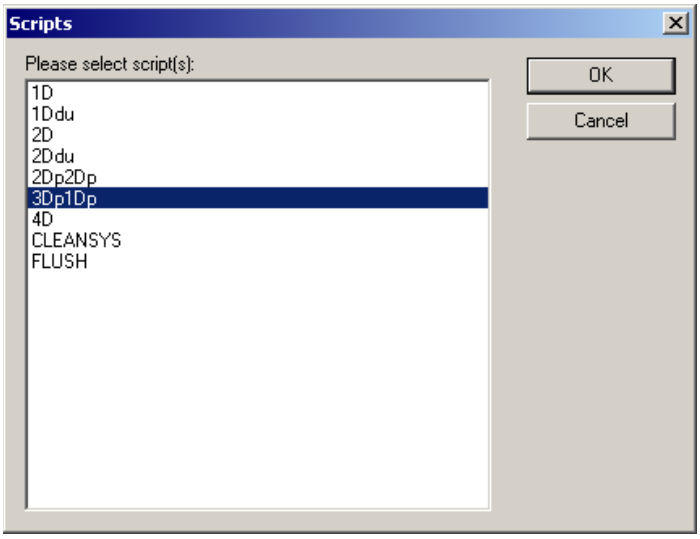
> **Notes:**
>
> · The Rapid Capture System software monitors the temperature of the incubator chambers. Probe mix addition will not begin until the set temperature of 65°C is achieved. At that time, the script will continue automatically, with no user intervention required.
>
> · Use caution in employing a fully automated, walk-away approach. If an instrument error occurs, the Rapid Capture System will sound an alarm, pause, and wait for user input, which may invalidate any timing sequences currently in progress.

22. In the event of a system interruption, the instrument will stop by itself and sound an alarm. An error message will be displayed. Immediately consult with your local QIAGEN Technical Services Representative for proper instructions.

Rapid Capture System Run Example 2: 3Dp1Dp Script

1. Use the RCS ScriptSelect Software to choose appropriate script.
2. From the Rapid Capture System Software Main Menu, click on the flag icon. 

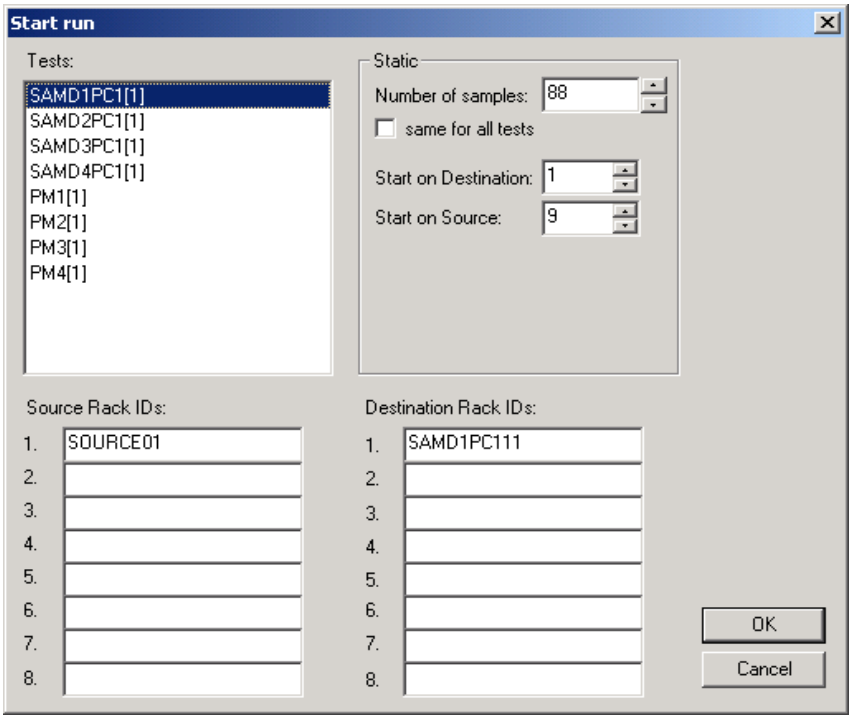
3. The **Scripts** dialog box appears, listing the scripts added to the Rapid Capture System Run list via the RCS ScriptSelect Software. Select the appropriate script for the Rapid Capture System run. The example given below shows the selection of a 3Dp1Dp Script.



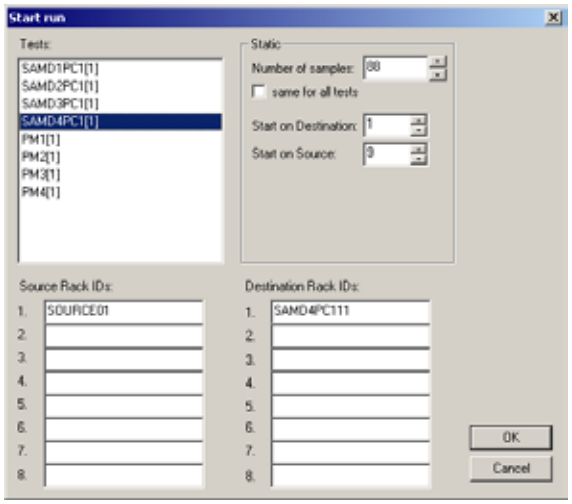
4. Highlight **3Dp1Dp**.

5. Click **OK**.

6. A window titled **Start run** appears.

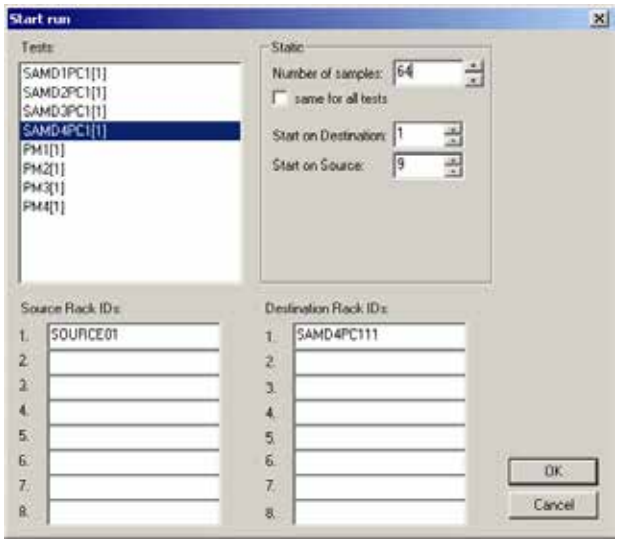


7. In the **Tests** list box, click on the **SAMD4PC1(1)** test corresponding to the partial plate number 4 for this specific example.

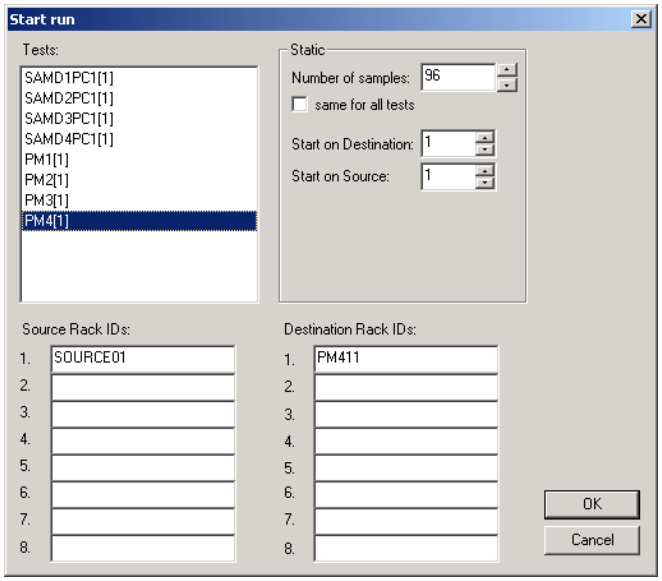


8. In the **Static Numbers of Samples** box, enter the number of specimens, **not** including Calibrators or Controls, to be run on the partial plate. The **SAMDPC** test determines the number of specimens to be transferred from the rack to the hybridization plate. The default number of samples for the **SAMD4PC1(1)** is 88.

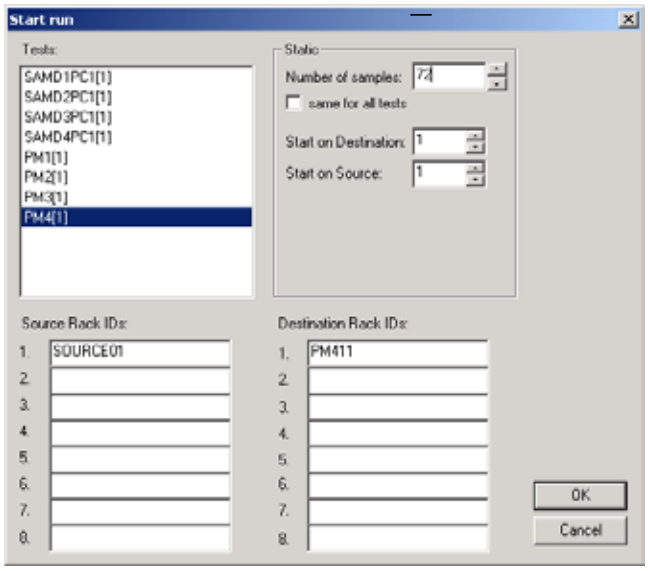
9. For this example, the last plate of a four-plate run has 64 specimens. Highlight test **SAMD4PC1(1)** then enter **64** for **Number of samples**. It is critical that the correct specimen number be entered for the appropriate plate. Entering a number that is less than the correct value will result in specimens not being transferred from the specimen collection tube. This may result in invalid assays and instrument failure due to the formation of precipitation that can clog the cannulas of the wash head. Entering a specimen number greater than the correct value is of less consequence, as it will only result in a longer than necessary time to transfer the rack; assay results and instrument performance will not be impacted.



10. Next, in the **Tests** box, click on the **PM4(1)** test corresponding to the partial plate number 4 for this specific example. The number of samples defaults to a full plate of 96. When running less than a full plate of specimens it is critical to type in the specific number of required wells for the Rapid Capture System run.



11. In the **Static** box, enter the number of specimens **plus 8** (for Calibrators and Controls). The **PM** test determines the number of wells to receive assay reagents. For the example above, highlight **PM4(1)**, then enter **72** for **Number of Samples**. It is critical that the correct specimen number be entered for the appropriate plate. Entering a specimen number that is less than the correct value will result in specimen wells not processed by the instrument. Entering a specimen number greater than the correct value can cause invalid assays and instrument failure due to the formation of precipitation that can clog the cannulas of the wash head.

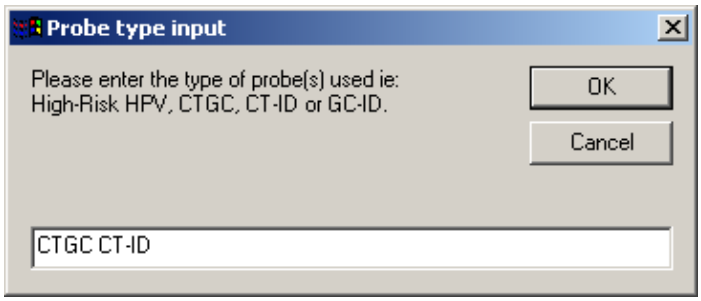


Caution: ⚠	Under no circumstances should the box “same for all tests” be checked while running any digene HC2 DNA Test. Checking this box can lead to the improper amount of reagent added to some patient specimens.

12. Click **OK** to begin the script.

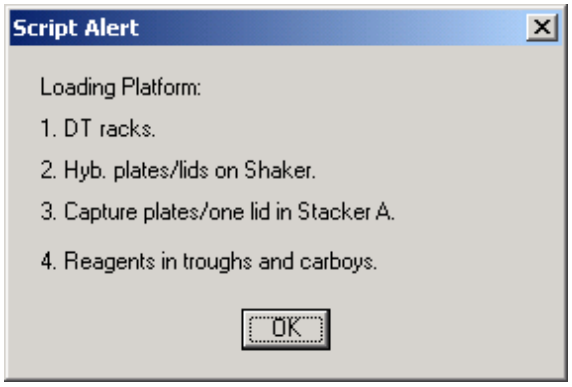
13. The **Probe type input** dialog box appears.

14. Type in the probe types to be utilized on the Rapid Capture System run. For this example CT/GC and CT Probes are to be tested. Type CTGC CT-ID. Click **OK**.



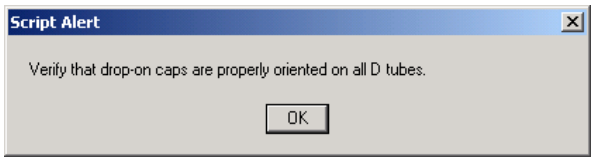
15. The Rapid Capture System software automatically prints the selected script and Probe types entered in the **Probe type input** dialog box. Retain for Rapid Capture System records.

16. All onboard components will initialize, and a window will appear reminding the user of the required deck preparation.



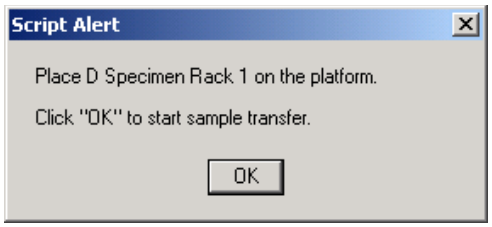
17. Click **OK** to allow the system liquid lines to prime and flush.

18. Another dialog box will then appear reminding the user to verify that drop-on caps have been placed on Rack 1 specimens.



19. Click **OK.**

20. Place Rack 1 on the deck, placing it so the notched corner of the rack is front and right and the base is positioned within the rack guides on the deck.



21. Click **OK** to begin specimen transfer.

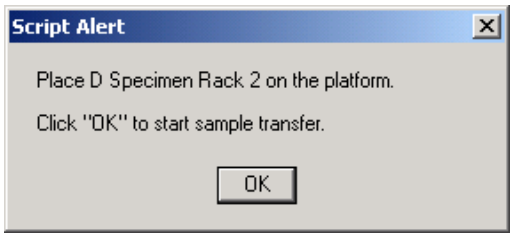
22. Add drop-on caps to remaining specimen racks during this time.

> **HINT:** For most efficient workflow, begin transfer of Rack 1 before placing drop-on caps on the remaining specimen racks.

23. Once rack 1 specimens have been transferred, the screen will display another alert window directing the user to verify that all specimens have been transferred. **After removing the rack and hybridization plate from the deck, visually inspect the hybridization plate for any empty wells that should have received specimen.** Any specimens that failed to transfer must be manually transferred using a single-channel pipettor (20-200 µl) and extra-long pipette tips. The transfer volume is 75 µl. The position of the well in the plate directly corresponds to the position of the specimen tube in the rack. The rack plate is removed from the deck to facilitate a manual transfer. **However, before continuing the run, it is critical that the plate be properly situated when returned to the pipetting position.**

24. Click **OK**. Another alert window will appear reminding the user to ensure that drop-on caps have been placed on Rack 2.

25. Place Rack 2 on the deck and click **OK**.



26. Repeat steps 15 through 25 until samples on all racks have been transferred.

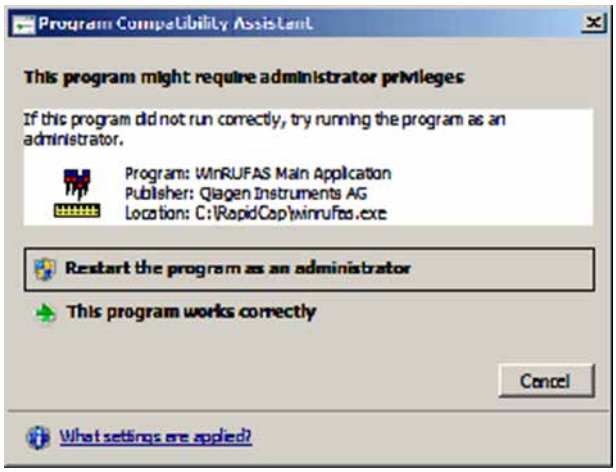
27. After the last rack of specimens has been transferred and been checked, a window will appear reminding the user to refill the DT racks.

28. At this time, refill all empty and partially empty disposable tip rack holders with full racks of tips. Empty the disposable tip waste container. It is important that these steps be completed before clicking **OK**. The operating software will control the timing of the Rapid Capture System once the next step, Probe Mix addition, begins. Any user interruptions after that point will interfere with assay incubation times.

29. Click **OK** and the Rapid Capture System will complete all subsequent steps of the assay through Detection Reagent 2 incubation, providing 3.5 hours of user-free run time. Set a timer for 3 hours and 20 minutes to ensure returning to the instrument in time to read the first plate.

> **Notes:**
> - The RCS software monitors the temperature of the Hybridization Incubator chambers. Addition of Probe will not begin until the set temperature of 65°C is achieved. At that time, the script will continue automatically, with no user intervention required.
>
> - If an instrument error occurs, the Rapid Capture System will sound an alarm, pause, and wait for user input. **Therefore, it is recommended that the user remain within hearing distance of the instrument during the run.** If an error occurs, immediately consult with your local QIAGEN Technical Services Representative for instructions.

30. In the event of a system interruption, the instrument will stop by itself and sound an alarm. An error message will be displayed. Consult with your local QIAGEN Technical Services Representative for proper instructions immediately. When exiting the RCS software after running a script, the Windows Compatibility Assistant may display. The RCS has been validated for use with Windows 7. This dialog can be closed without issue.



Reading the Microplates and Generation OF Results

Note:	The QIAGEN-approved luminometer must be turned on at least one hour prior to reading the first plate. It is recommended that the QIAGEN-approved luminometer be left on at all times. The user is required to retrieve the Microplates from the Rapid Capture System deck at the end of the Detection Reagent 2 incubation for each plate. Each plate is then placed in the QIAGEN-approved luminometer for result generation.

1. When plate 1 has completed its Detection Reagent 2 incubation and is ready for signal detection using the QIAGEN-approved luminometer, the Rapid Capture System will beep and a **Script Alert** window reading **Assay is completed. Read plate in luminometer** will appear.

1. Retrieve the plate from the deck.
2. Click **OK** to allow the RCS to continue processing the remaining plates.
3. Place the plate in the QIAGEN-approved luminometer and read. Refer to the applicable luminometer user manual and digene HC2 System Software User Manual for details regarding measuring a plate and generating result reports.
4. Repeat steps 1 - 4 above for all remaining plates.
5. Refer to the digene HC2 CT/GC DNA Test, digene HC2 CT-ID DNA Test, or digene HC2 GC-ID DNA Test IFU for quality control, assay verification, and instructions for interpretation of results.

Note:	Printing result reports, can, in some situations, cause a slowdown of the RCS that may affect assay timing. It is recommended that results from one plate be printed before results from subsequent plates are read in order to avoid this situation. Alternatively, all plates may be read but results should not be printed until the RCS run is completed.

Daily/System Cleanup

1. Discard the hybridization plates in Stacker B and the plate lid in Stacker A.

2. Clean reagent troughs and lids as follows:

   1j. Troughs: Wash and rinse with deionized or distilled water, and fill completely with sodium hypochlorite solution, 0.5% v/v. Allow the troughs to soak in the sodium hypochlorite solution overnight. The next day, rinse troughs thoroughly with deionized or distilled water for at least 60 seconds. Place inverted troughs on a paper towel to dry. Replace reagent troughs monthly.

   1k. Lids: Wash and rinse with deionized or distilled water, and soak overnight in sodium hypochlorite solution, 0.5% v/v. The next day, rinse thoroughly with deionized or distilled water for at least 60 seconds. Place on a paper towel to air dry. Reagent trough lids are not disposable and need not be replaced unless damaged or lost.

   NOTE: If a second Rapid Capture System run immediately follows the first run, it is recommended to use a second set of troughs and trough lids.

3. Discard the capture plates after reading and assay verification.

4. If the instrument will not be used the next calendar day, cover tip rack holders containing unused tips with a plate lid.

5. Empty the disposable tips waste container into an appropriate container.

6. **Empty the liquid waste container**. Rapid Capture System liquid waste has a relatively neutral pH. Dispose according to local, State, and Federal requirements.

7. Ensure that the quick-release fittings click securely in place when reconnecting the fittings to the waste container. Also, ensure that the bottle is located correctly with no kinks in the lines.

8. Wipe down the following surfaces with an alcohol-dampened soft cloth or low-lint paper towel:

   8a. Shaker platform and rollers.
   8b. Tip Eject Station.
   8c. Tip Eject Station Drip Guard (Guard must be removed and rinsed with deionized or distilled water).
   8d. Tip Rinse Station. Remove plastic cover and rinse with deionized or distilled water.
   8e. Trough Rack.
   8f. Inside of Stacker A and B.
   8g. Pipetting Positions 1 and 2.
   8h. All other deck surfaces.

9. Clean each pipette adapter with an alcohol wipe.

10. Remove Platewasher Boat and clean the Washer Platform and the top and bottom of the Washer Boat with an alcohol dampened soft cloth or low-lint paper towel.

Note:	**To prevent contamination of work areas with the alkaline phosphatase present in the waste solution, always change gloves after any possible contact with the waste solution including contact with the quick-disconnect fittings.**
Note:	**Reference the Routine Maintenance and System Shut Down sections of the Rapid Capture System User Manual.**

Limitations of the Procedure

1. Failure to visually observe the hybridization plate to ensure proper specimen transfer and failure to correct for any improperly pipetted specimens may result in false-negative results.
2. Refer to the digene HC2 CT/GC, CT-ID, and GC-ID DNA Test IFUs for additional limitations specific to the test method.

Expected Results

Refer to the digene HC2 CT/GC, CT-ID, or GC-ID DNA Test IFUs for expected results.

Performance Characteristics

Precision

A study was performed to determine the precision of the Rapid Capture System Application of the digene HC2 CT/GC, CT-ID, and GC-ID DNA Tests. Six-member simulated clinical specimen panels consisting of cultured epithelial cells suspended in Specimen Transport Medium (STM) were used for the precision evaluation. Separate panels were prepared for use with the digene HC2 CT/GC, CT-ID, and GC-ID DNA Tests, each consisted of two negative specimens, two low-positive specimens, and two midlevel-positive specimens, all containing a brush collection device. Each panel was tested in triplicate, two panels per plate, two plates per Rapid Capture System instrument, on two instruments, over the course of 5 days. To assess comparative precision performance, the manual method was performed using the same denatured sample panels on the same days following the same testing format. Two technologists, one per Rapid Capture System instrument, performed both the Rapid Capture System Application as well as the manual method.

The total precision results for the digene HC2 CT/GC, CT-ID, and GC-ID DNA Tests using the Rapid Capture System Application compiled for all 5 days of testing are presented in **Table 1**. This precision study demonstrated that the results generated using the Rapid Capture System method were equivalent to the corresponding results generated by the manual method. Although not evident from these tables, the qualitative interpretation of results was 100% in agreement with the expected result, when using an assay cutoff of 1.0.

Table 1
Total Precision for *digene* HC2 CT/GC, CT-ID, and GC-ID DNA Tests Using the Rapid Capture System Application Compiled for All 5 Days of Testing (n=120)

		CT/GC			
Panel Number	n	Mean RLU/CO	SD	%CV	Mean ± 2 SD
1	120	0.18	0.0675	38.44	0.04-0.31
2	120	0.17	0.0588	34.95	0.05-0.29
3	120	3.65	0.7690	21.04	2.12-5.19
4	120	3.93	0.6927	17.61	2.55-5.32
5	120	14.63	3.3627	22.98	7.91-21.36
6	120	17.91	3.2523	18.16	11.41-24.42

Panel Number	n	CT-ID Mean RLU/CO	SD	%CV	Mean ± 2 SD	GC-ID Mean RLU/CO	SD	%CV	Mean ± 2 SD
1	120	0.13	0.0213	16.35	0.09-0.17	0.12	0.0279	23.33	0.06-0.18
2	120	0.14	0.0240	17.63	0.09-0.18	0.12	0.0221	18.20	0.08-0.17
3	120	3.29	0.7237	21.97	1.85-4.74	3.13	0.5695	18.22	1.99-4.27
4	120	4.07	0.5814	14.27	2.91-5.24	3.93	0.6556	16.69	2.62-5.24
5	120	12.62	1.8652	14.78	8.89-16.35	14.05	3.2549	23.16	7.54-20.56
6	120	12.32	1.9653	15.95	8.39-16.25	13.14	3.3582	25.55	6.43-19.86

It is required that each laboratory evaluate the precision of the Rapid Capture System Application prior to routine clinical use of the digene HC2 CT/GC, CT-ID, or GC-ID DNA Test. To accomplish this, it is recommended operators use a sample panel, which enables each user to establish precision once the Rapid Capture System is properly installed.

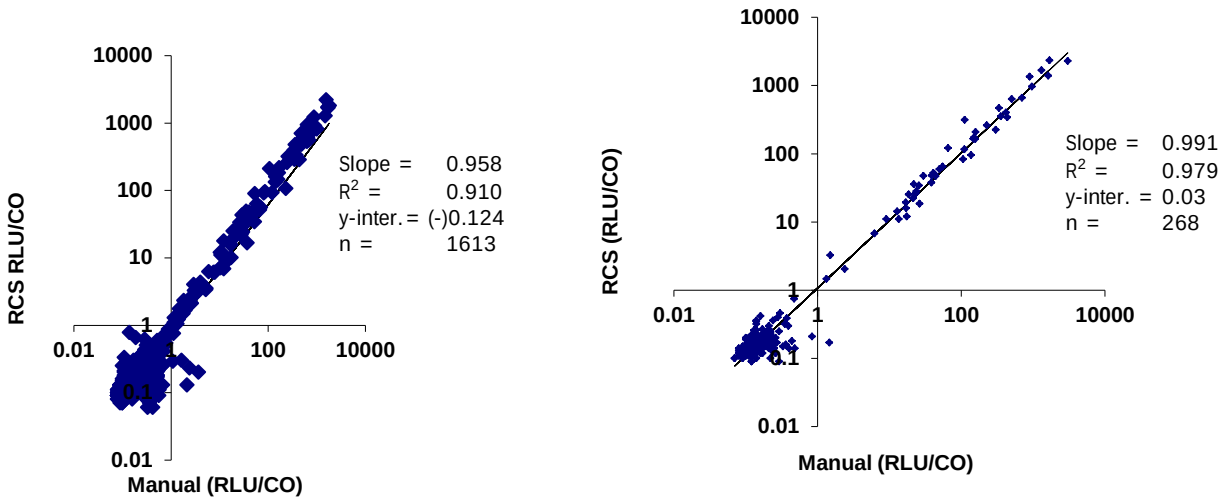
Rapid Capture System and Manual Methods Comparative Clinical Performance

Cervical specimens were tested using the digene HC2 CT/GC, CT-ID and GC-ID DNA Tests using both the Rapid Capture System application and the manual methods. The results generated for 1613 archived cervical specimens collected from a geographically and clinically diverse population are shown by the left side of **Figures 3, 4,** and **5** for testing with digene HC2 CT/GC, CT-ID, and GC-ID DNA Tests, respectively. The results generated for 268 prospective cervical specimens collected at one external site in the United States are shown by the right side of **Figures 3, 4, and 5** for each test, respectively.

Figure 3

Scatterplots of Rapid Capture System Application and Manual Method Initial digene HC2 CT/GC DNA Test Results Archived Cervical Specimens (n = 1613; left).

Prospectively Collected Cervical Specimens (n = 268; right)

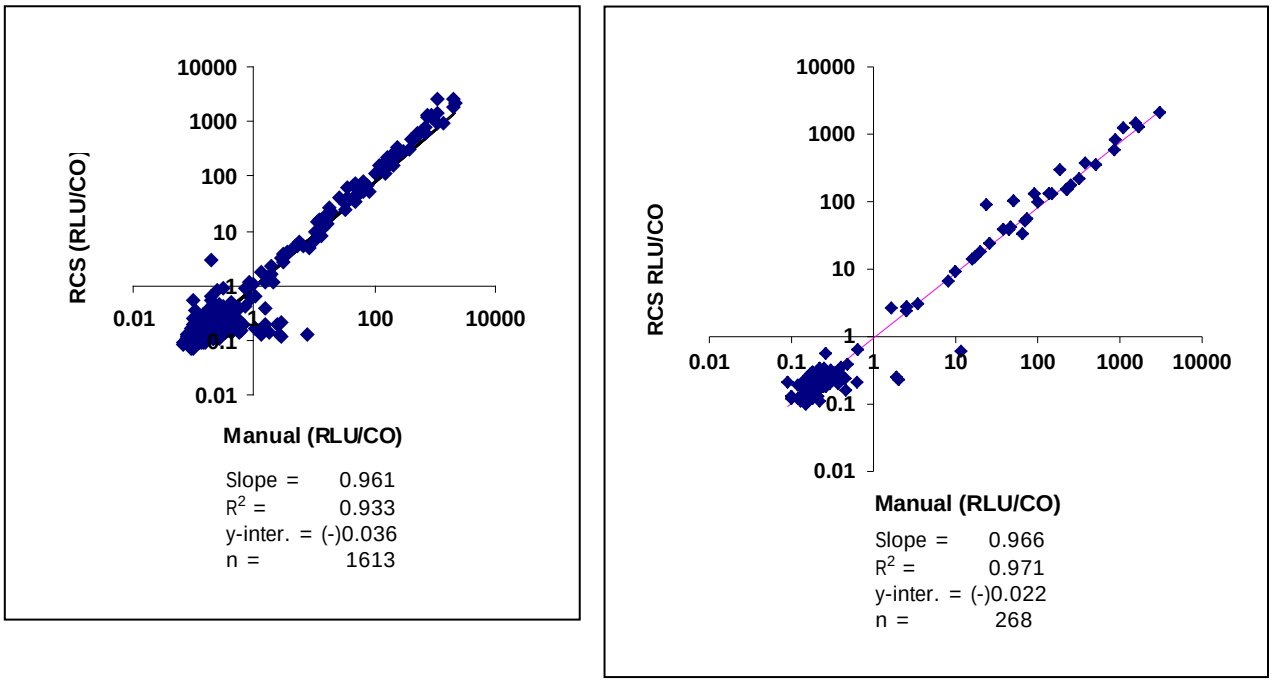


These linear regression analyses for the digene HC2 CT/GC DNA Test demonstrate that the Rapid Capture System Application and the manual method have a linear relationship. The overall agreement between the two methods was 99.6% (1607/1613; 99.2-99.9% 95% CI) for the archived specimen results and 99.6% (267/268; 97.9-100% 95% CI) for the prospectively collected cervical specimen results. Therefore, these data demonstrate that the two methods are equivalent in performance.

Figure 4

Scatterplots of Rapid Capture System Application and Manual Method Initial digene HC2 CT-ID DNA Test Results Archived Cervical Specimens (n = 1613; left).

Prospectively Collected Cervical Specimens (n = 268; right)

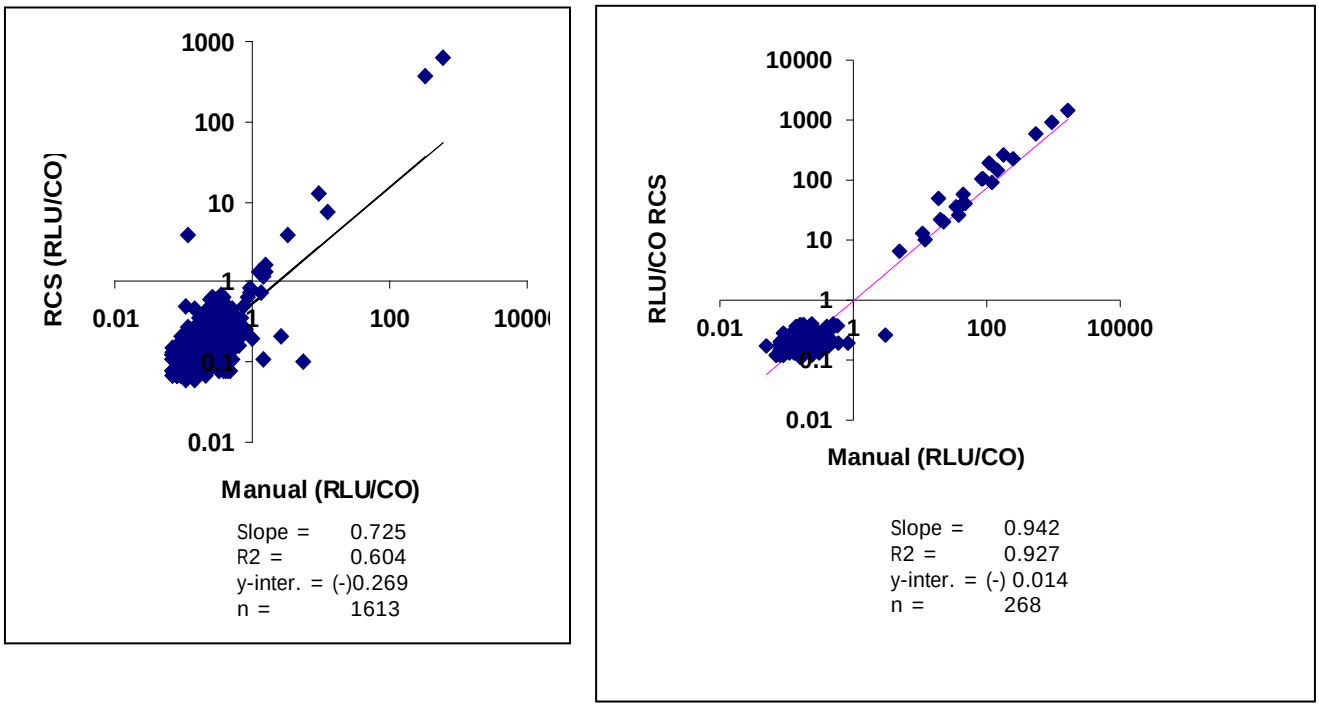


These linear regression analyses for the digene HC2 CT-ID DNA Test demonstrate that the Rapid Capture System Application and the manual method have a linear relationship. The overall agreement between the two methods is 99.7% (1608/1613; 99.3-99.9% 95% CI) for the archived specimen results and 98.9% (265/268; 96.8-99.8% 95% CI) for the prospectively collected cervical specimen results. Therefore, these data demonstrate that the two methods are equivalent in performance.

Figure 5

Scatterplots of Rapid Capture System Application and Manual Method Initial digene HC2 GC-ID DNA Test Results Archived Cervical Specimens (n = 1613; left).

Prospectively Collected Cervical Specimens (n = 268; right)



The linear regression analysis for the prospectively collected specimens tested using the digene HC2 GC-ID DNA Test demonstrate that the Rapid Capture System Application and the manual method have a linear relationship, suggesting that the two methods are equivalent in performance. The values for the slope and intercept obtained for the archived specimen results are lower than expected due to the low prevalence of GC in the population tested. The overall agreement between the two methods is 99.8% (1609/1613; 99.4-99.9% 95% CI) for the archived specimen results and 99.6% (267/268; 97.9-100% 95% CI) for the prospectively collected cervical specimen results.

Note: Refer to the *digene* HC2 CT/GC, CT-ID, or GC-ID DNA Test IFUs for additional performance characteristics.

References

1. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotrophic virus type III/lymphadenopathy-associated virus. J Infect. Dis 1985 Aug;152(2):400-3.

SUMMARY OF SEMI-AUTOMATED Rapid Capture System

FOR RUNNING *digene* HC2 CT/GC, CT-ID, AND GC-ID DNA TESTS

Important: **It is highly recommended that one be familiar with the detailed procedure before using this summary.**

	Procedure **USE THE RCS SCRIPTSELECT SOFTWARE TO DETERMINE THE Rapid Capture System SCRIPT AND PLATE SETUP.**
DENATURATION	Label digene Racks. Prepare Denaturation Reagent. Load Controls, Calibrators, and Specimens into the digene Rack. Enter Specimen ID into software while loading specimens in the rack. –
	Pipette Denaturation Reagent (volume is equivalent to half the sample volume) into Controls, Calibrators, and Specimens. Vortex the Controls, Calibrators, and specimens on the MST Vortexer 2 for 10 seconds at the highest speed (see application manual for details). **Controls, Calibrators, and specimens should be dark purple.** –
	Incubate in a 65 ± 2°C water bath for 45 ± 5 minutes. (Prepare Reagents) –
INSTRUMENT SETUP	Label Hybridization Plates. Place the hybridization plates with lids on the shaker. –
	Place the capture plates with a lid on the top plate in Stacker A. –
	Fill Wash bottle with 1X Wash Buffer. –
	Fill the appropriate reagent troughs with Probe Mixes, Detection Reagent 1, and Detection Reagent 2 and place in their designated location in the Reagent Trough Rack. –
	Fill the five tip rack holders with disposable tip racks. –
SPECIMEN TRANSFER/ASSAY PROCESSING	When the specimen racks have finished denaturing, remove from the water bath, dry excess water from rack, and vortex for 5 seconds. –
	Remove Specimen Rack lid, place drop-on caps on tubes, and place the rack on the deck. When ready, click **OK** for specimen transfer. –
	When the specimens have been transferred to the hybridization rack, check for any missing samples. Remove the rack; then repeat for the rest of the racks of samples to be tested. –
	After all the racks of samples to be tested have been transferred, replenish the disposable tip rack holders with new disposable tips. When ready to continue, click **OK** for probe addition. –
READING	Read Capture Microplate on QIAGEN-approved luminometer. –
	Validate assay and interpret specimen results. Generate Report.

High-Risk HPV Application Procedure

I. Reagent Preparation and Storage

Reference the digene Hybrid Capture 2 (HC2) High-Risk HPV DNA Test IFU for Warnings and Precautions specific to kit reagents.

To provide the required reagent volumes for processing multiple plates in a single run, it is necessary to combine certain components from multiple test kits of the same lot number.

A. Reagents Required:

- digene HC2 High-Risk HPV DNA Test [either REF 5199-1220 (1-plate kit) or REF 5199-00016 (4-plate kit)]
- digene HC2 DNA Collection Device [consists of one cervical brush and one tube containing 1 ml of Specimen Transport Medium (STM)]
- ThinPrep® Pap Test PreservCyt® Solution
- digene HC2 Sample Conversion Kit (Required for processing ThinPrep Pap Test PreservCyt Solution specimens)

B. Rapid Capture System HPV Reagent Testing Guidelines

- The performance of high specimen-throughput testing was not evaluated using the Low-Risk HPV Probe; therefore, the Rapid Capture System (RCS) is not intended to test low-risk HPV types 6/11/42/43/44.
- In order to optimize reagent usage it is recommended to test a minimum of 88 specimens, the equivalent of 1 plate, per Rapid Capture System run. Less then 88 specimens can be tested per Capture Microplate; however, the Rapid Capture System requires a minimum reagent volume for 1 full plate to be placed in the RCS reagent troughs. Full kit use may not be achieved when fewer than 88 specimens are tested.
- **Components from test kits of the same lot number may be combined to provide the required volumes for processing multiple plate runs. However, do not combine components of kits from different kit lots. The components have been tested as a unit. DO NOT interchange components from other sources or from different kit lots.**

II. SPECIMEN COLLECTION AND HANDLING

The types of cervical specimens recommended for use in the digene HC2 High-Risk HPV DNA Test are listed below. Specimens taken with other sampling devices or transported in other transport media have not been qualified for use with this assay. **The *digene* HC2 High-Risk HPV DNA Test performance characteristics with other specimen types and collection devices is unknown**. Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopic examination is being performed.

A. Cervical Brushes

The digene HC2 High-Risk HPV DNA Test is designed for use with specimens collected and transported using the digene HC2 DNA Collection Device (Cervical Brush and Specimen Transport Medium). Specimens may be held for up to two weeks at room temperature, after which specimens can be stored an additional week at 2-8°C. Specimens can be stored at -20°C for up to three months prior to testing. A preservative has been added to the Specimen Transport Medium to retard bacterial growth and to retain the integrity of DNA. It is **not intended** to preserve viability of organisms or cells.

Table 1
Endocervical Specimen Storage Requirements

Time Prior to Testing	Storage Duration	Storage Temperature
3 weeks	Up to 2 weeks	Room Temperature
	Up to an additional week	2-8°C
Greater than 3 weeks	Up to three months	-20°C

Specimens may be shipped without refrigeration to a testing laboratory; however, specimens should be shipped in an insulated container using either an overnight or 2-day delivery vendor.

B. Cervical Biopsies

Freshly collected cervical biopsies 2 - 5 mm in cross-section may also be analyzed with the digene HC2 High-Risk HPV DNA Test. The biopsy specimen must be placed immediately into 1.0 ml of Specimen Transport Medium and stored frozen at -20°C. Biopsy specimens may be shipped at 2-30°C for overnight delivery to the testing laboratory and stored at -20°C until processed. Do not use biopsies less than 2 mm in diameter.

Note:
To prevent caps from popping off specimens that are shipped or stored frozen (for STM specimens):

1. Cover caps with Parafilm® prior to shipping previously frozen specimens. Specimens may be shipped frozen or at room temperature.
2. When removing specimens from the freezer for testing, replace caps immediately with Specimen Collection Tube Screw Caps.

C. Specimens Collected in PreservCyt Solution

Specimens collected using a broom-type collection device and placed in PreservCyt Solution for use in making ThinPrep Pap Test slides can be used for the digene HC2 High-Risk HPV DNA Test. Collect specimens in the routine manner. Prepare the ThinPrep Pap Test slides according to Hologic instructions.

Note: A minimum of 4 ml of PreservCyt Solution must be processed for testing.

PreservCyt Solution specimens may be held for up to three months at temperatures between 2°C and 30°C following collection and before processing for the digene HC2 High-Risk HPV DNA Test. PreservCyt Solution specimens cannot be frozen. To process these specimens, the digene HC2 Sample Conversion Kit is required. For the processing procedure, see the digene HC2 Sample Conversion Kit or the **PreservCyt Solution Specimen Processing and Denaturation section of this Application Procedure.**

III. Specimen Processing

Multi-Specimen Tube Vortexer and Racks

The Multi-Specimen Tube Vortexer 2, the appropriate rack and lid, and the accessory components are required for specimen preparation, processing, and denaturation. Three different specimen rack designs are available. The specimen rack designs allow the laboratory to customize their testing. The rack names and usage are listed in the table below. The specimen racks are color coded to differentiate the rack designs.

Table 2
Description of Specimen Racks

Name of Specimen Rack	Rack Color Key	Type of Specimen Rack Accommodates
digene Specimen Rack	Blue	Specimens collected in STM (digene HC2 DNA Collection Device). This rack contains positions for two positive calibrators. For use only with the MST Vortexer 2.
Conversion Rack	Silver	Patient specimens collected in. PreservCyt Solution. These specimens require processing prior to digene HC2 DNA testing. PreservCyt Solution specimens are converted specimens. The rack accommodates VWR® or Corning® brand15-ml conical tubes. **For use only with the MST Vortexer 2.**

Specimen and Rack Setup

1. Remove the specimens and all required reagents from the refrigerator or freezer prior to beginning the assay. Allow them to equilibrate to room temperature (20-25ºC).

   Notes:
   - For PreservCyt specimens that have already been processed, denatured, and stored frozen, remove caps from the thawed tubes and reapply DuraSeal™ and the Conversion Rack Lid. The rack must be vortexed for 10 seconds on the MST Vortexer 2 prior to specimen transfer on the RCS.
   - The Conversion Rack and Lid cannot be used to vortex kit Calibrators or Quality Controls. The height of the STM tubes prevents adequate vortexing using the Conversion Rack. Place the denatured and vortexed Calibrators and Controls in the proper position on the Conversion Rack prior to placing the rack on the RCS pipetting station.

2. Label each digene Specimen Rack, Conversion Rack and corresponding lid according to the order to be tested on the RCS. Be sure to use a label and marker that will not wash off in a 65ºC water bath (see Reagent and Materials Required section).

   Notes:
   - Each digene Specimen Rack and Conversion Rack is serialized. The serial number is engraved on both the rack and lid. Serial numbers of the specimen rack and lid must match. Label accordingly.
   - Up to four racks of 88 specimens each may be tested per RCS run. For single-probe assays, the Racks are labeled 1 through 4 and must be filled and loaded onto the RCS in that order. Remember to include the kit calibrator and controls with each rack.

3. Use the digene HC2 System Software to enter specimen IDs and create plate layouts for each specimen rack. (Refer to the Hybrid Capture 2 System User Manual for instructions.) **It is critical that the specimen plate layout corresponds to the correct specimen rack to prevent reporting inaccurate specimen results.**

   Note: The digene HC2 Assay Protocols must be used to create the Calibrator/Control/specimen template. **For the Rapid Capture System instrument application, the RCS HPV HC2 Assay Protocol has been programmed to apply a Calibration Adjustment Factor (CAF) of 0.8 to the mean RLU value of the valid Positive Calibrator replicates. This CAF is necessary so that the performance characteristics of the assay remain equivalent to the manual test procedure.** This change only applies to assays performed using the Rapid Capture System instrument. Therefore, it is critical to select the correct assay protocol for use with each specific test method in order to generate accurate test results. See the digene H2 System Software User Manual for more detailed information.

4. Remove and discard caps from the Negative and Positive Calibrators, Quality Controls and specimens to be tested.

   Note: Caps removed from the specimen tubes are considered potentially infectious. Dispose of infectious material in accordance with local, State, and Federal regulations.

5. The Negative and Positive Calibrators and Quality Controls are required for each rack of specimens to be tested. The RCS distributes the Negative and Positive Calibrator in triplicate to the first column for each plate of specimens tested. The Quality Controls and specimens are tested individually.

6. **For each *digene* Specimen Rack (blue), and Conversion Rack (silver),** place the Negative Calibrator in the A1 and High-Risk HPV Calibrator (Positive Calibrator) in the D1 positions of the appropriate rack. Place the Low-Risk HPV Quality Control (QC1-LR) in the G1 and the High-Risk HPV Quality Control (QC2-HR) in the H1 positions of the appropriate rack. The RCS will distribute the Negative Calibrator, Positive Calibrator, Quality Controls, and specimens to be tested. Calibrators, Controls, and specimens are run in an 8-microwell column configuration. (The locations described in the following sentence refer to locations on a Microplate and not a vortexer rack.) The RCS places the Negative Calibrator (NC) replicates in A1, B1, C1; the Positive Calibrator (PC) in D1, E1, F1; QC1-LR in G1; and QC2-HR in H1. The RCS places specimens beginning in A2.

   See Example 1 for the *digene* Specimen Rack Layout, and Example 2 for the Conversion Rack Layout.

   Note: **The Conversion Rack and *digene* Specimen Rack** have a position for a second Positive Calibrator. It is important to place the Positive Calibrator in the D1 position to obtain valid assay results. **Do not** place the Positive Calibrator in the E1 position.

7. Proceed to PreservCyt Solution, Specimen Processing and Denaturation (Section C) or the Denaturation of digene HC2 DNA Collection Device specimens, Calibrators and Controls (Section D) after specimens are placed in the appropriate rack and the plate layouts are created.

   Note: The digene HC2 System Software will report both Calibrator and Control results based on their location in the plate to verify the assay run. Proper placement of Calibrators and Controls in the Rack and proper assay protocol selection are essential for valid assay results.

EXAMPLE 1

***digene* SPECIMEN RACK LAYOUT (1/2 Rack Displayed)**

Row	Column					
	1	2	3	4	5	6
A	NC	Spec. 1	Spec. 9	Spec. 17	Spec. 25	Spec. 33
B		Spec. 2	Spec. 10	Spec. 18	Spec. 26	Spec. 34
C		Spec. 3	Spec. 11	Spec. 19	Spec. 27	Spec. 35
D	PC 1	Spec. 4	Spec. 12	Spec. 20	Spec. 28	Spec. 36
E	EMPTY	Spec. 5	Spec. 13	Spec. 21	Spec. 29	Spec. 37
F		Spec. 6	Spec. 14	Spec. 22	Spec. 30	Spec. 38
G	QC1-LR	Spec. 7	Spec. 15	Spec. 23	Spec. 31	Spec. 39
H	QC2-HR	Spec. 8	Spec. 16	Spec. 24	Spec. 32	Spec. 40

- The digene Specimen Rack is designed to emulate a 96-well plate.
- Column 1 of the digene Specimen Rack is designed with five positions for 1 Negative Calibrator, up to two Positive Calibrators, and 2 Quality Controls.
- The remaining 11 Columns can accommodate up to 88 digene specimens.

EXAMPLE 2

CONVERSION RACK LAYOUT (1/2 Rack Displayed)

Row	Column					
	1	2	3	4	5	6
A	NC	Spec. 1	Spec. 9	Spec. 17	Spec. 25	Spec. 33
B		Spec. 2	Spec. 10	Spec. 18	Spec. 26	Spec. 34
C		Spec. 3	Spec. 11	Spec. 19	Spec. 27	Spec. 35
D	PC	Spec. 4	Spec. 12	Spec. 20	Spec. 28	Spec. 36
E	EMPTY	Spec. 5	Spec. 13	Spec. 21	Spec. 29	Spec. 37
F		Spec. 6	Spec. 14	Spec. 22	Spec. 30	Spec. 38
G	QC1-LR	Spec. 7	Spec. 15	Spec. 23	Spec. 31	Spec. 39
H	QC2-HR	Spec. 8	Spec. 16	Spec. 24	Spec. 32	Spec. 40

- The Conversion Rack is designed to emulate a 96-well plate for PreservCyt specimens processed in 15-ml conical tubes.
- Column 1 of the Conversion Rack is designed with five positions to accommodate the digene STM specimen tubes for 1 Negative Calibrator, up to two Positive Calibrators and 2 Quality Controls.
- The remaining 11 Columns can accommodate up to 88 specimens.
- The positions in columns 2-12 accommodate 15-ml conical tubes and do not hold digene Specimen tubes.
- The Calibrators and Quality Controls cannot be vortexed in the Conversion rack. They must be vortexed separately and placed in the rack following PreservCyt processing.

PreservCyt Solution Specimen Processing and Denaturation

Specimens may contain infectious agents and should be handled accordingly.

The following materials are required:

- Conversion Rack and Lid (silver)
- digene HC2 Sample Conversion Kit
- 15-ml VWR® or Corning® brand polypropylene conical tubes
- Multi-Specimen Tube Vortexer 2 and accessories
- For additional materials, see the digene HC2 Sample Conversion Kit IFU or the Reagents and Materials Required section of this user manual.

Notes:

- Processing and denaturation of PreservCyt specimens are performed off line from the RCS.
- A minimum of 4 ml of PreservCyt Solution must be processed and denatured to produce one test result on the RCS.
- Each 2 ml of PreservCyt Solution that is processed and denatured provides enough material for one digene HC2 High-Risk HPV DNA Test result with the High-Risk HPV RCS Application. However, to account for the void volume associated with the RCS specimen transfer step, an extra 2 ml of PreservCyt Solution must be processed, regardless of the number of tests to be performed. For example, processing the minimum acceptable 4 ml of PreservCyt Solution provides enough material for one digene HC2 High-Risk HPV DNA Test result.
- Prepare PreservCyt Solution specimens in batches of 36 or fewer through the centrifugation and decanting steps. This is important for maintaining the integrity of the cell pellet during the decanting step.
- The MST Vortexer 2 may not be used for the pre-centrifugation step of mixing PreservCyt Solution specimens with the Sample Conversion Buffer.
- Only VWR or Corning brand 15-ml conical tubes may be used with the Conversion Rack. During vortexing, only one of these two brands may be present in a Conversion Rack at any one time. This is due to slight differences in their height.
- The Conversion Rack and Lid cannot be used to vortex the kit Calibrators or Quality Controls. The height of the STM tubes prevents adequate vortexing using the Conversion Rack.

digene HC2 Sample Conversion Kit Denaturation Reagent Preparation

To prepare the Denaturation Reagent (DNR) from the digene HC2 Sample Conversion Kit, add 3 drops of Indicator Dye to the bottle of DNR and mix well. The solution should be a uniform, dark purple color. Once prepared, the DNR is stable for three months when stored at 2-8ºC. Label the bottle with the new expiration date. If the color fades, add 3 additional drops of Indicator Dye and mix well.

In the procedure below, 4-ml aliquots of PreservCyt Solution are processed, producing enough material for 1 test per specimen. Alternate volumes may be processed according to Table 3.

Table 3
PreservCyt Processing Volumes Required

No. of Tests	PreservCyt Volume	Conversion Buffer Volume
1	4 ml	**0.4 ml**
2	6 ml	**0.6 ml**
3	8 ml	**0.8 ml**
4	10 ml	**1.0 ml**
5	12 ml	**1.2 ml**

1. Label the VWR or Corning brand 15-ml conical tubes with the appropriate specimen identification number.
2. Handling one specimen at a time:
   2a. Shake the PreservCyt vial vigorously by hand or vortex for 5-10 seconds to resuspend cells and ensure homogeneity.
   2b. Immediately, as cells settle very quickly, pipette the appropriate volume of the PreservCyt specimen into the labeled tube. Deliver the PreservCyt Solution to the bottom of the conical tube to minimize cellular material from adhering to the inside of the tube.
3. Add the appropriate volume of Sample Conversion Buffer to each tube (see Table 3 above).
4. Place a screw cap on each tube. Label the caps to indicate the specimen ID.
5. Mix the contents of each tube thoroughly using a vortex mixer with cup attachment.
6. Centrifuge the tubes in a swinging bucket rotor at 2,900 ± 150 x g for 15 ± 2 minutes.
7. During centrifugation, prepare the Specimen Transport Medium (STM) / Denaturation Reagent (DNR) mixture in a 2:1 ratio, according to Table 4.
   Note: Solution must be prepared fresh each day.
   7a. To determine the total volume of STM / DNR mixture required, use the starting volume of the PreservCyt Solution specimen as a guide and then multiply the STM and DNR "per tube" volumes by the number of specimens to be processed.

Table 4
STM/Denaturation Reagent Volumes Required

No. of Tests	PreservCyt Volume	STM Volume per tube	DNR Volume per tube	STM + DNR Mixture added per tube
1	4 ml	120 µl	60 µl	150 µl
2	6 ml	170 µl	85 µl	225 µl
3	8 ml	220 µl	110 µl	300 µl
4	10 ml	270 µl	135 µl	375 µl
5	12 ml	320 µl	160 µl	450 µl

   7b. Mix the solution thoroughly by vortexing.
8. Remove tubes from the centrifuge one at a time and place into a Conversion Rack. A pink / orange pellet should be present in bottom of each tube.
9. Handling each tube individually:
   Remove the cap and set aside on a clean low-lint paper towel.
   9a. Carefully decant the supernatant.

9b. Maintain the inverted tube position and gently blot (approximately 6 times) on absorbent low-lint paper towels to remove the excess liquid. Use a clean area of the towel each time. **Do not** allow the cell pellet to slide down the tube during blotting.

9c. Place the tube in the Conversion Rack.

Notes:

- The Conversion Rack and Lid are designed to accommodate only VWR or Corning brand 15-ml conical tubes.
- Strict adherence to the specified vortexing times is required.

10. For the appropriate amount of PreservCyt Solution starting volume, add the appropriate amount of STM + DNR to each pellet. (Example: For a 4-ml PreservCyt starting volume, add 150 µl STM + DNR mixture to each pellet). See Table 4 above when processing volumes of PreservCyt Solution specimens other than 4 ml.
11. Cover the 15-ml conical tubes with DuraSeal® film by pulling the film over the tubes in the rack.
12. Place the rack lid over the film-covered tubes and lock the lid into place with the two side clamps. Cut the film with the cutter device after the lid is securely fastened.
13. Place the Conversion Rack and Lid on the MST Vortexer 2 so that the largest notched corner of the Conversion rack is located in the front right corner. Secure the rack with the clamp by pushing down on the red-handled lever.
14. Verify that the motor speed setting is at 100 (maximum speed) and the pulser button is in the OFF position.
15. Turn the vortexer power switch to the ON position and **vortex the tubes for 30 seconds**.
16. Lift up the red-handled lever to release the rack.
17. Place the rack in the 65 ± 2°C water bath for 15 ± 2 minutes.
18. After the 15-minute incubation, remove the rack from the water bath and set on paper towels to drain excess water to prevent splashing during vortexing.
19. Place the Conversion Rack on the MST Vortexer 2 so that the largest notched corner of the rack is located in the front right corner. Secure the rack with the clamp by pushing down on the red-handled lever.
20. Verify that the motor speed setting is at 100 (maximum speed) and the pulser button is in the OFF position.
21. Turn the vortexer power switch to the ON position and vortex the tubes for 1 minute.
22. Lift up the red-handled lever to release the rack.
23. Return the rack to the 65 ± 2°C water bath and continue denaturation for another 30 ± 3 minutes.
24. Remove the rack from the water bath and set on paper towels to drain excess water.
25. Place the Conversion Rack on the MST Vortexer 2 so that the largest notched corner of the rack is located in the front right corner. Secure the rack with the clamp by pushing down on the red-handled lever.
26. Verify that the motor speed setting is at 100 and the pulser button is in the OFF position.
27. Turn the vortexer power switch to the ON position and vortex the tubes for 10 seconds.
28. Immediately remove the rack lid and DuraSeal Film and either proceed to RCS testing or store as indicated below.

Optional Stop Point:

After denaturation, specimens may be stored at 2 - 8°C overnight or at -20°C for up to 3 months. For overnight refrigeration, specimens may be left in the Conversion Rack with new DuraSeal film and Rack Lid replaced. Prior to storage at -20°C, the Rack Lid and DuraSeal film must be removed, and caps placed on the tubes. If the manual vortex procedure was used, place the rack of capped tubes at the desired storage temperature. In either case, the specimens must be equilibrated to room temperature (20 - 25°C) and thoroughly vortexed before proceeding to the Hybridization step.

Note: Do not store or ship denatured specimens on dry ice.

***digene* HC2 High-Risk HPV DNA Test [REF 5199-1220 (1-plate kit)]**

A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. For specimens processed using the MST Vortexer 2, remove the Rack Lid and DuraSeal tube sealer film from the tubes, and cap each tube with a screw cap before storing specimens at -20°C.

***digene* HC2 High-Risk HPV DNA Test [REF 5199-00016 (4-plate kit)]**

A maximum of 1 freeze/thaw cycle may be performed for kit calibrators and controls with a maximum of 2 hours at room temperature during the thaw cycle. A maximum of 3 freeze/thaw cycles may be performed on specimens with a maximum of 2 hours at room temperature during each thaw cycle. For specimens processed using the MST Vortexer 2, remove the Rack Lid and DuraSeal tube sealer film from the tubes, and cap each tube with a screw cap before storing specimens at -20°C. **The kit is designed for high-volume use on the Rapid Capture System and must be consumed in ≤2 Rapid Capture System runs to obtain the full 384 tests.** Running partial plates outside of the suggested formats may result in fewer than 384 tests due to limited High-Risk HPV Probe and Probe Diluent volumes.

Denaturation of digene HC2 DNA Collection Device Specimens, Kit Calibrators and Controls

Notes:

- **Warning:** Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, and eye/face protection. Use care when handling. Dispose of corrosive material in accordance with local, State, and Federal regulations.
- **Important:** Some specimens may contain blood or other biological material that may mask the color changes upon addition of Denaturation Reagent and Probe Mix. Specimens that exhibit a dark color prior to the addition of Denaturation Reagent may not give the proper color changes at these steps. In these cases, failure to exhibit the proper color change will not affect the results of the assay. Proper mixing can be verified by observing the color change of the Calibrators and Controls.
- Do not remove the brush from the digene HC2 DNA Collection Device prior to denaturation.
- During the denaturation step, be sure that the water level in the water bath is adequate to immerse the entire volume of specimen in the tube.
- Specimens may be prepared up through the denaturation step and stored at 2-8°C overnight or at -20°C for up to 3 months. A maximum of 3 freeze-thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. Vortex well before using.
- To avoid false-positive results, it is critical that all Calibrator, Control, and specimen material come into contact with Denaturation Reagent. Vortexing after Denaturation Reagent addition is a critical step. **Make sure the MST Vortexer 2 is set on 100 (maximum speed) setting and that the Pulser Button is OFF.**

- Following denaturation and incubation, the specimens should no longer be considered infectious. However, lab personnel should still adhere to universal precautions.

Refer to the *Reagent Preparation* section of this user manual or instructions on Denaturation Reagent preparation.

1. Pipette Denaturation Reagent with Indicator Dye into each Calibrator, Control, or specimen using a repeating or adjustable pipettor. Take care not to touch the sides of the tube, as cross-contamination of specimens could occur. The volume of Denaturation Reagent needed is equivalent to half the specimen volume. The exact volume for each type of Calibrator, Control, and specimen is listed in the table below.

Table 5
Denaturation Volumes Required for Calibrators, Controls, and Specimens

digene HC2 High-Risk HPV DNA Test [REF 5199-1220 (1-plate kit)]

Calibrator, Quality Control, or Specimen	Vol. Of Denaturation Reagent Required
Negative Calibrator	1000 µl
High-Risk HPV Calibrator	**500 µl***
Low-Risk or High-Risk HPV Quality Controls	500 µl*
Cervical Specimen	500 µl*

digene HC2 High-Risk HPV DNA Test [REF 5199-00016 (4-plate kit)]

Calibrator, Quality Control, or Specimen	Vol. Of Denaturation Reagent Required
Negative Calibrator	1000 µl
High-Risk HPV Calibrator	**1000 µl**
Low-Risk or High-Risk HPV Quality Controls	500 µl*
Cervical Specimen	500 µl*

*If using an Eppendorf Repeater Pipette, use a 12.5-ml tip and a pipettor setting of 2.

- Dilute remaining Denaturation Reagent in bottle prior to disposing. Dispose of in accordance with local, State and Federal regulations.

2. Mix the specimens using the MST Vortexer.
   Cover the Calibrator/Control/Specimen tubes with DuraSeal film by pulling the film over the tubes in the rack(s). Place the rack(s) lid(s) over the film-covered tubes and lock into place with the two side clips. Cut the film with the cutter device after the lid is securely fastened.
3. Place the rack on the MST Vortexer 2 so that the largest notched corner of the rack is located in the front right corner. Secure the rack with the clamp by pushing down on the red-handled lever.
4. Verify that the motor speed setting is at 100 (maximum speed) and the pulser button is in the OFF position.
5. Turn the vortexer power switch to the ON position and vortex the tube for 10 seconds. The Calibrators, Controls, and specimens should turn purple.
6. Lift up the red-handled lever to release the rack.
7. Incubate the tubes in each rack in a 65°C ± 2°C water bath for 45 ± 5 minutes.
8. Prepare the reagents and set up the Rapid Capture System deck during the specimen denaturation.

9. After the 45-minute incubation, remove the rack(s) with specimens from the waterbath. Denatured Calibrators, Controls, and specimens can be vortexed and tested immediately, or stored as described in the **Optional Stop Point** below.
10. Immediately place the labeled Rack 1 on the MST Vortexer 2 and vortex for a minimum of 10 seconds with motor speed of 100 (maximum speed).
11. Immediately place the rack on the bench top and release the latches. Lift the rack lid ~1 cm and move it gently left and right to release any specimen tubes that may have adhered to the DuraSeal Film. Remove the lid by lifting it straight up until it clears the rack base.
12. Carefully peel the DuraSeal Film from the lid and discard.
13. Repeat steps 9-12 for the remaining specimen racks.

Optional Stop Point:

After denaturation, specimens may be stored at 2 - 8°C overnight or at -20°C for up to 3 months. For overnight refrigeration, specimens may be left in the digene Specimen Rack with new DuraSeal film and Rack Lid replaced. Prior to storage at -20°C, the Rack Lid and DuraSeal film must be removed, and caps placed on the tubes. If the manual vortex procedure was used, place the rack of capped tubes at the desired storage temperature. In either case, the specimens must be equilibrated to room temperature (20 - 25°C) and thoroughly vortexed before proceeding to the Hybridization step.

Note: Do not store or ship denatured specimens on dry ice.

***digene* HC2 High-Risk HPV DNA Test [REF 5199-1220 (1-plate kit)]**

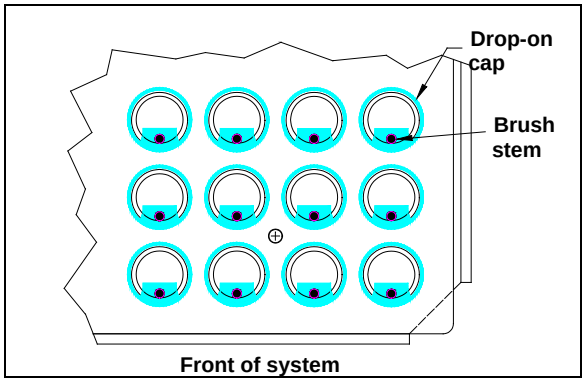
A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. For specimens processed using the MST Vortexer 2, remove the Rack Lid and DuraSeal tube sealer film from the tubes, and cap each tube with a screw cap before storing specimens at -20°C.

***digene* HC2 High-Risk HPV DNA Test [REF 5199-00016 (4-plate kit)]**

A maximum of 1 freeze/thaw cycle may be performed for kit calibrators and controls with a maximum of 2 hours at room temperature during the thaw cycle. A maximum of 3 freeze/thaw cycles may be performed on specimens with a maximum of 2 hours at room temperature during each thaw cycle. For specimens processed using the MST Vortexer 2, remove the Rack Lid and DuraSeal tube sealer film from the tubes, and cap each tube with a screw cap before storing specimens at -20°C. **The kit is designed for high-volume use on the Rapid Capture System and must be consumed in ≤2 Rapid Capture System runs to obtain the full 384 tests.** Running partial plates outside of the suggested formats may result in fewer than 384 tests due to limited High-Risk HPV Probe and Probe Diluent volumes.

14. When placing the rack on the RCS, orient the rack so that the Negative Calibrator is in the upper left corner. In Specimen Rack 1, place a drop-on cap onto each tube containing a brush. Ensure that the shaft of the collection device is trapped between the tab of the drop-on cap and the side of the specimen tube. The drop-on caps must be oriented so the tab is closest to the user as they face the rack (Figure 1).

Figure 1. Orientation of Drop-On Caps



REAGENT PREPARATION

The table below details volumes required for multi-plate runs for high-volume testing.

<table>
<tr>
<td>digene HC2 High-Risk HPV DNA Test Denaturation Reagent</td>
<td>PREPARE FIRST:

Warning: Denaturation Reagent is corrosive. Wear suitable protective clothing, gloves, eye/face protection. Use care when removing cap from bottle and when handling.

digene HC2 High-Risk HPV DNA Test [REF 5199-1220 (1-plate kit)]
· Add 5 drops of Indicator Dye to the bottle of Denaturation Reagent and mix thoroughly. The Denaturation Reagent should be a uniform, dark purple color.

digene HC2 High-Risk HPV DNA Test [REF 5199-00016 (4-plate kit)]
· Add 10 drops of Indicator Dye to each bottle of Denaturation Reagent and mix thoroughly. The Denaturation Reagent should be a uniform, dark purple color.

Once prepared, the Denaturation Reagent is stable for 3 months when stored at 2-8°C. Label it with the new expiration date. If the color fades, add 3 drops of Indicator Dye and mix thoroughly before using.</td>
</tr>
<tr>
<td>High-Risk HPV Probe Cocktail

(PREPARE FRESH DAILY)</td>
<td>CAUTION: Probe Diluent may cause eye irritation. Wear eye/face protection.

CAUTION: Extreme care should be taken at this step to prevent RNase contamination of Probe and Probe Mix. Use aerosol-barrier pipette tips for pipetting probe. Probe Diluent is viscous. Care should be taken to ensure thorough vortexing when preparing Probe Mix. A visible vortex must form in the liquid during the mixing step. Incomplete vortexing may result in reduced signal.

NOTE: Probes and reagents can only be combined if from the same kit lot.

NOTE: There are 384 tests in the digene HC2 High-Risk HPV DNA Test [REF 5199-00016 (4-plate kit)]. The smallest volume of wells that can be run in one use is 96. The kit is designed for high-volume use on the Rapid Capture System and must be consumed in ≤2 Rapid Capture System runs to obtain the full 384 tests. Running <1 plate or partial plates outside of the suggested formats may result in fewer than 384 tests due to limited Probe and Probe Diluent volumes.

IMPORTANT: SOMETIMES PROBE GETS TRAPPED IN THE VIAL LID

· Centrifuge each vial of the High-Risk HPV Probe briefly to bring liquid to the bottom of the vial. Tap tube gently to mix.
· Determine the amount of Probe Mix required for each probe cocktail using the table below. Extra Probe Mix is required to account for the void volume required in the Rapid Capture System reagent troughs and is included in the table. The smallest number of wells recommended for each use is 96 or one microplate.
· Transfer the required amount of Probe Diluent to a polypropylene conical tube. Make a 1:25 dilution of Probe in Probe Diluent to prepare Probe Mix using the table below

<table>
<tr><th>No. of Plates</th><th>Volume Probe Diluent*</th><th>Volume Probe*</th></tr>
<tr><td>1</td><td>5.0 ml</td><td>200 µl</td></tr>
<tr><td>≤ 1.5</td><td>6.0 ml</td><td>240 µl</td></tr>
<tr><td>≤ 2</td><td>7.5 ml</td><td>300 µl</td></tr>
<tr><td>≤ 2.5</td><td>9.0 ml</td><td>360 µl</td></tr>
<tr><td>≤ 3</td><td>10.0 ml</td><td>400 µl</td></tr>
<tr><td>≤ 3.5</td><td>12.0 ml</td><td>480 µl</td></tr>
<tr><td>≤ 4</td><td>13.0 ml</td><td>520 µl</td></tr>
</table>
</td>
</tr>
</table>

	*These values include the recommended extra volume. · Pipette High-Risk HPV Probe into Probe Diluent by placing the pipette tip against the inner wall of the tube just above the meniscus and expelling the contents. **Do not immerse the tip into Probe Diluent.** · Vortex for at least 5 seconds at maximum speed to mix thoroughly. **A visible vortex must be produced**. Label as "High-Risk HPV Probe Cocktail." **Unused Probe Mix should be discarded.** Do not store probe cocktail for future use.
Wash Buffer	**Warning: Wash Buffer Concentrate is toxic by ingestion. Wear suitable protective clothing, gloves, and eye/face protection. To minimize exposure, add water to Wash Buffer Concentrate when preparing.** For the Rapid Capture System, the Wash Buffer can be prepared as described below and stored in the Wash Bottle at 20-25°C. See the table below for mixing volumes: **No. of Plates** / **Amount of Wash Buffer Concentrate** / **Amount of Deionized/Distilled Water** / **Final Volume of 1 X Wash Buffer*** £ 2 / 100 ml / 2.9 L / 3 L > 2 / 200 ml / 5.8 L / 6 L *These values include the recommended extra volume needed to fill the 300-ml void volume of bottle. **Note:** Prepared Wash Buffer is stable for three months at 2-30°C. Label with the new expiration date. If Wash Buffer has been refrigerated, equilibrate to 20-25°C prior to use.

VOLUMES FOR READY-TO-USE REAGENTS

Detection Reagent 1 & Detection Reagent 2	Determine the volume of Detection Reagent 1 or Detection Reagent 2 required for the run. The minimum run size is 1 plate. For 1 plate plus partial plates, use the volumes indicated in the table below. Mix individual reagent bottles thoroughly, then combine the appropriate volume of Detection Reagent 1 or Detection Reagent 2 into a clean disposable polypropylene conical tube. Mix thoroughly. Pour entire contents into the designated reagent trough. To avoid contamination, these reagents **must not** be returned to the original bottles. **Discard unused residual material in the trough or conical after use**. **No. of Plates** / **Minimum Volume for Detection Reagents 1 and 2** 1 / 10 ml ≤ 1.5 / 14 ml ≤ 2 / 18 ml ≤ 2.5 / 22 ml ≤ 3 / 26 ml ≤ 3.5 / 30 ml ≤ 4 / 34 ml

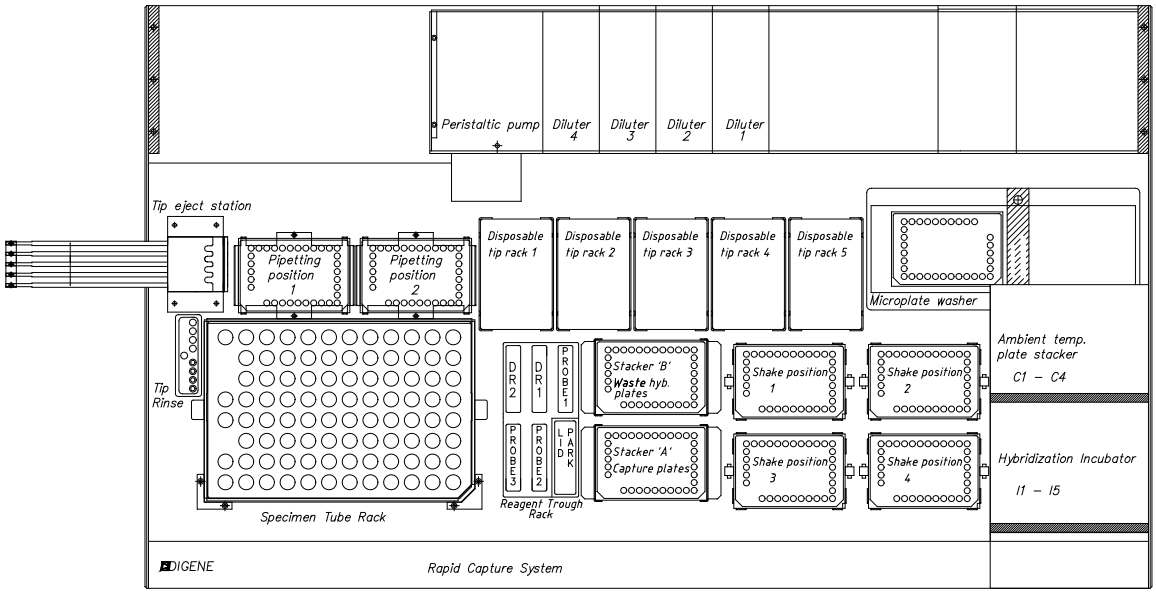
IV. Setup of the Rapid Capture System Deck

Flush with deionized/distilled water prior to first use each day by running the "FLUSH" script twice after initializing the system. Ensure that all air bubbles are removed from system lines. Failure to complete a system flush may result in improper aliquot volumes being dispensed.

Notes:

- Reference the Rapid Capture ScriptSelect Software Application Manual section of this Application Manual to aid in choosing the correct script for the specific RCS Run. The ScriptSelect Software allows the user to select the proper script and add it to the RCS Run list.
- Wear disposable, powder-free gloves during deck setup.

Figure 2: Deck Layout



A. Deck Preparation

1. Inspect the deck, including all stackers and incubators, and remove any plates, lids, or other miscellaneous items. If the preceding run was aborted, inspect the incubator by manually opening each chamber door using a disposable pipette tip. If plates are present, contact your local QIAGEN representative for instructions to remove the plate from the incubator. **Failure to remove all such items may result in damage to the RCS.**

   Caution: The Incubator reaches a set temperature of 65°C.

2. Wearing powder-free disposable gloves, fill all 5 disposable tip (DT) racks with disposable tip trays. **When loading the disposable tip tray, the "u-shaped" notch of the tray must be positioned in the front left of the rack**. The tray should snap into place. If it does not, remove the disposable tip tray from the rack and pull the center tabs on the front and back ends of the rack toward the center to increase the tension on the tray. Replace the disposable tip tray. Failure to load disposable tip trays will result in an audible alarm and a dialog box will appear indicating the requirement to load tips.

3. Number the front side of the Hybridization Plates 1 through 4. Place a lid on each plate.

4. Place the Hybridization Plates with lids on the Shaker in the corresponding labeled positions, S1 – S4. Ensure that the hybridization plates are correctly oriented, with A1 in the back left corner, and that the plates are **seated within the guides**.

5. Number the front side of the Capture Plates 1 through 4 to correspond. If any plate has fewer than 88 specimens, remove the appropriate number of capture strips or wells from the plate, return them to their original Mylar® bag, and store at 2-8°C. Replace **all** missing wells in the capture plate with RCS Microplate Well Strips.

6. Stack the capture plates in numerical order, with plate number 1 on top. Ensure that each plate is correctly oriented with the A1 well position in the back left corner. Place a lid on Plate 1 **only,** and set the plates in Stacker A.

   CAUTION: Plate Gripper Crash Hazard - If hybridization or capture plates are not present when the instrument attempts to retrieve them from the Shaker or Stacker A, the Plate Gripper will crash at the pipetting position. A crash may require the run to be restarted and/or may damage the RCS.

7. Empty the Liquid Waste bottle.

   Note: Ensure that the waste container is empty before starting each run! The waste container may overflow onto the deck causing flooding and alkaline phosphatase contamination. Always change gloves after handling the liquid waste bottle or any possible contact with the waste solution, including contact with the quick-disconnect fittings, to prevent contamination of work areas with the alkaline phosphatase.

8. Label reagent troughs and trough lids as required for the RCS. It is important to label the reagent troughs and segregate reagents to prevent possible contamination of reagents from run to run. Once labeled, do not use reagent troughs with other reagents. It is recommended to maintain two sets of reagent troughs so that a clean dry set is always available.

B. Reagent Preparation for the Rapid Capture System

1. Fill the Wash Bottle with 1x Wash Buffer (See Reagent Preparation section). Ensure that the quick-release valve clicks securely in place.

CAUTION: Ensure the Wash Bottle is adequately filled prior to each run.

2. Empty the System Liquid Bottle and refill with fresh deionized/distilled water. Ensure that the quick-release valve clicks securely in place.

CAUTION: Ensure the System Liquid Bottle is adequately filled prior to each run.

3. Add the prepared Probe Mix to the designated probe reagent trough(s) and place in the appropriate position(s) in the Reagent Trough Rack. Cover the trough(s) using the corresponding trough lid(s).

4. Add the required volume of Detection Reagent 1 to the designated reagent trough and place in the back center well of the Reagent Trough Rack. Cover the trough using the corresponding lid (see Reagent Preparation and Storage section and RCS Deck Layout, Figure 2).

5. Add the required volume of Detection Reagent 2 to the designated reagent trough and place in the back left well of the Reagent Trough Rack. Cover the trough using the corresponding trough lid (see Reagent Preparation section and RCS Deck Layout, Figure 2).

Notes:

- See Figure 2: RCS Deck Layout for the proper positioning of the probe for the specific RCS run.
- The RCS employs liquid-level sensing when dispensing reagents from the reagent troughs to a capture or hybridization plate. In the event of insufficient volume, the system will pause, display a dialog box indicating the problem, and signal the user with an audible alarm. The user can then place the filled reagent trough on the deck or add additional reagent, as appropriate.

6. When the specimens have completed the 45-minute denaturation incubation, retrieve the racks from the water bath and drain excess water onto paper towels.

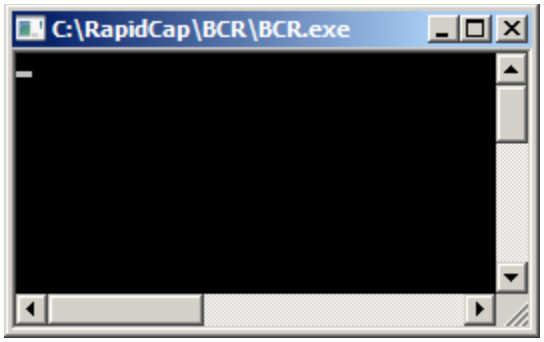
Note: DO NOT allow specimen racks to cool to room temperature before removing the rack lid. If cooling occurs, tubes may stick to the lid and subsequently spill. See Section C: PreservCyt Solution Specimen Processing and Denaturation; and D: Denaturation of digene HC2 DNA Collection Device Specimens, Kit Calibrators, and Controls.

V. Starting the RCS Run

Follow the examples below to initiate an RCS run.

CAUTION: Do not attempt to reach into the instrument while the plate handler is moving. Pause the RCS by pressing the **Escape** key or selecting the **Abort Run** icon and wait for a display dialog box to appear before readjusting or repositioning plates.

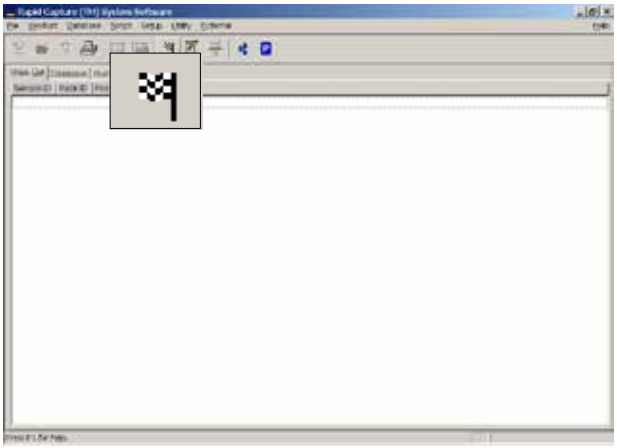
The barcode upgrade includes an application that saves the scanned barcodes for use by the digene HC2 System Software. While the barcode scanning application is running, a command window will be displayed. Do not close the command window. The window will close automatically after the barcode is saved. If the command window is closed by the user, then the scanned barcode will not be saved.



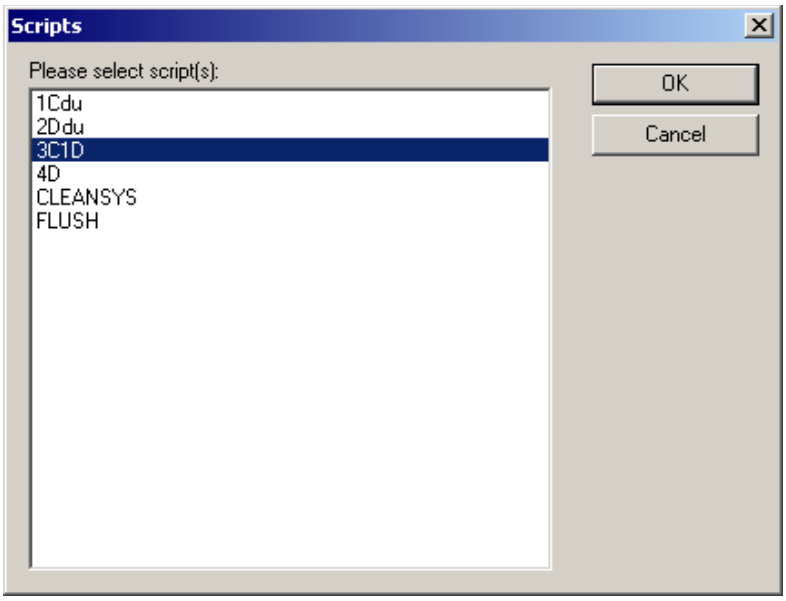
The barcode upgrade includes functionality to ensure that the scanned capture plate corresponds to the correct capture plate. However, it is important that users do not switch the sequence of plates on the RCS (for example, during error recovery) to ensure that the association of the capture plate and hybridization plate are correct. Incorrect plate association could lead to incorrect results.

Rapid Capture System RUN Example 1: 3C1D Script

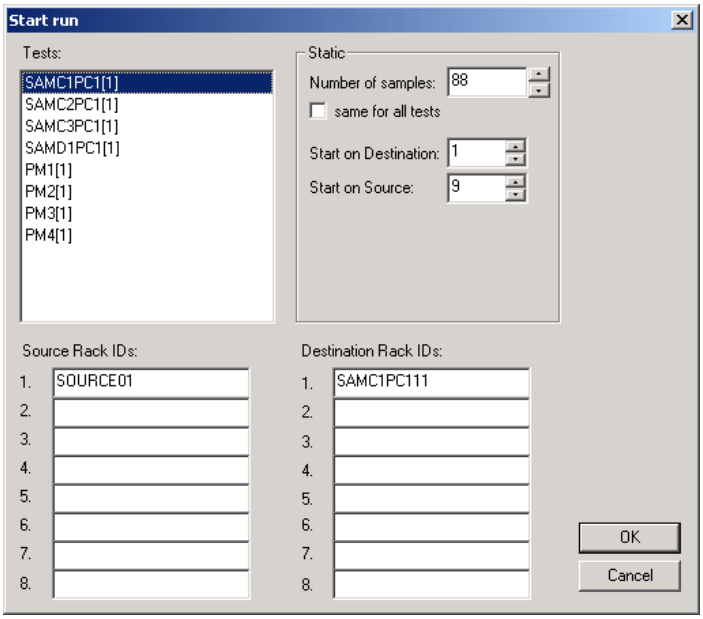
1. From the Rapid Capture System Software, select on the flag icon.



2. The **Scripts** dialog box appears, listing the scripts that have been added to the RCS run list via the Rapid Capture System ScriptSelect Software.
3. Select **3C1D**. This script is used for single-probe testing for 3 Conversion Racks and 1 digene Specimen.
4. Select **OK**.

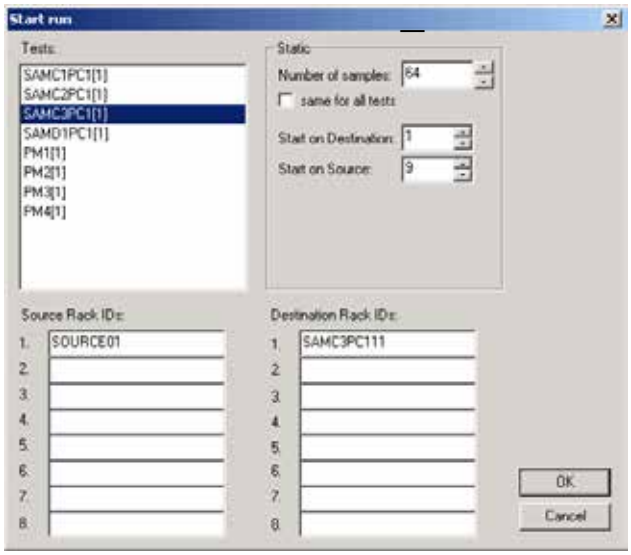


5. The **Start run** window appears (see below).

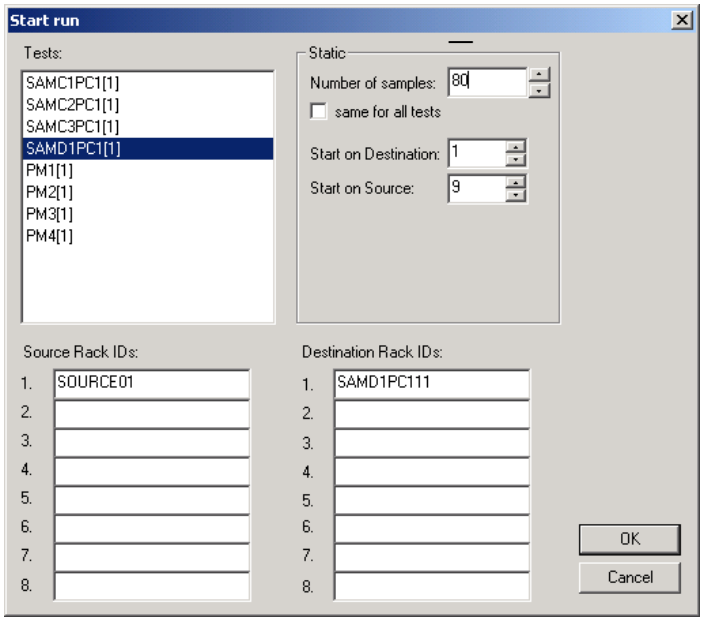


6. In the **Number of Samples** text box of the **Start Run** window, the default specimen numbers are for full plates of 88 specimens. If a partial plate is being run, this number can be changed from 88 to adequately reflect the number of specimens being run on that plate. To change the number of samples from the default of 88 for a specific plate, select the desired plate in the **Tests** area. In the **Test** area, the prefix SAM indicates the number of specimens to be transferred from the rack to the hybridization plate. For this example, the plate SAMC3PC1(1) is a partial plate of Converted specimens (PreservCyt specimens) and the SAMD1PC1(1) is a partial plate of digene Specimens.

7. For this example **SAM3CPC1(1)** is the third plate of the 4-plate run and contains 64 converted specimens. The **"C"** in the **SAMC3PC1(1)** denotes a Converted Specimen Rack**.** In the **Tests** area, select **SAMC3PC1(1).** In the **Number of Samples** text box, enter the number of specimens, not including calibrators or controls, to be run on the partial plate.Type in **64** for **Number of Samples**.



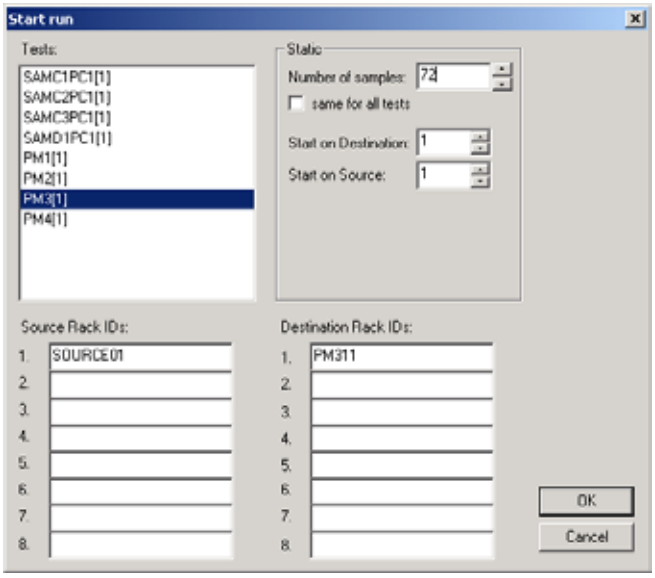
8. For this example **SAMD1PC1(1)** is the last plate of the 4-plate run and contains 80 digene Specimens. The **"D"** in the **SAMD1PC1(1)** denotes a digene Specimen Rack**.** In the **Tests** area, select **SAMD1PC1(1).** Type in **80** for **Number of Samples**.



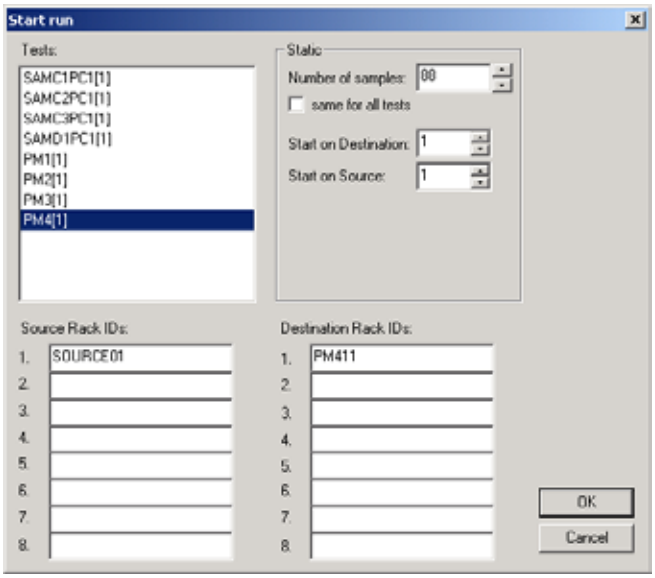
9. **It is critical that the correct specimen number be entered for the appropriate plate**. Entering a number that is less than the correct value will result in specimens not being transferred from the specimen collection tube to the hybridization plate. Entering a specimen number greater than the correct value will result in a longer than necessary time to transfer the rack. Assay results and instrument performance may also be impacted if a reagent is added to a well that does not contain a specimen. Instrument failure could occur due to the formation of precipitates that can clog the cannulas of the wash head.

10. In the **Tests** area, the PM1(1)-PM4(1) determine the number of wells to receive assay reagents for each plate. The PM1(1)-PM4(1) include the total number of specimens to be tested plus Calibrators and Controls. In the **Static** area, the number of specimens plus 8 (for calibrators and controls) is entered into the **Number of samples** text box. The default is a full 96-well plate. For this example, the correct number of specimens for the partial plates PM3(1) and PM4(1) (which correspond to SAMC3PC1(1) and SAMD1PC(1), respectively) must be entered.

11. Select **PM3(1),** then enter **72** for **Number of Samples** (64 specimens + 8 calibrators and controls). It is critical that the correct specimen number be entered for the appropriate plate. Entering a specimen number that is less than the correct value will result in specimen wells that do not have reagents added and are not transferred to the capture plate. Entering a specimen number greater than the correct value may cause Probe to be added to wells not containing specimen. The Probe will not be diluted by the specimen. This could cause the formation of precipitates that clog the cannulae of the wash head.



12. Select **PM4(1).** Enter "**88**" for **Number of Samples**. (80 specimens + 8 calibrators and controls). It is critical that the correct number of specimens be entered for the appropriate plate. Entering a specimen number that is less than the correct value will result in specimen wells that do not have reagents added and are not transferred to the capture plate. Entering a specimen number greater than the correct value may cause probe to be added to wells not containing specimen. The Probe will not be diluted by the specimen. This could cause the formation of precipitates that clog the cannulae of the wash head.



CAUTION: NEVER check the box "same for all tests" while running patient specimens. Checking this box will lead to a failure to add the appropriate amount of reagent to some patient specimens.

13. Select **OK** to begin the script.

14. All onboard components will initialize, and a Script Alert will appear reminding the user of proper deck setup.

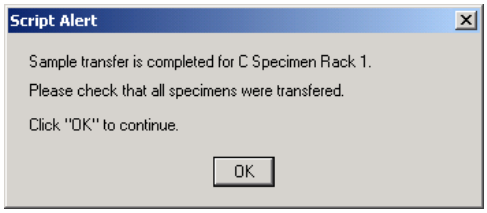
15. When deck setup is complete, select **OK**. The system lines will prime and flush.

16. A Script Alert appears instructing the user to place the C Specimen Rack 1 on the platform.

17. Place the Conversion (C) Rack 1 on the deck so the largest notched corner of the rack is located in the right front and the base is positioned within the rack guides on the deck.

18. Select **OK** to begin specimen transfer.

19. Once rack 1 specimens have been transferred, the screen will display a script alert directing the user to verify that all specimens have been transferred.



Remove the Conversion Rack from the deck. Visually inspect the hybridization plate for any empty wells that should have received specimen. Any specimens that failed to transfer must be manually transferred using a single-channel pipettor (20-200 µl) and extra-long pipette tips. The transfer volume is 75 µl. The position of the well in the plate directly corresponds to the position of the specimen tube in the rack. The Hybridization plate may be removed from the deck to facilitate a manual transfer.

CAUTION: Before continuing the run, it is critical that the plate be properly situated on the RCS deck when returned to the pipetting position.

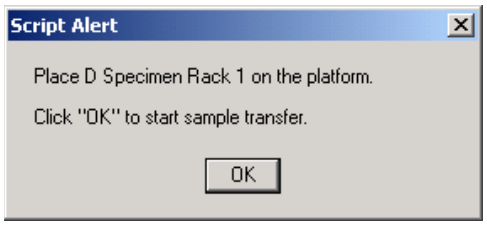
20. Select **OK**.

21. Follow prompts and repeat steps 15-19 for the remaining Conversion Racks.

    Note: The fourth rack is a digene Specimen Rack. The specimens loaded on the rack contain digene HC2 DNA Collection Devices. X, Y, Z collection location of the tip adapters on the RCS is different for the digene Specimen Rack and the Conversion Rack. Drop-on caps must be placed in the proper orientation on all digene specimens as displayed in Figure 2: Orientation of Drop on caps.

22. Ensure the digene Specimens have drop-on caps on the tubes and ensure the brush shaft will not obstruct the tip adapters during specimen transfer.

23. A Script Alert dialog appears instructing the user to place the "D" Rack on the RCS deck.



24. Select **OK** after ensuring all digene HC2 DNA Collection Devices have drop-on caps.

25. Once the D Specimen Rack 1 specimens have been transferred, the screen will display a script alert window directing the user to verify that all specimens have been transferred.

Remove the *digene* Specimen Rack from the deck. Visually inspect the hybridization plate for untransferred specimens. Any specimens that failed to transfer must be manually transferred using a single-channel pipettor (20-200 µl) and extra-long pipette tips. The transfer volume is 75 µl. The position of the well in the plate directly corresponds to the position of the specimen tube in the rack. The hybridization plate may be removed from the deck to facilitate a manual transfer.

WARNING: Before continuing the run, it is critical that the plate be properly situated on the RCS deck when returned to the pipetting position.

26. Select **OK**.

27. After the last rack of specimens has been transferred and checked, a script alert will appear reminding the user to refill the disposable tip (DT) racks.

28. At this time, refill all empty and partially empty disposable tip racks with full trays of tips. Empty the disposable tip waste container. It is important to follow the instructions in the Script Alerts before selecting **OK.** The Rapid Capture System Software will control the timing of the assay steps once the Probe Mix addition step begins. Any user interruptions after that point will interfere with assay incubation times.

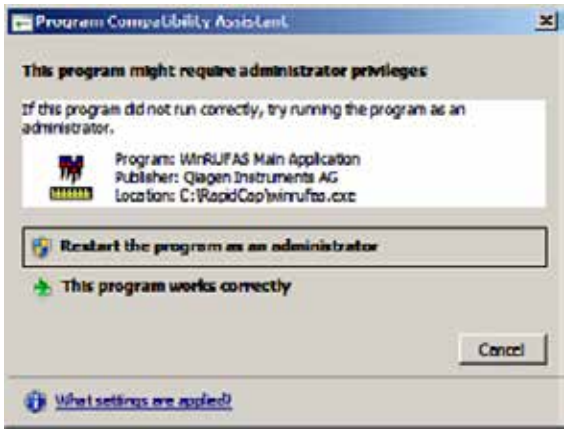
29. Select **OK** and the RCS will complete all subsequent steps of the assay through Detection Reagent 2 incubation, providing 3.5 hours of user-free run time. Set a timer for 3 hours and 20 minutes to ensure returning to the instrument in time to read the first plate.

Notes:

- The Rapid Capture System Software monitors the temperature of the Incubator chambers. Addition of Probe will not begin until the set temperature of 65°C is achieved. At that time, the script will continue automatically, with no user intervention required.

- If an instrument error occurs, the Rapid Capture System will sound an alarm, pause, and wait for user intervention. **Therefore, it is recommended that the user remain within hearing distance of the instrument during the run.** If an error occurs, immediately consult with your local QIAGEN Representative for instructions.

- When exiting the RCS software after running a script, the Windows Compatibility Assistant may display. The RCS has been validated for use with Windows 7. This dialog can be closed without issue.



VI. READING THE MICROPLATES AND GENERATING RESULTS

Notes:

- The luminometer must be turned on at least 1 hour prior to reading the first plate. It is recommended that the luminometer be left on at all times. The user is required to retrieve the microplates from the Rapid Capture System deck at the end of the Detection Reagent 2 incubation period for each plate. Each plate is then placed in the luminometer for result generation.
- Verify that RCS-specific protocols were used to create the plate layout.

1. When plate 1 has completed its Detection Reagent 2 incubation and is ready for signal detection using the luminometer, the Rapid Capture System will audibly alarm and a **Script Alert** dialog reading "**Assay is completed. Read plate in luminometer"** will appear.

2. Retrieve the plate from the pipetting position on the RCS deck.
3. Select **OK.** The RCS will continue processing the remaining plates.

   Note: The RCS HPV assay protocol in the digene HC2 System Software has been programmed to apply a Calibration Adjustment Factor (CAF) of 0.8 to the mean RLU value of the valid Positive Calibrator replicates. This CAF is necessary to ensure the performance characteristics of the assay performed on the RCS remain equivalent to the manual test procedure. This change only applies to assays performed using the Rapid Capture System. Therefore, it is critical to select the correct assay protocol for use with each specific test method in order to generate accurate test results.

4. Place the plate in the luminometer and read. Refer to the Hybrid Capture 2 System User Manual for details regarding measuring a plate and generating result reports.
5. Repeat steps 1 - 4 above for all remaining plates.
6. Refer to the digene HC2 High-Risk HPV DNA Test IFU for quality control, assay verification, and instructions for interpretation of results.

   Note: Printing result reports from the luminometer while generating additional result reports, can, in some situations, cause a slowdown of the RCS that may affect assay timing. It is recommended that results from one plate be printed before results from subsequent plates are read in order to avoid this situation. Alternatively, all plates may be read but results should not be printed until the RCS run is completed.

VII. Daily/System Cleanup

1. Discard the hybridization plates and plate lid in Stacker B.

2. Clean reagent troughs and lids as follows:

   2a. Troughs: Discard residual reagents in accordance with local, State, and Federal requirements. Wash with deionized/distilled water, rinse with deionized/distilled water, and fill completely with sodium hypochlorite solution, 0.5% v/v. Allow the troughs to soak in the sodium hypochlorite solution overnight. The next day, rinse troughs thoroughly with deionized/distilled water for at least 60 seconds. Place inverted troughs on a paper towel to dry. Replace reagent troughs monthly.

   2b. Trough Lids: Wash with deionized/distilled water, rinse with deionized/distilled water, and soak overnight in sodium hypochlorite solution, 0.5% v/v. The next day, rinse thoroughly with deionized/distilled water for at least 60 seconds. Place on a paper towel to air dry. Reagent trough lids are not disposable and need not be replaced unless damaged or lost.

   Note: If a second Rapid Capture System run immediately follows the first run, it is recommended to use a second set of troughs and trough lids.

3. Discard the capture plates after reading and assay verification.

4. If the instrument will not be used the next calendar day, disposable tip racks containing unused tips should be covered with a plate lid to prevent dust from contaminating the tips.

5. Empty the disposable tips waste container into an appropriate container.

6. Empty the waste container. Rapid Capture System waste has a relatively neutral pH. Dispose according to local, State, and Federal requirements.

7. Ensure that the quick-release fittings click securely in place when reconnecting the fittings to the waste container. Also, ensure that the bottle is situated correctly with no kinks in the lines.

8. Wipe down all surfaces with an alcohol dampened soft cloth or low-lint paper towel. These surfaces include:

   8a. Shaker positions and rollers. Rollers should not stick in position.
   8b. Tip Eject Station Drip Guard (Guard must be removed and rinsed with deionized/distilled water)
   8c. Tip Eject Slide (remove all tips and wipe between rails with alcohol to remove residual fluid)
   8d. Tip Rinse Station and Cover. Remove cover and rinse with deionized/distilled water.
   8e. Trough Rack.
   8f. Inside of Stacker A and B.
   8g. Pipetting Positions 1 and 2.
   8h. All other deck surfaces.

9. Clean each pipette adapter with an alcohol wipe.

10. Remove Plate Washer Boat and clean the Washer Platform and the top and bottom of the Washer Boat with an alcohol dampened soft cloth or low-lint paper towel.

Notes:

- To prevent contamination of work areas with the alkaline phosphatase present in the waste solution, always change gloves after any possible contact with the waste solution including contact with the quick-disconnect fittings.
- Reference the Routine Maintenance and System Shut Down sections of this user manual.

VIII. LIMITATIONS OF THE PROCEDURE

1. Failure to visually observe the hybridization plate to ensure proper specimen transfer and failure to correct for any inadequate specimen transfer may result in false-negative results.

2. To obtain the required reagent volumes for RCS testing, only reagents from the same kit lot may be combined.

3. Reference the digene HC2 High-Risk HPV DNA Test IFU for additional limitations specific to the test method.

4. Follow the warnings and precautions stated in the digene HC2 High-Risk HPV DNA Test IFU.

IX. PERFORMANCE CHARACTERISTICS

Refer to the digene HC2 High-Risk HPV DNA Test IFU for specific performance characteristics of HPV testing using Hybrid Capture 2 technology.

X. ADDITIONAL PERFORMANCE CONSIDERATIONS WHEN USING THE RAPID CAPTURE SYSTEM

When performing high specimen-throughput testing using the Rapid Capture® System, consider the following performance characteristics.

A. Carryover

The Rapid Capture System was designed to minimize specimen contamination or carryover of residual alkaline phosphatase through the use of disposable pipette tips for reagent and specimen aspiration. To confirm this design characteristic, QIAGEN conducted several studies to evaluate if use of the Rapid Capture System increased the potential for carryover or cross-contamination of specimens compared with the manual method. Multiple Rapid Capture Systems were used to assess carryover potential from system-to-system.

In one study, 2ng and 20ng of HPV DNA Plasmid was added to the Negative Calibrator material to prepare highly positive simulated STM specimens. The 20 ng/ml concentration yields RLU values approximately 3-5 times higher than those of the highest positive clinical specimen expected to be observed during routine clinical testing. These highly positive simulated specimens were placed throughout the microplate in a checkerboard pattern alternating with wells containing only Negative Calibrator (test wells). This design considers potential additive effects of sequential high-positive specimens. Microplates were then tested using both the manual method and the Rapid Capture System. After processing, the numbers of false positive test wells were compared. The Rapid Capture System did not produce more false-positive test wells than the manual method with these simulated STM specimens, even when an extremely high sequence of positive specimens were contained on the plate.

In a second carryover evaluation, HPV positive, patient PreservCyt specimens were combined to create a panel of specimens with differing levels of chemiluminescence to yield RLU/CO values representative of the range expected during routine clinical use of the Rapid Capture System. The positive specimens ranged from approximately 200 to 1800 RLU/CO. To assess the potential for carryover, including the potential additive effects of sequential high positives, these positive panel members were placed on microplates in a checkerboard pattern next to Negative Calibrator wells. These plates were then assayed using the Rapid Capture System.

The results of this carryover evaluation, using pooled patient specimens*, suggests a potential false-positive rate of 0.3% due to carryover effects when utilizing the Rapid Capture System for digene HC2 High-Risk HPV DNA Test.

* QIAGEN's experience conducting tests with pooled PreservCyt specimens suggests that pooling patient PreservCyt specimens creates specimens that do not exhibit characteristics similar to single patient specimens. Although the effects of this pooling on the carryover potential of the Rapid Capture System are unknown, additional pre-clinical testing of the Rapid Capture System indicated no increased potential for false-positive results due to carryover. These evaluations were conducted using artificial plasmid specimens with DNA concentrations nearly 5 times higher than observed in the clinical setting.

A third carryover evaluation created test specimens by adding a fluorescent dye, in concentrations representative of the dynamic RLU range of the assay, to background matrices that approximated the viscosity of clinical specimens and digene HC2 High-

Risk HPV DNA Test reagents. These test specimens were then processed using three separate RCS instruments and evaluated the carryover potential of each of the following key procedural steps of the RCS application: 1) specimen transfer, 2) plate-to-plate transfer, 3) probe addition, 4) microplate shaking, and 5) microplate washing. The resulting fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm and was sensitive enough to detect a carryover event on the order of 1:20,000, which would correspond to a false-positive result with the digene HC2 High-Risk HPV DNA Test (i.e. 1pg in 20ng). The results of this evaluation demonstrated no carryover event during any of the key procedural steps of the RCS application, which would lead to a false positive digene HC2 High-Risk HPV DNA Test result.

B. Onboard Reagent Stability

QIAGEN assessed Rapid Capture Assay performance characteristics when using reagents that remained onboard the system platform for extended periods. The reagents most likely to be subject to extended onboard placement include the Probe Mix, Detection Reagent 1, Detection Reagent 2, and the Capture Plates.

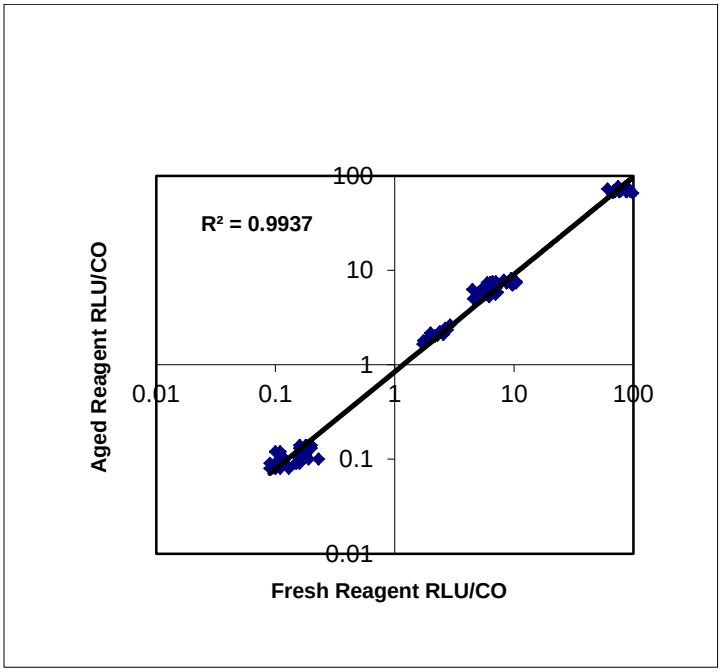
Assay performance was evaluated using both freshly prepared reagents and reagents that were allowed to age onboard the Rapid Capture System platform at room temperature for a period of 16 hours (to simulate 2 work shifts in the laboratory setting). Testing of simulated clinical specimens were performed using two Rapid Capture Systems on each of two testing days with reagents matrix as follows:

Table 6
Study Design for Onboard Reagent Stability Evaluation

Rapid Capture System	Day 1	Day 2
1	Aged Reagents	Fresh Reagents
2	Fresh Reagents	Aged Reagents

A plot of all RLU/CO data points is shown in Figure 3. The plot and regression analysis for aged versus fresh reagents indicate agreement between the aged and fresh reagents.

Figure 3
Scatter-Plot Comparing Assay Calibrator and Control Values Using Aged and Fresh Reagents



Further examining the agreement results shows that no qualitative results changed when using aged reagents:

Table 7
Agreement of Calibrator and Control Results
Fresh vs. Aged Reagents

Overall Agreement (95% C.I.)	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)	R2	Slope	Intercept	Kappa
100% 96/96 (97.97-100)	100% 64/64 (97.97-100)	100% 32/32 (97.97-100)	0.9937	0.97	0.47	1.0

The data analysis shows the results to be statistically identical for fresh and aged reagents, indicating that the reagents are sufficiently stable when placed onboard the instrument for a period of up to 16 hours.

C. Reproducibility with STM Specimens

To assess the within-run, day-to-day, and inter-laboratory reproducibility of digene HC2 High-Risk HPV DNA Test results using STM specimens tested with the Rapid Capture System, a 16-member panel of pooled patient specimens was tested using a single lot of reagents for each test run, two times per day on three different days. Each panel member was tested in quadruplicate. The panel was composed as illustrated in the following table.

Table 8
STM Specimen Reproducibility Panel Composition

Panel ID	Composition*	Expected *digene* HC2 High-Risk HPV DNA Test Result
1N	< 0.4	Negative
2N	0.4 – 0.8	Negative
3E	0.8 – 1.2	Equivocal
4E	0.8 – 1.2	Equivocal
5E	0.8 – 1.2	Equivocal
6P	1.2 – 2.0	Low Positive
7P	1.2 – 2.0	Low Positive
8P	1.2 – 2.0	Low Positive
9P	2.0 – 5.0	Low Positive
10P	5 - 10	Mid Positive
11N	< 0.4	Negative
12N	< 0.4	Negative
13N	< 0.4	Negative
14XR	LR HPV DNA Positive Clinical Material in STM clinical negative pool	Equivocal
15XR	LR HPV DNA Plasmid in STM clinical negative pool	Equivocal or Low Positive
16XR	Plasmid Vector DNA Control in STM clinical negative pool	Equivocal or Low Positive

*RLU/CO values represent the target values intended during preparation of the panel members and may not reflect the exact values observed during testing.

Panel members 14XR and 15XR were included to evaluate the potential for cross-hybridization of the High-Risk HPV Probe with specimens containing only low-risk HPV DNA types 6, 11, 42, 43, and 44. Panel member 16XR was composed of pGEM DNA at a concentration of 1.49ng/ml and served as a vector control for panel member 15XR. The results of this testing indicated no false-positive digene HC2 High-Risk HPV DNA Test results due to the presence of low-risk HPV DNA types in clinical specimens. These results are consistent with the manually performed assay.

The reproducibility of HR HPV results when testing STM specimens using the Rapid Capture System is described in **Table 9**. Variability was calculated according to the method described by NCCLS E5-A¦. This method requires the computation of variance components for each of the sources of variability: laboratory, day, run and error (defined as inter-assay and between-assay variation).

¦ NCCLS. *Evaluation of Precision Performance of Clinical Chemistry Devices*; Approved Guideline. NCCLS document E5-A (1999).

Table 9
Rapid Capture System: STM Specimen Reproducibility Study
Standard Deviations (SD) and Coefficients of Variation (CV)
By Laboratory, By Day and By Run**

Panel Member	N	Mean RLU/CO	Standard Deviation					Total CV%
			Within Run	Between Run	Between Day	Between Lab	Total	
1N	72	0.13	0.02	0.01	0.01	0.01	0.02	15.10
2N	72	0.36	0.03	0.01	0.03	0	0.04	11.69
3P	72	0.96	0.06	0.06	0.04	0	0.09	9.55
4P	72	1.03	0.06	0.18	0.06	0	0.19	18.81
5P	72	1.41	0.11	0.14	0.15	0.06	0.24	17.00
6P	72	1.73	0.10	0.27	0	0.11	0.31	18.10
7P	72	1.74	0.12	0.21	0	0	0.24	13.78
8P*	70	1.95	N/A	N/A	N/A	N/A	0.47	23.80
9P	72	5.21	0.34	0.44	0.21	0	0.59	11.36
10P	72	7.67	0.46	0.63	0.71	0	1.05	13.70
11N	72	0.13	0.01	0.01	0.01	0	0.02	16.89
12N	72	0.17	0.03	0.06	0.03	0	0.07	39.14
13N	72	0.15	0.02	0.02	0	0.01	0.03	17.01

*Two invalid replicates for panel member 8P precluded variance component analysis due to unequal size groups under comparison.
** Negative variance components are set equal to zero.
N/A: variance analysis not possible due to fewer replicates than other panel members

C. Precision with PreservCyt Solution Specimens

An in-house precision study of the Rapid Capture System HPV application was performed using clinical PreservCyt specimens obtained predominately from women with cytology of ASC-US or greater (HPV prevalence 57%). Specimens were divided into two aliquots; each aliquot was then processed individually using the digene HC2 Sample Conversion Kit and then tested in duplicate with the digene HC2 High-Risk HPV DNA Test. As with other qualitative IVDs, variability of digene HC2 High-Risk HPV DNA Test results obtained from clinical specimens is associated primarily with one or a combination of the following: 1) Specimen collection; 2) specimen processing prior to testing; and 3) the testing procedure. Variability due to specimen collection was controlled because comparative replicates were obtained from the same clinical specimen. The repeatability of results obtained from two individually processed specimen aliquots from the same clinical specimen (referred to as "Between Processed Aliquots") reflects variation due to the combination of PreservCyt specimen conversion processing and the digene HC2 High-Risk HPV DNA Test procedure.

In contrast, the repeatability of replicate results obtained from the same processed specimen aliquot (referred to as "Within Processed Aliquot") reflects variation from the digene HC2 High-Risk HPV DNA Test procedure alone.

Table 10
Qualitative Result Agreement Within and Between Processed PreservCyt Aliquots

	Analysis	Positive Agreement (%) (n/N) 95% CI	Negative Agreement (%) (n/N) 95% CI	Overall Agreement (%) (n/N) 95% CI
Within Processed Aliquot	All data	99.62 (261/262) 97.9, 100.0	94.7 (160/169) 90.1, 97.5	97.7 (421/431) 95.8, 98.9
	Strong Positive/ Negative Regions	100.0 (249/249) 98.5, 100.0	98.2 (160/163) 94.7, 99.6	99.3 (409/412) 97.9, 99.9
Between Processed Aliquot	All data	99.6 (264/265) 97.9, 100.0	98.2 (163/166) 94.8, 99.6	99.1 (427/431) 97.6, 99.8
	Strong Positive/ Negative Regions	100.0 (249/249) 98.5, 100.0	99.4 (161/162) 96.6, 100.0	99.8 (410/411) 98.7, 100.0

D. Quantitative digene HC2 High-Risk HPV DNA Test Reproducibility Results for Simulated PreservCyt Solution Specimens when using the Rapid Capture System

A study was conducted to evaluate the quantitative reproducibility of results obtained with the Rapid Capture System when testing simulated PreservCyt Solution specimens. Three testing sites, including QIAGEN, participated in the study.

Each testing laboratory performed the digene HC2 High-Risk HPV DNA Test twice per day on five different days, using both the RCS and manual test procedures, with a reproducibility panel provided by QIAGEN. Each simulated PreservCyt panel member was tested in quadruplicate. The panel was made up of six members consisting of two negatives, two low-positives, one mid-positive and one high-positive panel member. Each panel member was composed of cultured cells spiked into PreservCyt Solution intended to yield an approximate RLU/CO value as described in the table below.

Table 11

Composition of Six-Member Panel for Rapid Capture System High-Risk HPV Application Simulated PreservCyt Specimens

Panel ID #	Cell Type	Approximate RLU/CO	Expected Result
1	JurKat	< 1.00	Negative
2	JurKat	< 1.00	Negative
3	SiHa + JurKat	5.0-8.0	Low Positive
4	SiHa + JurKat	5.0-8.0	Low Positive
5	SiHa	30.0-50.0	Mid Positive
6	SiHa	200.0	High Positive

The HPV DNA positive panel members were prepared by adding varying amounts of HPV DNA positive SiHa cells (from a laboratory cell line) to generate low-, mid-, and high-positive panel members. The negative panel member was composed of HPV-negative JurKat cells (from a different laboratory cell line). The final cellular concentration of all six specimens (with or without HPV infected cells added) was approximately 5 x 10^4 cells/ml.

The reproducibility of HR HPV results when testing PreservCyt specimens using the Rapid Capture System is described in **Table 12**. Variability was calculated according to the method described by NCCLS E5-A ¦. This method requires the computation of variance components for each of the sources of variability: laboratory, day, run and error (defined as inter-assay and between-assay variation). The results of these analyses are presented for each specimen in the table below. Each of the six panel members was tested in quadruplicate in each of the 10 runs (two runs per day over 5 days of testing) at each of the three testing laboratories.

Table 12

Rapid Capture System: PreservCyt Specimen Reproducibility Study Standard Deviations (SD) and Coefficients of Variation (CV) By Laboratory, By Day and By Run* (N=120)

Panel Member	N	Mean RLU/CO	Standard Deviation					Total CV%
			Within Run	Between Run	Between Day	Between Lab	Total	
1N	120	0.20	0.04	0.01	0.01	0.08	0.089	44.4
2N	120	0.20	0.06	0.01	0	0.08	0.10	52.2
3P	120	4.05	0.76	1.17	0	0.26	1.42	35.1
4P	120	4.23	0.74	0.86	0	0.31	1.18	27.8
5P	120	28.6	5.00	5.61	4.41	0	8.71	30.5
6P	120	214.6	33.95	27.25	18.09	25.53	53.61	25.0

*Negative variance components are set equal to zero.

¦ NCCLS. *Evaluation of Precision Performance of Clinical Chemistry Devices*; Approved Guideline. NCCLS document E5-A (1999).

To supplement this initial reproducibility study with data from specimens very close to the assay cutoff, an additional precision study was conducted at a site external to QIAGEN using the Rapid Capture System. This external site completed HR HPV testing with the RCS application using a single lot of digene HC2 High-Risk HPV DNA Test reagents for each test run, performing the test twice per day on three different days with a five-member reproducibility panel of simulated PreservCyt specimens provided by QIAGEN. Each panel member was divided into four aliquots and all four aliquots were tested on the same microplate. The simulated PreservCyt precision panel consisted of one negative, two negative/low-positive, and two low-positive members. Each panel member was prepared by spiking cultured Jurkat and SiHa cells spiked into PreservCyt solution to yield RLU/CO values as follows:

Table 13

Rapid Capture System: PreservCyt Specimen Precision Study Target RLU/CO Values of Panel Members

Panel #	Approximate RLU/CO Value	Expected Result
1	0.2	Negative
2	0.8 – 1.2	Negative/Low Positive
3	0.8 – 1.2	Negative/Low Positive
4	1.2 – 2.0	Low Positive
5	1.2 – 2.0	Low Positive

Table 14

Rapid Capture System: PreservCyt Precision Standard Deviations (SD) and Coefficients of Variation (CV) By Day and Run*

Panel Member	N	RLU/CO Mean	Standard Deviation				%CV
			Within Run	Between Run	Between Day	Total	
1N	24	0.14	0.01	0.00	0.02	0.02	15.12
2E	24	1.39	0.14	0.15	0	0.21	14.84
3E	24	1.31	0.16	0	0.11	0.19	14.70
4P	24	1.74	0.13	0.21	0.18	0.31	17.73
5P	24	1.63	0.24	0.20	0.26	0.40	24.63

*Negative variance components are set equal to zero.

E. Rapid Capture System HPV Application Result Agreement with the Manual Method in Clinical Specimens

A multicenter study (n = 2270 patients) was conducted to evaluate the clinical results obtained with the Rapid Capture System compared with the results obtained using the manual method. Testing was performed a three sites, external to QIAGEN, with patient specimens collected from five collection sites. The data set consisted of 1269 cervical specimens collected in PreservCyt Solution and 1001 specimens collected in Specimen Transport Medium.

Statistical agreements, between matched specimens tested with the Rapid Capture System and with the manual test, were calculated for this patient population.

Table 15
Summary of Agreement: RCS vs. Manual HPV Test Method
STM Patient Specimen Data
N=1001

		Positive %Agreement (n/N) 95% CI		Negative %Agreement (n/N) 95% CI	
Cytological Classification	HPV Prevalence %	Overall	Strong Positive Region (≥2.5)	Overall	Strong Negative Region (<0.8)
WNL <30 years	21%	99.3 (139/140) 96.1, 100	99.1 (112/113) 95.2, 100	99.3 (538/542) 98.1, 99.8	100 (531/531) 99.3, 100
WNL 30+ years	15%	92.0 (23/25) 74.0, 99.0	93.8 (15/16) 69.8, 99.8	100 (143/143) 97.5, 100	100 (142/142) 97.4, 100
ASC-US	65%	98.1 (51/52) 89.7, 100	100 (47/47) 92.4, 100	96.4 (27/28) 81.7, 99.9	100 (26/26) 86.8, 100
LSIL+	96%	100 (65/65) 94.5, 100	100 (62/62) 94.2, 100	66.7 (2/3) 9.4, 99.2	66.7 (2/3) 9.4, 99.2
Other	33%	100 (1/1) 2.5, 100	100 (1/1) 2.5, 100	100 (2/2) 15.8, 100	100 (2/2) 15.8, 100
All STM	28%	98.6 (279/283) 96.4, 99.6	99.2 (237/239) 97.0, 99.9	99.2 (712/718) 98.2, 99.7	99.9 (703/704) 99.2, 100

Table 16
Summary of Agreement: RCS vs. Manual HPV Test Method
PreservCyt Clinical Specimen Data
N =1269

		Positive %Agreement (n/N) 95% CI		Negative %Agreement (n/N) 95% CI	
Cytological Classification	HPV Prevalence	Overall	Strong Positive Region (≥2.5)	Overall	Strong Negative Region (<0.8)
WNL <30 years	20%	96.2 (75/78) 89.2, 99.2	100 (64/64) 94.4, 100	98.4 (301/306) 96.2, 99.5	99.0 (293/296) 97.1, 99.8
WNL 30+ years	8%	88.7 (47/53) 77.0, 95.7	92.1 (35/38) 78.6, 98.3	99.1 (578/583) 98.0, 99.7	99.5 (571/574) 98.5, 99.9
ASC-US	36%	100 (48/48) 92.6, 100	100 (46/46) 92.3, 100	96.6 (84/87) 90.3, 99.3	96.5 (83/86) 90.1, 99.3
LSIL+	77%	100 (64/64) 94.4, 100	100 (62/62) 94.2, 100	89.5 (17/19) 66.9, 98.7	88.9 (16/18) 65.3, 98.6
Other Cytology	11%	100 (3/3) 29.2, 100	100 (3/3) 29.2, 100	100 (24/24) 85.6, 100	100 (24/24) 85.8, 100
All PreservCyt Clinical*	20%	96.4 (238/247) 93.2, 98.3	98.6 (211/214) 96.0, 99.7	98.5 (1007/1022) 97.6, 99.2	98.9 (990/1001) 98.0, 99.4

* Cytology data unavailable from 4 patients

A supplemental clinical study was performed using archived residual PreservCyt specimens collected from a subpopulation of women aged 30 years and older with normal cytology (digene HC2 High-Risk HPV DNA Test).

Table 17 indicates seven (7) discordant results between the manual and RCS methods in the strong positive region. The initial manual results for these seven specimens were outside of the recommended PreservCyt specimen retest algorithm; however, because the study design required testing all specimens in triplicate, repeat results were available for discrepant resolution. The repeat testing data for each of the seven discordant specimens is shown in **Table 18** and suggests that all of the discordant specimens are negative for HPV DNA. Based on the repeat negative results obtained for both replicates, each of the initially positive manual results was likely false positive.

Table 17
Rapid Capture System vs. Manual HPV Test Method *digene* HC2 High-Risk HPV DNA Test Intended Use Population (N=2077)

	Positive Agreement (n/N) 95% CI		Negative Agreement (n/N) 95% CI	
HPV Prevalence	Overall	Strong Positive Region (>2.5)	Overall	Strong Negative Region (<0.8)
4.8%	92.0 (92/100) 84.84, 96.48	91.8 (78/85) 83.77, 96.62	99.3 (1964/1977) 98.88, 99.65	99.7 (1944/1949) 99.40, 99.92

Table 18
Discordant PreservCyt Specimens in the *digene* HC2 High-Risk HPV DNA Test Intended Use Population (n=7)

		Manual (RLU/CO)			RCS (RLU/CO)		
Sample	Site	Initial	Repeat 1	Repeat 2	Initial	Repeat 1	Repeat 2
1	A	2.51	0.08	0.08	0.12	0.17	0.14
2	A	20.18	0.08	0.09	0.19	0.24	0.20
3	A	3.88	0.12	0.11	0.17	0.22	0.22
4	A	9.37	0.09	0.09	0.15	0.21	0.20
5	A	6.01	0.17	0.13	0.25	0.30	0.30
6	B	2.97	0.71	0.99	1.59	0.89	0.90
7	C	11.01	0.16	0.14	0.19	0.15	0.21

Results from this clinical study indicate the overall agreement between the Rapid Capture System and manual methods using either STM or PreservCyt specimens.