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Introduction

The cobas® 4800 HPV Test is for the qualitative in vitro detection of Human Paillomavirus in patients specimens using PCR. There are 118 types of HPV, and approximately 40 different HPVs that can infect the human anogenital mucosa. The cobas® 4800 HPV test specifically identifies types HPV 16 and HPV 18, and also detects high risk types (31, 33, 39, 45, 51, 52, 56, 58, 59, 66, and 68).
HPV is a small, non-enveloped, double stranded DNA virus with a genome of approximately 8000 nucleotides. The presence of HPV has been found in 99% of cervical cancers, with types 13 to 18 is considered high-risk for the development of cervical cancer, and its precursor lesions.

Specimen Collection, Transport & Storage

Cervical specimens collected in primary container: PreservCyt Solution, cobas® PCR Cell Collection Media, and SurePath Preservative Fluid. Minimum volume in primary container should be 3.0 mL. Primary containers for PreservCyt Solution, and cobas® PCR Cell Collection Media should be vortex prior to loading on the cobas® x480.

Cervical specimens in PreservCyt Solution, cobas® PCR Cell Collection Media, and SurePath Preservative Fluid can be transported at 2-30°C. Cervical specimens collected in SurePath Preservative Fluid should be refrigerated 24 hour after collection.

Cervical specimens collected in PreservCyt Solution, cobas® PCR Cell Collection Media, may be stored at 2-30°C for up to 6 months after collection date. Cervical samples collected in SurePath Preservative Fluid may be stored at 2-8°C for up to 6 months after collection.

For tissue samples, please submit extracted DNA or PE curls (5x10uM) indicating sample source.

Materials, Equipment and Facilities

cobas® 4800 System Liquid Cytology Preparation Kit – **240 tests (stored at 2-8°C)**
PK- Proteinase K
SDS- 4800 System SDS Reagent
LYS- 4800 Lysis Buffer
cobas® 4800 System Sample Preparation Kit – **240 test (stored at 2-8°C)**
MGP-Magnetic glass particles
EB-Elution buffer

cobas® 4800 System Wash Buffer Kit – **240 test kits (stored at 15-25°C)**
WB-Wash Buffer
cobas® 4800 HPV Amplification/Detection Kit – **240 test (stored in Clean Room at 2-8°C)**
HPV MMX-Master Mix
HPV Mg/Mn-Magnesium/Magnesium
cobas® 4800 HPV Controls Kit **(stored at 2-8°C)**
HPV (+) C - Positive Control
HPV (-) C - Negative Control
Reagent Reservoirs – 50mL and 200 mL containers
cobas® 4800 System Extraction (deep well) Plate
cobas® 4800 AD (microwell) Plate
CO-RE pipette tips (1000 uL) rack of 96
Plate carrier
Tip carrier
Sample and reagent racks

cobas® x 480 Instrument
cobas® z 480 Analyzer
Heater/Shaker Unit
cobas® 4800 System Control Unit

Sample Processing

Samples received in SurePath Preservative fluid

1. Pipette 1 mL of Cobas Sample Prep Buffer into a tall tube
2. Vortex the sample received in SurePath Preservative fluid
3. Pippette 1 mL of sample into the the tube with 1 mL Cobas Sample Prep Buffer and mix well
4. Heat the tube of mixture on the 120 ^{o}C heating block for 20 mins
5. The heated sample can be loaded onto Roche Cobas 4800 after cooling down

Offboard preparation for deparafinization of tissue for HPV testing on Cobas 4800

1. Place the sections in a 1.5 mL Eppendorf tubes.
2. Add 320 µl Qiagen Deparrafinization Solution and vortex vigorously for 10s. Centrifuge briefly to collect the sample at the bottom of the tube.
3. Incubate at 56 ^{o}C for 3 mins and allow to cool at room temperature (15-25 ^{o}C).
4. Add 180µl Qiagen Buffer ATL and mix by vortexing.
5. Centrifuge for 1 min at 11,000 g (10,000 rpm)
6. Add 20µl proteinase K to the lower clear phase. Mix gently by pipetting up and down.
7. Incubate at 56 ^{o}C for 1 hour.

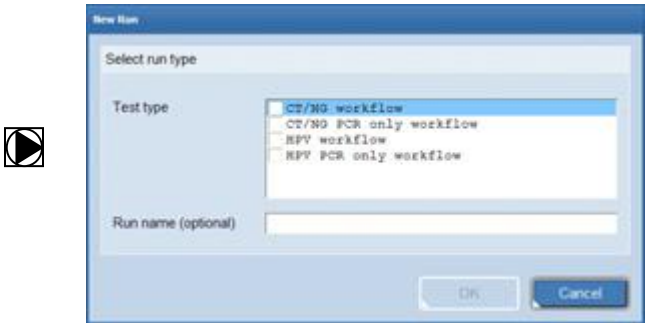
8. Incubate at 90 ^{o}C for 1h.
9. Briefly centrifuge the 1.5 mL tube to remove drops from inside the lid.
10. Pipet 180 µl of the lower clear phase into a pre-aliquoted 1.2 mL Thinprep solution Roche tube.
11. Load onto the Roche Cobas 4800

Procedure

NOTE: All reagents must be at <u>except HPV Amplification/Detection Kit</u> should be at room temperature before loading on the cobas® x480. HPV MMX and HPV Mg/Mn can be loaded directly on cobas® x480 from 2-8°C.
Specimens in PreservCyt Solution, cobas® PCR Cell Collection Media, and SurePath Preservative Fluid must be at room temperature before loading on the cobas® x480.

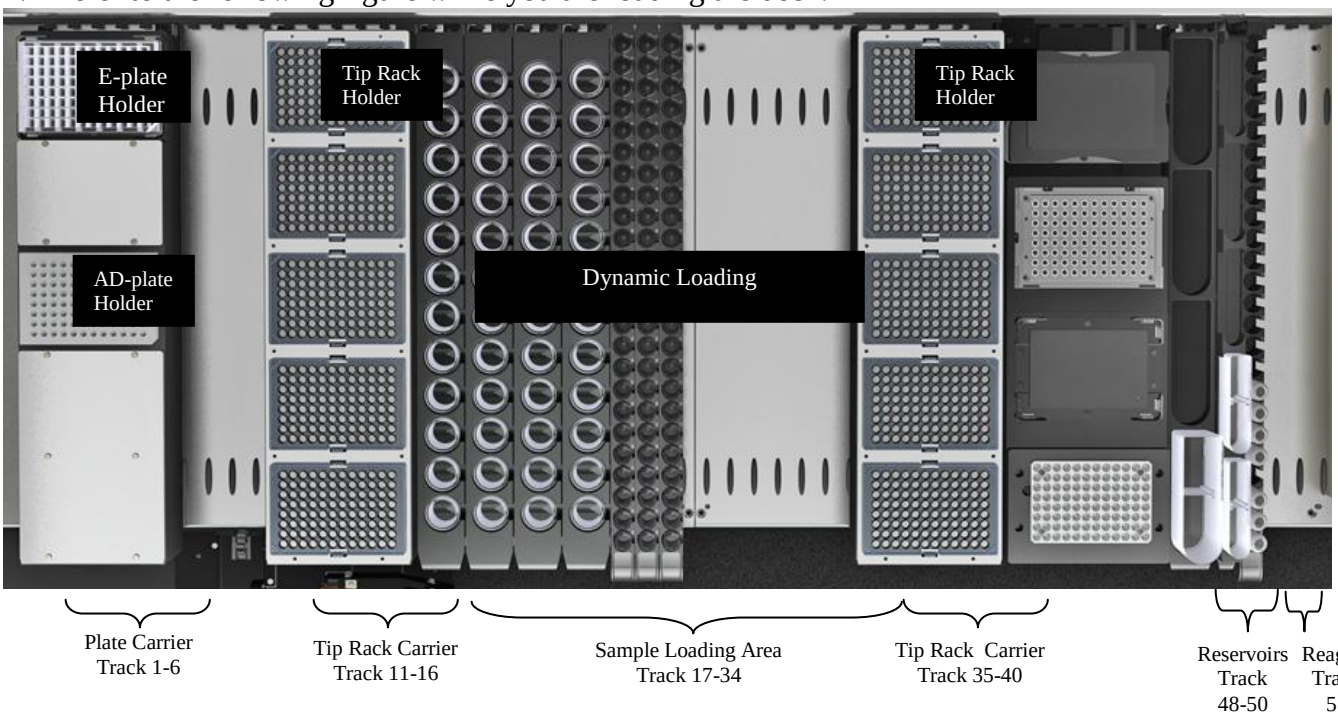
1. Turn on the system as the following sequence:
   1. cobas® z 480 **2**. Heater/Shaker unit **3.** cobas® x 480 instrument
2. When the left orange light on the cobas® z 480 turns to solid green, turn on the computer workstation.
3. Remove one set of reagents from cobas® 4800 System Liquid Cytology Preparation Kit, one set of reagents from cobas® System Sample Preparation Kit from fridge, and allow reagents to come to room temperature
4. Logon to the cobas 4800 software
   User ID: **LABOPERATOR**
   Password: **00labOPR**
5. Perform instrument maintenance: follow the online window dialogue boxes
   1) Select **Overview** tab and check maintenance status of the cobas x 480 instrument.
      - ❖ If weekly maintenance is due, click Run Weekly **Maintenance.**
      - ❖ If daily maintenance is due, click Run Daily **Maintenance.**
   2) Follow the online instructions.

6. Start New Run
   a. After the completion of the maintenance, in the overview tab, select **New Run** icon. on the right hand vertical bar
   b. The following window pops up. Select test type: **HPV workflow**.

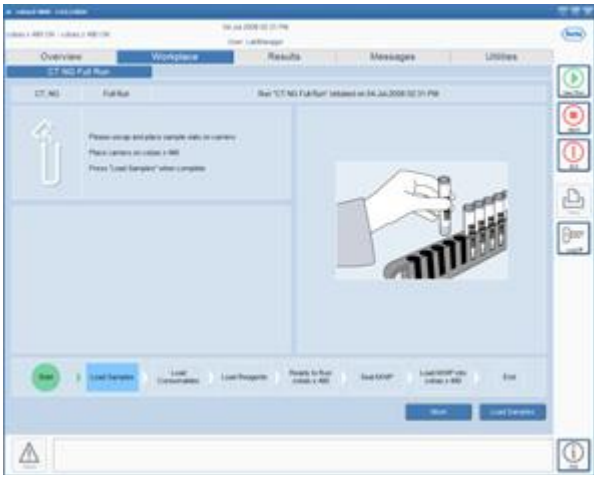


c. Type run name ie: HPV 2012.05.29-1
d. Click OK.

7. Refer to the following figure while you are loading the deck:

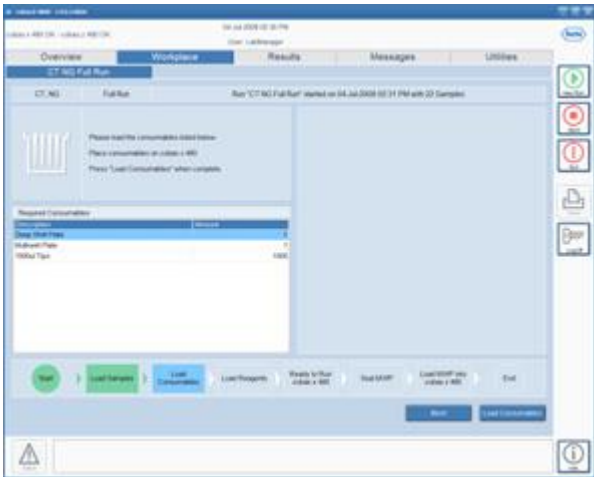


8. Load samples
Vortex the primary PreservCyt Solution samples prior to loading on the cobas® x480.



Un-cap samples.
Place samples on corresponding carrier.
Insert sample carriers on autoload tray when the LEDs for the designated track(17-34) are flashing.
Select **Load Samples**

9. Load consumables



Place listed consumables on appropriate carriers.

- **Both** tip carriers are fully filled and barcodes facing the right hand side
- Ensure deepwell plate is loaded flatly and properly –barcode facing the right hand side
- Ensure microwell plate is loaded flat and properly (barcode facing away from you)

Insert carriers on autoload tray.

Select **Load Consumables.**

10. Load reagents

200 mL reagent reservoir carrier

- Load Wash Buffer (WB) on 200 mL reagent reservoir carrier as indicated in the wizard (scan-scan-pour).
- Insert carrier on autoload tray.
- Click **Load Reagents**.

Note: Once the Wash Buffer is scanned you have one hour to complete the loading process and Press Start button.

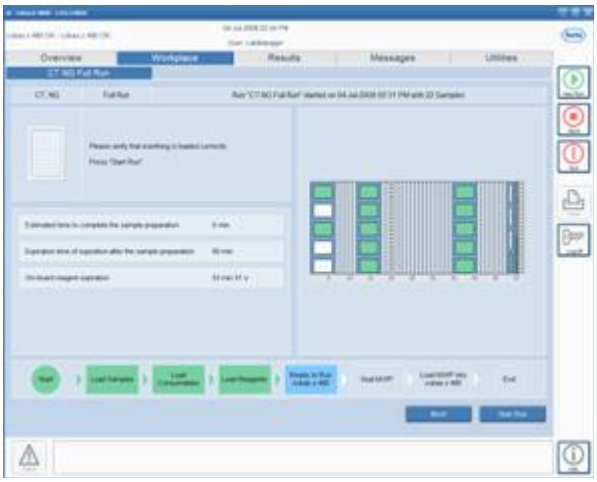
50 mL reagent reservoir carrier

- Load reagents on 50 mL reagent reservoir carrier as indicated in the wizard.(scan-scan-pour).
- Elution Buffer (EB), SDS Reagent (SDS), Lysis Buffer (LYS)
- **vortex MGP for 10s prior to scanning and loading**
- Insert carrier on autoload tray.
- Click **Load Reagents**.

Reagent carrier

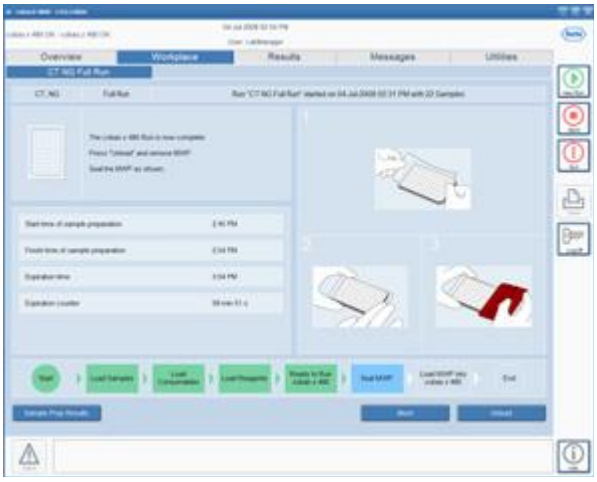
- Take out **HPV MMX** and **HPV Mg/Mn** from the clean room
- Open reagent vials and load them on reagent carrier as indicated in the wizard.
- **Change gloves after uncapping positive control**
- Insert carrier on autoload tray.
- Click **Load Reagents**.

11. Start sample preparation run



Select **Start Run**.
The sample preparation starts.
Check the timer in the wizard; timer should count down.

12. Unload AD (microwell) Plate. Seal AD plate.

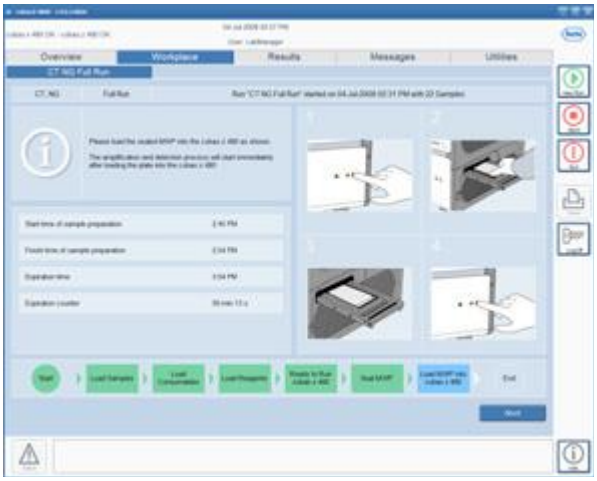


Select Sample Prep Results to review the results of the sample preparation.
Select **Unload**.

Seal AD (microwell) plate with Sealing Film and applicator as indicated on screen.
Be sure not to touch the seal with your fingers as it may affect detection. Hold the sides of the plate and use the smoothing applicator provided to properly seal.
The plate must be transferred from the cobas x 480 to the cobas z 480 within 90 minutes of completion

Select **NEXT**

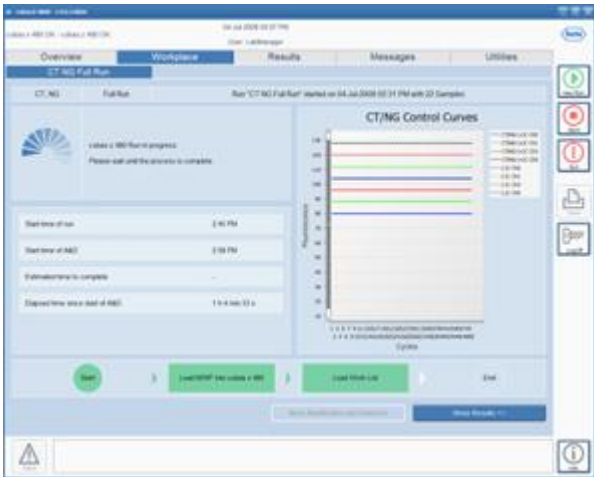
13. Load mirowell plate on **cobas® z 480** analyzer.



Press load button on the cobas z 480 analyzer.
Place the sealed AD (microwell) plate into the extended loader.
Select load button again.
The amplification and detection run starts automatically.
Check the timer in the wizard; timer should be counting down.

14. Remove used reagents, reservoirs, samples, and deepwell plate from the **cobas x 480** instrument. Discard waste appropriately, used reagents should be disposed of properly.
(Some reagents may contain Guanidine HCl and should be disposed of properly.) PCR waste should put in sealed waste bags into the biohazard boxes.

15. Detection is completed in the cobas® z 480. Run is finished.



Select **Show Results**.
Review and accept results in **Results.**
●DO NOT ACCEPT FAILED or INVALID results●

Select results and select printer icon 🖶 ; to print the results report.
Select results to Export.

16. Unload the AD (microwell) plate from the **cobas® z 480**. Discard the AD plate in a sealed waste bag into the biohazard box.

Log-off and Exit from the cobas® 4800 program. Shutdown the PC. Switch-off the cobas® z 480, switch-off the cobas® x 480, and switch-off the heater.

Reporting

All assay and run validation is determined by the Cobas® 4800 software. **Controls must be run with each sample run and valid results must be obtained for both the positive and negative control in order for sample results to be displayed.**

Cobas® 4800 HPV Test	Interpretation
Other HR HPV NEG, HPV16 NEG, HPV18 NEG	Specimen is negative for Other High Risk HPV, Negative for HPV16, Negative for HPV18
Other HR HPV POS, HPV16 NEG, HPV18 NEG	Specimen is **POSITIVE for Other High Risk HPV**, Negative for HPV16, Negative for HPV18
Other HR HPV POS, HPV16 POS, HPV18 NEG	Specimen is Negative for Other High Risk HPV, **POSITIVE for HPV16**, Negative for HPV18
Other HR HPV NEG, HPV16 NEG, HPV18 POS	Specimen is Negative for Other High Risk HPV, Negative for HPV16, **POSITIVE for HPV18**
INVALID or FAILED SAMPLE RESULTS	NO NOT ACCEPT the results in the results field. These results should not be exported. Review Details. Refer to trouble shooting guidelines.

Trouble Shooting

INVALID	Re-test the original specimen.
X3: Error Clot Detected	Vortex 30s to break-up excessive mucous. Re-test.
X4: Error Processing of the sample was aborted due to one or more pipetting error(s)	Check for excessive mucous. Vortex 30s to break-up excessive mucous. Re-test.

Quality Control

Each HPV run includes a set of HPV Positive and Negative Controls. The HPV controls must be valid, before specimens results are displayed, in the cobas 4800 HPV result field.
Each sample has an internal control, ß-globin, to monitor cell adequacy, extraction and amplification; internal control must be valid for specimen test to be valid.
CAP provides external proficiency testing.

All invalid failed runs should be brought to the attention of Charge/seniors for trouble shooting, and resolution.

Related Documents

PCR Reagent Inventory Log	T:\Microbiology\Virology\QC statistics\EXTERNAL QC and INVENTORY Logs\PCR Reagent QC & Inventory Current.xls

Reference

cobas® 4800 HPV Test 2010, Roche Molecular Systems, Inc.

Record of Edited Revisions

Manual Section Name: Human Papilloma Virus (HPV) PCR by Cobas® 4800

Page Number / Item	Date of Revision	Signature of Approval
Manual Transferred from Molecular Diagnostics Manual Policy # MI/MD/v51 archived 2015.12.02	December 2, 2015	Dr. T. Mazzulli
Annual Review	December 2, 2016	Dr. T. Mazzulli
Annual Review	December 2, 2017	Dr. T. Mazzulli
Annual Review	December 2, 2018	Dr. T. Mazzulli
Annual Review	December 2, 2019	Dr. T. Mazzulli

Full documents review included all updates. Bi-annual review conducted when no revision had been within 2 years.

Minor formatting updates to revisions section	April 13, 2021	Dr. T. Mazzulli
Updated Surepath preservative 2-8c storage to 6 months per package insert	July 12, 2021	Wayne Chiu
Replace “Formalin fixed paraffin embedded tissue block” with “For tissue samples, please submit extracted DNA or PE curls (5x10uM) indicating sample source”	February 04, 2022	Qin Liu