HPV TEST TECHNOLOGIES IN USE IN NEW ZEALAND

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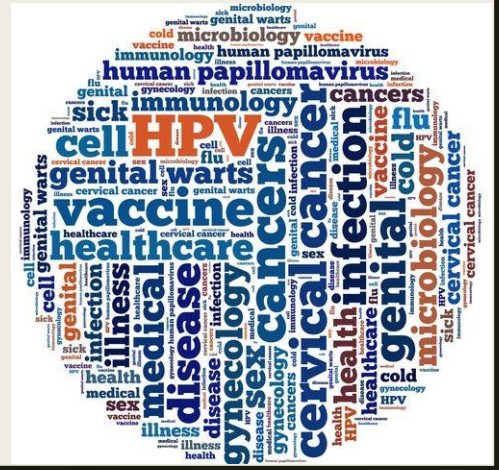
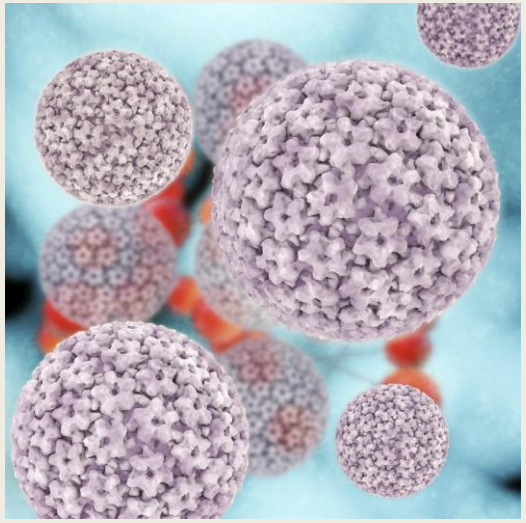
NCPTS Training Team
2018



Introduction

- High-risk HPV is recognised as the single major cause of cervical cancer
- High-risk HPV DNA is found in 99.7% of cervical carcinomas
- The risk of developing cervical cancer increases by 250 fold for women with persistent high-risk HPV infection
- Without HPV infection cervical cancer is rare
- There are more than 100 commercially available HPV assays
- Detection of 13-14 high risk HPV genotypes including HPV16 and HPV18 allows for improved risk assessment and patient management

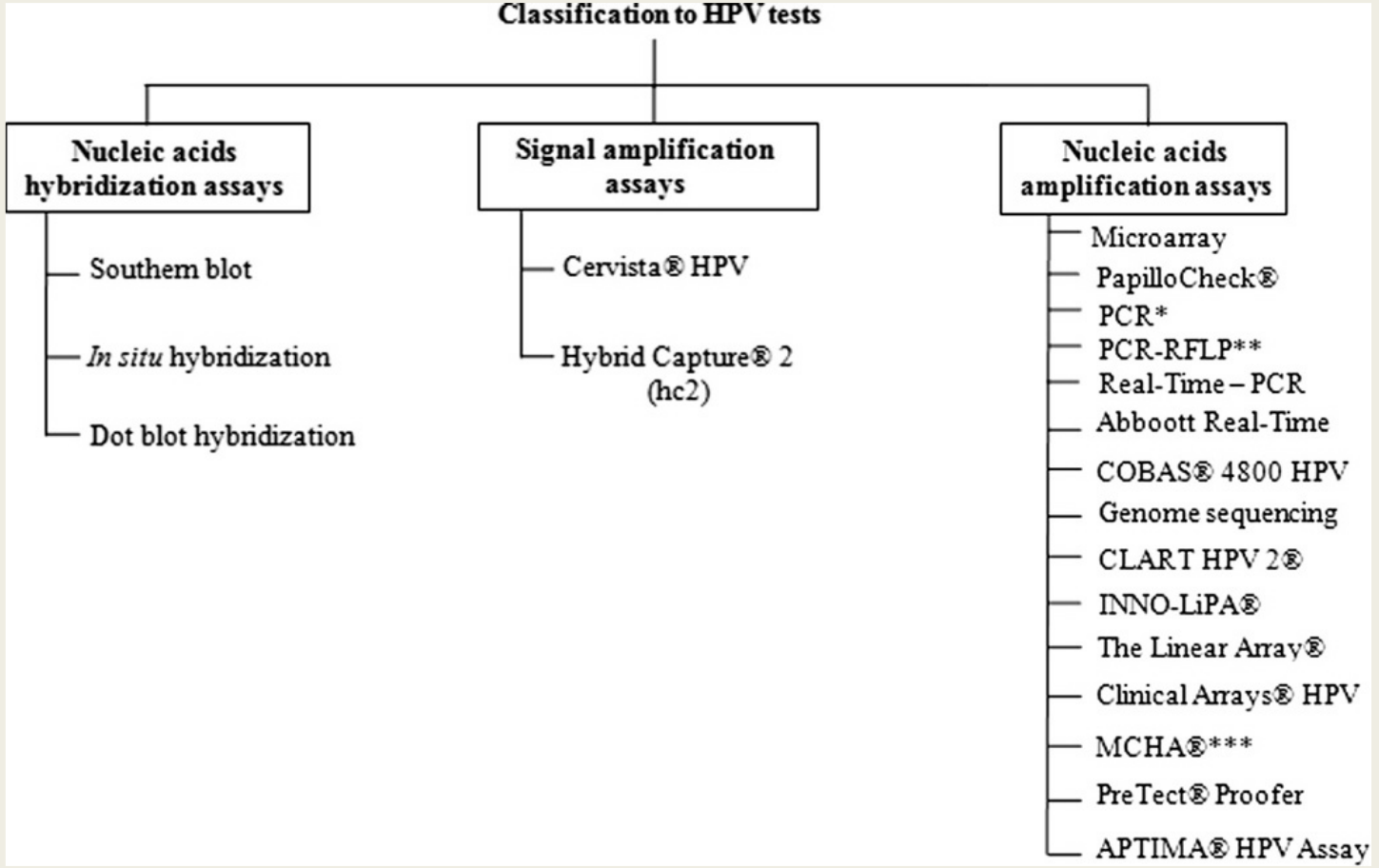


Introduction

- New technologies resulting from this increased understanding are changing our approach to cervical cancer prevention
- HPV test technologies will play a critical role in cervical screening in countries where HPV tests become the primary screening test
- Having robust technology will be critical for the continued success of cervical screening both through sensitive detection of women at risk of cervical cancer and by accurately excluding women who are at very low risk, with negative HPV test results
- These technologies are constantly evolving and will continue to evolve for future years

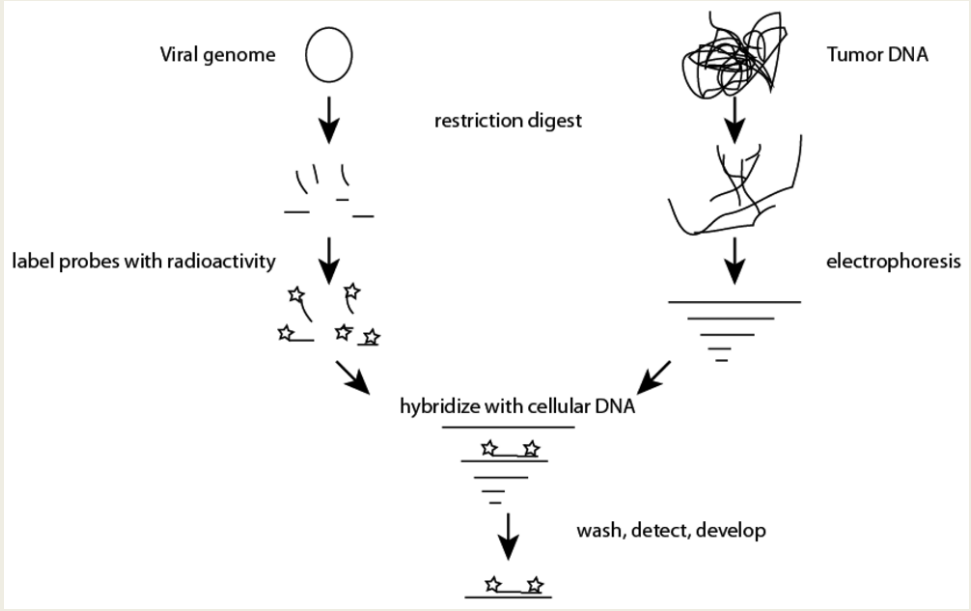
Testing Methodologies

- Three main HPV testing methodologies:



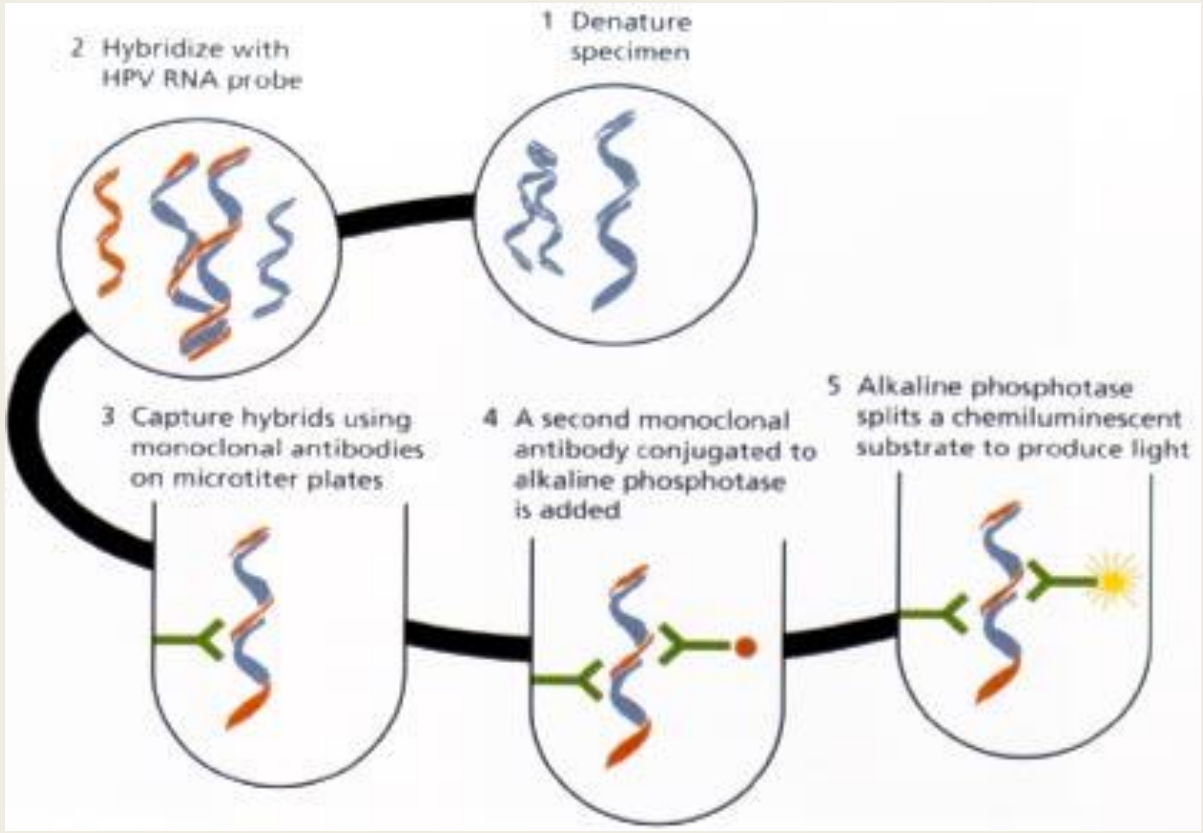
Nucleic Acid Hybridization Assays

- Techniques such as Southern blotting, *in situ* hybridization and dot-blot hybridization were previously used to detect HPV
- They used radio-labelled nucleic acid hybridization assays
- They generated high quality results but had low sensitivity, needed large amounts of purified DNA and were time consuming procedures



Signal Amplification Assays

- Non-radioactive signal amplification methods based on the hybridization of target HPV-DNA to labelled RNA probes in solution
- Chemiluminescent or fluorescent signal is amplified to aid detection
- Examples of this assay are the Qiagen Hybrid Capture 2 High-Risk HPV DNA Test and the Hologic Cervista HPV Assay



Nucleic Acid Amplification Assays

- Target amplification is the most flexible and sensitive of all HPV analysis techniques
- One of the most important in this group is the Polymerase Chain Reaction (PCR)
- PCR allows in-vitro multiplication of unique regions of DNA
- This technology can be used for detection, viral load quantitation, DNA sequencing, and mutation analysis
- These assays can also be performed in multiplex, whereby multiple target DNA sequences can be amplified simultaneously

HPV Assays used in New Zealand (2018)

Abbott Real Time High-Risk HPV Assay

Roche Cobas®4800 HPV test

BD Onclarity HPV Assay

All are Nucleic Acid Amplification Assays

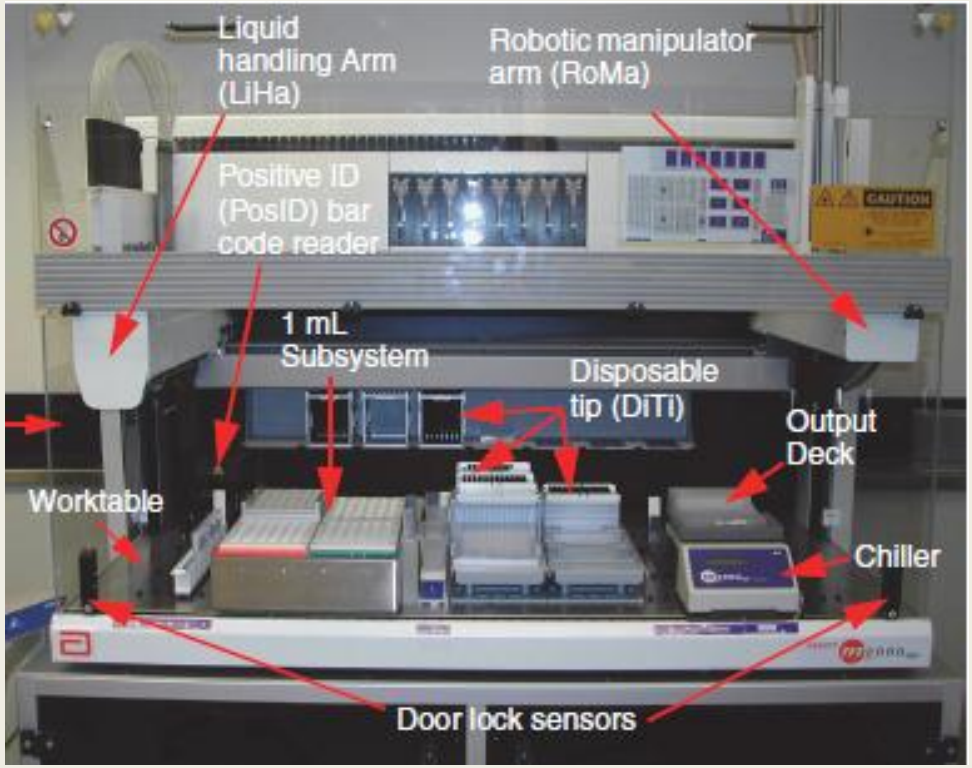
Abbott Real Time High-Risk HPV Assay

- One assay for the detection of 14 high-risk HPV genotypes while simultaneously identifying HPV16 and HPV18
- Clinically validated according to international consensus guidelines
- Multiplex real-time PCR for the separate detection of HPV16 and HPV18 and 12 other pooled high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, & 68)
- Human beta-globin is used as a cellular internal control for reliability in HPV-negative test results

ABBOTT *m*2000*sp* System

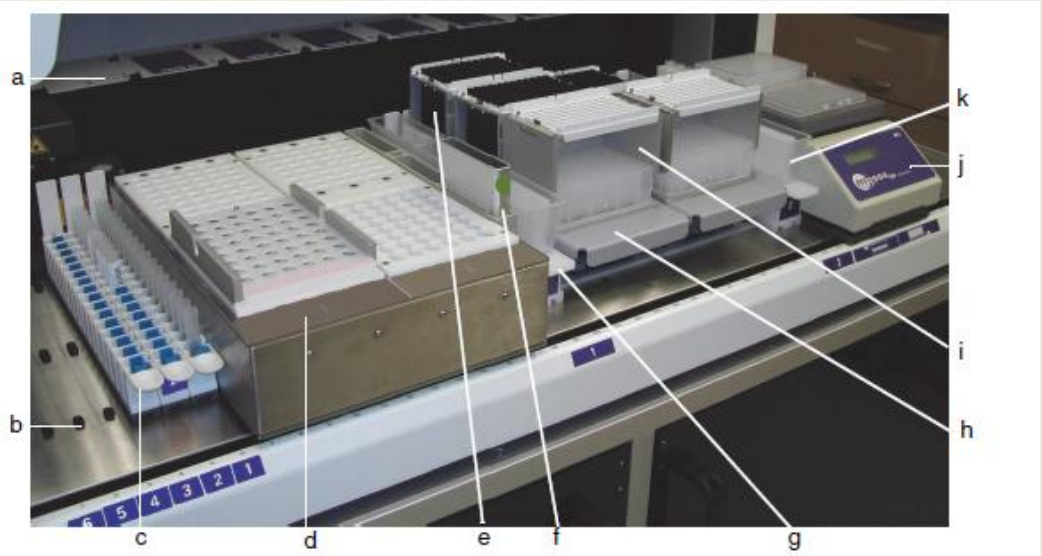
- The ABBOTT *m*2000*sp* is an automated system for performing sample preparation for nucleic acid testing
- The operator controls the system through the System Control Centre, using Abbott *m*2000*sp* software
- At the end of the Sample Extraction procedure, the user may select a Master Mix Addition protocol to automatically distribute the assay reagents and extracted nucleic acid samples into an ABBOTT 96-Well Optical Reaction plate
- This plate can be used on the Abbott *m*2000*rt* (Real-Time PCR instrument) for nucleic acid detection

ABBOTT *m*2000*sp* Instrument



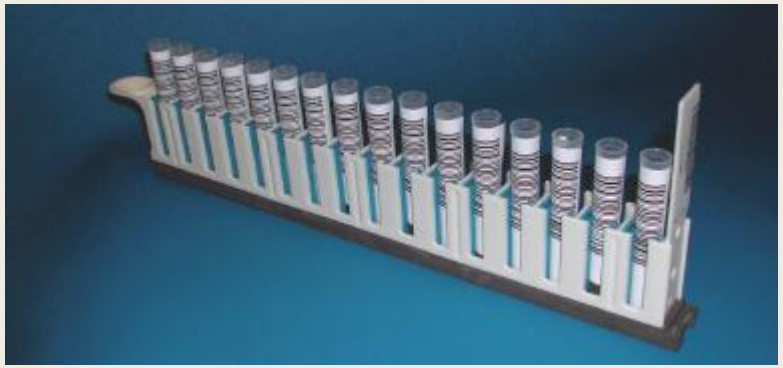
- The robotic arm moves the carrier and disposable tip racks to different positions on the worktable
- The Output Deck contains the chiller, amplification reagent packs, master mix tubes, and PCR and deep well plates
- The liquid handling arm pipettes, dilutes, and mixes samples and reagents, and dispenses liquid through eight different channels, using disposable tips
- The positive ID bar code reader is an optical device which reads information from the reagent vessels, carrier and rack labels, and specimen labels
- The 1ml Subsystem is a sub-assembly which performs magnetic separation and heating steps during the extraction

ABBOTT *m*2000*sp* Instrument



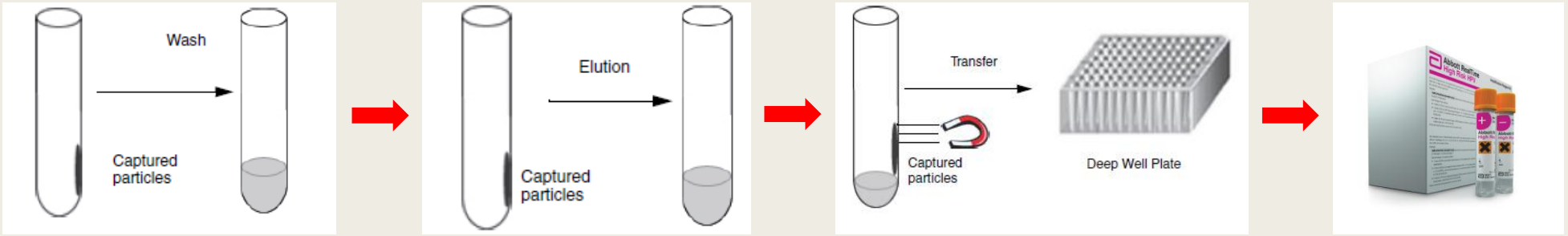
a. Shelf for 1000 µL disposable tips	g. Reagent vessel carrier
b. Positioning pins (guide pins)	h. DiTi rack carriers
c. Sample racks	i. Disposable tip reuse rack
d. 1 mL Subsystem	j. Output Deck
e. Disposable tip rack	k. 200 ml reagent vessel
f. Waste Station	

- The *m*2000*sp* worktable is configured to process 96 specimens, calibrators, and controls for the isolation of nucleic acids in one batch
- It can process 93 samples plus 3 controls in 6-7 hours from extraction through to PCR.
- Standard sample racks hold up to 16 primary or secondary specimen tubes with calibrator and control vials for sample preparation



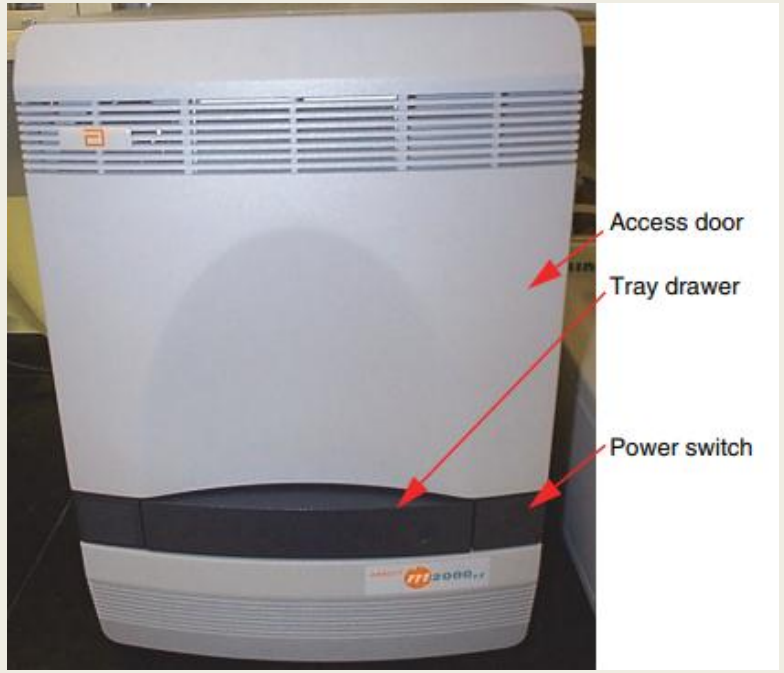
ABBOTT *m2000sp* Sample Preparation

- The sample preparation process consists of the following:
  - *releasing the nucleic acid target by lysing the cells*
  - *binding of nucleic acids to magnetic particles*
  - *separation of magnetic particles from the residual sample*
  - *washing to remove unwanted materials*
  - *elution of nucleic acid from the magnetic particles*
- The *m2000sp* automates this sample preparation process
- The operator continues the process by adding the PCR Master Mix to prepare the plate for PCR



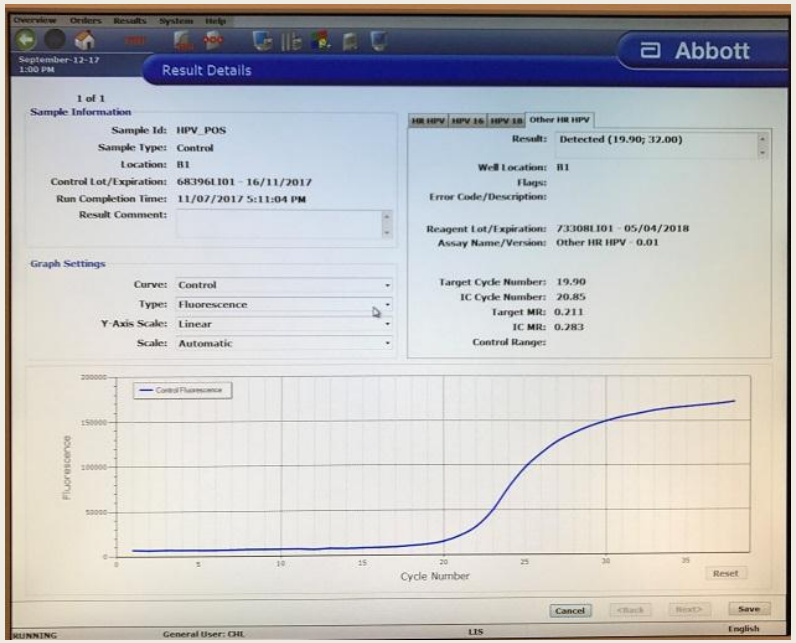
Abbott *m2000rt*

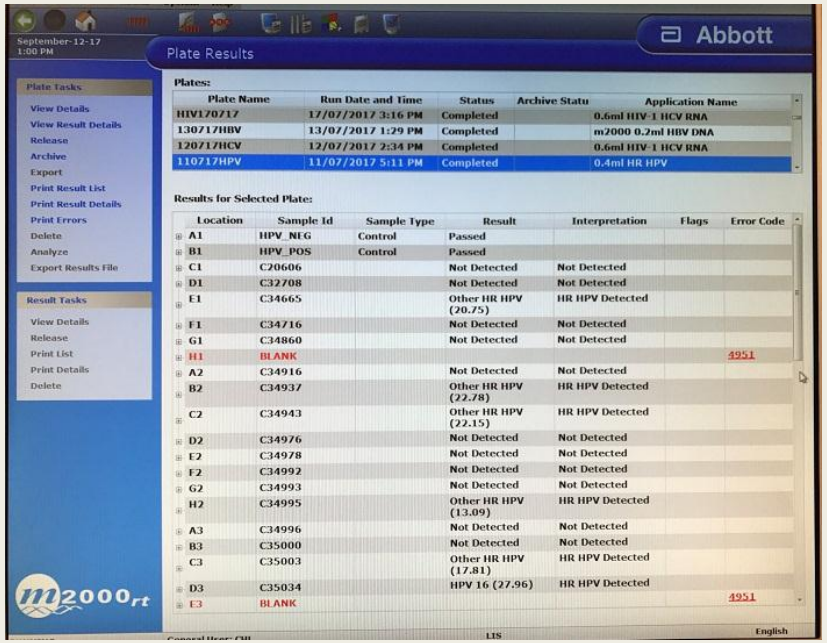
- The m2000rt System provides for real-time measurement of the stages of the PCR
- Real-time PCR measures DNA amplification as it occurs, cycle-by-cycle, allowing quantitative measurements to be made during the highly-reproducible exponential phase of PCR
- The front of the instrument features functionality for turning the instrument ON and OFF, and for accessing the tray that holds the PCR plates
- The computer (System Control Centre) controls the m2000rt System and stores real-time PCR data collected from the reaction plate



Abbott *m2000rt*

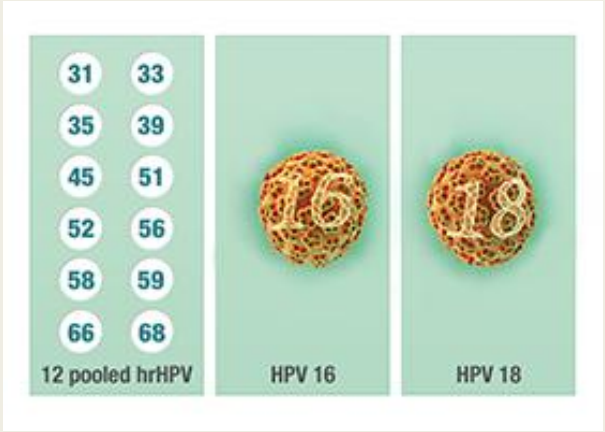
- Each run consists of a single plate (up to 93 samples plus 3 controls)
- The presence or absence of HPV DNA is determined by the PCR cycle at which the signal crosses a pre-established threshold.
- The graph on the top shows a HPV positive control curve
- The table on the bottom shows results from a run and you can see some samples have been found to be positive for “Other HR HPV” or “HPV 16” but most are “Not Detected”

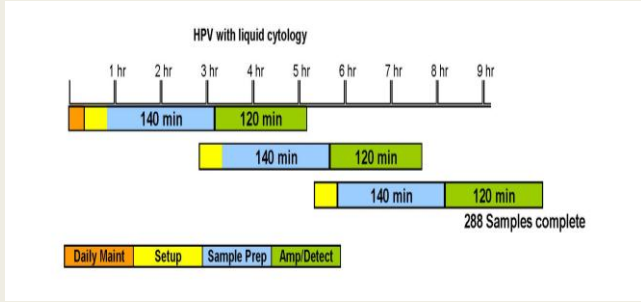




Roche Cobas®4800 HPV test

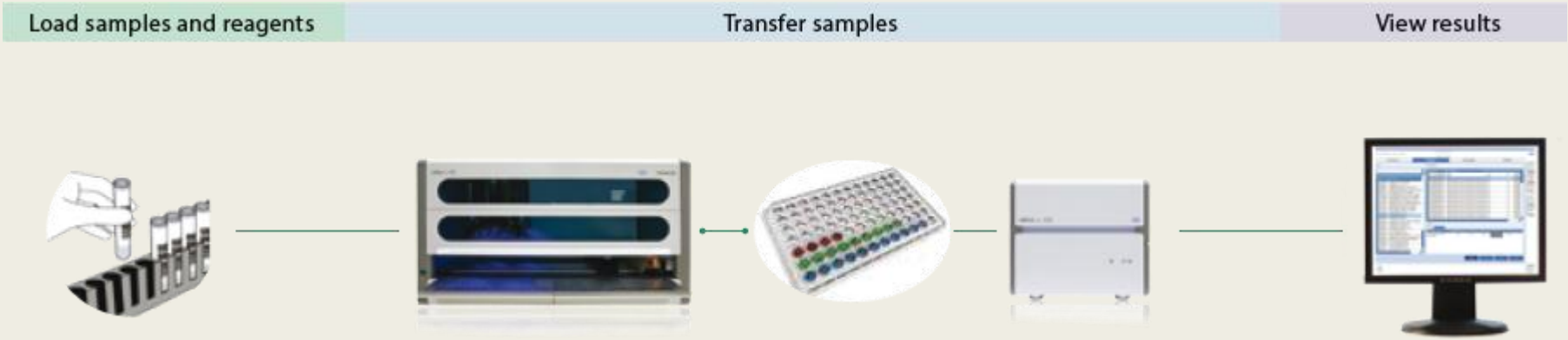
- The Roche Cobas® 4800 HPV test is fully automated using the Cobas 4800 system
- It consists of two separate instruments: the Cobas z 480 and the Cobas x 480 analyzers
- Roche Cobas®4800 HPV has individual genotyping of HPV16 and HPV18 and pooled detection of 12 hrHPV other subtypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).
- Primers in this test define a sequence of 200 nucleotides within the polymorphic *L1* region of the HPV genome.
- Four different fluorescent dyes are used for the detection of the PCR products; one each for HPV16, HPV18, β-globin and pooled other HPV subtypes.
- The Roche Cobas® 4800 system can process 96 samples in 5 hours or 288 samples in 9 hours.





Roche Cobas®4800 HPV test

- The Cobas system integrates automated total nucleic acid isolation, automated PCR setup, and real-time PCR
- It can be connected to a Laboratory Information System
- The Cobas software guides the operator from sample preparation to amplification, and detection and result interpretation

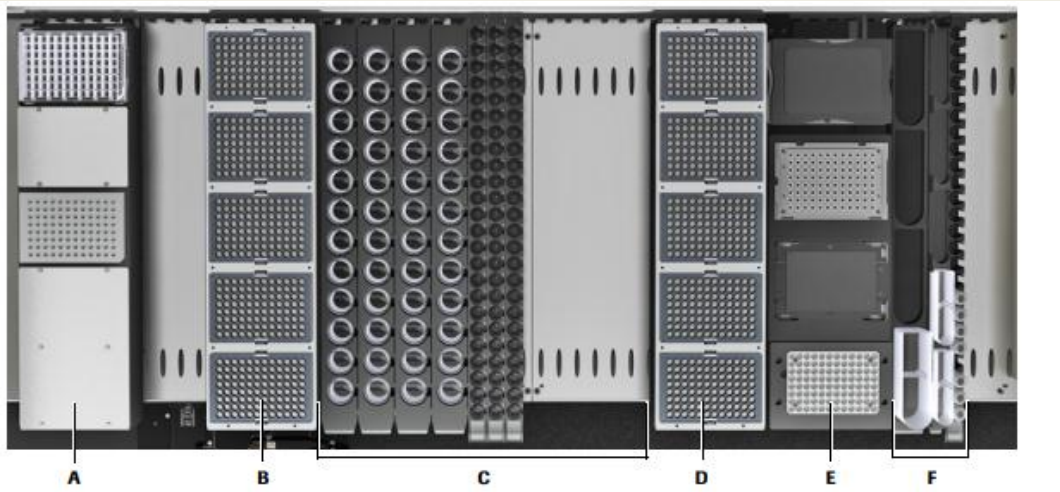


Cobas x 480 instrument

- The Cobas x 480 instrument is an automated multi-channel pipetting instrument used to extract, purify, and prepare target nucleic acid for subsequent PCR testing on the cobas z 480 analyzer
- It is loaded with samples, consumables and reagents
- It takes about 20 minutes to set-up for a full run of 94 samples
- Can process up to 384 samples per day on a single system
- After sample preparation, the microwell plate with the PCR-ready samples is unloaded, sealed, and transferred to the cobas z 480 analyzer for amplification and detection using real-time PCR

Cobas x 480 instrument

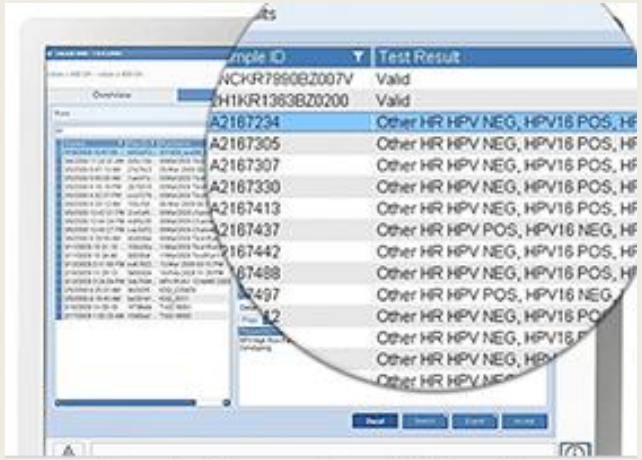
- The work area of the Cobas x 480 instrument is called the instrument deck
- The instrument deck holds:
  - *removable carriers for samples, reagents, plates, and consumables.*
  - *a stationary carrier used for sample processing which holds a heater and shaker unit, magnet plate, and the plate holders for the deepwell plate and the microwell plate.*



A Plate carrier for deepwell plate and microwell plate
B Tip carrier (left)
C Sample loading area
D Tip carrier (right)
E Stationary carrier used for sample processing
F Reagent area for reagent reservoir carriers (200 mL and 50 mL) and reagent carrier

Cobas z 480 Analyser

- The Cobas z 480 analyzer is a rapid thermal block cycler with integrated real-time detection capabilities
- It has simultaneous detection on four detection channels allowing analysis of signals from multiple dyes in multiplex real-time PCR assays
- It utilizes fluorescence signals to detect nucleic acids amplified by using real-time PCR methodology.
- Loading and unloading of the microwell plate is the only manual intervention
- Recording and interpretation of results is done by the Cobas 4800 System software



Easy-to-interpret results from the cobas 4800 System

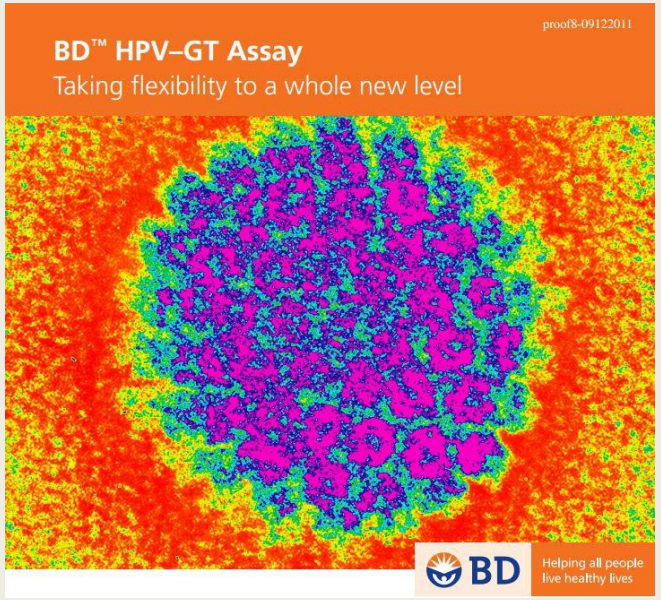
Cobas Control Unit

- A control unit runs the Cobas 4800 software, and controls the Cobas x 480 instrument and the Cobas z 480 analyser
- The handheld barcode scanner is used to scan reagents and reagent reservoir barcodes during loading as well as sample barcodes to setup the work order file
- The Cobas 4800 software is used to manage the Cobas 4800 system workflow

A Display
B Display gooseneck
C Control unit

BD Onclarity

- BD Onclarity is a Real-Time PCR based HPV screening test which has been clinically validated
- It targets *E6/E7* DNA regions on the HPV genome
- It enables specific identification of six hrHPV types (16, 18, 31, 45, 51, 52) by detecting type-specific regions of the virus
- The remaining eight high-risk genotypes are reported in three small groups: (33, 58), (35, 39, 68) and (56, 59, 66)
- The reagents are dried in three tubes (G1, G2, and G3) that are capable of detecting the 14 HPV genotypes and a specimen-derived internal control consisting of a fragment of DNA from the human beta globin gene
- Human beta-globin internal quality control



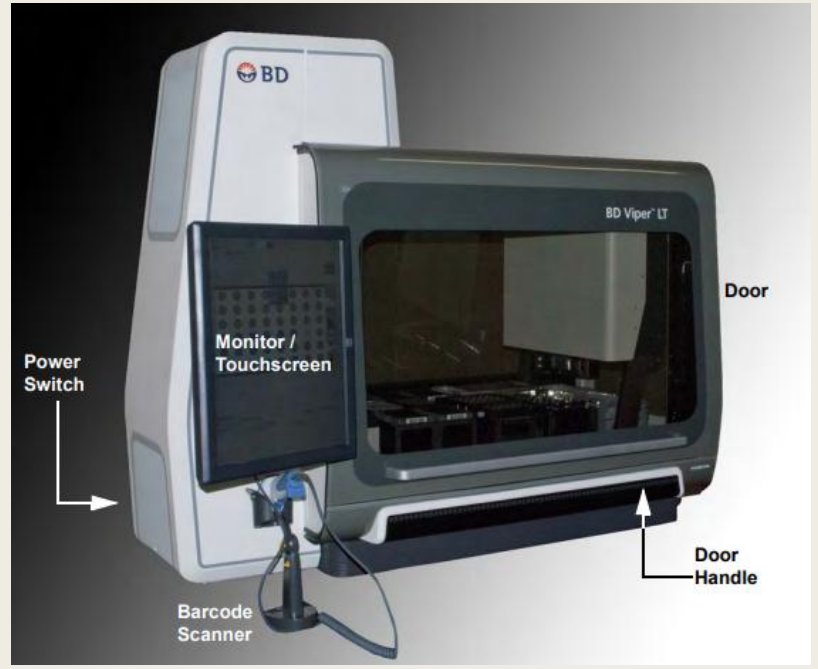
G1	G2	G3
• HPV 16	• HPV 33_58	• HPV 51
• HPV 18	• HPV 31	• HPV 52
• HPV 45	• HPV 56_59_66	• HPV 35_39_68
• IC	• IC	• IC

BD Onclarity

- The BD Onclarity HPV Assay is performed with the BD Viper™ LT System
- 120 samples can be processed in 8 hours
- It is based on two major processing steps
  - *Automated specimen preparation*
  - *PCR amplification of target DNA sequences*
- Specimens undergo a pre-warm step in the BD Pre-warm Heater to homogenize the matrix, lyse cells, and release the DNA
- After cooling, the specimens are loaded onto the BD Viper LT System which then performs all the steps involved in extraction and amplification of target DNA
- The purified cellular DNA solution from each specimen is transferred into PCR tubes containing PCR reagents

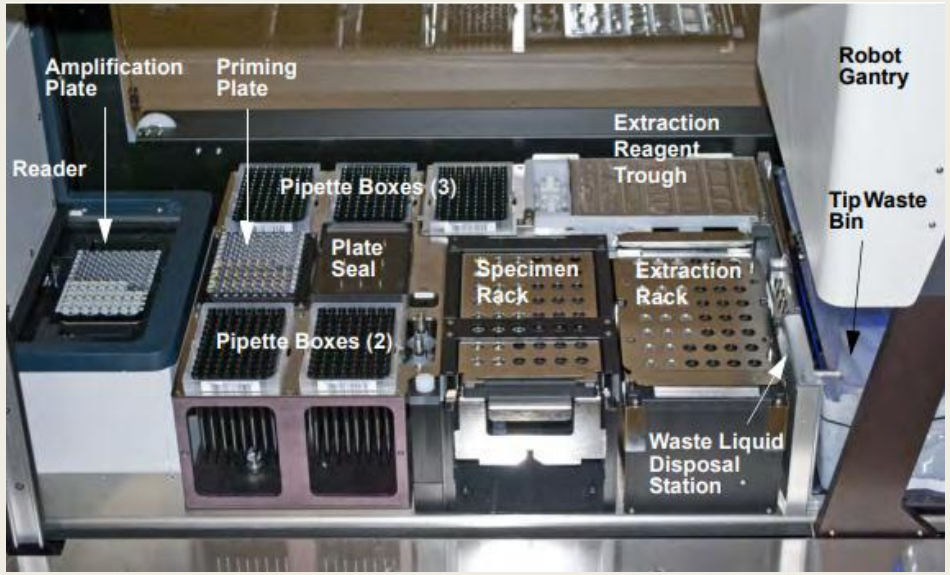
BD Onclarity Viper LT System

- The BD Viper LT instrument contains the following modules:
  - *a temperature control/heating system*
  - *a robotic pipetting arm to transfer samples, puncture troughs, and seal the PCR plate*
  - *an extractor that chemically extracts DNA from samples*
  - *a colour LCD monitor with touchscreen*
  - *an on-board reader, to measure the amplification reaction and report results*
  - *the main computer, which is responsible for instrument control, self-calibration and the user interface*



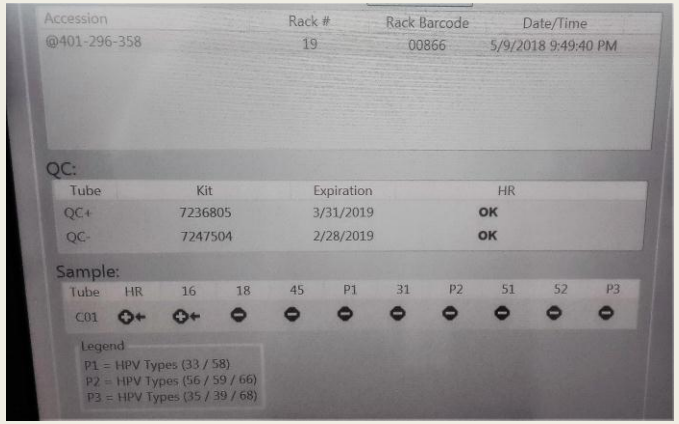
BD Onclarity Viper LT System

- Major components of the BD Viper system are:
  - *Liquid handling system - The robot performs all sample transfers*
  - *Extractor - Specimen lysis and DNA extraction take place in the extractor module located on the front right side of the instrument deck*
  - *Reader - The reader employed in the BD Viper LT System is a self-contained assembly capable of performing real-time PCR in a 96-well format*
  - *Onboard heating/cooling - The thermal subsystem includes a heat block*
  - *LCD monitor and touchscreen - The LCD monitor is mounted on the left side of the instrument exterior*
  - *Barcode scanners - Two barcode scanners are located on the instrument deck: one at the specimen rack station and one at the extraction reagent trough*



BD Onclarity HPV Assay

- The BD Viper LT System pipettes a portion of the purified DNA solution from each extraction tube into the three BD Onclarity HPV PCR tubes (G1, G2, and G3) which are then sealed to prevent contamination
- The on-board reader door closes over the plate and amplification and detection occurs
- The presence or absence of HPV DNA is determined by the PCR cycle at which the signal crosses a pre-established threshold
- The assay will also extract, amplify and detect a fragment of the human beta globin gene as an internal control to assess specimen processing, extraction, and amplification



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