HPV Testing – What are EQAS and QC data telling us?

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Pathology Update – 24 February 2019
HPV Screening

2017 – Molecular testing for human papillomavirus (HPV) replaced cytology as the screening method for cervical cancer.

HPV testing also recommended in other countries as the preferred screening method.
HPV Screening

- HPV testing helps to identify risk of developing cancer
  - Persistent HPV infection $\rightarrow$ Cervical Cancer$^1$
- Partial genotyping determines the pathway of treatment for the patient$^2$
- Also used in Test of Cure (ToC)

$^1$Meijer CJLM et al. Int. J. Cancer 2009
$^2$National Cervical Screening Program 2017
NRL Quality Assurance

- NPAAC Guidelines*
  - “For HPV NAT within the NCSP, externally sourced non-manufacturer supplied control material must be used at least daily when tests are being performed.”

- Two programs:
  - NRL External Quality Assessment Schemes (EQAS)
  - NRL Quality Control (QC) Program

*NPAAC: REQUIREMENTS FOR LABORATORIES REPORTING TESTS FOR THE NATIONAL CERVICAL SCREENING PROGRAM (First Edition 2017)*
NRL External Quality Assessment Scheme
NRL EQAS for HPV

- NRL released HPVN435 in 2017
- Consists of cultured cells suspended in a Liquid Based Cytology medium
  - HPV positive: types 16 and 18
  - HPV negative: uninfected cells
- Material is quantified using digital droplet PCR (ddPCR)
Why Quantify?

Quantified material:
- provides assay manufacturers and participants "standardised" feedback on overall assay performance
- allows for critical review of laboratory processes and staff training
Quantification using qPCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Crossing point</th>
<th>Standard</th>
<th>Calculated Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>QS 1</td>
<td>21.4</td>
<td>1x10^5 cop/µl</td>
<td>1.15x10^5 cop/µl</td>
</tr>
<tr>
<td>QS 2</td>
<td>24.9</td>
<td>1x10^4 cop/µl</td>
<td>1.26x10^4 cop/µl</td>
</tr>
<tr>
<td>QS 3</td>
<td>28.7</td>
<td>1x10^3 cop/µl</td>
<td>0.85x10^3 cop/µl</td>
</tr>
<tr>
<td>QS 4</td>
<td>31.9</td>
<td>1x10^2 cop/µl</td>
<td>1.12x10^2 cop/µl</td>
</tr>
<tr>
<td>QS 5</td>
<td>35.8</td>
<td>1x10^1 cop/µl</td>
<td>0.81x10^1 cop/µl</td>
</tr>
<tr>
<td>Unknown Sample</td>
<td>26.1</td>
<td></td>
<td>6.21x10^3 cop/µl</td>
</tr>
</tbody>
</table>

Used with permission from Scott Bowden, Department of Molecular Microbiology, VIDRL
Quantification using ddPCR

20,000 droplets created!

So, if...

1,000 droplets are positive, then 50 copies/μL (or 50,000 c/mL)

https://www.abmgood.com/marketing/knowledge_base/polymerase_chain_variation_system.php
Analysing HPVN435

- Six test events (from two years) were analysed
- Panels distributed to 32 participants from four countries
- Samples were tested in 12 different assays
Analysing HPVN435

For submitted results

- Includes all interpretations
- Aberrant results are those that are “not concordant” with reference results

ddPCR allows same concentration samples to be combined in a single analysis
## HPVN435: Panel Configurations

<table>
<thead>
<tr>
<th>ID</th>
<th>Year: 2017</th>
<th>Year: 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TE1</td>
<td>TE2</td>
</tr>
<tr>
<td>A</td>
<td>HPV-18 10^4 c/mL</td>
<td>HPV-16 10^4 c/mL</td>
</tr>
<tr>
<td>B</td>
<td>HPV-16 10^5 c/mL</td>
<td>HPV-16/18 10^5 c/mL</td>
</tr>
<tr>
<td>C</td>
<td>Uninfected cells</td>
<td>HPV-18 10^2 c/mL</td>
</tr>
<tr>
<td>D</td>
<td>HPV-16/18 10^5 c/mL</td>
<td>HPV-16 10^4 c/mL</td>
</tr>
<tr>
<td>E</td>
<td>HPV-16 10^4 c/mL</td>
<td>HPV-18 10^4 c/mL</td>
</tr>
</tbody>
</table>

Uninfected cells provided at a cell density of 1x10^4 cells/mL.
### HPVN435: Panel Configurations

<table>
<thead>
<tr>
<th>ID</th>
<th>TE1</th>
<th>TE2</th>
<th>TE3</th>
<th>TE1</th>
<th>TE2</th>
<th>TE3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPV-18 $10^4$ c/mL</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-16 $10^5$ c/mL</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-16/18 $10^5$ c/mL</td>
</tr>
<tr>
<td>B</td>
<td>HPV-16 $10^5$ c/mL</td>
<td>HPV-16/18 $10^5$ c/mL</td>
<td>Uninfected cells</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-18 $10^4$ c/mL</td>
<td>Uninfected cells</td>
</tr>
<tr>
<td>C</td>
<td>Uninfected cells</td>
<td>HPV-18 $10^2$ c/mL</td>
<td>HPV-16/18 $10^5$ c/mL</td>
<td>HPV-18 $10^4$ c/mL</td>
<td>HPV-16 $10^2$ c/mL</td>
<td>Uninfected cells</td>
</tr>
<tr>
<td>D</td>
<td>HPV-16/18 $10^5$ c/mL</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>Uninfected cells</td>
<td>HPV-16/18 $10^5$ c/mL</td>
<td>Uninfected cells</td>
<td>Uninfected cells</td>
</tr>
<tr>
<td>E</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-18 $10^4$ c/mL</td>
<td>HPV-16 $10^4$ c/mL</td>
<td>HPV-18 $10^3$ c/mL</td>
<td>HPV-18 $10^4$ c/mL</td>
<td>Uninfected cells</td>
</tr>
</tbody>
</table>

Uninfected cells provided at a cell density of $1x10^4$ cells/mL
Analysis: HPV-18 at $1 \times 10^4$ c/mL

Seegene Anyplex II HPV HR Detection (n=1)
Seegene Anyplex II HPV 28 Detection (n=1)
Sacace HPV High Risk Typing (n=1)
Roche Linear Array HPV Genotyping Test (n=1)
Roche cobas 4800 HPV Test (n=11)
Roche cobas (6800/8800) HPV Qualitative Assay (n=5)
OPERON High + Low Papilloma Strip (n=2)
Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)
Cepheid Xpert HPV Assay (n=6)
Becton Dickinson BD Onclarity HPV Assay (n=1)
Abbott RealTime High Risk HPV Assay (n=1)
AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)

Number of submitted results

- Aberrant
- Concordant
Analysis: HPV-16 at $1 \times 10^4 \text{ c/mL}$

- Seegene Anyplex II HPV HR Detection (n=1)
- Seegene Anyplex II HPV 28 Detection (n=1)
- Sacace HPV High Risk Typing (n=1)
- Roche Linear Array HPV Genotyping Test (n=1)
- Roche cobas 4800 HPV Test (n=11)
- Roche cobas (6800/8800) HPV Qualitative Assay (n=5)
- OPERON High + Low Papilloma Strip (n=2)
- Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)
- Cepheid Xpert HPV Assay (n=6)
- Becton Dickinson BD Onclarity HPV Assay (n=1)
- Abbott RealTime High Risk HPV Assay (n=1)
- AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)

Number of submitted results

![Bar chart showing number of submitted results for various HPV assays]
Analysis: HPV16/18 at 1x10^5 c/mL

- Seegene Anyplex II HPV HR Detection (n=1)
- Seegene Anyplex II HPV 28 Detection (n=1)
- Sacace HPV High Risk Typing (n=1)
- Roche Linear Array HPV Genotyping Test (n=1)
- Roche cobas 4800 HPV Test (n=11)
- Roche cobas (6800/8800) HPV Qualitative Assay (n=5)
- OPERON High + Low Papilloma Strip (n=2)
- Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)
- Cepheid Xpert HPV Assay (n=6)
- Becton Dickinson BD Onclarity HPV Assay (n=1)
- Abbott RealTime High Risk HPV Assay (n=1)
- AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)

Number of submitted results

- Aberrant
- Concordant
## Detection at low concentrations

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Aberrant</th>
<th>% Detection</th>
<th>Aberrant</th>
<th>% Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbott RealTime High Risk HPV Assay (n=1)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Becton Dickinson BD Onclarity HPV Assay (n=1)</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cepheid Xpert HPV Assay (n=6)</td>
<td>3</td>
<td>85</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)</td>
<td>1</td>
<td>66</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>OPERON High + Low Papilloma Strip (n=2)</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Roche cobas (6800/8800) HPV Qualitative Assay (n=5)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Roche cobas 4800 HPV Test (n=11)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Roche Linear Array HPV Genotyping Test (n=1)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Sacace HPV High Risk Typing (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seegene Anyplex II HPV 28 Detection (n=1)</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seegene Anyplex II HPV HR Detection (n=1)</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Test Event 3, 2018 comprised four samples (samples B to E) that contained uninfected human cells.

Designed to review reporting now that molecular testing has replaced cytology as the HPV screening assay.
## Test Event 3, 2018

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Problem code*</th>
<th>Aberrant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime High Risk HPV Assay (n=1)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Becton Dickinson BD Onclarity HPV Assay (n=1)</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>Cepheid Xpert HPV Assay (n=5)</td>
<td>2/20</td>
<td></td>
</tr>
<tr>
<td>Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)</td>
<td>0/12, 2/12</td>
<td></td>
</tr>
<tr>
<td>OPERON High + Low Papilloma Strip (n=2)</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td>Roche cobas (6800/8800) HPV Qualitative Assay (n=4)</td>
<td>14/16</td>
<td></td>
</tr>
<tr>
<td>Roche cobas 4800 HPV Test (n=10)</td>
<td>9/40</td>
<td></td>
</tr>
<tr>
<td>Roche Linear Array HPV Genotyping Test (n=1)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Seegene Anyplex II HPV 28 Detection (n=1)</td>
<td>0/4, 2/4</td>
<td></td>
</tr>
<tr>
<td>Seegene Anyplex II HPV HR Detection (n=1)</td>
<td>4/4</td>
<td></td>
</tr>
</tbody>
</table>

*Problem codes: Inhibited, Invalid, Compromised
Summary

2018 TE3 “Invalid” for negative samples
- High proportion – expectation of positive samples

Absolute quantification using ddPCR:
- allowed for combined data analysis
  - benefits smaller peer groups
- provided useful feedback in assay performance
  - includes assays not available in Australia
NRL QC Program
NRL QConnect

Main vehicle for NRL QC program

Consists of:

- QConnect QC samples
- World leading scientific ideals for serology/NAT
- Technical support
- EDCNet
EDCNet

- Web-based QC data analysis repository
- Incorporates NRL QConnect Limits*
- Allows for individual and peer group analyses
- Allows users to conduct their own analyses

EDCNet

- Levey-Jennings chart
- Highlight by colours
- Completely user definable

Legend

Reagent Lot Numbers

Reagent Lots in colour

Analyte
EDCNet

Peer Group data charts
Show comparative data
Completely user definable

Site data - scatter
QConnect Limit - Upper
QConnect Limit - Lower
Site IDs

NRL Science of Quality
QConnect HPV NAT

- Made available for NCSP NAT launch (Dec 2017)
- Three QC samples:
  - HPV16NAT (5,000 c/mL HPV gt16)
  - HPV18NAT (5,000 c/mL HPV gt18)
  - HPVNEG (no HPV nucleic acid)
- Matrix = PreservCyt (storage @ 2-8ºC)
- Each QC contains 75,000 c/mL β-globin
Lab 25

- All data submitted
  - HPV16NAT
Data by Reagent lot - HPV16NAT
Data by Instrument
- HPV16NAT
Data by Operator
- HPV16NAT
Data by Operator
- HPV16NAT
Variation Source

Operator Variation!

Different sample preparation techniques developed over time
Data by Operator

Lab manager monitored before/after retraining
New QC lot

‘Normalised’ practices continue
HPV18NAT
Data by Operator
Variation also present
HPV18NAT

Retraining date
Data by Operator

‘Normalised’ practices continue
Lab 1043

- Different lab
- Similar experiences

‘Normalised’ practices after retraining
Lab 1043

Consistency continues across three QC lots
Operator Variation

- Training and competency – critical
- QC samples different to patient samples
- Same preparation technique used for QC
- Understand importance of…
  - Recording data regularly
  - Actioning any suspicious trends
Summary

- Early days for HPV screening laboratories
  - Need to evolve and adapt to quality assurance practices

- NRL QConnect – change in QC format
  - Multimarker positive version more appropriate and will be implemented before end 2019

- QConnect Limits identified variation that could be addressed
Thank-you!

Acknowledgements

- NRL EQAS team
- NRL QC team
- All the program participants