Quality Assurance in HPV Testing – The First Two Years

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NRL, Melbourne, Australia

NRL Symposium – 14 October 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 Scheme (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
- Opens up opportunities for further analyses
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>6.21x10^3 cop/µl</td>
<td></td>
</tr>
</tbody>
</table>

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

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

- Eight distribution events were analysed
- Panels distributed to 39 participants from four countries
- Samples were tested in 16 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 for analysis
# HPVN435: Panel Configurations

<table>
<thead>
<tr>
<th>ID</th>
<th>Year: 2017</th>
<th>Year: 2018</th>
<th>Year: 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TE1</td>
<td>TE2</td>
<td>TE3</td>
</tr>
<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>
</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>
</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>
</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>
</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>
</tr>
</tbody>
</table>

Uninfected cells provided at a cell density of $1\times10^4$ cells/mL
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<tbody>
<tr>
<td><strong>ID</strong></td>
<td><strong>TE1</strong></td>
<td><strong>TE2</strong></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>HPV-18 10^4 c/mL</td>
<td>HPV-16 10^4 c/mL</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>HPV-16 10^5 c/mL</td>
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Uninfected cells provided at a cell density of 1x10^4 cells/mL
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<th>TE3</th>
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<tbody>
<tr>
<td>A</td>
<td>HPV-16 10⁴ c/mL</td>
<td>HPV-16 10⁴ c/mL</td>
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<td>HPV-16 10⁴ c/mL</td>
<td></td>
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<td>HPV-16 10⁴ c/mL</td>
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<td>???</td>
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<tr>
<td>B</td>
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<td>HPV-16 10⁴ c/mL</td>
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<td></td>
<td></td>
<td></td>
<td>HPV-16/18 10⁴ c/mL</td>
<td>???</td>
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<td>C</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td>???</td>
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<td>HPV-16 10⁴ c/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HPV-16 10⁴ c/mL</td>
<td>???</td>
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</tbody>
</table>

Uninfected cells provided at a cell density of 1x10⁴ cells/mL
Uninfected cells provided at a cell density of $1 \times 10^4$ cells/mL
# HPVN435: Panel Configurations

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<td>HPV-16 10^4 c/mL</td>
<td>HPV-16/18 10^5 c/mL</td>
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<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>
<td>HPV-18 10^4 c/mL SurePath</td>
<td>HPV-16 10^4 c/mL</td>
<td></td>
</tr>
</tbody>
</table>

## Year: 2018

## Year: 2019

Uninfected cells provided at a cell density of 1x10^4 cells/mL
No. samples provided

<table>
<thead>
<tr>
<th>HPV Gt</th>
<th>$1 \times 10^5$</th>
<th>$1 \times 10^4$</th>
<th>$1 \times 10^3$</th>
<th>$1 \times 10^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>7</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HPV 18</td>
<td>5</td>
<td>8 (+1 SurePath)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Uninfected cells provided at a cell density of $1 \times 10^4$ cells/mL
## Detection at low concentrations

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>HPV-16 1x $10^2$ c/mL</th>
<th>HPV-18 1x $10^2$ c/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)</td>
<td>Aberrant</td>
<td>% Detection</td>
</tr>
<tr>
<td>Abbott RealTime High Risk HPV Assay (n=1)</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>
</tr>
<tr>
<td>Cepheid Xpert HPV Assay (n=6)</td>
<td>3</td>
<td>85</td>
</tr>
<tr>
<td>Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td>OPERON High + Low Papilloma Strip (n=2)</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Roche cobas (6800/8800) HPV Qualitative Assay (n=5)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Roche cobas 4800 HPV Test (n=11)</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>
</tr>
<tr>
<td>Sacace HPV High Risk Typing (n=1)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Seegene Anyplex II HPV 28 Detection (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Seegene Anyplex II HPV HR Detection (n=1)</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
HPV-16 – All data

All instruments
HPV-16 – by instrument

- Roche cobas 6800
- Abbott m2000
- Roche cobas 4800
- Cepheid Xpert
HPV-18 – All data

All instruments
HPV-18 – by instrument

- Roche cobas 6800
- Abbott m2000
- Roche cobas 4800
- Cepheid Xpert
HPV 16 – $10^4$ c/mL – All samples
HPV 16 – $10^4$ c/mL – Instrument

**Roche cobas 6800**
- Ct ~ 27

**Abbott m2000**
- Ct ~ 24

**Roche cobas 4800**
- Ct ~ 31
- Ct ~ 29

**Cepheid Xpert**
Conclusions – EQAS

- Absolute quantification using ddPCR:
  - Allowed for combined data analysis
  - Benefits smaller peer groups
  - Provided useful feedback in assay performance
  - Included assays not available in Australia

- Evidence supports consistent performance for participants across multiple distribution events per assay

- Comparison across assays – interpret with caution!
  - Qualitative results (Ct values) not representative of concentration
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

Reagent Lot Numbers

Legend

Reagent Lots in colour

Analyte

NRL
Science of Quality
EDCNet

Peer Group data charts
Show comparative data
Completely user definable

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

Retraining date
New QC lot
‘Normalised’ practices continue
HPV18NAT

Data by Operator

Variation also present
Data by Operator

‘Normalised’ practices continue
Data by Operator

‘Normalised’ practices continue

All QC lots
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 kit controls
- Patient samples similar to QCs
  - same preparation technique used for QC
- Understand importance of...
  - Recording data regularly
  - Actioning any suspicious trends
Conclusions – QC

- Still 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 available in 2020

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

Acknowledgements

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