The early detection of cervical cancer in scraping population-based screening programs worldwide

1) cytology only
   *The classical approach*

2) primary cytology and HPV reflex testing
   *Presently used commonly (e.g., Dutch guidelines)*

3) cytology /HPV co-testing
   *Guideline in USA (Saslow 2012)*

4) primary HPV testing and reflex cytology
   *New guideline in NL starting in July 2016; interim guideline in USA 2015*

5) primary HPV testing and reflex CINTEC, methylation, hrHPV-typing and others
   *Presently validated*

HPV in scrapings of (pre)malignant cervical lesions

- ~4% high-risk HPV-positive
- ~85% high-risk HPV-positive

- Normal cervix
- CIN III
- Cervical Cancer
- 100% high-risk HPV-positive
Praktijkrichtlijn BVO cervixcytologie:
indicatie HPV-onderzoek bij Pap2/3A1

Pap 1 97.1%
HPV-

Retour BVO 5 jaar
Pap 2 70%
HPV+

Pap 3A1 2.1%
HPV-

Herhalen 6 maanden
Pap 2 49%
HPV+

+ HPV
Pap 3A1 25%
HPV-

Herhalen 12 maanden
Pap 2 49%
HPV+

Pap 3A2 51%
HPV+

Pap 3A2 > 5%
HPV-

Gynaecoloog

Bulkmans, the Lancet, 2007
Bais, Int J Cancer, 2005
Rebolj, Int J Cancer, 2007

Analytical and clinical sensitivity of HPV-detection assays

Clinical sensitivity of HC2 is
5000 HPV copies

Analytical sensitivity of PCR-
based methods detecting <10 HPV copies

Adapted from Snijders et al. Journal of Pathology 2003; 201:1-6

Clinical validated HPV-tests (in the Netherlands)

Digene HC2 HPV test (Qiagen)
GP5+/GP6+ PCR EIA (Vumc/Qiagen)
Cobas 4800 HPV test (Roche)
Cervista hrHPV test (Hologic)
GenProbe-Aptima hrHPV assay (Hologic)
Abbott realtime HR HPV assay (Abbott)
Kwantitatieve multiplex RT HPV test (PON)

FDA-approved clinical HPV tests

Digene HC2 HPV test (Qiagen)
Cervista HPV HR test (Hologic) > internal control
Cervista HPV 16/18 test (Hologic) > internal control
GenProbe-Aptima hrHPV assay (Hologic) > based on RNA
Cobas 4800 HPV test (Roche) > HPV16/18 separate and internal control

Analytical and clinical sensitivity of PCR-:

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Andere toepassingen voor HPV testen:

- Follow-up patienten behandeld voor CIN3
- Profylactische vaccinatie
- Therapeutische vaccinatie
- Diagnose RRP (recurrent respiratory papillomatosis)
- Klonale verwantschap

available HPV EQA platforms

1) QCMD HPVDNA:
   - using established cell lines in LBC (~4 HPV types)

2) WHO HPV panel:
   - Plasmid DNA spiked into cell line DNA (>30-45 types)

3) NEQAS UK:
   - Patient samples (~4 samples) Fagan, JClinVirol2010

Human Papillomavirus
2014 EQA Programme Report
QAV094130 (HPVDNA14)

Prof. Ed Schuuring
Scientific Expert on behalf of QCMD
Report authorised by the QCMD Executive in November 2014

A UKAS accredited proficiency testing provider (no. 4338)
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Any queries about this report should be addressed to the QCMD National Office.

6th QCMD HPVDNA EQA

ISO17043:2010 accredited

QCMD 2014 Human Papillomavirus DNA EQA Programme (HPVDNA14)

Primary goals:

- To assess the proficiency of laboratories in the detection of different high risk Human Papillomavirus (HPV) types
- To provide laboratories with an analytical performance based on the consensus qualitative results of all participants
- To provide feedback on the number and percentage of datasets reporting typing result

Secondary goal:

- To provide laboratories with information on clinical reporting based on the consensus qualitative results of all participants
QCMD 2014 Human Papillomavirus DNA EQA Programme (HPVDNA14)

**composition EQA panel**

- Door simulatie van klinische samples mbv “established” BMKH-celllijnen in dunne-laag-cytologie
- Core samples: voor proficiency testing (rapportage performance)
- Educational samples: lastige, uitdagende monster ter lering

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<tr>
<th>Sample code</th>
<th>Sample matrix</th>
<th>Sample content</th>
<th>Sample status</th>
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<td>HPV16 [Costi]</td>
<td>Positive</td>
<td>Core</td>
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**Human Papillomavirus 2014 EQA Programme**

**QCMD – QAV094130 – HPVDNA14**

Internationale rondzending:

UMCG: Ed Schuuring (Scientific Advisory Board)
UMCG: Lorian Slagter-Menkema (preparation/validation)
UMCG: MD-cytology lab (cobas-HPV testing)
Reference-labs: UMCG and 3 others (?)

QCMD: Paul Wallace, Catherina di Lorenze
QCMD = Quality Control for Molecular Diagnostics (Scotland)

UMCG = CCKL(ISO15189) accredited

* 10 samples in PreservCyt
* 4 core samples containing HPV16, 18, 45 or mix 16/18 in BSM using established cell lines
* 2 core samples with BSM only as HPV-negative controls
* viral load determined by both cobas and HC2 DNA testing
* 1 education sample with low viral HPV16 load (“clinical HPV-negative”)
* 3 education samples with other HPV genotypes (determined by LIPA) (provided by PON)
* all samples pre-tested and confirmed in reference-labs by HC2 (2x) and cobas (2x)
Primary goals using core samples:

• To assess the proficiency of laboratories in the detection of different high risk Human Papillomavirus (HPV) types

• To provide laboratories with an analytical performance based on the consensus qualitative results of all participants

• To provide feedback on the number and percentage of datasets reporting typing result

Secondary goal using educational samples:

• To provide laboratories with information on clinical reporting based on the consensus qualitative results of all participants
Qualitative performance of all versus Dutch participants

### Performance of all participants

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### Qualitative performance of Dutch participants

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**Qualitative performance of all versus Dutch participants**

**QCMD-HPVDNA2014**

Primary goals using core samples:

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- To provide laboratories with an analytical performance based on the consensus qualitative results of all participants
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Secondary goal using educational samples:

- To provide laboratories with information on **clinical reporting** based on the consensus qualitative results of all participants
In 2014 clinical performance is NOT reported individually:

- QCMD-report provides clinical reporting data separately
- Samples HPV14-10 is considered clinically HPV-negative
- For own interpretation only (educational case)
- Sample does NOT represent determination of real clinical outcome
- “Impossible” to prepare a clinical relevant sample (HPV-low-copy)
Rapportage van lab dat clinical testing uitvoert

**Performance-score**

Notes on Qualitative Panel Scoring for this EQA programme:
- Notes were awarded to 12 institutions based on qualitative sample results.
- For national members, QCMD 13 report must be delivered to the EU for educational purposes only.
- Total Qualitative Panel Score for all participants:

  | Score | Number of Datasets
<table>
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<tbody>
<tr>
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</table>

The sum of the Qualitative Panel Score for your dataset was 74.9% of all datasets.

Pre-test threshold van klinisch-relevante HPV assays

Clinical HPV-tests:

- **HC2**: 1 pg/ml = ~5000 kopieën
- **Abbott**: = ~5000 kopieën
- **Cobas**: = ~300 kopieën (afhankelijk van HPV-type)

Dus eigenlijk kunnen we geen panel definieren met een klinische threshold omdat HC2 niet meer de standaard is.
Vanaf 2013 alleen toetsing performance van analytische interpretatie
Educatief samples alleen als aparte reportage (eigen interpretatie op basis van performance van andere met vergelijkbare testen)
Vanaf 2015 tabel bij individual reporting cores en educational samples apart
Kleinere panels en meer rondzendingen/jaar (>2016)
Andere matrices (nu een pilot SurePath)
Ontwikkelen van referentie/calibratie-sets (pilot 2015)

Dank voor uw aandacht
Vragen?
e.schuuring@umcg.nl