Experience from a prequalification reference lab: challenges and opportunities

NHS Lothian

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1

Role & dimensions of WHO Pre-qualification

" Promote and facilitate access to safe , reliable and appropriate in vitro diagnostic technologies and laboratory services in an equitable manner"

Various aspects to this

- Review of a product dossier
- Performance evaluation
- Manufacturing site(s) inspection
- Labelling review
- LAB SUPPORT

2

PQ Evaluating Labs

- **Labs that express interest are audited by WHO for compliance to relevant quality standards and capacity to undertake evaluations according to protocol**
- Currently 14 PQ labs overall - **2 labs listed for evaluation of HPV assays**
  - Scottish HPV Reference Laboratory, Edinburgh, United Kingdom
  - National AIDS Research Institute, Pune, India
- **AUDIT**
  - Submission of documentation
  - On site inspection 2-3 days
  - Findings /observations agreed - remedial actions(s) undertaken and submitted to audit team

*https://www.who.int/diagnostics_laboratory/evaluations/ale_listing.pdf?ua=1
**https://www.who.int/diagnostics_laboratory/evaluations/alter/protocols/en/

3

Challenges and Opportunities: Audit

- **Challenge**
  - **Inspection audit is comprehensive – having existing accreditation to CAP or ISO15189 does not mean a "fast track" to acceptance**
  - **Ensure time/staff to devote to the audit**
  - **Engagement and support of your local quality manager very important**
- **Opportunity**
  - **Motivating for team /department when approved**
  - **Like any audit – highlights areas for improvement**
  - **Opens the door to new collaborations (technologies, partner labs, clinical teams, quality experts)**
  - **Opportunity for there to be more international labs that are interested in obtaining PQ status– provide resilience and flexibility for future evaluations**

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4

Prequalified HPV products

- 2 products evaluated at Scottish HPV Reference Lab; summary reports available online

https://www.who.int/diagnostics_laboratory/evaluations/pq-list/180713_pqpr_pqdx_0085_028_00_carehpv_with_labelling.pdf?ua=1

https://www.who.int/diagnostics_laboratory/evaluations/pq-list/hiv-vrl/171221_final_pq_report_pqdx_0268_070_00.pdf?ua=1

5

Cepheid Xpert HPV

- 1 cartridge per sample
- 1 hour
- 14 high-risk HPV types detected (delineation of 16 and 18/45 separately)
- PCR HPV E6/E7 target
- Internal, endogenous control

6

96 well plate

13 high-risk HPV types

3.5 hours

DNA-RNA hybridization

Positive & Negative Calibrators

7

Prequalified HPV products

- Xpert and Care Assay evaluated according to the ***previous*** version of the protocol*:
  - Assessment of **analytical performance confined to limit of detection for HPV 16 and HPV 18 (international standards)**
  - **Virologic performance assessed (relative to a clinically validated reference test according to Meijer 2009 criteria) using n=500 clinical samples collected in LMIC**. Clinical performance relative to CIN2+ not assessed.

Comparator assay : rT HPV Test (Abbott)

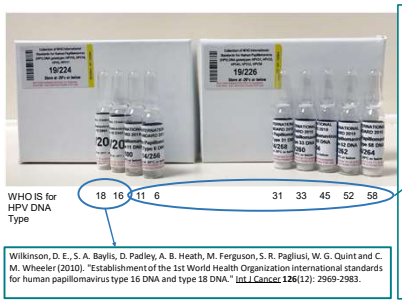
* Under review as per presentation by AL Page

8

Challenges and Opportunities: Analytical Validation

- **Challenge**
  - **The way manufactures report on analytical sensitivity/limit of detection in their instructions for use is not consistent… and rarely relates to an international standard**
  - **WHO international HPV DNA standards are plasmids (circularised DNA) do not approximate a matrix for extraction.**

9



World Health Organization

WHO/BS/2019.2360
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 21 to 25 October 2019

WHO International Standards for Human Papillomavirus (HPV) DNA for Low-Risk Types HPV6 & HPV11 and High-Risk Types HPV31, HPV33, HPV45, HPV52 & HPV58

Wilkinson, D. E., S. A. Baylis, D. Padley, A. B. Heath, M. Ferguson, S. R. Pagliusi, W. G. Quint and C. M. Wheeler (2010). "Establishment of the 1st World Health Organization international standards for human papillomavirus type 16 DNA and type 18 DNA." Int J Cancer 126(12): 2969-2983.

The HPV DNA ISs are synthetic, plasmid-based, cell-free preparations of purified DNA, **their utility in standardisation/evaluation of HPV DNA assays is limited to the amplification and detection steps of the assays**

Thanks to Dianna Wilkinson for this slide

10

Extract from summary reports

Analytical performance	
Limit of Detection	LOD for HPV either genotype could not be estimated by probit analysis. Last dilution detected was 1 x 10^6 IU/ml.
Clinical performance	

careHPV

Performance characteristics	
Analytical performance	
Limit of Detection	HPV genotype 16: 2903 IU/ml (95% fiducial limits: 1081-20 463). HPV genotype 18: 50 493 IU/ml (95% fiducial limits: 10 711-5 267 264)

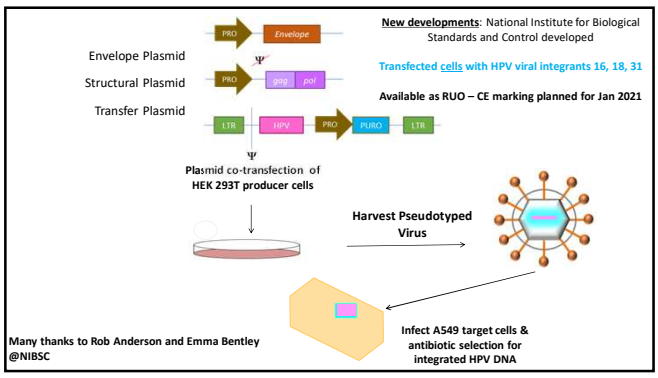
Xpert

11

Challenges and Opportunities: Analytical Validation

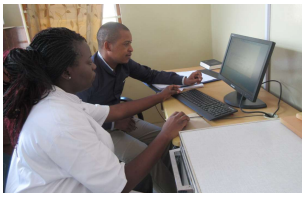
- **Opportunity**
  - **Analytical validation to have a broader scope than just limit of detection (e.g. reproducibility, analytical specificity)**
  - **Create simulated materials to support analytical validation that are more representative of clinical samples (eg)**
    - **International standards seeded into a context of cells**
    - **HPV containing cell lines (including transduced cells) – ideally linked to traceable standard**
  - **The above have potential applications beyond PQ**

12



13

Challenge & Opportunity: virological evaluation



Malawian team (Nkhoma) - sample transported to Edinburgh every 2 weeks in small tranches (approx. 60-70 samples) **until 500 samples obtained**.

Identifying collection sites; ideally from LMIC who can engage in

Governance; Material Transfer, Ethics, Consent
Fieldwork: Planning and delivery/sample taking
Admin: Completion of in-house database & transit proforma, liaison with collaborating team
Logistics: Sample dispatch

14

Challenge & Opportunity: virological evaluation

Xpert(vs rtHPV Assay)

Reports based on previous protocol; 500 samples vs rTHPV test

careHPV (vs rtHPV Assay)

Clinical performance		
	Initial (95% CI)	Final (95% CI)
Positive percent agreement %	82.76% (73.16-90.02)	88.51% (79.88-94.35)
Negative percent agreement %	97.63% (95.54-98.91)	98.68% (96.95-99.57)
Invalid rate	0.52% during the analytical testing (limit of detection) and 7.17 % during the testing of clinical specimens.	

Clinical performance		
	Initial (95% CI)	Final (95% CI)
Positive percent agreement %	70.93% (60.14-80.22)	74.42% (63.87-83.22)
Negative percent agreement %	96.68% (94.40-98.22)	97.45% (95.36-98.77)
Invalid rate	0%	

System for virological evaluation under review

Will still be based on agreement with a validated comparator method(s)

Measure(s) of "success" updated & the power required to achieve this success. Collaboration with Prof M Arbyn, Belgian Institute of Public Health

Operational characteristics such ease of use will be considered

15

Final perspectives

- Interest from collection sites and new laboratories willing to support PQ evaluations welcome. https://www.who.int/diagnostics_laboratory/evaluations/alternative/en/
- WHO elimination goals for cervical cancer will require more HPV tests/testing; important that tests are fit for purpose
- WHO PQ can provide important information on a test suitability for global purposes but for robust evaluation in a particular setting –field testing remains of key value

16

Thanks to

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Prevention and Control Board Organisers

17