Ensuring quality in cervical screening programmes based on molecular HPV testing

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Running title – Quality assurance for primary HPV screening

Word counts: *Manuscript: 4036* **,** *Tables: 2* **,** *Figures: 1*

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Acknowledgements:

The authors would like to thank Drs Camille Busby-Earle and David Nuttall for their insightful and constructive comments on the manuscript.

Abstract:

The increased use of HPV testing within cervical screening programmes necessarily brings about changes to the laboratory services which are required to support them. A crucial element of such services is to demonstrate initial and ongoing quality of the test (and associated processes). In this review we outline some of the quality-considerations and challenges with an emphasis on the laboratory including assay and platform validation, internal quality control selection and strengths and weaknesses of external quality assurance schemes. The influence and role of key external entities, including regulatory agencies, guideline-groups, programme commissioners and commercial providers are also discussed.

Keywords

Human Papillomavirus, Molecular Testing, Quality Assurance, Quality Control, Verification

Background

The transition from cytology to molecular testing for high-risk HPV for primary cervical screening continues apace. Evidence for implementation is based on a number of randomised clinical trials and health technology assessments which indicate that a greater sensitivity, negative predictive value and inter-operator consistency is conferred by HR-HPV testing as a screening test compared to cytology.[1,2] HR-HPV testing also provides the opportunity to extend the time between screening rounds.[3] Additionally, the hypothesised quality reduction in the performance of cytology for the detection of significant lesions in immunised women has now been demonstrated, strengthening the argument for an objective test.[4]

There remain several challenges with respect to implementation of HPV based primary screening that require consideration. As articulated in the European guidelines for quality assurance in cervical cancer screening; optimal screening performance is achieved through a national approach, implicit in which are guidelines for training, recall & management, key performance indicators and external and internal quality assessment.[5] Accordingly, it is important that we develop strategies that reflect the new era of cervical screening not least of which ensure the quality of the HPV test applied.

For comprehensive quality monitoring; various "levels" of input are required. Figure one illustrates some of the key contributions at three levels (A) the international contribution, (B) the requirements of the (particular) national screening programme and (C) the responsibility of the individual laboratory. With respect to A, commercial platform providers drive regulatory compliance and international, evidence based guidelines have and will continue to be highly influential. Regarding B, National guidelines which outline performance requirements for the test and the laboratories that deliver it are essential . Finally, with respect to C the laboratory itself is obliged to deliver testing of a quality which meets the standards in a particular programme but also those required to meet external accreditation bodies. In the present article, we will describe key considerations for assessment, validation, application and quality monitoring of molecular HPV testing in a primary screening

International contribution

The commercial landscape

An advantage of using commercial HPV tests (rather than home-brews) is that the associated regulatory "stamps" such as CE marking and/or FDA approval assure the user that certain performance criteria have been demonstrated. As the process for CE marking is more rapid and less demanding than FDA approval, a greater number of CE marked tests exists but those that are FDA approved have a larger market share at least in Europe and the US. In a review of commercially available HPV tests; Poljak and colleagues described "*193 distinct commercial HPV tests and at least 127 test variants available on the market in 2015*.[6] Certainly, there is a considerable variety in chemistry, platform, output and crucially, in the level of evidence associated with their application; indeed the authors described that 65% were not associated with any peer reviewed publication(s). It is therefore essential that the clinical performance of a test is clearly demonstrated for a cervical screening application and systems/metrics for achieving this are described in the section below. This is particularly relevant for HPV tests as unlike other molecular viral assays, exquisite analytical sensitivity is not desirable given the high prevalence of transient infection.[7,8] Notwithstanding this important issue, a highly competitive commercial landscape provides benefits to the user and programme in terms of price, options and the pace of innovation.

International performance metrics/guidelines

The now well established validation criteria of Meijer et al (2009) outline a system for validation of new HPV tests by comparison to a gold standard on which longitudinal performance for the detection of CIN2+ has been demonstrated.[9] Requirements for inter and intra-laboratory reproducibility are also incorporated. In 2015 Arbyn et al undertook a systematic review to determine which tests fulfilled the criteria, which then numbered eight .[10]

Said guidelines demonstrate how international collaboration (the authorship has input from 8 countries) can support best practice. However, they are not entirely comprehensive which is perhaps unsurprising given their age. Strict interpretation excludes assessment of assay-performance in women under 30 and also of non-DNA based tests. However several programmes screen below the age of 30 and also, mRNA tests have been used for screening and disease management including within national programmes.[11,12] Furthermore, the criteria do not cover key operational aspects, including platform throughput; so an assay may theoretically fulfil the clinical performance and reproducibility criteria but not reconcile with throughput demands associated with primary screening.

Finally, the guidelines do not incorporate assessment of type-specific performance although the use of genotyping as a triage of primary HPV infection is supported in several country-specific guidelines .[13] The international VALidation of HPV GENotyping Tests - (VALGENT) project has several objectives, with one being to provide an evidence based framework for the clinical validation of HPV genotyping assays - and so should be helpful in this regard.[14] Thus, an update to the Meijer criteria to better accommodate recent developments in HPV technologies, both pre-analytical and analytical would be welcome. In the absence of this the onus on the national programme (and associated laboratories) to consider aspects that are not currently reflected in international guidance becomes ever more relevant.

National input

HPV platform selection and quality exercises

Although there is global consensus that HR-HPV testing will replace cytology as a primary screening test, there is variation between countries as to application with respect to age range, time between screening rounds and triage strategies.[15] National programmes should therefore create protocols which set out a pathway to acceptance/approval in addition to producing specifications for HPV platforms. In some countries a single system is procured for the whole programme as is the case for the Netherlands.[16] Comparatively in England, a list of approved platforms[17] was created after internal review and assessment which incorporated a three-step process of "dry" assessment of key technical and analytical parameters, "wet" evalulation to ensure the assay and associated pre-analytical platform performed as expected (in situ) and a comprehensive assessment of available literature on assay performance. Oversight of the process/outputs by a specific working group co-ordinated by the programme led to ratification of eligible assays.

National programmes should also support test validation by resourcing exercises that confirm adequate laboratory performance prior to initiation of a new service and to determine adequate performance in view of system change, repair or release of new reagents. This may include an inter-laboratory assessment, as was carried out in England before the roll out of HPV testing for triage of low grade abnormalities and as a test of cure. Briefly, laboratories were tasked with testing a blinded validation panel composed of anonymised pooled specimens where a minimum agreement (87%) of observed vs expected HPV results (at the qualitative level) was required. Table 1 shows overall performance relating to this scheme in 35 separate laboratories (2 operators per laboratory). Performance was reassuringly high, irrespective of platform used.

In a similar vein in the Netherlands, the Rijksinsttuut voor Volksgenondheid en Milieu supported a national quality exercise prior to roll out of primary HPV testing. A panel independently created by a reference laboratory composed of HPV-containing cell line material(s) was tested initially to obtain a reference set of results based on the semi quantitative output of the assay (Ct value). This panel was disseminated to the relevant service laboratories with acceptable performance based on compliance with the reference set. [18]

In addition to constructing and resourcing exercises to support laboratories embed a test– national programmes may provide advice on best practice for other quality measures. For example, the UK CSP has created a guidance document on laboratory quality control and assurance for HPV testing.[19] Having guidance endorsed at the programme level can be constructive & supportive, particularly at the earlier stages of a significant "change" to a programme. Such guides may also add to the public's confidence in a HR HPV laboratory based screening programme.

Evaluation and monitoring, key performance Indicators and audit

Setting out detailed metric(s) for comprehensive performance evaluation of HPV based programmes is beyond the scope of the present article and clearly, international collaboration for the creation of these will be helpful. This said, a key challenge in defining universal parameters relates to the adoption of different triage and management protocols for women with positive screening tests across the various programmes.[15] Consequently, KPIs should be cognisant of evolving international guidance while also involving national, multi-disciplinary input from primary care, laboratory and clinical services to ensure all dimensions of the programme and patient pathway are represented.

The current European guideline for quality assurance in cervical cancer screening 2015 (supplement) acknowledge the "diversity in the development of HPV primary screening protocols…."concluding "further work is required to develop standard monitoring tables and data input formats.[5] However there is an acknowledgement that core parameters for determining the performance of HPV based screening are arguably similar to those currently used for cytology and can be grouped into 3 elements (i) screening intensity, (ii) screening test performance and (iii) diagnostic assessment treatment and post treatment follow up. Table 2 includes the 20 suggested parameters therein. .

With respect to the "screening test performance" element a somewhat contentious area is what to consider as true disease. The utility of CIN2 as a robust outcome/measure of significance has been questioned for some time. However as the threshold for treatment is still generally CIN2+ measures which rely exclusively on this outcome will continue to be used unless there is widespread change in clinical practice. The recent meta-analysis by Tainio et al (2018) which details regression rate of

CIN2+ may precipitate such a change.[20] In the meantime it is clearly possible to evaluate performance, separately, according to both CIN2 and CIN3 outcomes and it should be noted that some systems work to the separate terminology of low grade or high grade squamous intraepithelial lesions (LSIL or HSIL).

Audits

Retrospective audits of invasive cancers (whether detected clinically of via screening) are recommended in several countries which offer cervical screening, particularly those with organised programmes and national registers. Stakeholder/staff education through workshops, regularly updated communications with FAQs and dedicated websites are fundamental to the success of any changes to the screening programme. Public education that ensures awareness and clarity in respect of the process, aims, benefits and limitations of cervical screening is essential. Audit should be undertaken for the purposes of education, learning, continuous service evaluation and improvement, quality assurance and clinical governance. Cognisant of public opinion and patient preference at the prevailing time, if disclosure is considered and undertaken, it must be done in a sensitive manner at an appropriate time in the patient's journey by members of a dedicated team that understand the principles goals and limitations of the programme skilled in delivery of this type of information. The move to HPV primary screening should not alter the requirement for such, although the operational aspects are likely to change. Cytology slides can be reviewed retrospectively and are often kept for decades; they are also relatively compact and can be stored at room temperature. Comparatively, storing an aliquot of sample (to enable re-test of the HPV assay) requires significant biobanking capacity in addition to quality monitoring of sample stability. A systematic costs to benefits analysis would be required if this approach were to be considered nationally. In a recent article by Hortlund et al (2016), the authors suggested that estimation of the clinical sensitivity of CIN3+ could be achieved by obtaining "pre-diagnostic" biobanked samples taken up to 2 years before.[21] This is an interesting approach however requires suitable infrastructure and resource. Additionally, HPV assays (like all molecular microbiology assays) are validated according to a finite testing window so re-testing outside that window affects the credibility of the result.

Relevant to this; in Australia, national guidance stipulated that HPV prevalence (in routinely taken screening samples) should remain within anticipated parameters and not exceed +/- 2 SD of this level. To support this, laboratories were initially asked to work in operating batches of 2000 samples which should remain accessible in the service laboratory should retesting be required. This constituted a considerable operational burden in terms of storage capacity and record keeping.[21]

Furthermore, as geographic but nevertheless explainable differences in HPV prevalence have been observed within country (due to rurality, deprivation, age profile, vaccine uptake etc),[23] exceeding a national average may trigger alarm bells whilst actually representing natural epidemiological phenomena. Retesting also brings challenges particularly in screening settings where disease prevalence is low and where oscillation around a qualitative result can occur.[24,25] It is notable that at time of preparation of this manuscript the requirement for Australian labs to store 2000 samples has since been removed.

The role of the laboratory

The delivery of a quality laboratory service is clearly supported by formal accreditation. Accreditation behoves a raft of practice (and evidence thereof) which relate to the test itself, the staff performing it and the environment. For the purposes of this manuscript we will focus on aspects of the test itself.

Initial platform validation by the host laboratory and subsequent verification

Even for (very) well evidenced commercially available HPV assays, there is still a requirement for the test laboratory to provide evidence that the assay performs in the anticipated way for all relevant biospecimens and that this is evidenced in an initial "validation" (sometimes referred to as verification) report. The design of the validation exercise and the materials required for its execution could feasibly be defined by the programme or set by the laboratory who would communicate the results to the relevant quality group(s). There is flexibility around the size/scope of the validation-and a large exercise designed to replicate the power and outcomes of studies that have informed product regulatory approval is arguably excessive. One that takes into account the available literature and also incorporates a bench/wet assessment is practical. Validation reports should include how success/failure is measured.

Ongoing validation/verification is also required, at least annually, to demonstrate that the assay is working consistently. This can incorporate a summary of other longitudinal quality measures such as performance in EQA schemes and IQC. Yearly verification should also incorporate aspects such as turnaround, machine down-time and supplier issues and regular minuted meetings with commercial suppliers can support this. Again, criteria for success and failure should be described in the report and exceptions/issues clearly articulated.

Internal Quality Assessment (IQA)

Internal quality assessment (IQA) represents one component of the laboratory quality monitoring process which can help assess uniformity/consistency of testing results, in addition to other laboratory quality indicators including interpretation of results, patient management recommendations etc. At time of preparation of this manuscript, there are no explicit guidelines from accreditation agencies that indicate the amount of IQA that should be performed. One approach is to select a clinical sample at random which can be split into two aliquots at specimen reception, with the duplicate sample de-identified before processing. However, this may be operationally challenging for certain media where less volume is available. An alternative is to re-test a previously reported clinical sample. The issue with IQA, is how to handle discrepant results, particularly those which would invoke a management change, particularly as the justification for a result change is questionable given that HPV assays do not have 100% intra-laboratory reproducibility, particularly around assay cut-off in samples with no underlying disease.[25] The use of anonymised pooled material obviates some of the issues of discrepant IQA results .

User defined control or internal quality control (IQC)

Controls independent of those provided by the manufacturer should be included in each test run, referred to as internal quality controls or user defined controls they should ideally represent the whole process from extraction to detection and resemble the biological matrix of the sample under evaluation. Anonymised, pooled clinical material produced internally can be used as can material sourced from companies or reference laboratories/facilities. HPV containing-cell line material (and HPV negative cervical cancer cell lines) can be of use but may be challenging to produce/maintain in laboratories which exclusively provide diagnostic services.

Currently, although HPV assays are applied at the qualitative level to inform management decisions, the semi-quantitative measure/output of assays can allow trending of IQCs relative to an established expected mean and increasingly commercial test providers offer IT capacity to allow longitudinal monitoring of IQC data. Consistent monitoring of IQC performance and exception reporting should ,as a minimum, be performed by the laboratory but there are examples where national oversee has been implemented. In the Netherlands the IQC is run by each of the service laboratories with results overseen by an independent quality executive, supported by a national reference officer.

There is an increasing demand for IQCs to be linked, where possible, to traceable standards. International standards exist for HPV 16 and 18, established by the WHO HPV LabNet and available via the National Institute for Biological Standards and Controls (NIBSC) which represent an international unit (IU).[26] Standards that reflect IU for HPV types: 31,33,45 52 and 58 are also in development. However, these standards which contain lyophilised plasmid material were not

designed to work with pre-analytical and extraction platforms so their inclusion as day-to-day IQC is not suitable. Certainly, endeavours which can link the semi-quantitative output of an assay to an international unit would be helpful as this could support standardisation and a better assessment of inter-laboratory performance. To this end collaboration between laboratories and accredited providers of quality materials will be helpful.[27]

External Quality Assessment

External quality assessment is an essential component of any clinical laboratory service and participation is a requirement for ISO15189:2012 and specific national accreditation bodies. There are several certified (to ISO17043:2010) EQA schemes available which assess laboratory proficiency in the detection of HR HPV nucleic acid. These were reviewed by Carozzi et al (2016) when HPV testing was primarily used as triage for cytology and for test of cure.[28] Certified EQA schemes include those supplied by UK NEQAS, QCMD, WHO HPV LabNet, Instand and (College of American Pathologists) CAP.

In general terms, EQA schemes relevant for molecular virology assays/laboratories are typically designed to assess analytical performance in individual laboratories. Analytical performance in terms of sensitivity and specificity is a reasonable end point for most clinical virology investigations. However the assessment of clinical performance through current HPV EQA schemes is challenged by the fact that there is no viral load correlate of clinically significant infection. Thus expectations of HPV EQA schemes have to be limited to the insight they give into technical performance of a platform/laboratory rather than clinical performance of the assay and screening process. Arguably clinical performance is revealed more optimally through longitudinal quality monitoring which takes into account results after the initial screening test. This said HPV EQA schemes are still an important part of quality monitoring in that under-performance can highlight operational issues

The optimal components of an EQA scheme for a molecular HPV test should ideally; (i) include representation of all 12-14 hrHPV types, individually or in pools at some point within the cycle of the scheme, (ii) reflect the sample collection matrix used in screening (ie cytology media or self-collection media), (iii) be suitable for use in different nucleic acid amplification technologies (conventional PCR, real-time PCR, DNA array and transcription mediated amplification), (iv) have the ability to assess analytical performance of the assay, (v) have at least an annual distribution and (vi) assess the end to end process of the assay including pre-analytics, extraction (if performed) detection and reporting.

At present, EQA programs use a mix of material including lyophilised cell cultures, pooled clinical samples and plasmid derived material. Each scheme has its strengths and limitations and no existing scheme fulfils all criteria described above.[28] Pooled clinical samples reflect the reality of an actual sample more readily than plasmid, however, controlling for specific type(s) is challenging. HPV containing cell lines have been incorporated into EQA schemes; these can be manipulated more easily than clinical samples and reflect cervical epithelia more closely than plasmids, however cell-lines that represent all HR-HPV types are not readily available and the per cell copy numbers of HPV genomes in these cell lines may be high and lack clinical comparison.

The majority of the EQA schemes described above formally score on qualitative performance (ie presence or absence of HR-HPV types), however, as genotyping is increasingly incorporated into screening pathways this may well change. WHO Labnet scores on type specific performance but is not set up to evaluate the extraction element of an assay.

It should be noted that given the variety of tests available, some assays will be more challenged by a particular EQA scheme than others. The nature of pooled, clinically derived material makes it more reconcilable to assays that involve target amplification by PCR and although we describe optimal elements of an EQA scheme, one that proves perfect for all platforms may prove lofty particularly given the pace of change in HPV test developments.

Endogenous controls and interpretation

Endogenous controls frequently involve the amplification of a housekeeping gene present in every human cell such as beta-globin and control for extraction and inhibition. At present, several molecular diagnostic assays for HPV do contain an internal control, and this aspect may be mandated by a particular programme. While endogenous controls can be helpful, they do not confirm that relevant cervical cells are necessarily in the sample, just that human cells are. A positive aspect is that they guard against the possibility of media-only false negatives where, in error, a sample has not been taken. The magnitude of this issue is questionable, however, and work is ongoing within the UK screening programme to quantify it more precisely. It is also worth reflecting on the fact that most frequently used (to date) gold standard reference/comparator assay as detailed in Meijer 2009 guidelines described above did not contain an endogenous control, although this may reflect the vintage of the gold standard assay used therein.[9] It is feasible that the issue of acellular samples may be higher in a self-taken component and audits and research that consider this will be of value, particularly as the use of self-sampling is likely to increase.[29,30]

Discussion

The move from cytology based screening to molecular HPV testing has necessitated a steep learning curve for national screening programmes and the laboratories that support them. Our aim was to outline key processes and resources that can support quality assurance in addition to some of the current limitations of existing processes.

National guidance on recommended platform, metrics for validation/verification and quality monitoring can support laboratories and may be particularly apposite at the "start" of an HPV based programme. How extensively the guidance is applied will be dependent on setting and influenced heavily by whether a programme is organised or opportunistic. In addition some aspects of quality monitoring (such as trending internal quality controls) will be harder to achieve in programmes where more than one HPV test platform is used. A practically achievable and robust quality framework should incorporate a system which ensures monitoring of operational, analytical and clinical performance through its constituent and varied parts, rather than expecting a particular aspect (whether it be IQC, IQA, EQA) to satisfy all of the above. Quality monitoring of a programme must crucially be built around protection against cancer which necessitates longitudinal assessment against relevant disease endpoints. Such monitoring should also be reactive to – and take into account - influencing factors including vaccination.

There is an opportunity and perhaps an obligation to learn from each other's experience through national and international laboratory networks such as WHO Labnet and other forums which benefit from the interdisciplinary input of clinical virology, cancer screening and molecular pathology. This is particularly relevant given the skill mix of laboratories that ultimately deliver the service. Knowledge gaps may exist within microbiology laboratories (with respect to contextualisation of the results for cervical screening) and cyto-pathology laboratories (with respect to high-throughput molecular testing and associated processes). The meeting of the International Papillomavirus Society and the European Organisation of Genital Infections and Neoplasia benefit from representation of those with laboratory, clinical, screening and molecular biology expertise and specific sessions dedicated to quality challenges and monitoring of HPV based programmes - with outputs that could be disseminated to the community - would be of value. Information from countries which have moved to full roll out of HPV screening and who have the ability, increasingly to determine the success of real-time quality processes over time will be of particular use.

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