Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays—an update

Sonia R. Pagliusi\textsuperscript{a,*}, Joakim Dillner\textsuperscript{b}, Michael Pawli\textsuperscript{a}, Wim G.V. Quint\textsuperscript{d}, Cosette M. Wheeler\textsuperscript{e}, M. Ferguson\textsuperscript{f}

\textsuperscript{a} Department of Immunization, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland
\textsuperscript{b} Department of Medical Microbiology, Lund University, Malmö, Sweden
\textsuperscript{c} Research Program Infection and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany
\textsuperscript{d} Delft Diagnostic Laboratories, Delft, The Netherlands
\textsuperscript{e} Department of Molecular Genetics and Microbiology, School of Medicine, University of New Mexico, Albuquerque, NM, USA
\textsuperscript{f} WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control (NIBSC), South Mimms, UK

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Abstract

International reference materials such as International Standard reagents facilitate quality assurance of essential biopharmaceutical products and related \textit{in vitro} diagnostic tests. Standardization of antibody and DNA measurements and harmonization of laboratory procedures are key to the success of cancer prevention strategies through screening methods as well as for development and implementation of vaccination against the human papillomavirus (HPV). The WHO supported the preparation and initial analysis of a panel of candidate serological and DNA reference reagents aimed at facilitating inter-laboratory comparisons and detection of HPV worldwide. Two international collaborative studies assessed the performance of various HPV antibody and HPV-DNA detection assays and examined the feasibility of generating HPV antibody and DNA standard reagents. These studies showed that improvement in performance and comparability of assays is urgently needed and that the use of the same International Standard reference reagent could significantly improve performance and comparability. It is hoped that the establishment of International Units and International Standards for HPV antibody and DNA analysis will be pursued with high priority.

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1. Introduction

International standardization is intended to assure purchasers and users that products meet their expectations and are of high quality, reliable, safe, effective, as well as providing benefits to individuals and populations, thereby ensuring reliable products at an economical cost worldwide. The quality assurance of essential biopharmaceutical products and devices such as vaccines, blood products, and related \textit{in vitro} diagnostic tests helps to save lives during routine medical care worldwide. Furthermore, the increasing sophistication of biotechnology products presents a considerable challenge regarding production consistency, analytic characterization, and safety and efficacy evaluation for both regulatory authorities and manufacturers.

To facilitate the work of national health and regulatory authorities, the World Health Organization (WHO) has a mandate from member states, as set out in its Constitution,
to “develop, establish and promote International Standards with respect to food, biological, pharmaceutical and similar products” (http://www.who.int/about, WHO Constitution, Chapter II, Article 2). These Norms and Standards are based on wide scientific consultation and on international consensus and are intended to assist WHO Member States in ensuring the consistent quality and safety of biological medicines and related in vitro biological diagnostic tests worldwide. The Organization develops this work through the WHO Expert Committee on Biological Standardization, in close collaboration with the international scientific communities, regional and national regulatory authorities, manufacturers, and expert laboratories.

Over the past few years the WHO has worked with the scientific community and national regulatory authorities, through a series of consultations, to review the scientific basis of HPV-associated cancer and develop reference materials or reagents for ensuring the quality of future HPV vaccines [1–3]. The WHO has played a key role for over 50 years in developing WHO guidelines and recommendations on the production and control of biological products and technologies and in establishing the WHO International Biological Reference Preparations. These activities include:

- The development of written standards or guidelines for quality assurance and control of biological products for human use. This guidance covers the manufacture, control, and clinical and pre-clinical assessment of products and the substrates from which they are derived. The WHO does not regulate or legislate but some countries may adopt the WHO recommendations as the basis of their national regulations for individual products. These written standards and regulatory activities are addressed in Chapter 22.
- The establishment of global measurement standards or international reference materials for use in laboratory evaluation and monitoring the effects of biological products upon administration. Many of these global measurement standards are designated as International Standard reagents, which are physical preparations to which an International Unit (IU) of activity has been assigned that is traceable to a defined part of the content of an ampoule. International Standard reagents must fulfill several criteria, including consistent performance, long-term stability under defined storage conditions, and performance in a variety of detection assays. The use of biological standards facilitates comparisons between laboratories, the determination of the sensitivity of assays, and the standardization of vaccines and other biologicals from different manufacturers. Their use enables the development of internationally agreed criteria for acceptability, standardization, and control of products.

A number of International Standard reagents for viral antibodies already exist, for example for poliomyelitis, measles, rubella, rabies, smallpox, hepatitis B, hepatitis A, parvovirus B19, and varicella zoster. Antibody standards calibrated in IU are used in many epidemiological studies and clinical trials of vaccines and comparisons of GMTs (geometric mean titers) with IUs are published. Assays for markers of immunity (e.g. to infectious agents) are often defined in terms of agreed IUs of antibodies and this provides a basis for an international consensus on the measurement of the immunological status of individuals or populations following vaccination or infection. In addition, minimum levels of antibodies (in IUs) that are indicative of immunity are available for many viruses, and antibody concentrations (also in IUs) serve as relative potency requirements for therapeutic immunoglobulins.

International Standards for use in nucleic acid amplification and detection tests have also been established for blood-borne viruses such as HCV, HBV, and HIV-1. These have enabled minimum levels of sensitivity of assays to be set for regulatory purposes. The published catalogue of WHO biological references includes over 300 materials (a list can be found at www.who.int/biologicals).

Because cervical cancer prevention is a high priority for public health interventions in many countries, the WHO supported the preparation of a panel of candidate reference reagents aimed at facilitating inter-laboratory comparisons and detection of human papillomaviruses (HPV) worldwide. Standardization of antibody and DNA measurement and harmonization of laboratory procedures are key to conducting state-of-the-art epidemiological studies and to the success of cancer prevention strategies through screening methods as well as for development and implementation of vaccination against HPV.

HPVs are a family of related epitheliotropic viruses that comprises many genotypes. The HPVs infecting genital mucosa can be divided into oncogenic and low-risk types, depending on their association with cancer development [4–6]. HPV types classified as carcinogenic to humans can differ by an order of magnitude in risk for cervical cancer [7]. The genetic diversity of HPVs and the common infection of individuals with multiple HPV types present a challenge in accurate, sensitive, and specific HPV detection. International collaborative studies on the standardization of detecting antibodies for oncogenic HPV types and for detecting HPV-DNA have involved several expert laboratories [8,9]. These studies, which aimed to assess the specificity and sensitivity of assays currently in use for measuring antibodies to L1, the major HPV capsid protein, and for measuring HPV genomic DNA, are summarized below.

2. Developing International Standard reagents for the evaluation of HPV serological assays

The detection of genotype-specific HPV capsid antibody in serum suggests past or current HPV infection. Although not all individuals infected with HPV develop measurable HPV antibodies, more than half of all infected subjects will seroconvert, and thus detection of HPV capsid antibodies
has been widely used in natural history and epidemiological studies of cumulative HPV infection in groups. HPV capsid antibody levels also correlate strongly with the level of serum neutralizing activity, which appears to correlate with vaccine-induced protection against infection, at least in animal models.

The development of reliable and standardized HPV capsid serology methods would therefore be highly useful in the development, implementation and evaluation of HPV vaccines. Examples of major applications of HPV serology are:

- Determination of prior HPV exposure, which is a criterion for non-eligibility for evaluation of HPV vaccination trials. If different HPV vaccine trials define HPV seropositivity at baseline differently, estimates of efficacy will be difficult to compare.
- Search for immune correlates of protection. As mentioned, animal studies indicate that the level of neutralizing antibodies correlates with protection. Defining the minimum amount of neutralizing antibodies that mediate protection in humans could greatly facilitate the monitoring of population immunity against HPV and would potentially enable the assessment of whether booster vaccinations are required (both on the individual level and on the vaccination program level).
- Epidemiological studies to guide vaccination program design, such as age at vaccination. Populations may differ greatly in their extent of HPV exposure, depending, for example, on their sexual behavior. Simple seroprevalence surveys may be used to determine the age groups where HPV infections start to appear and therefore should be reached before by vaccination programs.

These important applications are today hampered by the fact that there is no agreed definition of what level of response indicates effective sero-reactivity, and there is no possibility of comparing levels of antibodies detected in different laboratories. The issue is further complicated by the fact that a variety of different assay formats exist for measuring HPV antibodies. Based on data from animal experiments as well as on theoretical grounds, assays that specifically measure functional neutralizing ability may be more likely to reflect protective immunity. This is, however, limited by the fact that neutralizing epitopes for HPVs have not been fully characterized and human responses are likely to be heterogeneous with respect to the major and minor neutralizing epitopes. There is also evidence to suggest that such assays may be more type-specific. Improving the comparability of assays for HPV capsid antibodies with specific tools will therefore assist with HPV vaccine development and epidemiology.

Neutralization assays for HPV have historically been very laborious, difficult to perform, and available only for a few HPV types. However, recent methodological developments that enable efficient production of HPV “pseudovirions” that encapsidate a marker gene (secreted alkaline phosphatase) have resulted in neutralization assays being used as the major assay for both epidemiological studies and vaccination trials rather than a rarely used reference method. However, the complexity of these assays makes it likely that simpler antibody assays will continue to be used for many years to come, especially in resource-poor settings. Studies of antibody classes will also continue to use serological assays based on binding to HPV capsids.

Despite the availability of several different types of HPV capsid antibody assays [10–17], the major difficulty lies in consistently obtaining high-quality virus-like particles (VLPs) in each batch that can be used as substrates for capsid antibody assays. Poor quality VLPs expose epitopes that are not HPV type-specific and not neutralizing. The simplest possible assay format, namely direct binding of VLPs to ELISA plates, therefore strictly requires that only high quality VLPs are used. Several assay formats have been described that select for high quality VLPs. Binding neutralizing antibodies to the ELISA plate will mean that only VLPs containing the respective neutralizing epitopes bind to the plate, thus providing only partial information. An alternative is the use of heparin-coated plates, where only intact VLPs will bind, which is based on the finding that heparan sulfate is a major HPV receptor. Further, the use of competitive assays (cELISA or cRIA) can measure antibodies in human serum that block the binding of well-characterized neutralizing antibodies.

An assay format that is gaining increasing popularity is the use of Luminex, a system where differently colored beads are coated with up to 100 different antigens and incubated with serum in a single well. Fluorescent readout will then determine if antibodies have bound to which antigen they have bound, thus enabling simultaneous testing of antibodies to many different HPV types. These assays exist both in direct format with HPV L1 fusion proteins coated on the beads and in competitive formats using neutralizing antibodies and different VLPs coated on the beads.

As it is evident that the field of HPV serology is under continuous development, the goal of a standardization program should not primarily be to encourage the use of exactly the same method and exactly the same standard operating procedures, but also to ensure that all different assays used report results in the same units. As all proficient laboratories use reporting of results in relation to an in-house standard, the task at hand is to ensure that these standards are calibrated in IUs.

An international collaborative study was undertaken to investigate the specificity and sensitivity of assays in current use for measuring antibody to the major viral capsid protein L1 of HPV. Ten laboratories from eight countries each analyzed twelve coded serum samples, which were derived from an uninfected woman, from naturally infected women, and from individuals immunized with different vaccine candidates under early clinical development. Nine assays involved the use of VLPs as substrates, but these VLPs were from different sources and were produced by several different methods. The majority of the assays undertaken by participants were direct enzyme-linked immunosorbent assays (EIAs), which involve coating EIA plates with VLPs, whereas...
Table 1
Reactivity for antibodies to HPV-16 as determined in a neutralization and immunoassays. (Reprinted from [9] with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reciprocal of end-point dilution in neutralization tests</th>
<th>Range of titers for HPV-16 obtained by different laboratories using immunoassays</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16 natural infection</td>
<td>100</td>
<td>100–640</td>
</tr>
<tr>
<td>HPV-16 natural infection</td>
<td>40</td>
<td>&lt;20–160</td>
</tr>
<tr>
<td>Natural HPV-6 + HPV-11 + HPV-16 + HPV-18</td>
<td>30</td>
<td>&lt;20–80</td>
</tr>
<tr>
<td>Pool HPV-6 + HPV-11 + HPV-16 + HPV-18</td>
<td>20</td>
<td>&lt;100–640</td>
</tr>
<tr>
<td>HPV-18 natural infection</td>
<td>&lt;20</td>
<td>Negative</td>
</tr>
<tr>
<td>HPV-6 + HPV-11 natural infection</td>
<td>&lt;20</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;20</td>
<td>Negative</td>
</tr>
<tr>
<td>Vaccine</td>
<td>1600</td>
<td>100–2560</td>
</tr>
<tr>
<td>Vaccine</td>
<td>400</td>
<td>384–2560</td>
</tr>
<tr>
<td>Vaccine</td>
<td>5445</td>
<td>400–2560</td>
</tr>
<tr>
<td>Vaccine</td>
<td>10240</td>
<td>1600–10240</td>
</tr>
<tr>
<td>Vaccine</td>
<td>87040</td>
<td>5381–40960</td>
</tr>
</tbody>
</table>

others used capture assays. One participant used an RIA, and one participant used bacterially expressed and affinity-purified L1 capsid proteins fused to glutathione S-transferase as antigens [15]. One laboratory performed HPV-16 neutralization assays on the samples. This assay involves the neutralization of pseudovirions carrying a reporter gene to cells in vitro and was previously characterized to be specific and sensitive. Samples were scored positive or negative for each assay based on the cut-off determined by each participant. Where participants returned data for dose-response curves from a series of dilutions, the immunoassays were analyzed at the National Institute for Biological Standards and Control (NIBSC) as parallel-line assays. Potencies were expressed relative to one of the samples that was randomly selected as a reference sample for the study and assigned a unitage of 1.0. The range of titers for antibodies to HPV-16 in each sample determined in immunoassays is given in Table 1. The titers were in general agreement with the HPV-16 neutralization test endpoints in that samples which were of highest titer in these tests also gave higher titers in the immunoassays. All participants scored the sera from vaccinees as positive/reactive. These samples had higher titers than the samples from naturally infected individuals. There was considerable inter-laboratory variation in estimated antibody levels. However, expression of HPV-16 antibody levels relative to that of a single serum sample from an HPV-16-infected subject considerably improved the inter-laboratory assay comparability, as can be seen in Table 2. In addition, the participants ranked the 12 test sera generally in the same order when antibody concentration was expressed relative to a single serum. The potencies calculated from neutralization titers generally agreed with this ranking. Overall, all participants described the low-titer samples as low and the samples of high antibody concentration as high (Fig. 1).

Taken together, establishment of an International Standard for antibodies to HPV-16 would therefore facilitate the agreement of a meaningful and internationally comparable HPV antibody level indicative of a post-infection seroconversion in epidemiological studies, as well as the antibody level in vaccinees that is indicative of vaccine-induced protection. Such a preparation is being characterized and assessed for its suitability to serve as the International Standard for HPV-16 antibody.

Table 2
HPV-16: potency relative to sample 01, which was assigned a unitage of 1.0 units. (Reprinted from [9] with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.)

<table>
<thead>
<tr>
<th>Lab.</th>
<th>Sample</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>14</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.07</td>
<td>4.93</td>
<td>2.05</td>
<td>4.14</td>
<td>0.24</td>
<td>0.09</td>
<td>16.44</td>
<td>24.68</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.10</td>
<td>6.70</td>
<td>4.03</td>
<td>3.88</td>
<td>0.45</td>
<td>0.54</td>
<td>18.10</td>
<td>53.85</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>4.04</td>
<td>3.91</td>
<td>5.06</td>
<td>0.37</td>
<td>0.41</td>
<td>16.28</td>
<td>74.21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.22</td>
<td>5.13</td>
<td>3.32</td>
<td>4.79</td>
<td>0.37</td>
<td>0.27</td>
<td>16.20</td>
<td>74.21</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.29</td>
<td>4.45</td>
<td>2.35</td>
<td>5.12</td>
<td>0.54</td>
<td>0.36</td>
<td>12.76</td>
<td>26.14</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.26</td>
<td>5.21</td>
<td>3.11</td>
<td>4.15</td>
<td>0.50</td>
<td>0.29</td>
<td>10.20</td>
<td>55.02</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.11</td>
<td>6.71</td>
<td>3.67</td>
<td>6.19</td>
<td>0.42</td>
<td>0.18</td>
<td>29.99</td>
<td>91.51</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
<td>9.95</td>
<td>1.79</td>
<td>6.65</td>
<td>0.40</td>
<td>0.10</td>
<td>17.40</td>
<td>25.03</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.19</td>
<td>8.24</td>
<td>4.63</td>
<td>7.24</td>
<td>0.68</td>
<td>–</td>
<td>24.23</td>
<td>59.30</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.26</td>
<td>3.26</td>
<td>2.20</td>
<td>2.92</td>
<td>0.74</td>
<td>0.30</td>
<td>3.03</td>
<td>3.13</td>
<td></td>
</tr>
<tr>
<td>Neutralization test data</td>
<td>0.2</td>
<td>16</td>
<td>4</td>
<td>54</td>
<td>0.4</td>
<td>0.3</td>
<td>102</td>
<td>870</td>
<td></td>
</tr>
</tbody>
</table>
3. Developing International Standard reagents for HPV nucleic acid assays

HPV cannot be routinely cultured in vitro and serological assays cannot distinguish between current and past infection. Diagnosis of HPV infection therefore relies entirely on the detection of the viral DNA in clinical samples, which means that accurate molecular diagnostic tools are required for detection and identification of HPVs. This becomes particularly important when performing longitudinal epidemiological studies and in assessing long-term vaccine efficacy, which both require sensitive type-specific HPV-DNA tests.

The choice of HPV-DNA test will vary depending on the application. For instance, in natural history studies and clinical trials of prophylactic HPV vaccine candidates [23–25], where women are under evaluation for HPV infections and related disease, highly sensitive HPV-DNA genotyping assays are desirable. Phase IV and post-marketing vaccine evaluations may similarly require highly sensitive type-specific DNA assays. On the other hand, for the management of genital HPV-related clinical disease it has been demonstrated that less-sensitive HPV detection limits may be appropriate [26,27]. The design of screening or triage tests for HPV
must consider important differences between viral types and be able to accurately discriminate at the genotype level. For genital HPV infections, the high prevalence of HPV-DNA versus clinical disease has demonstrated that overly sensitive HPV detection and inclusion of various HPV genotypes would result in excessive triage of women for diagnosis and treatment [28]. The establishment of appropriate sensitivity for any HPV assay used in clinical settings requires evaluation in very large, preferably randomized, trials, and issues of cost-effectiveness as related to use in public health settings must be considered. With the introduction of highly sensitive technologies to detect oncogenic HPVs, quantitative assays may be useful for establishing clinically relevant sensitivity. An intrinsic part of using such a variety of technologies in a large number of different laboratories should be the availability of well-characterized International Standard reference reagents and of proficiency panels. The development of these quality control (QC) materials is important for the harmonization of HPV laboratory assays. Ideally, these standards should mimic properties of actual biological samples under measurement and allow evaluation of the full laboratory sample-processing procedures. However, cervical scrapes or small genital biopsy specimens obtained for diagnosis of HPV-infected individuals often harbor multiple HPV types and provide only low numbers of HPV genomes. In addition, human cell-lines harboring a broad spectrum of episomal HPV genotypes are not readily available to generate reproducible epithelial cell-based HPV standards. Recombinant HPV genomes are, in turn, readily available for consideration as nucleic acid standards.

An international collaborative study was initiated to assess the performance of various HPV-DNA detection assays and examine the feasibility of generating HPV-DNA standard reagents consisting of recombinant HPV-DNA plasmids placed into a constant background of an HPV-negative human cervical carcinoma derived cell-line (C33A available from ATCC). Cloned HPV-DNA standards such as those used in the WHO organized study allow assessment of the analytic HPV assay sensitivity and specificity but do not provide standards capable of evaluating biological specimen processing. Cloned HPV-DNA mixed with a cellular genomic matrix does not model intracellular viral HPV genomes appropriately. However, the constructed standards minimally provide the host cellular genomes expected in average clinical specimens that might compete during detection assays.

HPV types 16 and 18, in two separate dilution series, were the main focus of the WHO panel samples because they are responsible for the majority (approximately 70%) of cervical cancer cases worldwide [29,30] and are the primary targets of current prophylactic HPV vaccines [23–25]. A sample containing low-risk HPV-6 was also included, as was an HPV-negative sample containing human C33A cell-line DNA. Other carcinogenic HPV types, including HPV types 31, 33, 35, 45, and 52, were also included as a mixture and examined alone or in combination with HPV-16 or -18 (see Ref. [8] for further details).

Here we review the results of 27 laboratories in 12 countries who participated in a study to assess the performance of various HPV detection assays using the panel of recombinant HPV-DNA reagents. The results of this study are summarized in Table 3, where it can be seen that the majority of participating laboratories accurately detected HPV-16 and -18 at the highest DNA concentrations represented in the panel. A laboratory was considered proficient for HPV-16 and -18 detection in the panel samples if the following three criteria were met: (1) HPV-16 and -18 were detected when present at a dilution of 10^-5, which corresponded to 10^6 HPV genome equivalents per milliliter or 10^4 HPV genome equivalents per assay; (2) no false-positive results were observed; and (3) no detection was observed out of a logical dilution order. Qualitative assays were generally consistent across laboratories, and most invalid results reflected a lack of HPV test sensitivity. Both the individual laboratory proficiency with a given test and the HPV-DNA detection system itself were contributors to the inter-laboratory variations observed. For instance, a single HPV detection method used by seven laboratories demonstrated a variation of several orders of magnitude in sensitivity for HPV-16 detection (Fig. 1). The combined data sets had proficiency for HPV-16 of about 63% (15/24) and 74% (17/23) for HPV-18. HPV-31 was the least accurately detected by participating laboratories; approximately half of the participating laboratories failed to detect high concentrations of HPV-31.

The goal of accurate detection and characterization of HPV is to provide consistent and meaningful results in research and clinical settings to help target and focus

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Table 3
Proficiency of detecting HPV types 6, 16, 18, 31, 33, 35 and 52 (adapted from [8])

<table>
<thead>
<tr>
<th>HPV type data sets</th>
<th>16a</th>
<th>18a</th>
<th>6b</th>
<th>31b</th>
<th>33b</th>
<th>35b</th>
<th>45b</th>
<th>52b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proficiency results (%)</td>
<td>63</td>
<td>74</td>
<td>71</td>
<td>43</td>
<td>95</td>
<td>71</td>
<td>95</td>
<td>71</td>
</tr>
<tr>
<td>Rate of false-positives (%)</td>
<td>21</td>
<td>26</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.5</td>
</tr>
<tr>
<td>Rate of false-negatives (%)</td>
<td>25</td>
<td>0</td>
<td>5</td>
<td>57</td>
<td>5</td>
<td>29</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Total number of data sets (n)</td>
<td>24</td>
<td>23</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

a Detection of HPV types 16 or 18 at a dilution of 10^6 genome equivalents per milliliter, no false positive results, and no detection out of logical order.
b Detection of HPV types 6, 31, 33, 35, 45 and 52 at a dilution of 10^6 genome equivalents per milliliter and no false-positive results.
c Determination of false-positive percentage is calculated by dividing the number of data sets with false positives by total number of data sets.
d False negatives are data sets with negativity at 10^6 genome equivalents per milliliter.
resources in disease prevention and control. The results of the WHO-sponsored HPV-DNA detection study demonstrated a clear need to develop and implement HPV-DNA International Standards. The international collaborative study group recommended that the focus of International Standard reagents be first on HPV types 16 and 18 rather than on low-risk HPV types not related to cancer, and then to expand to the other most prevalent high-risk HPV types 31, 33, 35, 45, 52, and 58. Furthermore, the group recommended that HPV-DNA International Standards should be monovalent or individual HPV type standards as this will allow unequivocal calibration of individual HPV-DNA material and will allow the assessment of potential detection interference when multiple HPV types are present. The HPV-DNA International Standard unit remains to be established. IU’s should be defined as traceable to a defined part of a defined ampoule, therefore it would be desirable to determine as accurately as possible the amount in previously used units (e.g. genome equivalents, micrograms, or copy numbers) that would correspond to the IU.

The current HPV-DNA standards discussed above represent recombinant plasmids; however, it will be important to consider future exploration of systems that are capable of better representing an appropriate biological specimen. This is primarily required if HPV-DNA standard reagents are to be used to assess the full spectrum of HPV-DNA testing, including specimen processing. One such possible source of material is the organotypic (raft) culture systems capable of supporting the full HPV life cycle. HPV types 16, 18, 31 and 45 have been successfully propagated in organotypic cultures where intracellular episomal HPV’s as well as encapsidated virion are produced within differentiated epithelial cell layers [31–34]. More recently, a transient-transfection-based system that produces over 1000 times more infectious virus per cell culture dish than the much more labor-intensive organotypic culture has been developed [35]. This newly acquired capacity would potentially enable the generation of HPV-DNA standards across a broad spectrum of oncogenic HPV types at a reasonable cost. Although adequate batches of intracellular HPV-DNA can be produced, it will be necessary to determine if these systems have the capacity to generate HPV-DNA that can be reproducibly and quantitatively implemented as a standard reference reagent.

The potential benefits of available HPV-DNA reference reagents are many. For example, the sensitivity and specificity of HPV-DNA assays can be determined, validated, and monitored, and the performance of HPV-DNA detection methods relative to International Standards will facilitate comparisons of data from multiple studies. Improving the comparability of assays for type-specific HPV-DNA detection through the use of International Standard reagents will assist with HPV vaccine development and epidemiology. Laboratories charged with monitoring outcomes of HPV vaccination must therefore achieve high proficiency and reproducible high type-specific sensitivity.

The availability of international HPV-DNA standards will contribute to the field of HPV prevention, diagnosis, and treatment. In particular, such standards, if available worldwide, will allow for reference calibration of HPV-DNA tests, thereby enabling manufacturers to further validate and develop HPV detection reagents and kits, which will allow reliable disease and vaccination monitoring worldwide.

4. Conclusion

International Standard reagents are the key to ensuring that the quality of products is measured in a comparable manner worldwide, thus ensuring equity among populations that will be able to benefit from the same degree of quality of interventions.

It is most unfortunate that current assays do not relate to an internationally defined antibody level or HPV-DNA amount, both for measuring HPV antibodies and HPV-DNA. Thus, current definitions of HPV naïve subjects (antibody and DNA negative) to be evaluated in vaccination trial are not comparable, nor are the immunogenicity of the vaccines in terms of induced (neutralizing) antibodies or the assessment of efficacy for preventing HPV infection easily comparable. The WHO studies in this area have both shown that improvement in performance and comparability is urgently needed and that the use of the same International Standard reference reagent significantly improves performance and comparability. The development and establishment of International Standards by the WHO biological standardization program will enable the use of assays traceable to a common IU. This will contribute to better evaluation of HPV vaccines in the pre-licensing stage of development as well as in monitoring HPV vaccination programs at a later stage.

Disclosed potential conflicts of interest

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References


