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International collaborative proficiency study of Human Papillomavirus type 16 serology

Carina Eklund[a,b], Elizabeth R. Unger[c], Denise Nardelli-Haefliger[d], Tiequn Zhou[e,1], Joakim Dillner[a,b,*]

[a] WHO HPV LabNet Global Reference Laboratory, Departments of Clinical and Medical Microbiology, Laboratory Medicine Skåne and Lund University, Malmö, Sweden
[b] Departments of Laboratory Medicine, Medical Epidemiology & Biostatistics, Karolinska Institutet, Stockholm, Sweden
[c] WHO HPV LabNet Global Reference Laboratory, Centers for Disease Control and Prevention, Atlanta, GA 3033, USA
[d] WHO HPV LabNet Europe Regional Reference Laboratory, Institute of Microbiology and Department of Urology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, CH-1011 Lausanne, Switzerland
[e] Quality, Safety and Standards Team, Immunizations, Vaccines and Biologicals Department, World Health Organization, Geneva, Switzerland

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ABSTRACT

We performed an international proficiency study of Human Papillomavirus (HPV) type 16 serology. A common methodology for serology based on virus-like particle (VLP) ELISA was used by 10 laboratories in 6 continents. The laboratories used the same VLP reference reagent, which was selected as the most stable, sensitive and specific VLP preparation out of VLPs donated from 5 different sources. A blinded proficiency panel consisting of 52 serum samples from women with PCR-verified HPV 16-infection, 11 control serum samples from virginal women and the WHO HPV 16 International Standard (IS) serum were distributed. The mean plus 3 standard deviations of the negative control serum samples was the most generally useful "cut-off" criterion for distinguishing positive and negative samples. Using sensitivity of at least 50% and a specificity of 100% as proficiency criteria, 6/10 laboratories were proficient. In conclusion, an international Standard Operating Procedure for HPV serology, an international reporting system in International Units (IU) and a common "cut-off" criterion have been evaluated in an international HPV serology proficiency study.

1. Introduction

Cervical cancer is the second most common type of cancer among women, each year causing over 250,000 deaths, approximately 80% of which occur in developing countries [1].

More than 150 HPV genotypes are known, but only about 14 HPV types can cause cervical cancer as well as other anogenital and oropharyngeal cancers [1]. The distribution of HPV types in cervical cancers varies between regions, but HPV16 is the dominant cancer cause in all regions [2]. Today, prophylactic HPV vaccines play a key role in cervical cancer control strategies. So far, two HPV vaccines have been licensed, one quadrivalent vaccine containing L1 Virus-Like Particles (VLPs) for HPV types 6, 11, 16 and 18 [Gardasil (Merck and Co., Whitehouse Station, NJ, USA)], and one bivalent vaccine containing L1 VLPs for HPV types 16 and 18 [Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium)]. As of September 2008, each HPV vaccine was licensed for prevention of cervical precancer and cancer in at least one country in each WHO Region, the bivalent vaccine in 71 countries and the quadrivalent in 105 countries [3]. WHO is recommending universal HPV vaccination [4]. At the end of 2009, 27 countries had introduced HPV vaccination in their national immunisation programs [5].

HPV serology is an essential technology for both HPV vaccinology and HPV epidemiology. Definitions of HPV-naïve subjects eligible for HPV vaccination trials include seronegativity for HPV. Immunogenicity of HPV vaccines has been used to bridge results from efficacy trials in adolescents to children and to evaluate different batches of HPV vaccines. Antibody measurements are also important in vaccinology research, e.g. for characterizing the immune response with respect to studies of seroconversion and antibody increases, cross-reactions, immune memory and immune persistence as well as kinetics of antibody responses and establishment of correlates of protection. Finally, HPV seroepidemiology is also useful for understanding the epidemiology of HPV infections in populations to be targeted by HPV vaccination programs [6]. The lack of a standardized assay to measure HPV antibody levels has hindered both epidemiological studies of HPV infection and comparison of results from different HPV vaccine trials [7].

* Corresponding author at: Department of Laboratory Medicine, Karolinska University Hospital Huddinge, F68 S-14186 Stockholm, Sweden. Tel.: +46 40 338126; fax: +46 40 337312.
E-mail address: joakim.dillner@ki.se (J. Dillner).

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WHO Guidelines for HPV vaccines suggest that "initial assessment of immune responses to HPV VLP vaccines should be based on measurement of neutralizing antibodies in serum". Once the neutralizing antibody response has been well characterized, the use of alternative assay methods, such as Enzyme Linked ImmunoSorbentAssay (ELISA), may be proposed [6].

The available data [7–10] suggest that neutralizing and ELISA antibody titres are usually highly correlated when the ELISA antigen target is conformationally intact VLPs. Due to the complexity and labour-intensiveness of neutralization assays, VLP-based ELISAs have been preferred in large epidemiological studies. E.g., a study of HPV seroprevalences was conducted by measuring HPV 16 antibodies with an HPV16 L1 VLP-based ELISA to estimate the public-health impact of HPV vaccination strategies [11].

WHO has been coordinating work to develop standard assays that will help in assessing vaccine quality and monitoring impact after vaccination [12]. In 2006, WHO established a global HPV laboratory network (LabNet) with a main focus being the harmonization and standardization of laboratory testing procedures to support consistent laboratory evaluation of regional disease burden and monitoring of the performance of HPV vaccines. At a WHO consultation in January 2008, a group of experts recommended that the HPV LabNet should develop or identify standardized assays for general use and that efforts towards standardization on VLP-ELISA should be a high priority of the WHO HPV LabNet [13].

Following the recommendation, the WHO HPV LabNet launched a serology standardization program encompassing: (i) an international collaborative study to evaluate and refine a direct HPV 16 VLP-ELISA suggested Standard Operating Procedure (SOP), (ii) an international request for donations of VLPs to be used as international reference reagents for serology, followed by characterization and selection of optimal reagents, and (iii) an international collaborative proficiency study on HPV 16 serology, where participating laboratories used the same standardized SOP and the same VLP reference reagent.

2. Materials and methods

2.1. Materials

The SOP evaluation study was based on a single source of HPV 16 VLPs (kindly provided by Dr. John T. Schiller, NCI, Bethesda, USA). These VLPs had been used in previous serological studies and had been characterized for adequate epitope exposure using monoclonal antibodies [11]. For selection of a reference reagent to be used in the proficiency study, VLPs from five different sources were donated and validated: two commercial sources, Biotrin International (Dublin, Ireland) and Xiamen Innovax Biotech (Xiamen, China) and three academic sources (Dr J Dillner, Lund University, Sweden; Dr E Unger, CDC, US and Dr I Kukimoto, National Institute of Infectious Disease, Japan).

Validation of the donated VLPs used a type specific (H16.V5) and a cross-reactive (H16.D9) monoclonal antibody (kindly provided by Neil Christensen, Pennsylvania State University Collage of Medicine, Hershey, USA) in a direct ELISA.

The SOP evaluation study used 18 serum samples. Six negative control samples were taken from women who reported no sexual experience and had previously been found to be HPV16 seronegative using ELISA [14]. Eleven samples were selected from cervical cancer patients whose tumors had been shown to be positive for HPV type 16 DNA by PCR [15]. One of these samples was included twice in the testing panel, to evaluate the assay variability when testing exactly the same sample. The WHO International Standard for HPV 16 antibodies (NIBSC 05/134, http://www.nibsc.ac.uk) [7] was included to enable calculations of IU.

The proficiency study used 63 samples. Eleven samples from virginal women and 52 samples from cervical cancer patients whose tumors had been shown to be positive for HPV type 16 DNA by PCR [16]. The WHO International Standard for HPV16 antibodies (NIBSC 05/134) [7] assigned 5 International Units (IU)/ampoule, as established by the WHO Experts Committee on Biological Standards, was included to enable the calculations of IU. All laboratories were entirely blinded to the identity of the samples.

2.1.1. Participating laboratories

Nine WHO HPV LabNet laboratories distributed around the world participated in the SOP evaluation study. The SOP was provided by the Global Reference Laboratory (GRL), Sweden and modified in accordance with comments from the other members of the LabNet. This final SOP was adopted by the WHO HPV LabNet and included in the WHO HPV Laboratory Manual [16]. Ten laboratories participated in the proficiency study, and were directed to use the provided VLP reference reagents and the final WHO SOP for HPV serology were used. All shipments, including VLP-coated plates and serum samples, were sent by courier at ambient temperature. Sending and arrival dates were tracked.

2.2. Methods

2.2.1. Preparation of VLP coated plates for interlaboratory comparison

In the SOP evaluation study, 2 different methods for preserving and storing coated VLPs were investigated. Plates were coated with 100 μl of 1 μg/ml HPV 16 VLP in cold PBS over night at +4 °C where after the coating solution was discarded. One set of plates was immediately stored in sealed bags ("moist storage"), whereas the other set of plates were dried at room temperature for 8 h ("dry storage"). The coated plates were first stored for 2 weeks at room temperature (to mimic the requirement for worldwide shipping at ambient temperature), the remainder of time the storage was at +4 °C. One plate from each set was tested with a panel of 18 sera in 3 dilutions using direct ELISA described below on day one, after 2 weeks and after one, two, three and six months of storage.

After 4 weeks, the VLP coated plates that were immediately stored in sealed bags ("moist storage") had stable reactivity and were selected for this study. Drying of plates resulted in loss of reactivity. The stability of the plates remained acceptable up to 3 months of storage at +4 °C, with an average International Units (IU) antibody level of positive samples of 95% compared to freshly coated plates and similar results for the negative control sera. After 7 months of storage, all samples that tested positive at day one were still positive with an average IU level of 87% compared to freshly coated plates. However, at 7 months of storage the background reactivity in the negative control was almost doubled (increased with 182%). Because of the impaired specificity, we concluded that the expiry date of plates coated with HPV16 VLPs is 3 months post coating.

2.2.2. Direct VLP ELISA

Plates were coated with HPV 16 VLP in cold PBS and control plates with PBS in the GRL in Sweden using the moist storage procedure described above. The coated plates were distributed to the participating laboratories and stored at +4 °C until use. Direct ELISA to detect HPV 16 antibodies was performed as described [11,16]. Briefly 10% horse serum in PBS (HS-PBS) was used to block the plates for 1 h at room temperature. Serum samples were prepared by making 1:10, 1:31 and 1:100 dilutions in HS-PBS, added to the plates and incubated for 2 h at room temperature. Following five washes using the standardized wash-buffer PBS–0.5% Tween (PBS-T), with detailed instructions on a manual washing procedure, anti-human IgG HRP (Dako, diluted 1:1000 in HS-PBS) was