Diagnostic Microbiology and Infectious Disease xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Analytical performance evaluation of Anyplex II HPV28 and Euroarray HPV for genotyping of cervical samples

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 22 March 2016
Accepted 15 April 2016
Available online xxxx

Keywords:
Anyplex II HPV28
Euroarray HPV
Human papilloma virus
HPV genotyping
Method comparison
Vaccine monitoring

ABSTRACT

Analytically accurate human papillomavirus (HPV) genotyping methods are required to assess the impact of HPV vaccination. The aim of this study was to evaluate the analytical performance of Anyplex II HPV28 (Seegene, Korea) and Euroarray HPV (Euroimmun, Germany) genotyping kits, for conducting a future HPV vaccine efficacy monitoring study in Luxembourg. A total number of 150 cervical swabs were collected from women with mean age 31.4 years. Agreements for detecting any HPV between Aptima/Anyplex (88.0%) and Aptima/Euroarray (90.7%) were similar. Agreement of Anyplex/EuroArray with Aptima was higher for Genotypes 16, 18 or 45 than for the other 11 HPVs. The average number of HPV genotypes detected per sample was similar with 2.6 and 2.5, for Anyplex and EuroArray, respectively. In conclusion, Anyplex and Euroarray showed high agreement in general and in particular for detecting genotypes contained in HPV vaccines.

1. Introduction

Cervical cancer is the fourth most common female cancer affecting 528,000 women each year and leading to 266,000 deaths worldwide (Ferlay et al., 2015). In Luxembourg it remains the third most common cancer in women (Ferlay et al., 2013). Epidemiological studies have shown that invasive cervical cancer (ICC) is strongly linked with persistent infection with oncogenic human papillomavirus (HPV) types (de Sanjose et al., 2010; Guan et al., 2012; IARC, 2012). According to the International Agency for Research on Cancer (IARC), 12 genotypes are carcinogenic (Group 1), 13 genotypes are probably/possibly carcinogenic (Group 2) and Group 3 genotypes have not been classified as to the carcinogenicity (Bouvard et al., 2009; IARC, 2012). Genotypes 16 and 18 account for 70% of all ICC at a global level, and the other ten carcinogenic types cause about one quarter (de Sanjose et al., 2010; Munoz et al., 2004). Some of the HPV types belonging to Group 2 of carcinogenic types may also rarely cause cervical cancer (Arbyn et al., 2014).

Efficacious prophylactic HPV vaccines have been developed due to the strong etiological link between HPV infection and carcinogenesis (Schiller et al., 2012). Moreover, HPV testing has been useful for screening and management of screen-positive women (Arbyn et al., 2012). While Luxembourg does not have an organised screening program, cervical screening has been conducted since 1962 by a single national cytology laboratory. In 2014, the liquid-based ThinPrep® Pap Test and Imaging System by Hologic (Marlborough, MA) replaced conventional cytology for cervical screening at this national facility. At the same time, HPV testing using the Aptima HPV test (Hologic, Marlborough, MA) was introduced, mainly on samples showing atypical squamous cells of undetermined significance or upon a physician's request. The Aptima test is FDA-approved for detecting HPV targeting E6/E7 messenger RNA (mRNA) using the same sample used for cytology, but has only limited genotyping ability for types 16, 18, or 45. From July 2014 until December 2015, 163,321 cervical samples from 121,027 women were investigated and HPV testing was conducted on 11,582 samples.

The HPV vaccination program in Luxembourg was introduced in 2008 offering a choice of Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) or Gardasil (Merck & Co., Whitehouse Station, NJ USA) to a target population of 12–17 year old girls. Between March and November 2008, the vaccination program covered 29% of the target population (Arbyn et al., 2010). In a future research study we aim to evaluate the impact of HPV vaccination by comparing the HPV prevalence in vaccinated and unvaccinated young women. Therefore, we

Abbreviations: CI, confidence interval; GE, genome equivalents; HPV, human papillomavirus; IARC, International Agency for Research on Cancer; ICC, invasive cervical cancer; IU, International Units; mRNA, messenger RNA; PCR, polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; TPTIS, ThinPrep® Pap Test and Imaging System.

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http://dx.doi.org/10.1016/j.diagmicrobio.2016.04.011
0732-8893/

conducted a pilot study to assess the analytical performance of two commercially available genotyping assays, the Anyplex II HPV28 (Seegene, Seoul, Korea) and the Euroarray HPV (Euroimmun, Luebeck, Germany).

The Anyplex II HPV28 is a multiplex real-time polymerase chain reaction (RT-PCR) assay designed to detect 28 HPV genotypes. In several comparative studies, the Anyplex had high agreement with other HPV assays (Estrade & Sahli, 2014; Kwon et al., 2014; Lillsunde Larsson et al., 2015; Marcuccilli et al., 2015). Recently introduced, the Euroarray is based on a microarray system and can identify 30 HPV types.

To our knowledge, no studies have evaluated the Euroarray assay before. We investigated HPV genotype-specific concordance between the Anyplex II 28HPV, the Euroarray HPV and the Aptima HPV assay.

1.1. Objectives

The objective of this study was to evaluate and compare the analytic performance of the Anyplex II 28HPV and the Euroarray HPV genotyping assays in order to use it for vaccine impact monitoring.

2. Materials and methods

2.1. Nomenclature

HPV genotypes were divided into three groups according to IARC carcinogenicity Group 1 (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), probable/possible carcinogenic IARC Group 2 (26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, 97) and IARC Group 3 (6, 11 and others) (Bouvard et al., 2009; IARC, 2012).

2.2. Samples

A total number of 150 selective cervical swabs were included in the study. The samples collected in ThinPrep Pap test vials containing PreservCyt solution were obtained from women undergoing routine cervical and HPV testing with a mean age of 31.4 years (range, 16–70 y). Indications for requesting HPV tests were unknown. These samples were initially tested by the APTIMA HPV assay which targets 12 Group 1 genotypes and two Group 2 genotypes (66 and 68), and, if positive, followed by a separate APTIMA 16, 18/45 genotype assay. In order to have a diverse spectrum of positive and negative samples, our pilot study panel consisted of three categories. The first category consisted of 50 consecutive samples positive for either HPV Types 16, 18, or 45. The second category included 50 consecutive samples negative for 16, 18 or 45, but positive for any of other genotypes (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68). The last category consisted of 50 consecutive samples negative for all 14 genotypes detectable by the APTIMA assay.

2.3. Sample preparation

After a maximum of 5 days storage at room temperature, total DNA was extracted from ThinPrep vials using the QIAamp DNA mini kit according to manufacturer's instructions (Qiagen, Hilden, Germany).

2.4. Amplification and detection of HPV DNA

2.4.1. The Anyplex II HPV28 (Seegene, Seoul, South Korea)

The Anyplex II HPV28 identifies simultaneously 12 IARC Group 1 genotypes, 8 IARC Group 2 genotypes (26, 53, 66, 68, 69, 70, 73, 82) and 8 IARC Group 3 genotypes (6, 11, 40, 42, 43, 44, 54, 61) in two multiplex reactions on the CFX96 real-time thermocycler (Bio-Rad, Hercules, CA). The L1 gene of HPV DNA was amplified and simultaneously the human housekeeping gene (human *beta-globin*) was co-amplified as an internal control to monitor DNA purification efficiency, RT-PCR inhibition, and sample adequacy. Reactions were performed as recommended by the manufacturer (5 μL input volume for both multiplex reaction), data recording and interpretation were automated with the Seegene viewer software according to the manufacturer's instructions (Estrade & Sahli, 2014; Kwon et al., 2014; Marcuccilli et al., 2015).

2.4.2. The EUROArray HPV (Euroimmun, Luebeck, Germany)

The Euroarray HPV test detects 12 IARC Group 1 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), 7 IARC Group 2 genotypes (26, 53, 66, 68, 70, 73, 82), and 11 IARC Group 3 genotypes (6, 11, 40, 42, 43, 44, 54, 61, 72, 81, 89). The test is based on the detection of oncogenes E6/E7 via a hybridisation reaction with immobilised DNA probes. An input volume of 5 μL is amplified by conventional multiplex PCR using Applied Biosystems 2720 Thermal Cycler (Thermo Fisher Scientific, Waltham MA, USA). The fluorescing PCR products are detected with an oligonucleotide microarray using a special Scanner. The use of subtype-specific primer systems and probes allows the detection and typing of 30 HPV types in one test run. Data analysis and data interpretation were performed fully automated using the EUROArrayScan software according to the manufacturer's instructions.

2.5. WHO HPV LabNet Proficiency Study

The 2014 WHO HPV LabNet Proficiency Study was provided by Equalis (Uppsala, Sweden) consisting of 41 specimen of DNA plasmids and 3 cell samples. The WHO proficiency panel is designed for evaluating HPV assays used in HPV vaccine research and HPV surveillance (Eklund et al., 2014). Samples contained single or multiple HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a and 68b), at concentrations of 5 to 500 International Units (IU) or genome equivalents (GE) per 5 μL of DNA (Marcuccilli et al., 2015). DNA Extraction of cell samples was performed as described above. All samples were tested by the Anyplex and the Euroarray assays and submitted to EQUALIS for evaluation.

2.6. Statistical analysis

Analysis was performed using STATA 12.1 software (Texas, USA). Four genotypes were excluded from the analysis, because they were not shared by both assays: 69 (Group 2) is not detectable by the Euroarray, whereas 72, 81, and 89 (Group 3) are not detectable by the Anyplex. Concordance of HPV typing results was assessed by the percentage of agreement and Cohen's kappa (k) which was classified as follows: 0.00–0.19, poor; 0.20–0.39, fair; 0.40–0.59, moderate; 0.60–0.79, strong; 0.80–1.00, excellent (Estrade & Sahli, 2014). Post hoc power calculations using the function N.cohen.kappa of the R package irr (http://www.R-project.org/) show that with a sample size of 150, there was 80% power (type I error alpha 5%) to detect a difference of 0.13 assuming a true kappa of 0.9 and a prevalence of 0.3. The McNemar test was used to assess discordance. Concordant samples were defined when all genotypes were detected by two methods. Partially concordant samples were defined as those which shared at least one but not all genotypes, whereas discordant samples were defined as those with no shared genotypes.

3. Results

3.1. Comparison of Aptima with Anyplex and Euroarray

When restricting analysis to the 14 HPV types shared by all three assays, the overall agreement for detecting any HPV between Aptima and Anyplex (88.0%) and between Aptima and Euroarray (90.7%) was strong (Table 1) with kappa values of 0.727 and 0.790, respectively. When restricting analysis to Genotype 16, agreement between Aptima and Anyplex (98.0%) and between Aptima and Euroarray (98.0%) was excellent with kappa values of 0.949 and 0.950, respectively. Similarly, when restricting analysis to Genotypes 18/45, the agreement between Aptima and Anyplex (99.3%) and between Aptima and Euroarray (98.7%) was

Please cite this article as: Latsuzbaia A, et al, Analytical performance evaluation of Anyplex II HPV28 and Euroarray HPV for genotyping of cervical samples, Diagn Microbiol Infect Dis (2016), http://dx.doi.org/10.1016/j.diagmicrobio.2016.04.011