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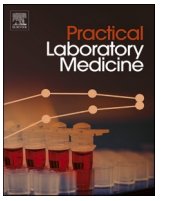

External quality assessment program for human papillomaviruses DNA testing in Thailand

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: Since 2020, the National Health Security Office includes the human papillomavirus DNA testing for cervical cancer screening in the government's healthcare schemes. HPV DNA testing has become primary screening in many laboratories in Thailand. External quality assurance scheme is crucial for assessment of laboratory performance.
Objectives: The aim of this study was to develop a pilot program using LBC samples for the EQA of molecular methods and to review the methods used by participants to detect the presence of high risk HPV genotypes.
Study design: Four pilot distributions were shipped between December 2021 and May 2023, six months apart of two panels, each consisting of five different specimens.
Results: All participants achieved 100 % accuracy in correctly identifying the presence or absence of high-risk genotypes in all 5 EQA samples. The most used HPV DNA test for detecting the presence of high-risk HPV DNA was the two specific high-risk genotypes and 12 other high-risk HPV genotypes. There was an observed increase in the use of assays that could detect 14 HPV HR genotypes. It suggests expanding testing methods to include a broader range of high-risk HPV genotypes, which could improve the comprehensiveness of the testing.
Conclusions: The HPV DNA testing scheme provides a standardised, homogeneous and characterised clinical specimen. These results indicate that the LBC samples are suitable for utilisation in an EQA scheme. EQA of HPV molecular screening programme is essential for monitoring the performance of laboratory networks.

1. Background

Cervical cancer is the 3rd leading cause of female cancer in Thailand, with an estimated 9158 new cervical cancer cases annually in Thailand (estimations for 2020) [5]. Human papillomavirus (HPV) infection is the viral sexually transmitted infection associated with cervical cancer. HPV consists of several different types of five groups (alpha, beta, gamma, mu, and nu) [1,2]. More than 200 HPV types are known to exist in the basis of phylogenetic species with 2 groups, 'high risk' (HR) and low-risk HPV types. Low-risk HPV types include types 6, 11, 42, 43, and 44. High-risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. The common types associated with cancer are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, 68, and 70 [3,4]. HPV-16 and 18 are considered to be the most frequent HPV types worldwide and are responsible for approximately 50 % of high grade cervical pre-cancers and nearly 70 % of all cervical cancer cases [6,7].

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HPV-based cervical cancer screening has demonstrated the high sensitivity and thus the greater negative predictive values compared with cytology-based screening. Molecular assays are the main technique for HPV identification because of the cultivation is hard while the sensitivity of serological tests based on VLPs (virus-like particles) is about 50 % [8,10]. Nucleic acid amplification-based methods including target amplification, signal amplification, and nucleotide hybridization have known the potential of the direct detection of HPV from cervical specimens [1]. These molecular techniques supply an alternative attitude to cervical screening and patient management. In Thailand, the Royal Thai College of Obstetrics and Gynaecology (RTCOG) released a practice guideline of cervical cancer screening for women between 25 and 65 years with cervical cytology every 2 years, or co-testing or primary HPV testing every 5 years. With a history of sexual intercourse, the screening should start at age of 25 years [9]. Since 2020, the National Health Security Office (NHSO) includes the human papillomavirus (HPV) DNA testing for cervical cancer screening in the government's healthcare schemes. HPV DNA testing has become primary screening in many laboratories in Thailand. External quality assurance (EQA) scheme is essential for assessment of laboratory performance. The EQA panels from clinical specimens demonstrated the variety of genotyping results reported by testing laboratories and different genotypes were not associated with any specific approach [11]. The great variety of HPV genotypes in the clinical specimens is of especially useful in the cervical screening where great variety in HPV genotypes and viral load may be encountered. EQA scheme for HPV DNA testing was proceeded in 2021–2022 by the Department of Medical Sciences, national laboratory of the Ministry of Public Health.

2. Objectives

To develop a pilot program using liquid based cytology (LBC) samples for the EQA of molecular methods and to review the methods used by participants to detect the presence of high risk (HR) human papillomavirus (HPV) genotypes.

3. Study design

EQA samples were prepared using liquid based cytology (LBC) samples from the routine cervical screening in Thailand and performed testing by extraction with the DNA extraction kit based on Magtration technology and amplification using the Anyplex™II HPV HR Detection, multiplex real-time PCR simultaneously detects and differentiates 14 high-risk HPV genotypes. A large volume of EQA specimens were prepared with pooling/diluting and re-testing to ensure the results of laboratory tests. Homogeneity assessment involved randomly selected 10 vials of each EQA samples using a function of microsoft excel and tested in duplicate. Stability assessment involved two vials of samples. The stability of control materials was assessed at room temperature and cold temperature. Linear regression analysis was performed to assess long-term stability. The specimens were then shipped to participants with a request to perform the HPV DNA detection by molecular techniques. The EQA panel included 4 samples with a various HPV genotypes and 1 negative sample. Each 1000 ml of all EQA sample aliquots were kept at room temperature until shipment. Thailand Post, a delivery service was used to transport EQA samples at room temperature to participating laboratories within 48 h. The user instructions given to participants accompany each distributed specimen to ensure that it meets conditions on storage, handling, timing, and assay. The participating laboratories submitted all laboratory results online through the DMSc-PT program website. The EQA provider delivered a preliminary performance report to all participating laboratories within 48 h following the deadline for result return. The association between the two variables, extraction and multiplex real-time PCR. Each participating laboratory were requested to complete a survey for evaluation of satisfaction.

4. Results

The EQA materials included pooled specimens consisting of 4 positive and 1 negative for high-risk (HR) HPV genotypes. This mixture of specimens was used for testing accuracy. The homogeneity assessment indicated that 10 vials of samples showed uniformity, ensuring that the EQA materials were consistent and suitable for distribution to participating laboratories. The stability of EQA materials was found to be good at both room temperature and in cold storage, ensuring that the materials remained suitable for testing over time. EQA materials were distributed to participating laboratories in four pilot distributions: December 2021 (1st-2022), May 2022 (2nd-2022), December 2022 (1st-2023), May 2023 (2nd-2023). Results from these distributions were returned to the EQA provider within two weeks. The number of participating laboratories increased over the two years: In 2022, there were 58 and 73 laboratories across countries that participated in the first year pilot schemes. In 2023, the number of participants increased to 151 in the first round of the second-year pilot schemes, with the remaining 150 laboratories participating in the second round. The most commonly used HPV DNA test for the detection of high-risk (HR) HPV DNA in the first year was the 16, 18, and other HR genotypes assay. However, in the second year, the use of assays detecting 14 HPV HR genotypes increased (Table 1). The HPV DNA testing

Table 1
Information on the number of participating laboratories for a particular testing program

Round	14 HPV HR genotypes	16, 18 and other HR
1st-2022	22	36
2nd-2022	28	45
1st-2023	69	82
2nd-2023	74	76

protocol used by participating laboratories was divided into two main steps: DNA extraction and DNA amplification. There were variations among laboratories, with participants reporting the use of 25 different pairs of DNA extraction and amplification methods. This included the use of 12 different DNA extraction kits and 14 different detection kits (Table 2). It's noted that no laboratory implemented in-house PCR assays, suggesting that laboratories relied on commercially available DNA extraction and amplification kits for their testing.

The participating laboratories achieved excellent results, with 100 % of the participants reporting correct results in all specimens, as indicated in Table 3. Laboratories reported detecting HPV DNA genotypes using two platforms:Platform 1: Detected genotypes 16, 18, and other high-risk (HR) types. Platform 2: Detected 14 genotypes, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Incorrect results were associated with the 16, 18, and other 12 HR HPV assay. Internal investigations in laboratories reporting incorrect results revealed two possible causes of error: Low volume of External Quality Assurance (EQA) samples, Over-dilution of samples. Upon retesting by the laboratory using new aliquots of EQA samples provided by the EQA provider, laboratory was able to return correct results. This suggests that the errors were related to sample handling and not necessarily the testing method itself. A satisfaction survey was conducted, and the majority (89 %) of the participating laboratories expressed overall satisfaction with the EQA process. This indicates that the laboratories found the external quality assurance process to be beneficial and satisfactory.

Overall, it seems that the participating laboratories performed well in the study, with only a small number experiencing errors that were later identified and corrected. The high satisfaction rate suggests that the EQA process was effective in ensuring the accuracy of HPV DNA genotyping results.

5. Discussion

This study demonstrates good concordance among 73 and 150 laboratories in 2022 and 2023, respectively. The use of multiplex real-time PCR assays both 14 genotypes and 2 genotypes +12 other HR was the most commonly used assay for genotyping by those participating in the pilot distributions. This study showed the good concordant in the specimens containing one or two genotypes. No results varied when multiple genotypes were present even between laboratories using the same method. The reference laboratories

Table 2
Extraction kits and Detection kits applied by the participating laboratories

	Reagent for DNA extraction	Reagent for DNA Detection	No. of test reports	
			2022	2023
1	Abbott Alinity m HR HPV AMP Kit	Abbott Alinity mHR HPV AMP kit	5	3
2	Biogerm Nucleic Acid Extraction and Purification Kit	0001 Huanji gene technology Microarray Detection kit	0	1
3	Chemagic Viral DNA/RNA Extraction kit	Anyplex II HPV HR detection	1	0
4	Cobas 4800 System Sample Preparation Kit	cobas® 4800 HPV Amplification/Detection Kit	36	51
5	DX32 Viral Necleic Acid Extraction Kit	MolecuTech REBA HPV-ID	0	1
6	DX32 Viral Necleic Acid Extraction Kit	Human Papillomavirus Real Time PCR Kit	0	1
7	MagDEA® Dx SV	Anyplex II HPV HR detection	8	13
8	Nucleic Acid Extraction Rapid Kit	Anyplex II HPV HR detection	0	1
9	Nucleic Acid Extraction Rapid Kit	Human Papillomavirus Real Time PCR Kit	0	0
10	Nucleic Acid Extraction Rapid Kit	Human Papillomavirus Real Time PCR Kit	0	2
11	QIAAmp DNA mini kit	High-Risk HPV DNA Test [Macro Micro test]	1	0
12	Sanity 2.0 HPV DNA Extraction kit	High Risk HPV Genotyping kit (Sanity 2.0)	0	4
13	STARMAG 96X4 Universal Cartridge kit	Anyplex II HPV HR detection	0	3
14	TIANLONG® Nucleic Acid Extraction Kit	Anyplex II HPV HR detection	0	1
15	TIANLONG® Nucleic Acid Extraction Kit	15 High-risk Human Papillomavirus DNA (Genotype) Diagnostic Kit (PCR-Fluorescence Probing)	0	1
16	TIANLONG® Nucleic Acid Extraction Kit	Tellgen HPV2+12	2	7
17	TIANLONG® Nucleic Acid Extraction Kit	Human papillomavirus Nucleic Acid Amplification test kit	0	1
18	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Anyplex II HPV HR detection	9	13
19	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Neoplex HPV HR Multiplex Real-time PCR	0	6
20	Zybio Nucleic Acid extraction kit (Magnetic beads method)	15 High-risk Human Papillomavirus DNA (Genotype) Diagnostic Kit (PCR-Fluorescence Probing)	10	29
21	Zybio Nucleic Acid extraction kit (Magnetic beads method)	High Risk HPV Genotyping kit (Sanity 2.0)	0	1
22	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Tellgen HPV2+12	1	6
23	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Human papillomavirus (HPV) DNA Diagnostic kit	0	1
24	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Human Papillomavirus Real Time PCR Kit	0	1
25	Zybio Nucleic Acid extraction kit (Magnetic beads method)	Human papillomavirus Nucleic Acid Amplification test kit	0	3
			73	150

Table 3
The number of assay distributions between 2022-2023.

Assay	Distribution				No. of data sets	% correct results (n)
	1st-2022	2nd-2022	1st-2023	2nd-2023		
14 HPV HR genotypes	22	28	69	74	193	100 (193/193)
16, 18 and other HR HPV	36	45	82	76	239	100 (239/239)

Distribution	Samples	Code	Results	% correct results (n)
1st-2022	1	HPV 01	HPV 16	100
	2	HPV 02	HPV 16	100
	3	HPV 03	HPV 18	100
	4	HPV 04	HPV 33	100
	5	HPV 05	Negative	100
2nd-2022	1	HPV 01	HPV 68	100
	2	HPV 02	Negative	100
	3	HPV 03	HPV 16	100
	4	HPV 04	HPV 18	100
	5	HPV 05	HPV 16, 52	100
1st-2023	1	HPV 01	HPV 18	100
	2	HPV 02	HPV 16	100
	3	HPV 03	Negative	100
	4	HPV 04	HPV 31	100
	5	HPV 05	HPV 66, 68	100
2nd-2023	1	HPV 01	HPV 52	100
	2	HPV 02	HPV 16	100
	3	HPV 03	HPV 18	100
	4	HPV 04	Negative	100
	5	HPV 05	HPV 16	100

found that sample 05-2/2022 contained HR genotypes 16 and 52 and the 28 participants concurred with this result. For sample 05-1/2023, the reference laboratories detected HR genotypes 66 and 68 with 69 participants reporting the same result as the reference laboratories.

Robust external quality assessment (EQA) is crucial for monitoring the effectiveness and impact of HPV vaccine programs. EQA helps ensure that the testing methods used in these programs are accurate and consistent across different laboratories or participants. Using clinically relevant materials is essential in cervical screening situations because HPV infections can manifest in various ways, involving different genotypes, levels of infection, and viral loads. The testing methods should be able to detect and distinguish among these variations accurately. This study introduces a preliminary initiative that leverages Liquid-Based Cytology (LBC) samples for EQA in molecular methods. The program not only demonstrates feasibility but also ensures a guarantee of accuracy and comparability in HPV detection across different laboratories.

Highly sensitive DNA detection assays have greatly improved the diagnosis of HPV infections. However, these assays need to be carefully validated in the laboratory to ensure their accuracy and reliability. This includes verifying their sensitivity, specificity, and ability to detect different HPV genotypes. Degradation of viral DNA by endogenous endonucleases can lead to false-negative results. Therefore, ensuring the stability of samples during transport and storage is a critical part of the testing process. To assess the suitability of a specimen for molecular analysis, controls such as β-globin gene amplification or spiking the sample with known positive material can be used. This helps ensure that the DNA extracted from the sample is of sufficient quality for accurate testing.

In summary, the quality and reliability of HPV testing are essential for effective cervical cancer screening and monitoring the impact of HPV vaccination. Standardized testing methods, proper sample handling, and thorough laboratory validation are key components in achieving accurate results and, ultimately, improving public health outcomes in the context of HPV-related diseases.

CRediT authorship contribution statement

Pilailuk Akkapaiboon Okada: Writing – original draft, Principle Investigator, Project Manager, Project administrator, Supervision. **Suratchana Mitrat:** Project officer. **Archawin Rojanawiwat:** General support.

Declaration of competing interest

The authors declare no potential conflicts of interest.

Data availability

I opened my finding for all researcher

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