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Collaborative study Brazil-Portugal:
VALIDATION OF HPV LYOPHILIZED SAMPLES FOR THE CONTROL OF MOLECULAR TESTS

Maria Elizabeth Menezes[1], Edson Natal Fedrizzi[5], José Abol Correa[1], Daniela Cochicho[3], Luis Martins[3], Mario Cunha[3], Carmo Ornelas[3], Nuno Verdasca[4], Ana Paula Faria[4].

Programa Nacional de Controle de Qualidade[1]/ Instituto de Biotecnologia Aplicada[2]/Instituto Nacional de Saúde de Lisboa - Programa Nacional de Avaliação Externa da Qualidade[4]/Instituto Português de Oncologia de Lisboa, Serviço de Patologia Clínica – Laboratório de Virologia[3]/Universidade Federal de Santa Catarina[5].

1- Introduction:

There are about 120 types of Human Papilloma Virus (HPV). The major importance about it is the oncogenic potential of some types. They are referred as a high and low risk for oncogenic potential. Given the importance of the disease it causes, and the use of different laboratorial techniques, mainly techniques of molecular biology, it is mandatory to have an External Quality Assurance (EQA) program base on harmonized standards that rely on consistent controls. The participation in EQA's programs is mandatory in laboratories with an ISO 15189/17025 accreditation implemented.

The control of Molecular Biology techniques is absolutely necessary for the quality assurance of the results, the tracking and monitorization of the performance of the reagents. However, one of its limitations is the stability, homogeneity of the material, (ISO/IEC 17043:2010 requisite) as well as the availability of control samples adequate for the parameter to be analyzed. The PNCQ, in order to fill this gap, lyophilised the HPV samples control. In order to do the validation and to test the reproducibility of these lyophilized samples, PNCQ sent to IBIOTECNO , INSA-PNAEQ -DDI and the IPOLFG-SPCLV to be analysed by different methods and reagents in two different countries and different laboratories.

2- Objective:

Validation of lyophilized samples by different molecular techniques to have harmonized standards that relies on consistent control.

3- Material and Methodology

Screening for positive HPV was done by IBIOTECNO. 1,500 samples were collected from patients (women) and tested for HPV using Hybrid Capture technique in agreement with the manufacturer's Protocol (Qiagen). Negative samples were selected to be used as an HPV negative control. The positive samples for HPV, were divided into three groups:

1-Samples containing HPV of High Risk; 2-Samples containing HPV of Low Risk 3- Samples containing HPV of High and Low Risk. The samples after lyophilisation were tested for group specific again by Hybrid Capture technique, to make sure the concentration was not lose on the process of lyophilisation.

The liophilizaded sample was sent at room temperature to INSA (Laboratório Nacional de Referência das Infecções Sexualmente Transmissíveis - vírus do papiloma humano , vírus herpes genital) and IPOLFG SPCLV (Instituto Português Oncologia de Lisboa Franscisco Gentil, Serviço de Patologia Clinica Laboratório de Virologia) had HPV of high oncogenic potential (HPV-B) and was used for validation of the process, in order to be used as a control. The sample was reconstituted with water free of DNAse and RNAse, DNA extraction and detection/genotyping were done using methodologies described in Table 1.

Institution	DNA Extraction	Detection/ Genotyping
INSA (PT)	Automatic – NucliSENS™ bioMerieux easyMAG	**Clart HPV 2 (Genomica)** ·Primers: MY09/11 (amplification of 450pb, L1 region)
IPO (PT)	Manual – Qiagen Vírus Mini kit	**Real Time PCR SYBR Green (In House assay):** ·Detection (screening) ·Primers: SPF 10 (amplification of 75 bp, L1 region) ·Accreditation ISO 15189 **INNO-LIPA HPV (Innogenetics):** ·Primers: SPF 10 (amplification of 65 bp, L1 region) ·Accreditation ISO 15189 **Papillocheck (Greiner):** ·Primers: NA (amplification of 350 bp, E1 region)
PNCQ /IBIOTECNO (BR)	Qiagen Kit	**Hybrid Capture 2** ·Discrimination between High Risk and Low Risk

Table1: Methodologies used in DNA extraction and detection/genotyping

4- Results:

The data shows that the viral concentration of the sample was preserved after lyophilisation: 2.65 RLUs before lyophilisation and 2.33 RLUs after. These results reflect the preservation of DNA. In terms of DNA quantification, both Portuguese laboratories obtained good values: at INSA was 21.09 ng/µl and in IPOLFGSPCLV were 43.09 ng/µl. What is important is that the DNA was preserved although there is a difference between the two quantifications. Also, the genotypes detected were similar in all laboratories, despite the different techniques used. In table 2 and 3 are the results of the genotyping and screening tests.

		HPV screening
IPO	**Real Time PCR SYBR Green**	**Result:** Detected (Ct= 26,17 Tm = 73ºC)
PNCQ	**Hybrid Capture II**	**Result:** High Risk and Low Risk

Table 3: Results obtained with the screening assays

		HPV Genotypes																	
IPO	**Inno-Lipa**	6	11	16	18	26	31				51			56					69/71
	PapiloCheck	6		16			31	39	40	42	51	52	53	56		59	66	82	
INSA	**Clart**	6		16	18		31				51	52	53	56	58	59	66	82	

Table 2: HPV genotypes detected in the lyophilized sample by the different methodologies used. Genotypes were classified according to Munoz et all, NEJM 2003 – 348 (518-27)

Legend: Low risk | High risk | Possible high risk | Indeterminate risk

5-Conclusion

This validation between three different laboratories was important for the establishment of a standardized control. Regardless of the DNA extraction and amplification, it was possible to detect HPV DNA. The differences noted in the quantification of DNA are possibly due to the DNA extraction methodology and it doesn't compromise the main goal of this collaboration.

The discrepancies observed between the different kits used for genotyping can be due to the fact that we have several genotypes present in this sample and the different sensibility for each HPV type of the different kits. The presence of so many genotypes in a single sample is due to the sample used is a pool of HC2 positive samples. If genotyping test will be used in the Quality Control, a better sample selection is needed.

The use of liophilizaded samples in the quality control had the advantage of no especial condition of shipments is needed and a better conservation of the samples is obtain.

It is sheduled the first Pilot scheme in 2014, which will include 10 laboratories with large experience in HPV detection in Portugal.

Contacts:
pnaeq@insa.min-saude.pt;
biomol@pncq.org.br