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USE OF FTA ELUTE CARD IMPREGNATED WITH CERVICOVAGINAL SAMPLE DIRECTLY INTO THE AMPLIFICATION REACTION INCREASES THE DETECTION OF HUMAN PAPILLOMAVIRUS DNA

Carla R. Santos[1,2], Laura G. Franciscatto[2], Regina B. Barcellos[1], Sabrina E. M. Almeida [1,3], Maria Lucia R. Rossetti[1,2*]

[1] Centro de Desenvolvimento Científico e Tecnológico, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS, Brasil; [2] Universidade Luterana do Brasil, Canoas, RS, Brasil; [3] Universidade Feevale, Instituto da Saúde, Novo Hamburgo, RS, Brasil.

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ABSTRACT

This study aimed to evaluate the use of the FTA elute card™ impregnated with cervicovaginal sample directly in the PCR amplification for detection of HPV-DNA. The results were compared to a reference technique. This method was more efficient than the protocol indicated by the manufacturer, identifying 91.7% against 54.2% of the positive samples.

Key words: HPV, cervical cancer, FTA elute, molecular detection.

Human papillomavirus (HPV) infection has been associated with cervical cancer, and is considered a required prerequisite for the development of cervical cancer (15). This malignancy is the second most common type of cancer among women worldwide with incidence rate of 500,000 cases per year, of which 80% come from developing countries (17). In Brazil, for the year of 2010, it is expected 18,430 new cases of cervical cancer, with a total risk figure of 18/100,000 women (10).

There are more than 100 types of HPV, and they are described and classified according to their oncogenic potential (15). Routine preventive exams, as well as screening exams are performed anually, being the citopathological exam (Papanicolaou) the method of choice. In spite of the fact that this test contributed to a great decrease in the mortality rates, it simply identifies cellular changes that are usually associated with the viral infection, instead of directly detect the presence of HPV, allowing the latent infections to remain undiagnosed (10). A study by Fernandes et al. (2008) has shown that women who have HPV infection are 4.5 times more likely to develop cervical lesions than women presenting HPV negative results (5).

Several protocols based on DNA-detection have been developed for the identification and typing of HPV (3, 7, 13, 20). Generally, the samples used to perform these tests are from cervical cells collected by a citobrush placed in a liquid based transport medium, and DNA extraction protocols should be suited for large-scale HPV testing (9, 11). Filter paper cards, such as FTA elute card™ (Whatman, Inc., Clifton, NJ), make the collection,transport and archiving of DNA from several

***Corresponding Author. Mailing address:** Centro de Desenvolvimento Científico e Tecnológico, Fundação Estadual de Produção e Pesquisa em Saúde, CDCT/FEPPS; Av Ipiranga 5400, 3°andar, CEP 90610–000 – Porto Alegre, RS, Brazil.; Tel/Fax.: 55 51 33520336.; E-mail: mrossett@terra.com.br

types of biological materials possible. Gustavsson and colleagues (2009) reported that the FTA elute card™ could be an attractive alternative to a liquid based collection system for detecting HPV (9). The aim of this study was to verify the effectiveness in the direct use of the FTA elute card™ impregnated with self-collected cervicovaginal sample in the PCR for the molecular detection of HPV.

Cervicovaginal sample was obtained through self-collection from 39 women assisted at *Divina Providência* Hospital, in the city of *Frederico Westphalen*, southern of Brazil. These samples were collected by inserting a sterile swab into the vagina and rotating it 5 times before placing it into a HPV DNA specimen collection tube. These samples were resuspended in 2 mL 1X TE (10mM Tris pH 7.5, 1mM EDTA) and divided in two aliquots. One aliquot was used to perform the DNA extraction based on silica resin as described by Boom et al. (1990) (2) with modifications. Briefly, the samples were concentrated by centrifugation at 13 000 rpm for 10 minutes. One hundred microliters of a lysis buffer (guanidine hydrochloride 8 M, Tris HCl 0.08 M, EDTA 0.04 M and Triton X-100 2%) was added, followed by incubation at 100°C for 10 min. The samples were then centrifuged for 1 min at 13 000 rpm, and the supernatant was transferred to a microtube containing 2.5 µl of silica (12) and vortexed. After 1 min centrifugation at 13000 rpm, the supernatant was disposed by suction and the pellet was washed twice using a washing buffer (guanidine hydrochloride 8 M and Tris HCl 0.08 M) and once with ethanol 70%. The microtubes were dried out in a heath block at 56ºC for 10 min with closed lids and at room temperature for 5 min with open lids. The DNA was resuspended with 50 ul of 1x TE and incubated for 10 min at 56°C. After 1 min centrifugation at 13 000 rpm, 45 µl of the supernatant was collected and stored at -20°C. The other aliquot was spotted on a FTA elute card™ (Whatman, Inc.). The samples were spotted on the card using a pipet with a sterile filter tip, dried overnight and stored at room temperature. In order to getthe DNA from the samples in the cards, two different procedures were performed. The first procedure included DNA extraction of five punchers (Ø 2 mm each) from the spotted area of the card according to the FTA elute card™ manufacturer's protocol. This protocol involves sequential washing with ultrapure water for eliminating impurities and inhibitors; then, DNA release from the cards as a final product was diluted in 50 µL water after heating. Five microliters of the eluted DNA from the cards was added in the PCR. Alternatively, another procedure was performed adding 5 punchers (Ø 2 mm each) directly into the PCR mix, skipping the elution step.

The amplification of a 150 bp fragment of the HPV L1 region was achieved using the GP5+/GP6+ consensus primers (4). The PCR reaction was performed at 50 µL final volume containing 50 mM KCl, 10 mM Tris-HCl pH 8.5, 2.5 mM $MgCl_2$, 0.1 mM of each deoxynucleoside triphosphates, 50 ng of each primer, 2.5 U of Platinum®Taq DNA Polymerase (Invitrogen®) and 5 µL of elluted DNA. SIHA cells were used as a positive control for reactions. Negative control was composed by PCR mix without the template DNA. The PCR conditions included an initial denaturation step of 95°C for 5 minutes; 40 cycles of 95°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute; and final elongation step of 72°C for 10 minutes. Amplification of human β-globin gene, with primers GH20 and PCO4 (6), was performed to confirm the DNA extraction. Amplified products were detected by electrophoresis in 2.0% agarose gel with subsequent visualization under ultraviolet light. The results obtained for the DNA extraction by FTA cards were compared with the DNA extraction using silica resin (2), that was considered as a reference technique for successful studies had been performed in this centre (1, 18). The results were analyzed using the SPSS 16.0 statistical software (SPSS Ins. Chicago, IL, USA). Agreement between the techniques was evaluated using the *kappa* score.

Of the 39 analysed samples, 24 (61.5%) were HPV positive when using the resin technique; 25 (64.1%) tested

positive when the punchers were placed directly into the PCR tubes; and only 16 (41.0%) tested positive when performing the elution step as in the manufacturer's manual. All samples tested positive for the β-globin gene.

When comparing the results obtained by the use of cards for the 24 positive samples identified by the reference technique, the direct use of the card in the amplification reaction allowed the detection of 22/24 (91.7%) positive samples for HPV-DNA, while the use of the cards eluted allowed the identification of only 13/24 (54.2%) positive samples. Statistical analysis showed *Kappa* value of 31.1% (p=0.035) when comparing the resin technique to the eluted one and 83.5% (p>0,001) with the direct use of the punchers.

The use of cards for transporting, storing and preserving of genetic material has been successfully performed for various purposes. The maize streak virus can be successfully amplified after fixation in commercial cards in large-scale (16). *Escherichia coli*, in samples of faeces, was amplified from cards used for searching occult blood, and transported from Mexico to the U.S.A. (8). *Mycobacterium tuberculosis* was amplified from samples set in Genocard® (19), and human cells were amplified after transported on FTA® Elute Card, the same card tested in this study, under different conditions (21). Two recent studies about the use of cards in HPV have shown that elute cards presented satisfactory results for the detection of HPV-DNA in cervical specimens (9, 11).

The specimens used in this study were self-collected cervicovaginal samples, and this is the first report ofthis kind of sample using cards. The experiments performed here aimed to assess the use of the FTA elute card as recommended by the manufacturer protocol, and the use of the card directly into the PCR mix, skipping the elution step, to detect HPV-DNA. The amount of DNA presented in the card was crucial for the amplification step, as also described in a study by Morrison et al. (2007) (14). When comparing the results obtained from samples extracted using the conventional technique, the direct use of the card in the mix was more effective in identifying HPV-DNA, detecting 91.7% of the HPV-DNA positive samples, against 54.2% achieved by using the elution step. The heating of the PCR mix while in thermocycler was probably sufficient for the release of DNA into the mix, allowing the reaction to occur efficiently. Considering the low number of samples amplified when performing the elution step, the concentration of DNA obtained in the final product may have been a limiting factor. A larger volume of eluted product or the use of a higher number of punchers could improve this sensitivity. In this study, it was observed that the direct use of the cards into the PCR mix was more effective for the amplification of HPV-DNA, when compared to the technique performed according to the FTA elute card™ protocol. Even with the low number of samples analyzed in this study, these findings could collaborate with future large-scale screening programs aiming the prevention for HPV infection and cervical cancer.

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