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Long-term storage and safe retrieval of human papillomavirus DNA using FTA elute cards

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A B S T R A C T

Biobanking or collection and storage of specimens for future research purposes have become an essential tool in many fields of biomedical research and aims to provide a better understanding of disease mechanisms as well as the identification of disease-specific biomarkers that can navigate in complex diseases. In this study, we assessed the use of Flinders Technology Associates (FTA) cards as a long-term storage device for cervical specimens with suspected human papillomavirus (HPV) infections. HPV detection and genotyping results in liquid-based transport media were compared to HPV results from FTA cards. The overall agreement for the presence of any HPV infection between liquid-based medium and FTA cards stored for 1 year at ambient temperature was 100%. Reproducibility analysis of HPV detection and genotyping from FTA cards demonstrated that FTA cards are a reliable medium to store and preserve viral nucleic acids. Biobanking of cervical cells on FTA cards may provide a key resource for epidemiological and retrospective HPV studies.

1. Introduction

Although most human papilloma virus (HPV) infections are transient and asymptomatic, causing no clinical manifestations, persistent infection by certain types of HPV has been causally linked to the development of precancerous lesions and invasive cervical cancer (Schiffman et al., 2007). The most common HPV types associated with cervical cancers worldwide are 16, 18, 31, 33, 35, 45, 52 and 58. Of these high-risk HPV types, HPV type 16 is the most commonly found in cervical cancers (Munoz et al., 2003; Pretet et al., 2008). Current preventive HPV vaccines protect against the two high-risk HPV types 16 and 18 and were implemented in several countries worldwide in 2007. Recently, a systemic review and meta-analysis was conducted to investigate the efficacy of HPV vaccination programmes in a real-world setting (Drolet et al., 2015). In countries with HPV vaccination coverage of at least 50%, HPV type 16 and 18 infections in females aged 13–19 years are significantly reduced. Significant reductions of HPV types 31, 33 and 45 have been also recorded, suggesting cross-protection to other closely related HPV types (Drolet et al., 2015). Although these findings are promising, continued surveillance of HPV infection is required to identify signs of waning vaccine efficacy and the potential type replacement. This is of great importance, since co-infection with multiple HPV types occurs more frequently than previously thought (Chaturvedi et al., 2011; Trottier et al., 2006). Interestingly, positive and negative relationships between different HPV types have been reported suggesting that multiple type infections do not

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occur randomly, although further research is needed to understand the mechanisms behind it (Dickson et al., 2013; Rousseau et al., 2003; Trottier et al., 2006).

Flinders Technology Associates (FTA) cards (Whatman) provide a simple solution to collect, preserve and purify biological samples at room temperature for downstream DNA or RNA applications. The indicating FTA elute card contains an inert dye that changes from purple to white indicating the location of the applied sample. The FTA cards are impregnated with a patented chemical formula that lyses cells and denatures proteins upon contact but protects nucleic acids from nucleases. FTA cards have been successfully used for sampling, storage and characterisation of bacteria and viruses, human specimens for genetic studies and analysis in forensics (Abdelwhab et al., 2011; da Cunha Santos et al., 2010; Devost and Choy, 2000; Fontaine et al., 2007; He et al., 2007; Hedman et al., 2008; Kraus et al., 2011; Pagano et al., 2005; Picard-Meyer et al., 2007; Rajendram et al., 2006; Saieg et al., 2012). Specimens for cervical cytology and HPV testing are usually collected and preserved in liquid-based transport media. In developing and resource-limited countries, collection of cervical epithelial cells on FTA cards provides an alternative, simple and more cost-effective method compared to the liquid-based collection system. Several studies have reported that FTA card dry collected samples are suitable for the subsequent PCR-based HPV testing and typing (de Bie et al., 2011; Geraets et al., 2013; Gonzalez et al., 2012; Guan et al., 2013; Gustavsson et al., 2009; Gustavsson et al., 2011; Gyllensten et al., 2012; Lenselink et al., 2009; Phongsavan et al., 2012; Wang et al., 2014). Among those, five studies compared HPV results in samples collected in liquid-based media and placed onto FTA cards. In some studies, cervical brushes were first applied to the FTA card and then rinsed in different liquid-based media or frozen dry. In other studies, two samples were collected from each participant and placed on the FTA card or the liquid-based transport medium and sent to a laboratory for HPV analysis. Taken together, the studies found a good overall agreement of HPV DNA detection, ranging from 84 to 100% (de Bie et al., 2011; Gonzalez et al., 2012; Gustavsson et al., 2009; Lenselink et al., 2009; Wang et al., 2014). However, it has not yet been investigated whether FTA cards are an acceptable device for long-term storage of cervical cells and subsequent HPV testing. To address this issue, we evaluated the performance of FTA cards for HPV detection and genotyping following storage of FTA cards for 1 year at room temperature compared to liquid-based media. Additionally, we assessed in a subgroup the reproducibility of the FTA results and the viral loads over a 1-year period.

2. Materials and methods

2.1. Samples

Thirty cervico-vaginal samples were obtained from women (mean age 42.3 years; age range 22–70 years) participating in EVE, a regional cervical cancer-screening programme, Strasbourg, France. Cervico-vaginal samples were collected in different transport media, including Cytyc ThinPrep-Pap Test PreservCyt solution ($n = 6$), TriPath Imaging SurePath ($n = 7$) and Easyfix transport medium ($n = 17$). In some cases (13c14122, 13c14130, 13c14697, 13c15487, 13c15613), aliquots have been taken for a liquid-based cytological analysis before the remaining sample was shipped to the virology laboratory.

2.2. DNA extraction from liquid-based medium

Total DNA was isolated from cervico-vaginal samples collected in liquid-based media by using the QIAamp DNA Mini Kit that contains a ready-to-use proteinase K solution. Briefly, 1 mL of the liquid was spun down and the cell pellet was resuspended in 200 μL of buffer ATL working solution containing 20 μl of proteinase K. The samples were then vortexed and incubated for 2 h at 56 °C, followed by a 10 min incubation at 80 °C to inactivate proteinase K. Samples were then purified using silica membrane containing columns and eluated in a final volume of 50 μL, as described by the manufacturer. Finally, 5 μL of the eluate was used for PCR.

2.3. FTA sample preparation

Four milliliters of cervico-vaginal samples collected in liquid-based media were vortexed and transferred onto Prep-Stain density gradient reagent (BD). Following centrifugation of the tubes at 200 g for 2 min, blood, mucus and cell debris are partially trapped in the gradient and removed. The remaining fluid is then centrifuged at 800 g for 10 min in order to pellet the cellular material. The supernatant was decanted and the cell pellet was washed once with PBS. After centrifugation, the cellular pellet was resuspended in 100 μL of PBS, applied to the indicating FTA elute card and allowed to dry. After drying, each card was inserted in a paper envelope and envelopes were then placed in a plastic box to prevent moisture absorption. The plastic box was stored at room temperature in a dedicated lockable dark cabinet. Laboratory rooms' (ambient) temperature (20–25 °C) and humidity are recorded by an automated analytic measuring system.

2.4. DNA extraction from FTA cards

A Harris Uni-Core device (Whatmann) was used to produce a 3-mm punch after 1, 6 and 12 months of storage of the FTA card. The punch was transferred into a 1.5-mL Eppendorf tube, and 500 μL of sterile water was added and immediately pulse vortexed three times for a total of 5 s. Then, the water was removed and 30 μL of sterile water was added to the punch. The tube was transferred to a heating block at 95 °C for 30 min and after the end of the incubation, the tube was pulse vortexed approx. 60 times (one pulse/s). Following an additional centrifugation for 30 s, the eluted DNA was transferred into a new Eppendorf tube and 5 μL of the eluate was used for PCR. To avoid any cross-contamination, two blank filter paper discs were performed between FTA cards.

2.5. HPV PCR and genotyping

HPV PCR and genotyping was performed using the CLART HPV2 assay (Genomica, Madrid, Spain). The CLART HPV2 assay is based on PCR amplification of genotype-specific HPV L1 gene fragments and specific hybridisation between the amplicons and their specific probes on a microarray with automated reading of results. The assay has two internal controls, a DNA control (human CFTR gene) for sample sufficiency and an amplification control (plasmid) for PCR process control. CLART HPV2 detects 35 HPV genotypes, including 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).

2.6. HPV16 load quantification

Liquid-based and FTA card samples were analysed by quantitative real-time polymerase chain reaction (qPCR) for HPV type 16 viral load, as described previously (Saunier et al., 2008). The viral load was determined using the HPV16 copy number (qPCR targeting HPV16 E6 gene) normalised according to the number of cells (qPCR targeting the human albumin gene). Results are expressed as HPV16 copy numbers per 10^3 cells and the detection limit of this assay is 1 copy/10^3cells.