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Time and temperature dependent analytical stability of dry-collected Evalyn HPV self-sampling brush for cervical cancer screening

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ABSTRACT

As a new initiative, HPV self-sampling to non-attenders using the dry Evalyn self-sampling brush is offered in the Capital Region of Denmark. The use of a dry brush is largely uncharted territory in terms of analytical stability. In this study we aim to provide evidence on the analytical quality of dry HPV self-sampling brushes as a function of time and temperature.

We assessed the analytical stability of dry stored Evalyn brushes at three different temperatures, (4 °C, room temperature, 30 °C) and five different storage time points; T = 0 (baseline), 2, 4, 8, 16, and 32 weeks prior to HPV analysis using the BD Onclarity HPV assay.

Mean Ct value of the Onclarity internal control was used as comparator of cellularity across time and temperatures, with no or only borderline statistical differences observed. HPV detection was stable throughout the five time points. In addition, analytically amplifiable DNA copy numbers and DNA fragmentation was assessed using the Agena iPLEX Exome QC assay, with no or only borderline statistical differences observed.

In conclusion, the Evalyn brush is analytically stable with respect to human genomic material and HPV detection for up to 32 weeks at temperatures ranging from 4 °C to 30 °C.

1. Introduction

Human papillomavirus (HPV) based cervical cancer screening [1–7] in combination with self-collected samples is increasingly being investigated as an alternative to clinician collected samples aiming at increasing the coverage of cervical screening worldwide [8–12]. In organized screening programs, HPV self-sampling is considered as a potential alternative to screening non-attending women [13–15]. In Denmark, women aged 23–65 years are invited for screening for cervical cancer free of charge as a public cancer prevention program. In Denmark approximately 50% of all cervical cancers are diagnosed amongst the 25% women who do not participate in screening after being invited [16,17], as also observed in similar North European countries with nationwide cervical cancer screening programs [18,19]. In qualitative studies evaluating women´s preferences in screening, the main reasons for non-participation are reported as a combination between the discomfort/embarrassment of the associated gynecological examination and the general inconvenience of the doctor's visit [20–22]. To address the screening non-attendance, the Capital Region of Denmark launched a pilot implementation program in 2014, the Copenhagen Self-sampling initiative (CSi), offering HPV self-sampling brushes to approx. 24,000 screening non-attenders [9,14,23]. In CSi, we distributed the Evalyn self-sampling brush to invited women who actively opted in after invitation using a purpose designed and developed self-sampling kit [9]. After sampling in the privacy of the woman´s own home, the women returned the brush in a dry state to the laboratory for HPV analysis using a postage pre-paid envelope.

Multiple approaches to self-sampling have been described but two types of self-sampling devices have predominantly been used for larger self-sampling initiatives; a “wet” brush that requires the woman to re-suspend the brush in a supplied media immediately after sampling or a “dry” brush, shipped directly to the laboratory after sampling without further interaction by the woman. Comparing these two approaches, the use of a dry shipped self-sampling brush in our opinion holds a number of logistically and safety related advantages independently of the self-sampling device. Firstly, a dry brush can be transported by mail between the women and the laboratory without the potential for spillage and leakage during collection and transport. Secondly, any risk of potential skin irritation and harm by accidental consumption e.g. by a child in the household, is eliminated. Thirdly, shipment of liquid

Abbreviations: HPV, Human Papillomavirus; CSi, Copenhagen Self-sampling initiative; RT, Room temperature; HBB, Human Beta globin Control of the BD Onclarity HPV assay; UTM, Universal Transport Medium; CBD, BD CytoBrush in Diluent; MALDI-TOF MS, Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry; SAP, Shrimp Alkaline Phosphatase; Ct-value, Cycle Threshold value; ANOVA, Analysis of Variance statistical tests; Bp, Base pair; QA, Quality Assurance

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biological samples is more expensive and subject to more strict regulations than shipment of dry samples, at least in the EU. Fourthly, re-suspended, liquid samples represent a diagnostic quality assurance challenge as spillage by the woman or during transport reduce the analytical volume available for analysis after reception of sample at the laboratory [11]. Variable analytical volumes challenges the validity of the clinical cut off of the HPV assay used. Yet, despite these advantages, the use of dry collected and shipped brushes is largely uncharted territory in terms of analytical stability of the resulting human and viral material received for analysis in the laboratory. The objective of this study was therefore to provide data on the analytical quality of dry brushes for HPV self-sampling.

The central question asked was; what is the analytical stability of dry brushes under low, normal and extreme temperatures or during prolonged storage and transportation after sampling?

Little if any information is available in the literature concerning the stability of dry collected HPV self-sampling brushes even though HPV self-sampling is in the process of being implemented in several countries, including the Capital Region of Denmark.

2. Material and methods

2.1. Sample collection

Fully anonymized cervical swab samples from women undergoing HPV testing in the Danish cervical screening program were used. The swab samples were received in universal transport medium (UTM, Copan Diagnostics INC. Murriette, CA, USA) where the residual sample typically contains more than 1.0 ml after routine diagnostic testing. UTM (Hank's Balanced Salts Bovine Serum Albumin L-Cysteine Gelatin Sucrose L-Glutamic Acid HEPES Buffer Vancomycin Amphotericin B Colistin Phenol Red pH 7.3 +/− 0.2 @ 25 °C) contains no fixatives affecting genomic stability of the sample. Only samples of cervical origin were included. A total of 183 swab samples were used in the study.

2.2. Study design

Biospecimens for an index (baseline, T0) and five different storage points were evaluated (2, 4, 8, 16 and 32 weeks) at three different temperatures: 4 °C, uncontrolled room temperature (RT, approximately 20–22 °C), and 30 °C (Fig. 1). In total, 639 individual Evalyn brushes were analyzed.

To allow for direct comparison at different time points, up to four individual brushes were inoculated with each swab sample creating the biospecimens for analysis. Material limitations necessitated that not all time-points could be derived from the same swap samples, which resulted in the separation of the 8 (0, 2, 4, & 8 weeks) and 32 (0, 16, and 32 weeks) week time points into two separate study elements, now designated the 8 week and the 32 week study element. Both elements contained an index test at T = 0. For the 8 week time point (baseline plus 2, 4 & 8 weeks), 90 swab samples were used with four brushes per swab sample, resulting in a total 360 brushes, with approximately 30 brushes per evaluation point. For the 32 week time point (baseline plus 16 and 32 weeks), 93 swab samples were used with three brushes per swab sample, resulting in a total of 279 brushes or approximately 30 brushes per evaluation point.

2.3. Sample processing

The swab sample material was transferred to a 5 ml Eppendorf tube (Eppendorf, Hamburg, Germany). The Evalyn brushes were dipped briefly into the swab sample, swirled around three to four times and left to dry to mimic a home-taken cervical self-sample. The brushes were randomly allocated for the different temperatures and time points. Samples for baseline testing were stored overnight at the designated temperature and subsequently processed for HPV analysis to generate a T = 0 time point.

After incubation at the designated time and temperature points, the brush heads were removed and placed in an empty 5 ml Eppendorf tube. Three ml BD “CytoBrush in Diluent” medium (CBD, BD Diagnostics, Sparks, USA) were added and the samples were subsequently vortexed for 5 s and left for 15 min at room temperature. Afterwards, the brush heads were discarded and the samples were vortexed for an additional 5 s. Finally, 1.0 ml of re-suspended sample material was transferred to an empty BD sample tube for BD Onclarity HPV testing (Onclarity), and 0.2 ml was transferred to a 96 well plate for DNA extraction using Roche MagNA Pure 96 System.

2.4. BD Onclarity HPV assay

The CBD collected samples were tested using the Onclarity assay on the BD VIPER LT system [24,25] which has previously been described in details [14]. The Onclarity assay report nine different genotypes groups (16, 18, 31, 45, 51, 52, 33/58, 35/39/68, 56/59/66) and harbors an internal human beta globin control (HBB). In summary, 1.0 ml aliquots of the re-suspended CBD material were transferred to an empty sample tube before being preheat treated for 30 min at 120 °C on the VIPER pre-warm station. The pre-warmed samples were subsequently transferred to the fully automated VIPER LT platform and tested with the Onclarity assay according to manufacturer´s recommendations. Two samples were excluded due to technical failure during processing.

2.5. Agena iPLEX Pro Exome QC

The Agena iPLEX Pro Exome QC assay (Agena Bioscience, Hamburg, Germany) is a relatively new quality assurance assay, not previously used on cervical screening samples. The Exome QC panel is a quantitative assay that evaluates the amount of available DNA in a sample and the number of amplifiable copies at five different target amplicon sizes (100, 200, 300, 400 and 500 base pair (bp.).). The assay harbors 21 SNP, 3 markers for gender identification and 25 copy number controls, and five markers per amplicon length in a single multiplexed assay. The assay uses the Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) technology. All 639 samples were tested with the Exome QC assay to evaluate the relative amount and fragmentation of the DNA in the samples.

DNA was purified from the residual re-suspended brush CDB diluent, 200 µl was transferred to a microtiter plate for DNA purification on the Roche MagNA Pure 96 platform, using the MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics). Elution volume was 100 µl.

An initial multiplex amplification PCR was set up with 2 µl DNA, followed by a Shrimp Alkaline Phosphatase (SAP) reaction (which removes excess Nucleotides). The iPLEX Pro single base extension PCR reaction was then performed, where a mix of oligonucleotide extension primers designed to anneal to the amplified DNA fragments were added together with extension enzyme and mass-modified dideoxynucleoside terminators. The extension products were subsequently de-salted with Clean Resin prior to being loaded into the MassARRAY Dx Nanodispenser RS1000 (Agena, Hamburg, Germany), which transfers the analyte to a spectroCHIP. Here the samples crystalizes with the matrix on the chip, which was analyzed on the MassARRAY Dx Analyzer 4 (MA4). The analyte crystals are irradiated by a laser, inducing desorption and ionization. The MA4 accelerates the samples to a detector that differentiates genetic variants by molecular mass.

2.6. Statistics

The Onclarity assay has a three well design with nine HPV genotype read-outs, the internal HBB control is included in each well, and the Ct-value of HBB in this study was calculated as an average of the three