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Accurate measurement of female genital tract fluid dilution in cervicovaginal lavage samples

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ABSTRACT

An ion chromatographic method with conductivity detection for the precise and accurate analysis of lithium ions in phosphate-buffered saline, used as a cervicovaginal lavage (CVL) fluid, was developed and validated. The lithium ion dilution factor during the CVL is used to calculate the volume of cervicovaginal fluid (CVF) collected. Initial CVL Li^+ concentrations of 1 mM and 10 mM were evaluated. The method is robust, practical, and afforded an accurate measurement (5% of the measurement, or better) at 24 μL of vaginal fluid simulant collected per mL of CVL fluid, as low as 5 μL mL^{-1} using 10 mM Li^+ with a measurement accuracy of 6.7%. Ion chromatograms of real-world CVL samples collected *in vivo* from common animal models (sheep and pig-tailed macaque) and a human volunteer demonstrate that the analysis is interference-free. The method is readily transferrable and should enable the accurate measurement of CVF volume collected during CVLs benefitting a broad range of research disciplines, including pharmacokinetic, pharmacodynamic, metabolomic, and microbiome studies.

1. Introduction

Cervicovaginal lavage (CVL) is a frequently used sampling method in preclinical and clinical studies. A lavage fluid – usually 2.5–10 mL in clinical trials [1,2], but lower for small animal models (*e.g.*, 1 mL for macaques) – is used to rinse the lower reproductive tract and collect the components of the mucosal surface, including small molecules (xenobiotics and metabolites), extracellular polymeric substances, proteins and peptides, microbial cells, and host cells. Sterile saline or phosphate-buffered saline (PBS) are the most commonly used lavage fluids, as water is hypotonic and can lyse cells, confounding measurements of extracellular and intracellular vaginal fluid components [1]. The CVL method provides the significant advantage of collecting a sample integrated over the entire lower female genital tract, rather than the local sample obtained with swabs, sponges, and tear test strips. Consequently, CVLs collect larger cervicovaginal fluid (CVF) volumes that do not need to be recovered from the sampling device at the time of analysis. CVL is a safe and simple procedure that can be carried out easily in the clinic and self-sampling by trial participants also has been reported. A new self-sampling device was evaluated by Rwandan women and found to be acceptable for CVL collection [3], increasing the potential for sample collection in longitudinal studies.

The advantages sample collection *via* CVL have been exploited in numerous, diverse studies aimed at measuring the CVF concentration of one or more target analytes. Vaginal proinflammatory cytokines and other markers of immune activation are routinely measured in CVL samples [4–9]. More recently, a multiplexed assay to analyze antimicrobial peptides in CVL has been reported [10]. Proteomic studies also have been conducted using CVL samples [11]. Molecular methods have been used to measure the abundance of viral pathogens in CVL samples, including: human papillomavirus (HPV) [12,13], hepatitis C virus (HCV) [14], herpes simplex virus type 2 (HSV-2) [15–18], human immunodeficiency virus type 1 (HIV-1) [7,15,19], and cytomegalovirus (CMV) [20]. CVL samples from women volunteers have been analyzed to determine whether topical zinc deficiency is a risk factor in recurrent vulvovaginal candidiasis [21] and if dissolved nitric oxide gas is associated with bacterial vaginosis (BV) [22]. CVL samples even have been used to isolate bacterial DNA in culture-independent vaginal microbiome studies [23,24] and metabolomics studies [25,26]. The concentration of antiviral agents is measured in CVL samples as part of pharmacokinetic studies aimed at developing regimens for the prevention of vaginal HIV-1 [27–32] and HSV-2 [29,33] infection.

Abbreviations: BSA, bovine serum albumin; BV, bacterial vaginosis; CVF, cervicovaginal fluid; CVL, cervicovaginal lavage; HPW, high purity water; MSA, methanesulfonic acid; VFS, vaginal fluid simulant.

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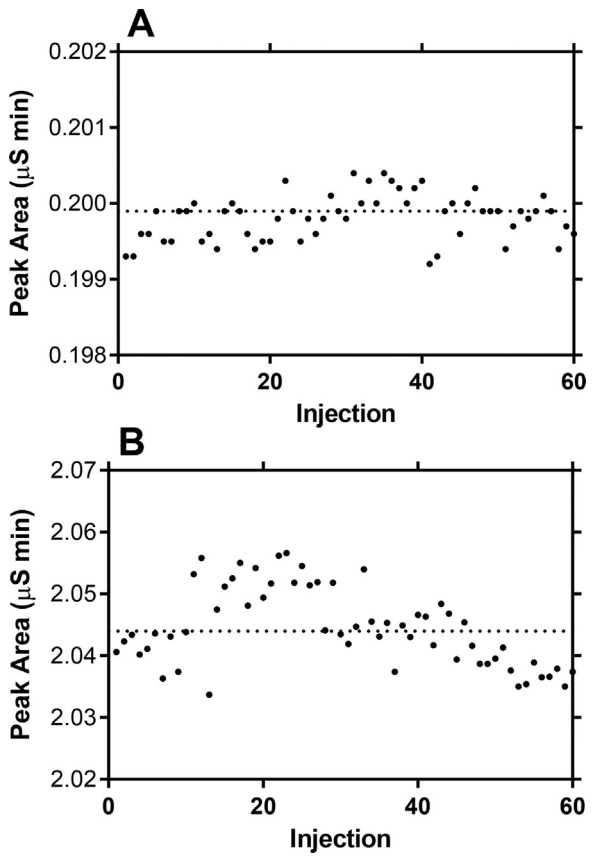

Fig. 1. Lithium ion peak areas measured in sixty sequential injections of PBS spiked with (A) 1 mM LiCl and (B) 10 mM LiCl after dilution with HPW (0.1 mL with 1.0 mL).

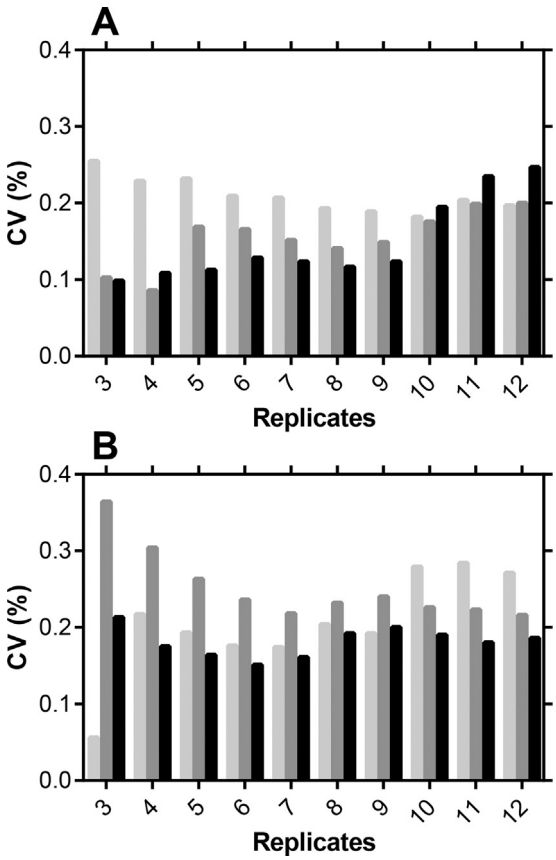

Fig. 2. Lithium ion measurement precision (CV) as a function of the number of replicates per sample at (A) 1 mM LiCl and (B) 10 mM LiCl in the CVL fluid. Pale grey bars, f_v = 5 μL mL^{-1} VFS; dark grey bars, f_v = 50 μL mL^{-1} VFS; black bars, f_v = 250 μL mL^{-1} VFS.

Recently, the analysis of CVL exosomes carrying microRNAs has been proposed as new biomarker for cervical cancer screening [34].

One fundamental drawback of current studies involving CVL sample analysis lies with the unknown amount of CVF collected, which can vary over more than one order of magnitude in women [2,35]. This broad uncertainty can lead to large errors in the measurements, confounding the interpretation of results. The inclusion of an additive in the CVL fluid that is not endogenous to the vaginal mucosa could be used to calculate the dilution of CVFs. The additive should be selected according to the following criteria: does not interfere with downstream assays (*e.g.*, ELISA) at the employed concentration; does not partition significantly into the vaginal mucosa during the lavage procedure; and can be analyzed with high measurement accuracy and precision. Both lithium chloride (LiCl) [2,35] and gluconate [1] as inert CVL additives have been used to measure this dilution factor, but the analytical methods suffer from insufficient precision. This significant limitation explain why the dilution factor in CVL samples is not measured routinely.

Here, a validated ion chromatography (IC) method for accurate and precise Li^+ analysis in the determination of CVF dilution factors in CVL samples is described. The method is robust across lavage fluid volumes up to 10 mL and Li^+ concentrations from 1 to 10 mM, and is applicable to a broad set of disciplines involved in the quantitative measurement of analytes in the vaginal mucosa.

2. Material and methods

2.1. Chemicals

Lithium chloride (molecular biology grade, L9650) and bovine serum albumin (BSA, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO) and methanesulfonic acid (MSA, 98 +%) was purchased from Alfa Aesar (Haverhill, MA). Sodium chloride (USP-grade) and potassium hydroxide (USP-grade) were obtained from BDH through VWR International (Radnor, PA) and calcium hydroxide (98%, extra pure), D,L-lactic acid (85%), and glycerol (Reagent ACS, 99.6%) were obtained from Acros Organics through Thermo Fisher Scientific (Waltham, MA). D-Glucose, monohydrate (biotechnology grade) was obtained from Amresco (Solon, OH) and acetic acid (Certified ACS), urea (reagent grade), and phosphate-buffered saline (PBS, 10 × solution, DNase-, RNase-, and protease-free) were purchased from Thermo Fisher Scientific. High purity water (HPW, >18 MΩ-cm) was obtained from a Milli-Q UF Plus ultrapure water system (EMB Millipore, Billerica, MA). Vaginal fluid simulant (VFS) was prepared according to the recipe by Owen and Katz [36].

2.2. Optimized IC method for Li^+ analysis

The analytical procedure was based on published methods [37,38]. The ion chromatography (IC) system consisted of a Model G1329A autosampler (Agilent Technologies, Santa Clara, CA) using an injection volume of 10 μL, a Model AS50 chromatography compartment (Dionex, Sunnyvale, CA), a Model GP50 gradient pump (Dionex) operating at a flow rate of 1.0 mL min^{-1}, a Model ED40 electrochemical detector (Dionex) in conductivity mode with the conductivity cell contained in a DS30 temperature stabilization compartment. The Dionex IC components and data acquisition was controlled by Chromeleon software version 6.80 (Dionex). The autosampler was controlled by Chemstation software version B.01.03 (Agilent Technologies) that was synchronized with the Dionex components through external relay contacts on the