UNIVERSITY OF KWAZULU-NATAL ™

INYUVESI YAKWAZULU-NATALI

MEASUREMENT OF VAGINAL MICROBICIDE ADHERENCE USING VISUAL INSPECTION AS COMPARED TO ULTRA VIOLET LIGHT ASSESSMENT OF RETURNED EMPTY GEL APPLICATORS

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Submitted as the dissertation component in fulfilment for the degree of Masters in Pharmacy (Pharmacy Practice) in the School of Health Sciences, University of KwaZulu-Natal

19 November 2015

Revised Version Submitted 29 February 2016

DECLARATION

In fulfilment of the requirements of the coursework degree of Masters in Pharmacy in the Discipline of Pharmaceutical Sciences, University of KwaZulu-Natal, Durban, South Africa, I, Michele Upfold, declare that: -

1. The research reported in this dissertation, except where referenced, is my original work.

2. This dissertation has not been submitted for any degree or examination to any other university

3. This dissertation does not contain other person's text, tables, data, graphs or other information, unless specifically acknowledged as being sourced from other persons

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    a. their words have been re-written but the general information attributed to them has been referenced;

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5. Where reference to a publication for which I am a principal author is made, I have referenced the "In Press" publication.

6. That my contribution to the project was as follows: I, with guidance from my Supervisor, devised the methodology for this research project. I collected the necessary data for this project and performed preliminary data analysis which was subsequently verified by a statistician. I am the primary author of the manuscript that emanated from this research project, and I have written this thesis on my own, under the guidance of my supervisor and co-supervisor.

7. That the contributions of others to this project were as follows:
   Dr Leila E Mansoor - Supervisor, Co-author of manuscript
   Prof Fatima Suleman - Co-supervisor, Co-author of manuscript
   Dr Anneke Grobler - Statistician, responsible for statistical guidance, and verification of calculations, Co-author of manuscript

Dr Nonhlanhla Yende - Statistician, responsible for extracting baseline and behavioural data from the CAPRISA 008 database

Londeka Lucia Zondi – Trainer on VIREA technique

Chanelle Smith – Primary gel assessor

Signed:  Date: 19 November 2015

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my Supervisor, Dr. Leila E Mansoor, for all her guidance, advice and troubleshooting. Right from conceptualization through to reviewing my draft manuscripts, you were able to bring my thought processes back into focus when I had lost my vision of the project. Also for being so supportive, and extremely patient throughout this long journey.

I also wish to express my gratitude to my Co-Supervisor, Prof. Fatima Suleman, for her guidance, support and encouragement along the way, and for taking the time during your sabbatical to review my draft manuscript and dissertation.

Dr Anneke Grobler, CAPRISA statistician, for statistical guidance and insisting I did my own analysis. I thought I couldn't, but I could because I tried.

Dr Nonhlanhla Yende-Zuma, CAPRISA statistician, for efficiently extracting the baseline and behavioural data from the CAPRISA 004 database.

Londeka Lucia Zondi, the CAPRISA 004 Vulindlela site gel assessor, for training pharmacy staff on VIREA inspection. Your experience in CAPRISA 004 truly benefited us in mastering the technique.

Dr Sarah Cohen, Wits Reproductive Health and HIV Institute, for lending the UV light viewing box for the purpose of this project, and for initial guidance and advice in proposal development stages.

To my colleague and friend, Chanelle Smith, who kept cracking the whip despite my many efforts at avoidance: thank you! Your determination to see our dissertations submitted timeously in 2015 was truly the motivation I, as a true procrastinator, needed to see this through to completion. Also for being the primary gel assessor for this project – many would have balked at the idea of this task, but you did so with utmost grace.

Andrew W. Smith, Research Fellow at CAPRISA Vulindlela CRS, for proof-reading my dissertation.

To my husband, Peter Upfold, for his time and diligence in photographing applicators, and for his patience and unending support during both my coursework and dissertation phases of my Master's degree: Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BAT 24	**B**efore sex, **A**fter sex, not more than **T**wo gels in **24** hours
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CI	Confidence Interval
CVF	Cervicovaginal Fluids
DNA	Deoxyribonucleic Acid
DSA	Dye Stain Assay
EDM	Electronic Dose Monitoring
FACTS	Follow-on African Consortium for Tenofovir Studies
FP	Family Planning
HEC	Hydroxyethyl Cellulose
HIV	Human Immunodeficiency Virus
MTN	Microbicide Trials Network
NPV	Negative Predictive Value
nm	Nanometres
PHC	Primary Health Care
PID	Participant unique Identifier
PrEP	Pre-Exposure Prophylaxis
PPV	Positive Predictive Value
SMS	Short Message Service
TFV	Tenofovir
UV	Ultra Violet
UVL	Ultra Violet Light
VIREA	Visual Inspection of Returned Empty Applicators
VOICE	Vaginal and Oral Interventions to Control the Epidemic

ABSTRACT

Introduction

Finding a safe, effective and acceptable HIV prevention method is key to preventing new infections in women. Vaginal microbicide trials aim to do so, but adherence to study product remains a challenge in interpretation of study product effectiveness. Accurate and objective measures of adherence are critical in microbicide trials.

Methods

We compared two applicator tests, visual inspection of returned empty applicators (VIREA) and ultraviolet (UV) light assessment of empty applicators returned as used within a tenofovir (TFV) gel implementation trial. Sensitivity and specificity in a small pilot sample was assessed at two time points, approximately three months apart. Reliability and concordance of the techniques was also assessed.

Results

Sensitivity and specificity analysis of 24 sample applicators at time point 1 was 75.0% and 66.7% for VIREA and 83.3% and 91.7% for UV light assessment, respectively; Sensitivity and specificity at time point 2 was 100% and 58.3% for VIREA and 100% and 66.7% for UV light assessment, respectively. Participants (n=115, median age 28 years) enrolled in the implementation trial at the Vulindlela Research Clinic, returned 1316 empty TFV applicators as used in January 2015. Assessment outcomes showed 78.8% agreement between VIREA and UV light techniques. Methods concurred that 22% of the returned empty applicators did not appear to be used. UV light assessment identified about 28% less product used, as compared to that returned as used by women.

Conclusion

UV light assessment appears to be a more accurate and less subjective measure of adherence as compared to VIREA. Further studies are needed to verify accuracy of UV light inspection against available DNA/protein biomarkers. UV light assessment can be used in combination with other biomarkers to identify potential challenges to adherence and inform targeted adherence interventions with the intention of optimizing adherence during microbicide clinical trials.

LAYOUT

This dissertation was written in accordance with the guidelines for presentation of Masters Dissertations provided by the College of Health Sciences, University of KwaZulu-Natal, 2015. There is a single reference list in the Vancouver format for references cited in chapters one, two, four and appendix A.

This dissertation comprises, the following chapters:

Chapter One describes the background and highlights the need for objective measures of adherence in microbicide trials that can facilitate optimization of adherence during trial conduct. The literature review describes key concepts surrounding adherence in microbicide trials, with a focus on the two measurement methods investigated in this research project, namely VIREA and UV light assessment. The problem statement, research question, aims and objectives of this research project are clearly outlined, followed by the conceptual framework that formed the basis of this research project.

Chapter Two describes in detail the methods utilized in this research project.

Chapter Three is the manuscript titled "*Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators*", as submitted for publication to the journal *AIDS and Behavior*. This is followed by a declaration of the Masters student's contribution to the manuscript.

Chapter Four is a comprehensive discussion of the major findings of the research project, including limitations and recommendations for future research.

CHAPTER ONE: Introduction

CHAPTER 1: INTRODUCTION

1.1 Background

In 2012, the South African National HIV Prevalence, Incidence and Behavioural Survey estimated that 12.2% of the population (6.4 million persons) were HIV positive, an increase of 1.2 million more people living with HIV than the previous survey in 2008 (1). South Africa's programme of increasing access to treatment has led to a decrease in AIDS related mortality and an increase in life expectancy. New infections, however, remain a particular concern, especially amongst female youth aged 15 - 24 years where HIV incidence rates in 2012 were over four times higher than the HIV incidence rates in males of the same age group (2.5% vs. 0.6%) (1). Amongst the key behavioural determinants of HIV infection (low age at sexual debut, age-disparate relationships amongst young people, multiple sexual partnerships and inconsistent condom usage), women often find negotiating condom usage in their relationships a challenge. HIV prevention methods that can be utilised and controlled by women are key to our efforts in getting to zero new HIV infections in this vulnerable population in the developing world. Vaginal microbicide trials aim to develop a safe and effective microbicide product that is acceptable to women and that can be used by women without their partner's knowledge (if need be) to prevent HIV acquisition. Experience has shown that the success of any prevention method under investigation in clinical trials depends not only on the actual efficacy of the product, but even more so on the study participant's willingness and ability to use the product correctly and consistently as instructed (2, 3). Adherence, or rather the lack thereof, can adversely affect the outcome and interpretation of study product efficacy in clinical trials and can result in the abandoning of potentially effective HIV prevention methods (4).

The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 Tenofovir (TFV) Gel Efficacy trial not only showed that pericoital TFV gel reduced HIV acquisition by 39% overall, but that there was a 54% protective effect in those who were able to use two doses of gel for more than 80% of their sex acts (termed high adherers) (3). The hopes raised by this proof of concept trial were recently dashed by the overall outcome of the Follow-on African Consortium for Tenofovir Studies (FACTS) 001 study, which showed no effect of pericoital TFV gel in preventing HIV acquisition in women (5). Although the FACTS 001 trial's overall analysis did not confirm the efficacy of TFV gel, a case-cohort substudy analysis showed that high levels of TFV detected in genital fluids was significantly associated with a 52% reduction in HIV acquisition (5, 6). However, while a protective effect was shown in women who used the TFV gel correctly and consistently, overall adherence in the FACTS 001 study was too low to show overall TFV efficacy. With FACTS 001 unable to confirm the results of the CAPRISA 004 TFV Gel Efficacy trial, efforts to move TFV gel to licensure may be hindered.

Measurement of adherence to study product in microbicide trials is critical to validating study product efficacy (4, 7). Most microbicide trials rely on participant self-report of study product use (8). However, self-report relies largely on participant recall, and has been shown to overestimate actual product use when multiple measures of adherence were used (8, 9). In clinical trial settings, due consideration must be given to a real-time objective measure of vaginal microbicide study product adherence that will inform adherence support interventions during trial conduct, without compromising study blinding.

In the CAPRISA 004 TFV gel efficacy trial, a new technique for assessing applicators, known as Visual Inspection of Returned Empty Applicators (VIREA) was introduced in an attempt to better quantify adherence to study product in the trial (10). The applicator test involved visually inspecting returned empty gel applicators for evidence of vaginal insertion according to set criteria. Although the applicator test was subjective, VIREA identified about 16% more applicators, amongst those returned as used and assessed, that had no visual evidence of vaginal insertion and may not have been vaginally inserted as compared to that reported by participants. The technique was also validated by linking VIREA assessments to detectable TFV levels in vaginal fluids at visits where vaginal specimens were taken from women in the TFV gel arm (10).

Another applicator test, that has shown promise as a method of measuring adherence is inspection of returned empty applicators under ultraviolet (UV) light. The technique has been used primarily in assessing empty hydroxyethyl cellulose (HEC) placebo gel applicators, either in daily vaginal use or before-sex / after-sex application (11-14). The sensitivity and specificity of assessment by UV light has been shown to be comparable, and sometimes even better than other applicator tests like the dye-stain assay (DSA) and visual inspection, with some authors suggesting UV light assessment be considered as an objective measure of product use in clinical trials (14).

Little is known about UV light assessment of returned empty TFV gel applicators. In an attempt to find a more objective method of measuring adherence, this research project, as an ancillary project to the CAPRISA 008 TFV Gel Implementation trial, aimed to assess the accuracy of both VIREA and UV light assessments, as well as assess the reliability and concordance of the two methods of measurement. Women enrolled in the CAPRISA 008 TFV Gel Implementation trial used 1% TFV gel in a pericoital based regimen viz. BAT 24 (insert one gel up to 12 hours **B**efore sex, one gel as soon as possible but within 12 hours **A**fter sex, and not more than **T**wo gels in **24** hours), and were required to return empty (used) and unopened (unused) applicators at each visit to the pharmacy for reconciliation (15). A subset of empty applicators returned as used in the CAPRISA 008 TFV Gel Implementation trial, will be assessed in this project.

1.2 Literature review

1.2.1 Key Concepts

1.2.1.1 Adherence in microbicide trials – need for an accurate and objective measure of product adherence

There are two factors which determine the observed level of effectiveness of any candidate microbicide under investigation in clinical trials: the actual efficacy of the product, as well as the participant's willingness and ability to use the product correctly and consistently as instructed (2, 3). Poor adherence and inability to accurately measure product adherence can compromise interpretation of microbicide trial results and can negatively impact the outcome of a clinical trial (4, 7).

Studies have also shown that product effectiveness is often higher in women who achieve correct and consistent product use. In the CAPRISA 004 TFV Gel Efficacy trial adherence to study product was defined as "the estimated proportion of reported sex acts covered by two gel doses" (3). The study not only showed that TFV gel reduced HIV acquisition by an estimated 39% overall, but that there was a 54% protective effect in those who were able to use two doses of gel for more than 80% of their sex acts (termed high adherers). The early termination, due to futility, of the daily 1% TFV gel and daily oral TFV arms in the Microbicide Trial Network (MTN) 003 / VOICE trial was in part attributed to low adherence. Adherence was estimated to be about 90% based on participant self-report and 86% based on counts of returned product (unused applicators and leftover pills). However, drug level analysis in a random sub-cohort revealed that TFV was only detected in about a quarter of samples, and more than half of the women had no TFV detected at any quarterly visit (16). The FACTS 001 trial, in an effort to gather more information on the efficacy of 1% TFV gel used pericoitally (BAT 24), was unable to corroborate the CAPRISA 004 TFV Gel Efficacy trial results, largely due to low overall adherence to study product (5, 6). FACTS 001 showed overall no effect of TFV gel in preventing HIV infection; however, analysis of a subset of participants in the TFV-treated group showed that TFV detected in the genital fluids of women who used the product consistently and covering more than 72% of their sex acts, conferred a protective effect with a 52% reduction in HIV acquisition. However, this subgroup represented only 20% of participants and was insufficient to confirm the efficacy of pericoital TFV gel in preventing HIV acquisition. The FACTS 001 study did, however, highlight the need for more research to understand the barriers to and motivators of product use (6). As can be seen, inadequate adherence poses a serious challenge to accurately estimating product efficacy.

Many researchers agree that optimising adherence (17, 18), as well as objective and accurate measurements of adherence in future microbicide trials is a key challenge that needs to be addressed (3, 9, 18). Challenges to accurate measurement of product adherence can compromise interpretation of microbicide trial results (4, 9), highlighting the need for more respondent–independent behavioural

and biological measures for future microbicide trials (9, 18). The choice of the most appropriate adherence measurement tool depends on the usefulness and reliability of the method in light of the goal of the study: whether the researcher wants to understand, quantify, or influence adherence, as well as available resources (7, 9). The shift in microbicide research towards antiretroviral (ARV) based products has allowed for direct measurement of drug levels as a marker of product exposure, and an indicator of compliance. Measurement of drug levels in body tissues such as the vaginal tract or plasma, enables insight into the level of drug required to confer a protective effect, and is crucial in understanding trial results (17, 19). However, in an efficacy trial, to avoid unblinding, this is only possible at study completion, and cannot assess adherence in the placebo arm. In order to achieve the necessary drug levels at the site of infection, correct (as per product use instructions) and consistent (at every sexual encounter) use of study product during efficacy studies is essential. Rapid onsite assessment of product adherence during trial conduct will enable real-time identification of participants with adherence challenges, and allow for targeted adherence support interventions to meet the required adherence threshold.

The focus of this research project is on two applicator tests, visual inspection and ultraviolet light assessment of returned empty applicators, as methods of measuring adherence in a microbicide trial that can also optimize adherence during trial conduct.

1.2.2 Applicator tests as Measurement Methods

1.2.2.1 VIREA in CAPRISA 004

The primary adherence measure in the CAPRISA 004 TFV Gel Efficacy trial was applicator based – defined as the number of reported sex acts covered by two doses of gel (3). Other measures of adherence in this trial included a count of returned used applicators, as well as self-reported adherence to study product (20). In an attempt to improve measurement of adherence in the CAPRISA 004 TFV Gel Efficacy trial, an additional measure of adherence was introduced after month 15, whereby returned empty applicators were, in a standardised manner, subjectively assessed as “appears used” or “appears unused” by visual inspection for residue on the outside of the applicator as an indicator of vaginal insertion (10). The technique, coined VIREA, showed that 77.5% of the 59800 empty applicators returned as used and assessed using the stipulated assessment criteria appeared to have been vaginally inserted. The other 22.5% of empty applicators returned as used did not have any visual evidence of vaginal insertion (no visible residue, no mucous, gel, secretions, or hair on the applicator barrel nor any residue besides gel on the applicator tip) and may not have been inserted into the vagina. VIREA identified about 16% more applicators that may have not been vaginally inserted as compared to that reported by the study participants, leading the researchers to conclude that estimates of adherence based solely on counts of returned empty applicators without physical

inspection using the VIREA technique may very well be over-estimates. Linking VIREA assessments to detectable TFV concentrations in vaginal fluid at 375 study visits where vaginal specimens were collected in women assigned to the TFV gel arm, showed that TFV was four times more likely to be detected in vaginal fluid in women who had more than half their applicators assessed as "used" by VIREA as compared to those who had half or less than half assessed as "used", thereby validating the process of VIREA. Even though the process of VIREA was standardised, the subjective nature of the technique remains a limitation (10).

1.2.2.2 UV Light

Visual inspection of applicators under 365 – 385nm UV light for evidence of vaginal insertion has been used as an adherence measurement method. Bodily fluids, including semen and cervicovaginal fluid (CVF), fluoresce under UV light. A streaked fluorescent pattern is considered to be a positive indicator of vaginal insertion (12). Moench *et al.* evaluated four microbicide gel adherence monitoring methods, namely 1) staining with Alcian Blue, 2) microscopic detection of vaginal cells after staining applicators with Iodine, and 3) direct inspection without staining under both ambient and 4) UV light. Results showed that UV light inspection of applicators had the highest mean sensitivity (84%) and specificity (83%) (12). The researchers found that the sensitivity of the method may be reduced to 65% without prior gel application and may increase to 95% with prior gel insertion. This was attributed to the observation that both the accuracy of the methods and intensity of the signal increased after prior insertions, possibly because accumulated gel from prior doses in the vagina supported retention of mucous and cells being on the surface of the inserted applicator. Hence the authors expressed concern over the accuracy of the method in coitally based dosing strategies, and concluded that UV light inspection of polypropylene applicators should provide a quick, reliable and quantitative assessment of whether applicators have actually been vaginally inserted especially in daily dosing regimens. Moench's research also showed that the mean reading time to read the full set of 250 applicators was only 15 minutes for UV light assessment (compared to a mean of 32 minutes under ambient light assessment). This rapid assessment rate was possible because the UV viewing box utilised by the researchers allowed for loading of 36 applicators onto a tray which was slid over rails into the viewing box allowing for multiple applicators to be viewed at a time (12).

Further investigations into the fluorescent properties of 1% TFV gel and placebo gel, and an enhanced UV system with optimised UV light source were conducted for the FACTS 001 trial (personal correspondence, Sarah Cohen, Research Operations Manager: FACTS, 23 November, 2014). In comparing the fluorescent intensity of TFV gel, placebo gel and CVF as viewed under 360nm UV light, Moench found that although both TFV and placebo gel had modest fluorescent properties, CVF had approximately 20-fold greater fluorescent intensity, and neither gels caused detectable quenching of CVF fluorescence (21). Hence, there is little potential for unblinding if UV light assessment of

empty TFV gel and empty placebo gel applicators was used to assess adherence in a blinded, placebo-controlled TFV vaginal microbicide gel study. In his initial publication using the original UV light system, Moench *et al.* expressed concern over the utility of UV light assessment of pre-sex applicators in coital-dosing strategies due to the reduced sensitivity of the method without prior-gel application (12). Subsequent evaluation of an enhanced UV system with optimised UV light source and filter which substantially improves illumination, allows for better detection of vaginally inserted applicators from "gel-naïve" participants (without prior gel dosing). Previous concerns about the accuracy of the method in pericoital dosing regimens, such as BAT24, appear to be overcome with this enhanced UV system (21).

In comparing three approaches for assessing adherence to vaginal gel in clinical trials, namely two applicator tests (DSA and UV light Assessment), Wisebag, and self-reported adherence to a daily vaginal dosing schedule of HEC placebo gel, van der Straten *et al.* found that UV light assessment and DSA performed similarly with 95% sensitivity and 79% specificity of UV light assessment as compared to 97% and 79% with DSA. The study confirmed that compared to these applicator tests, self-report overestimated adherence; whereas Wisebag was found to underestimate adherence, likely due to the practice of "pocket dosing", where more than one applicator is retrieved from the bag per opening event. The authors found UV light assessments to be faster than DSA (which required a 5-hour dye-drying time), and enabled possible immediate adherence feedback to participants during study visits, and also concluded that DSA and UV light assessments should be considered objective measures of product use in future microbicide trials (14).

Keller *et al.*, in comparing DSA and UV light techniques to assess empty placebo applicators before and after sex, supported Moench's findings in that the sensitivity of UV light assessment was higher for post-sex applicators than for pre-sex applicators (11).

Thurman *et al.*, in comparing visual and UV light inspection versus DNA/protein biomarkers of HEC placebo gel applicators found the sensitivity and specificity of both inspection methods to be higher in applicators inserted in the presence of vaginal gel (as in the post-coital dose of the BAT24 regimen) compared to those inserted in the absence of vaginal gel (as in pre-sex applicator) (13), even when using an optimised UV light source for UV light assessment. In assessing the inspection methods after 30 days of storing the applicators, the study found a statistically significant increase in specificity of visual inspection assessments and a statistically significant increase in overall sensitivity of UV light assessment (13).

1.2.2.3 Influence of training and experience on assessment outcomes

The influence of factors such as assessor experience is discussed by Moench *et al.*, who found that the accuracy of assessments by UV light or other methods was unaffected by the assessors' prior laboratory experience (12). However, the study design did not allow for investigation into the effect of

assessor experience in the technique itself on assessment outcomes. Moench *et al.* believe that training by practical experience of reading a set of known inserted and non-inserted applicators allows assessors to establish assessment thresholds which are key to achieving accurate assessment outcomes (12). Thurman *et al.*, by assessing applicators at two time intervals, found that assessor experience in the UV light assessment technique did influence the assessment outcomes, with improvements in both sensitivity and specificity at 30-day readings as compared to 7-day readings. The study noted significant variability between assessors in several of the UV light assessments, concluding that UV light assessment remains subjective (13). However, there is insufficient information on training in the methodology section of this publication to elaborate further.

A summary of the literature reviewed for the purpose of this dissertation is presented in appendix A.

1.3 Problem Statement

Objective methods of measuring adherence in clinical trials, although critical to assessing the effectiveness of the candidate microbicide under investigation, are lacking. Ideally, these should be reliable, inexpensive to implement, be able to be performed in real-time to inform adherence support interventions and without the risk of compromising study blinding.

Research of UV light assessments of returned applicators has primarily been done with HEC placebo gel applicators under strict study conditions, either where the majority of applicators were inserted under direct observation, and only a subset were inserted at home (12); or where the dosing regimen was daily dosing (14). Sensitivity and specificity of UV light assessment of empty TFV gel applicators has not yet been established.

UV light assessment of returned empty TFV gel applicators from an implementation study using a pericoital dosing strategy (BAT24), has, to the best of my knowledge, not yet been studied.

1.4 Research Question

Is assessment of returned empty TFV gel applicators by UV light comparable (in terms of accuracy, reliability and practicality) to assessment of the same applicators by VIREA? Is UV light assessment of returned empty TFV gel applicators less subjective than VIREA?

1.5 Aim and objectives

1.5.1 Aim

The aim of this research proposal is to assess the accuracy, reliability and practicality of UV light assessment of used TFV gel applicators as compared to assessment by VIREA as an adherence measurement technique for implementation in future microbicide gel trials.

1.5.2 Objectives

Objective One: To undertake a pilot study to estimate the accuracy of both VIREA and UV light assessments of empty TFV gel applicators by calculating the sensitivity and specificity of the techniques, within four months of applicator receipt.

Objective two: To assess the effect of storage on the sensitivity and specificity of the two techniques after an additional three months of storage.

Objective Three: To assess the reliability and concordance of UV light assessment of a subset of returned used / empty TFV gel applicators in a select one-month period for agreement, with the VIREA assessment of the same applicators, in terms of evidence of vaginal insertion.

Objective Four: To measure the time taken to assess a set of returned used / empty applicators for each technique in order to evaluate the practicality and efficiency of the measurement methods.

1.6 Conceptual Framework

Methods to measure adherence in microbicide trials can be classified into 3 broad categories (figure 1), viz. 1) behavioural, 2) biologic or surrogate markers and 3) biomarkers (9). Each method measures a different dimension of adherence; and the triangulation approach to adherence measurement employs methods with complementary strengths, to enable a researcher to capture the most relevant adherence information for a given candidate microbicide product.

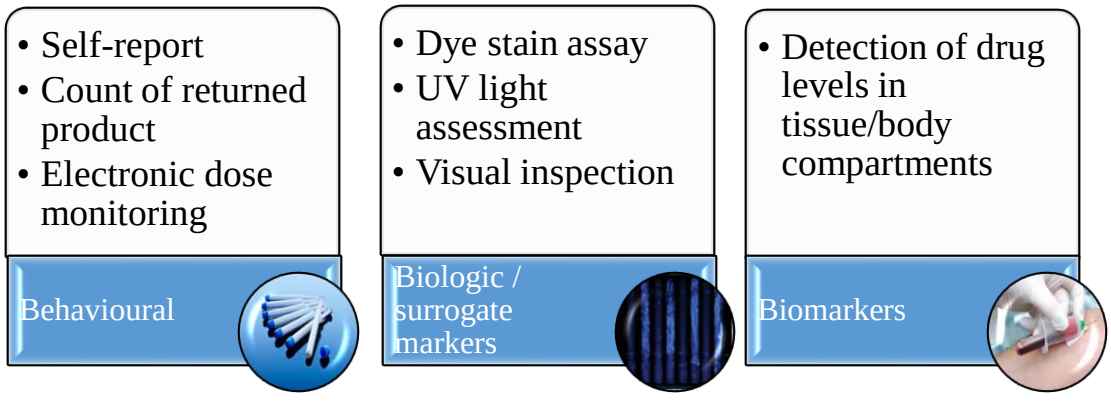

Figure 1. Methods of measuring adherence in microbicide trials

1.6.1 Behavioural measurement methods

1. *Participant self-report* of adherence to product, whether prospectively (e.g. paper diaries) or retrospectively (e.g. face to face interviews) is prone to error, both unintentionally (recall bias)

and intentionally (social desirability bias) (9, 18). Errors may also occur on the part of staff documenting the participant's response (recording bias), and also if confusion exists in what is actually being measured (instruction bias). Self-report, by any means, is relatively low-cost, versatile and simple to implement. However, self-report of adherence in microbicide and oral pre-exposure prophylaxis (PrEP) studies, has been shown to be upwardly biased (8, 9) i.e. self-report, as a measure of adherence, often overestimates adherence or product usage.

2. *Clinic counts of returned product* (returned empty and unused applicators) enables full applicator accountability, and since it does not rely on participant self-report has been considered an easy "objective" measure of adherence; but this method is still open to bias in accuracy of counts, and is dependent on the participant returning empty and unused applicators at every visit. Counting of returned empty applicators as proxies for use may overestimate adherence (18), since simple tallies of empty applicators as used cannot account for gel expelled ex-vivo without vaginal insertion.

3. *Electronic dose monitoring* (EDM) is by means of an electronic device that monitors container openings as a proxy for product use and adherence (e.g. EDM-adapted Wisebag, a lunch-bag style container – for storage of applicators – fitted with a battery-operated electronic device and chip that transmits an electronic signal for each opening event). In studies assessing this technology, participants are usually given instructions to open the unit only to retrieve an applicator for use (or in some cases return a used applicator for storage), so that each opening event theoretically provides data on applicator use in real time (14). EDM of gel usage may well overcome some of the limitations of participant self-report, providing detailed information on date / time of product retrieval, but cannot truly measure actual product use (whether the applicator was merely taken out of the bag or actually inserted, or even if gel was expelled vaginally) (9). EDM is limited primarily by expense in large scale trials; may present other challenges to participants in terms of acceptability (bulkiness, impracticality, and low portability); and may also underestimate adherence due to "pocket dosing" or overestimate adherence in "curiosity events" (with additional unintentional opening of the Wisebag for reasons other than retrieval of gel) (22). A potential benefit of the Wisebag is that it may be set up to send a Short Message Service (SMS) reminder to a user either when no opening event is detected, or perhaps even when more opening events are detected than expected, to alert the user to possible overuse of study product (9, 22, 23). The latter is especially useful where there may be a restriction on the amount of product that should be used in a given period, as in BAT24 dosing.

1.6.2 Biologic / surrogate markers

1. *Dye-stain assay (DSA)* involves staining of returned used applicators with a food dye, such as FD&C Blue dye No.1, in order to assess whether the applicators were actually vaginally inserted by visually detecting the dye-stained vaginal mucous. The test is relatively easy to implement, and has been validated with different gels, applicator types and dosing strategies. Although DSA appears to perform well with polyethylene applicators, sensitivity of the technique with multiple gel applications was significantly lower for polypropylene applicators, such that authors do not recommend DSA for polypropylene applicators that are currently used in microbicide trials (24). The time taken for the dye to dry after staining and prior to visual inspection, together with safety and toxicity concerns of certain dyes (e.g. trypan blue) limit utility of this technique (9).

2. *UV light assessment* technique is based on the practice of using UV light in forensic detection of bodily fluids, and in microbicide trials involves inspecting returned empty applicators under UV light at 365-385nm for signs of vaginal insertion. Semen and CVF fluoresce under UV light and so too may microbicide gels, and the test is unable to differentiate between them. It is therefore pertinent to understand the fluorescent properties of microbicide gels under investigation, and their effect on the fluorescent signal intensity of CVF if this technique is to be considered for use in future microbicide gel trials. Gel dosing regimen may affect the test sensitivity of UV light inspection of returned empty applicators. Reduced sensitivity without prior gel dosing or pre-sex application has been shown in several studies: Moench *et al.* reported sensitivity of 65% without prior gel application (as may occur in coital based regimens), and 95% with prior gel application (as in daily dosing regimens) (12); Keller et al. reported 87% sensitivity for pre-sex applicators and 97% for post-sex applicators (11). The UV light viewing box is relatively small and portable. With training, the technique can provide an accurate assessment of vaginal insertion, and has been shown to have the highest accuracy in correctly identifying the insertion status of an applicator as compared to other applicator tests (12).

3. *Ambient light assessment* involves visual inspection, according to predefined criteria, of returned empty applicators under ambient light for residue (secretions and/or gel) that may be indicative of vaginal insertion. The technique is cheap, requiring little to no ancillary equipment, and is easy to implement as an adherence measurement technique (10). This technique also allows for real-time feedback to guide targeted adherence interventions especially with regards to the mechanics of gel insertion (e.g. rapid identification of used applicators with incomplete / partially expelled gel). The technique has been shown to have a statistically significant lower specificity as compared to UV light assessment and staining

with Alcian Blue or Iodine (12). The specificity of the technique has also been shown to significantly increase after storage of applicators for 30 days as compared to assessment at 7 days (13).

Applicator tests, such as DSA, ambient and UV light assessments, all have inherent limitations. The following are to be noted: a) applicator tests cannot definitively assess actual product use (timing of use in relation to sexual activity or HIV exposure, or the amount of product used); b) applicator tests are unable to differentiate between an applicator vaginally inserted with gel expelled outside the vagina, or those truly inserted vaginally with gel expelled vaginally as per instructions for use (9); and, c) applicator tests may not be able to correctly identify applicators that have been used and wiped or washed after use, as may be a common practice in the field for reasons of discretion, as used.

1.6.3 Biomarkers

The use of ARV-based study products allow for study drug levels to be quantitatively measured in plasma, genital and rectal compartments; and these biomarkers may be crucial to help in interpretation of trial results. However, they have several limitations in that they can only be used in the active arm and, due to the potential to unblind, can only be used retrospectively in blinded studies, and hence are less useful to guide targeted adherence interventions (9). They also may be of limited value in intermittent pericoital dosing regimens (since a drug may only be detected for a limited time after last use, and the interval between last use and sampling may be variable) (9). Greater access to PrEP, such as Truvada for HIV prevention, and possible inclusion as standard of care in microbicide trials, may limit the utility of measured drug levels as a marker of adherence (25). In addition, cost may prove prohibitive.

The choice of the most appropriate adherence measurement tool in microbicide trials is guided by the candidate microbicide and product formulation; the utility and reliability of the method considering the goal of the study; what the researcher aims to achieve in term of adherence measurement; and ultimately, choice is often dictated by available resources.

CHAPTER TWO: Methodology

CHAPTER 2: METHODOLOGY

2.1 Study Setting and considerations

This research project was undertaken as a substudy within the CAPRISA 008 TFV Gel Implementation trial, an open-label randomized controlled trial to assess the implementation effectiveness and safety of 1% TFV gel provision through family planning (FP) services in KwaZulu-Natal, South Africa, which provided post-trial access of TFV vaginal gel to HIV-uninfected women who previously participated in the CAPRISA 004 TFV Gel Efficacy trial (15). Eligible women were randomly assigned to receive their TFV gel either through FP services coinciding with their contraceptive schedule (intervention arm), or monthly though the CAPRISA research clinics (control arm). They were required to return all unused and used/empty applicators to pharmacy for reconciliation at each study visit – either every two or three months in the intervention arm or monthly in the control arm. Determination of adherence to study product or regimen in CAPRISA 008 TFV Gel Implementation trial was three-fold: brief interviewer-administered questions at each study visit, counts of returned used and unused applicators at each study visit, and post-trial analysis of TFV concentrations in genital specimens collected at four bi-annual visits and study exit will aid in assessing product adherence and interpretation and understanding of trial results (15).

For the purpose of this research project, a subset of empty applicators returned as used by women enrolled in the CAPRISA 008 TFV Gel Implementation trial at the CAPRISA Vulindlela Clinic were assessed.

The aim of this research proposal is to assess the accuracy, reliability and practicality of UV light and VIREA assessment techniques. The accuracy of a test procedure is best measured in terms of sensitivity (the ability of a test to accurately identify a condition when present) and specificity (the ability of a test to accurately identify those in whom the condition is absent). Reliability is defined in the Merriam-Webster dictionary as “the extent to which an experiment, test, or measuring procedure yields the same results on repeated trials”. In terms of this research proposal, reliability of the assessment criteria for each assessment technique will be assessed by comparing the inter-assessor agreement, and furthermore, by assessing concordance of assessment outcomes by each technique. Practicality of the methods will be assessed in terms of efficiency (time-taken to complete assessments) as well as the feasibility of incorporating these assessment techniques into workflow procedures.

2.2 Data Collection

2.2.1 Training

Prior to conducting any assessments, two pharmacists underwent training on the techniques of VIREA and UV light inspection. The CAPRISA 004 Vulindlela site VIREA assessor, with prior experience in the VIREA assessment technique developed and utilised in CAPRISA 004 TFV Gel Efficacy trial, conducted a training and practical demonstration session of the VIREA technique for the new VIREA assessors. The CAPRISA 004 Vulindlela site VIREA assessor then set up a competency test for the new VIREA assessors with a selection of applicators returned as used.

Training in the technique of UV light assessment was conducted by the Masters student through images (figure 3) shared by Sarah Cohen, the Research Operations Manager for FACTS, WITS Reproductive Health & HIV Institute, as well as practical viewing of applicators returned as used. A competency test consisting of known to be vaginally inserted applicators and sham-inserted applicators (gel expelled ex-vivo, applicator handled but not vaginally inserted) was conducted.

Pharmacy staff had to pass both these independently conducted competency tests with 100% to qualify as an assessor (appendix B and C).

2.2.2 Sample for assessing sensitivity and specificity

Since there is limited sensitivity or specificity data on the use of VIREA and UV light assessments of empty TFV gel polypropylene applicators, this project aimed to estimate sensitivity and specificity as a pilot in a limited sample. Sample size as calculated using NQUERY advisor, Version 7.0: assuming the sensitivity of UV light will be between 73% and 95% then a sample of 12 used and 12 unused applicators will lead to a 95% 2-sided confidence interval that will extend 0.251 from the observed proportion if the observed proportion is 0.73 and 0.123 if the observed proportion is 0.95.

Assuming the specificity of UV light to be between 66% and 95% then a sample of 12 used and 12 unused applicators will lead to a 95% 2-sided confidence interval that will extend 0.268 from the observed proportion if the observed proportion is 0.66 and 0.251 if the observed proportion is 0.95.

The known to be vaginally inserted applicators (positive controls) for this pilot assessment were provided by volunteers within the CAPRISA 008 TFV Gel Implementation trial. The volunteers came to the clinic to insert either their before sex or after sex gel dose under direct observation. Sampling methodology was purely convenience sampling. Negative controls comprised of "sham-inserted" applicators - TFV gel applicators which had been handled, with gel expelled ex-vivo, but not inserted vaginally.

To further assess the effect of storage on the sensitivity and specificity of the techniques, the same set of applicators, after assessment by VIREA and UV light at time point one, were then retained and

stored at controlled room temperature for subsequent assessment at time point two after an additional three months of storage.

2.2.3 Sample for assessing concordance of techniques

A sample of approximately 1000 applicators (based on the median monthly number of applicators returned as used in the CAPRISA 008 TFV Gel Implementation trial of 1008 (IQR 841 – 1172)) is adequate to assess the reliability and concordance of the methods of measurement under examination. Hence, a convenience sample of all empty CAPRISA 008 study gel returned by study participants as "used" during January 2015, were first subjected to assessment by VIREA, and subsequently and independently by UV light inspection, by two assessors in a standardised manner. Assessments were performed by both assessors independently to verify the reliability of the assessment criteria. However, for the purpose of assessing concordance of the techniques, only the assessment outcomes of the primary assessor were taken into consideration.

2.2.4 Assessment procedures

Assessors were blinded to the positive and negative controls, where used. Applicators were assessed according to standardised criteria for each method independently, so that assessment outcomes for one technique did not influence the other. Prior to assessment, every returned applicator was identified by a consecutive number on the outside of its resealable plastic packaging. VIREA inspection preceded UV light assessments, and every effort was made to minimise contamination of the returned empty applicators between these assessments. This included the use of gloves to handle the returned empty applicators, only handling the applicators by the ridged end of the applicator barrel, and carefully returning each applicator to its numbered resealable plastic bag after assessment.

The outcomes of assessments of returned empty applicators by the processes of VIREA and UV light assessment were documented on the logs developed for this purpose. The log for sensitivity and specificity analysis (appendix D and E) document the date of assessment, time taken for assessment, initials of assessor and assessment outcome. The log for assessment of concordance of the techniques (appendix F and G) records the participant unique identifier (PID), the visit code and date the study product was issued / dispensed, the date and quantity of empty applicators returned by the participant, the date assessed by VIREA or UV light and the initials of the assessor, and the assessment outcome.

To compare the efficiency of each technique, the time taken to process a set of returned empty applicators by each technique was also documented. This together with the assessors' perspective on feasibility of incorporating the technique into workflow procedures, will enable evaluation of the practicality of each technique.

2.2.4.1 VIREA Assessments

The following VIREA assessment guidelines, as utilised in CAPRISA 004 TFV Gel Efficacy trial, used to assess the appearance of the returned 'used' applicators, were applied:

1. Each used applicator is individually removed from its resealable plastic bag packaging.
2. The applicator barrel is inspected for any visible residue, mucus, gel, secretions or hair (figure 2). If any substance is visible on the barrel, besides the tip where the gel is expelled, then the applicator should be categorised as **used.**
3. If in step 2, the applicator barrel appears to have no residue of any kind, then the tip should be inspected. If the applicator is capped, then the cap should be removed and the tip of the applicator should be inspected. If the tip is discoloured or has residue **that is not gel**, then the applicator is regarded as **used**.
4. If in step 2 the barrel is clean and in step 3, the tip is clean or has a small point of gel as one would find if the gel was squirted out or if a small amount of 'clean gel is visible' then the applicator is regarded as **unused**.

The outcomes of assessments of returned empty applicators by the processes of VIREA were documented on the appropriate log.

Figure 2. Applicators with visible residue, mucus, gel, secretions or hair are assessed by VIREA as "used" *(Pictures courtesy of Dr Leila E Mansoor, CAPRISA)*

2.2.4.2 UV Light Assessments

A viewing box, based on that proposed by Moench *et al.* (12) was utilised to view the returned empty applicators under UV illumination (appendix H). UV light was provided by a UVL-21 Compact UV Lamp (365-nm). Applicators were viewed through a 76- x 102-mm Tiffen UV Haze-2A filter to block transmission of reflected source illumination.

The following guidelines in assessing the appearance of the returned 'used' applicators by means of UV light inspection were applied:

1. Push test applicator through the applicator porthole.
2. Turn on the UV light and view the applicators through the viewing window.
3. Score according to the following criteria:

   "Appears used" – a streaked pattern of blue-green fluorescence on the barrel of the applicator (figure 3a, 3b)

   "Appears possibly used" – a generalised fluorescence with or without fluorescent speckles (an indication of possible usage / insertion of gel e.g. applicator may have, for reasons of discretion, been wiped post insertion and prior to returning to the clinic) (figure 3c, 3d)

"Appears unused" – absence of streaked or generalised fluorescence, with or without fluorescent speckles especially if these are limited to the area around the cap (figure 3e, 3f)

The outcomes of assessments of returned empty applicators by UV light assessment were documented on the appropriate log.

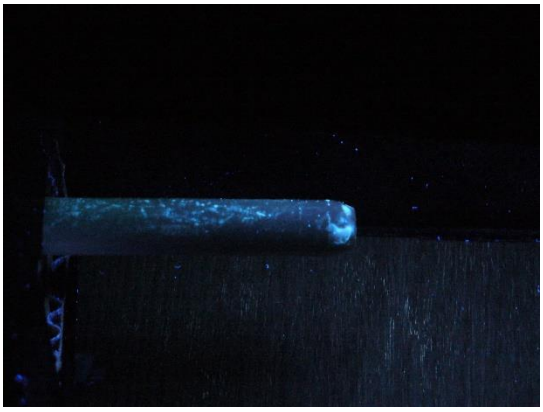

Figure 3a

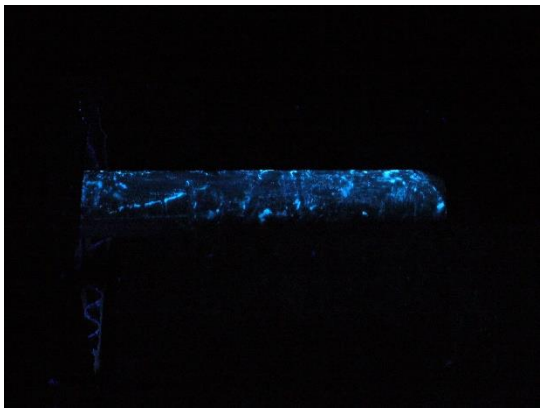

Figure 3b

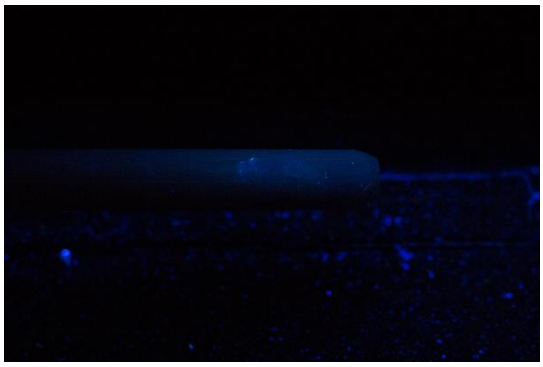

Figure 3c

Figure 3d

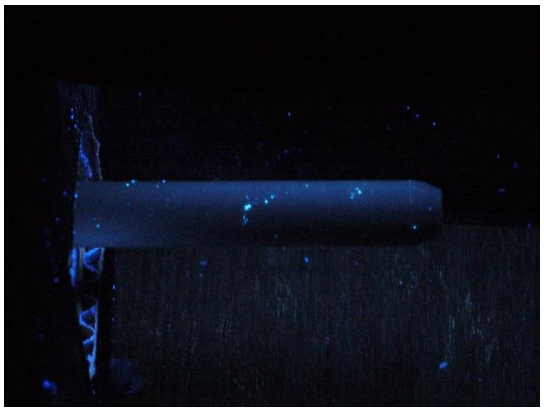

Figure 3e

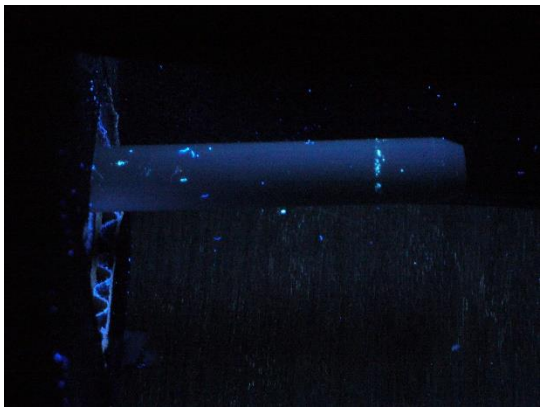

Figure 3f

Figure 3. Applicators as viewed under 365nm UV light. Figure 3a and b are assessed by UV light as "appears used"; Figure 3c and d are assessed by UV light as "appears possibly used"; Figure 3e and f are assessed by UV light as "appears unused".

(Pictures 3a, b, e and f courtesy of Dr Sarah Cohen, Wits Reproductive Health and HIV Institute; Pictures 3d and e courtesy of Peter Upfold)

2.3 Data Analysis

The sensitivity (proportion of applicators known to have been inserted vaginally which are correctly identified as such) of VIREA and UV light techniques were estimated using the positive controls. The specificity (proportion of applicators known to have not been inserted vaginally which are correctly identified as such) of the two techniques were estimated using "sham-inserted" applicators.

The sensitivity and specificity, as well as the 95% confidence intervals, were calculated using the standard formulas:

Sensitivity (%) = 100 x true positive / (true positive + false negative),

Specificity (%) = 100 x true negative / (true negative + false positive).

For the purpose of assessing reliability and concordance of the techniques, data captured on the assessment logs (appendix F and G) was subjected to a quality control process prior to capturing in an Excel database, and then imported to IBM SPSS Statistics Version 23 for further analysis. Percent agreement and the Kappa statistic was performed to assess interrater (inter-assessor) reliability, as well as reliability of the assessment techniques.

Average times taken to assess 100 empty applicators by each technique were calculated to assess efficiency of the techniques.

2.4 Ethical Considerations

Prior to commencement of this research project, ethical approval was obtained from the University of KwaZulu-Natal's Biomedical Research Ethics Committee (BREC), reference number BE083/15 (appendix I).

The assessment, and outcome of assessment, of empty TFV gel applicators returned by participants in the CAPRISA 008 TFV Gel Implementation trial, in no way affected their participation in the study or post-trial access to study product. Since study participants enrolled in the CAPRISA 008 TFV Gel Implementation trial were required to return both their used / empty and unused study product at every visit, this research project placed no additional burden on them.

The only participant identifiers utilised in this research project were the participant unique identifier (PID), a number which links the participant unique number (last 4 digits) to study and site; there is no linkage to participant name.

CHAPTER THREE: Manuscript

CHAPTER 3: MEASUREMENT OF VAGINAL MICROBICIDE ADHERENCE USING VISUAL INSPECTION AS COMPARED TO ULTRA VIOLET LIGHT ASSESSMENT OF RETURNED EMPTY GEL APPLICATORS

The manuscript below was formatted according to author guidelines for the journal *AIDS and Behavior*. Proof of submission of this manuscript to *AIDS and Behavior*, comprising of e-mail acknowledging receipt of submission, screenshot of first page of approved PDF, and cover letter to the journal, can be found in Appendix J.

3.1 Manuscript

Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators

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Running Head: Measurement of Vaginal Microbicide Adherence

Key words: Adherence · Visual Inspection · Ultraviolet Light · Returned applicators · Microbicides

ABSTRACT

Accurate and objective measurement of adherence is critical in microbicide trials. We compared 2 applicator tests: visual inspection of returned empty applicators (VIREA) and ultraviolet light (UVL) assessment in terms of sensitivity and specificity, and for concordance. Sensitivity and specificity analysis of 24 control applicators at 4-months after receipt was 75.0% and 66.7% for VIREA and 83.3% and 91.7% for UVL, respectively. After an additional 3 months of storage sensitivity and specificity was 100% and 58.3% for VIREA and 100% and 66.7% for UVL, respectively. In January 2015, 1316 empty applicators were returned as used by 115 participants enrolled at one site in a randomized controlled trial. Assessment outcomes showed 78.8% agreement between the techniques. Methods concurred that 22% of the returned empty applicators did not appear to be used. By UVL assessment, 40% of returned empty applicators had no evidence of vaginal insertion, translating to 28% less product used as compared to that returned as used by women. UVL assessment may be considered a more accurate and less subjective measure of adherence as compared to VIREA.

INTRODUCTION

Finding an effective and acceptable HIV prevention method that can be controlled by women is key in efforts to getting to zero new HIV infections in this vulnerable population in the developing world, where negotiating condom use is oftentimes challenging. Research has shown that the effectiveness of any prevention method under investigation in clinical trials depends not only on the true efficacy of the product, but more importantly on the participant's willingness and ability to use the product both correctly and consistently as instructed (1, 2). Adherence, or rather the lack thereof, can adversely affect the outcome of clinical trials and can result in the abandoning of potentially efficacious prevention methods (3).

The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir (TFV) gel for the prevention of HIV infection in women in South Africa, not only showed that pericoital TFV gel reduced HIV acquisition by 39% overall, but that higher gel adherence (using two doses of gel for more than 80% of sex acts) conferred a greater protective effect, reducing HIV acquisition by 54% (2). However, disappointingly low adherence to TFV gel in two other recent studies contributed to a failure to demonstrate a protective effect of TFV gel (4, 5), potentially hindering the path to licensure of TFV gel as an HIV prevention method.

Added to the deleterious effect of low adherence on clinical trial outcomes, challenges in accurately measuring adherence can also adversely compromise the interpretation of trial results (6). As such, measurement of adherence to study product is critical for validating study product efficacy (3, 7). Finding the most appropriate adherence measure, or combination of measures, is an essential consideration in trial design. The choice of the most appropriate adherence measure depends on the usefulness and reliability of the method in light of the goal of the study, and available resources (6, 7).

The shift in the microbicide development pathway towards antiretroviral based products allows for measured drug levels to be used as markers of exposure. Directly measuring drug levels in body tissues is crucial in interpretation of trial results, however utility is limited by cost and invasiveness, as well as timing in relation to study product use in pericoital dosing, and retrospective use in placebo

controlled trials (6). A real-time objective measure of microbicide product adherence that will not compromise study blinding, but that will inform adherence support interventions during trial conduct, is an important consideration especially in a blinded clinical trial assessing effectiveness of a candidate microbicide product.

Most microbicide trials rely on participant self-report of study product use (8). Self-reported adherence, although relatively low cost and simple to implement (6), may be subject to recall and social desirability bias (9), and has been shown to overestimate actual product use when multiple measures of adherence were used (8). Other more costly measures like electronic drug / dose monitoring (EDM) may not accurately measure actual product use (6), and EDM-adapted technologies such as the Wisebag™ may underestimate product usage as it is unable to monitor the practice of “pocket-dosing” where more than one applicator is retrieved per bag opening, or may overestimate product usage with curiosity openings of the bag (10, 11). Directly observed insertion (DOI), used in intravaginal microbicide ring trials at clinic visits is best suited for controlled drug release dosage forms, but does not monitor participant-initiated ring removals (6). Clinic counts of returned applicators, considered to be objective, are easily incorporated into clinic procedures at minimal extra cost, but are also subject to numerous forms of bias, and counts of returned empty applicators may overestimate adherence when used as primary proxies for adherence (9, 12). Biologic or surrogate markers such as applicators tests may be relatively inexpensive and easy to implement in clinical trial settings, enabling optimisation of adherence during trial conduct and providing another measure of study product adherence. However, the utility of dye-stain assay (DSA) is limited in terms of accuracy of assessing polypropylene applicators commonly used in recent microbicide trials, and concerns regarding the safety of some dyes (6, 13).

In CAPRISA 004, an applicator test coined the Visual Inspection of Returned Empty Applicators (VIREA) examined applicators under ambient light for residue on the outside of the applicator as an indicator of vaginal insertion, and identified about 16% less product adherence as compared to participant self-report (12). VIREA also enabled identification of participants with potential adherence challenges at their clinic visit. Visual inspection of returned empty applicators under 365 – 385nm ultraviolet (UV) light for evidence of vaginal insertion has also been used as an adherence

measurement method, with some studies suggesting UV assessments should be considered an objective measure of product use in future microbicide trials (14). Various studies of methods used to measure adherence have shown UV Light assessment of empty hydroxyethyl cellulose (HEC) placebo applicators inserted vaginally on a daily basis or pericoitally performs as well or better than other applicator tests, with higher sensitivity of UV light assessment both with prior gel dosing and post-sex application (15-17). Little is known about the technique in assessing empty TFV gel applicators in a clinical trial setting using a coitally-dependant dosing strategy.

This study aims to assess the accuracy of visual inspection and UV light inspection by calculating the sensitivity and specificity of the methods in a pilot assessment of control applicators, and furthermore to compare the techniques for reliability and concordance in a subset of empty TFV gel applicators returned as used in an implementation-effectiveness trial. The purpose of this study was to ascertain whether assessment of applicators under UV light can be considered a more accurate and less subjective measure of product adherence than VIREA.

METHODS

CAPRISA 008 Tenofovir Gel Implementation trial, was an open-label randomized controlled trial to assess the implementation effectiveness and safety of TFV gel provision through family planning (FP) services in KwaZulu-Natal, South Africa, which also provided post-trial access of TFV gel to HIV-uninfected women who previously participated in CAPRISA 004. CAPRISA 008 was conducted at the urban eThekwini and rural Vulindlela CAPRISA Research Clinics that participated in the CAPRISA 004 study and their neighbouring public sector primary health care (PHC) clinics where FP services are provided in KwaZulu-Natal, South Africa (18). Eligible women were randomly assigned to receive their TFV gel either through FP services coinciding with their contraceptive schedule (intervention arm), or monthly though the CAPRISA research clinics (control arm).

As in CAPRISA 004, the CAPRISA 008 study protocol required women to insert one dose of 1% TFV gel within 12 hours **b**efore sex and a second dose of gel as soon as possible within 12 hours **a**fter sex and no more than **t**wo doses of gel in a **24** hour period (BAT24) (2, 18). Study product was issued in quantities sufficient to cover their needs until their next scheduled visit according to their reported

frequency of sexual acts. Women were issued opaque resealable plastic bags to hygienically store one used empty applicator per bag until their return to the clinic. They were required to return all unused and used/empty applicators to the pharmacy for reconciliation at each study visit (two – three monthly according to contraceptive schedule at the FP clinic, or monthly at the CAPRISA clinic), or more frequently if required. Applicators assessed were a subset of empty applicators returned as used to the CAPRISA Vulindlela Research Clinic pharmacy in the CAPRISA 008 trial.

Pharmacy staff were trained in the technique of assessing returned empty applicators by visual inspection by the CAPRISA 004 Vulindlela clinic gel assessor, and in the technique of UV light inspection, and had to qualify as an assessor by scoring 100% on both a VIREA and a UV Light proficiency test.

Applicators were independently assessed by each technique according to standard procedures and pre-defined criteria for inspection (table 1) so that assessment outcomes by one technique did not influence the other. VIREA preceded UV light assessments, and every effort was made to minimise contamination of the returned empty applicators between these assessments. This included only handling applicators with gloves by the ribbed end of the barrel, and carefully inserting the applicators back into their numbered resealable plastic bags post assessment.

The criteria for assessing empty TFV gel applicators by VIREA (table 1) were as per those utilised in CAPRISA 004 (12) (figures 1a-c). The procedure involved inspecting the barrel of each empty applicator under ambient light for evidence of vaginal insertion. If the barrel appeared clean, the cap was removed and the applicator tip was inspected. Applicators with no visible residue of any kind on barrel and a clean tip were assessed as “appears unused”. A viewing box, based on that proposed by Moench et al (16), was utilised to view the returned empty applicators under UV illumination. UV light was provided by a UVL-21 Compact UV Lamp (365-nm). Applicators were viewed through a 76-x 102-mm Tiffen UV Haze-2A filter to block transmission of reflected source illumination. The criteria for assessing empty applicators by UV light (table 1) were as suggested by Moench et al (16), where those applicators with a streaked pattern of blue-green fluorescence, predominantly from adherent vaginal fluids, were scored as positive for evidence of vaginal insertion and assessed as “appears

used" (figure 1d). To accommodate assessment of those applicators which may have been wiped by participants post insertion, an additional category of "appears possibly used" (applicators with a generalised fluorescence) was included in the criteria for UV light assessment (figure 1e).

To determine the sensitivity and specificity of the two assessment methods, a small sample of 12 applicators that were known to be vaginally inserted (positive controls) and 12 sham applicators that had gel expelled ex-vivo, handled, but not vaginally inserted (negative controls) were independently assessed by a blinded primary assessor using VIREA and then UV Light inspection at time point one (120 days after receipt of applicators at the pharmacy). After storage at controlled room temperature for a further three months, the same sample was reassessed to determine if storage had any effect on the sensitivity and specificity of the adherence measures. The positive controls for this pilot assessment were provided by volunteers within the CAPRISA 008 study, who inserted either their before sex or after sex gel dose under direct observation in the research clinic.

To better understand the concordance of the two techniques, VIREA and UV light inspection was also performed on the empty TFV gel applicators returned over a selected one-month period (05 – 29 January 2015) by those participants who were enrolled in the CAPRISA 008 trial at the Vulindlela Research Clinic. To evaluate the reliability of the assessment criteria, assessments were performed by two independent assessors, however only the assessment outcomes of the primary assessor were considered for the interpretation of concordance of the techniques. For the purpose of this study and for more accurate comparison with VIREA technique, applicators assessed as "appears possibly used" by UV light were considered to be "appears used".

Descriptive statistics were used to describe the baseline and behavioural characteristics of the women whose applicators were assessed. Sensitivity and specificity as well as 95% confidence intervals for these were calculated by standard methods. Analysis using percent agreement and the Kappa statistic was performed to assess interrater (inter-assessor) reliability as well as reliability of the assessment techniques. Statistical analysis was performed using IBM SPSS Statistics Version 23. The University of KwaZulu-Natal's Biomedical Research Ethics Committee reviewed and approved this study (Reference #: BE083/15).

RESULTS

The CAPRISA 008 trial enrolled a total of 382 participants across the CAPRISA eThekwini and CAPRISA Vulindlela sites, with 266 of these enrolled at the CAPRISA Vulindlela Research Clinic where this study was undertaken. During the month of January 2015, a total of 146 women were seen for 152 follow-up visits at the Vulindlela Research Clinic. Of these, two were on product hold due to pregnancy, two reported either lost gels or leaving their gels at home, and 27 returned only unopened, unused applicators. The 115 participants who had reported gel usage since their previous study visit returned a total of 1880 applicators, of which 1316 were returned empty as used, and 564 returned unopened as unused.

The baseline and behavioural data of these 115 participants show the median age to be 28 years, with 100 (87%) women reporting to be in a stable relationship, and 15 (13%) women married (table 2). At their visit in January 2015, 108 (95.6%) women reported using gel at their last sex act as per BAT24 instructions.

Sensitivity and Specificity of techniques

At the 4-month assessment of the positive and negative controls, both the sensitivity (83.3% vs. 75.0%) and specificity (91.7% vs. 66.7%) of assessment by UV light was higher as compared to that of the technique of VIREA. After storage for a further 3 months, the assessments showed similar results, with sensitivity and negative predictive value (NPV) for both methods increasing to 100%. The specificity decreased for both methods (table 3).

Concordance of techniques

Substantial inter-assessor agreement for both VIREA and UV light techniques is evident in the percentage agreement (85.0% and 78.6% respectively) and, is supported by the kappa statistic, confirming reliability of the assessment criteria by each technique (table 4).

The assessment outcomes by the primary assessor showed 78.8% agreement between the two assessment techniques (Kappa statistic calculated as 0.529 ($p<0.001$), 95% CI 0.484-0.574). Of the

983 applicators assessed as used by VIREA, 747 (56.8%) were assessed as used or possibly used by UV light inspection. Assessments by VIREA and UV light concurred that 290 (22%) of the returned empty applicators did not have any visual evidence of vaginal insertion, and may not have been inserted into the vagina. In addition, 236 of the 983 applicators assessed as “appears used” by VIREA did not have any evidence of vaginal insertion by UV light inspection, and were classified as “appears unused” (table 5). UV light assessment identified a total of 526 applicators as unused. This, added to the 30% (564/1880) unopened applicators returned as unused, potentially increases the proportion of unused applicators to 58%, suggesting 28% less product used as compared to that returned as used by this subset of participants in January 2015.

On average it took the primary assessor less than 30 minutes to assess 100 empty applicators, irrespective of the measurement method employed.

DISCUSSION

Preliminary investigations into UV light assessment of empty TFV gel applicators returned as used suggests that the technique may be a more reliable measure of microbicide gel adherence and a less subjective measure as compared to the technique of VIREA. Inspection by UV light identified 526 of all returned empty applicators that may not have been inserted vaginally – this is about 28% less product used as compared to that returned as used by this subset of women.

The higher sensitivity and specificity of UV light assessment as compared to VIREA at 4-month assessment is similar to findings of other studies assessing empty HEC placebo applicators (16, 17), and suggests that the UV light assessment technique is more likely to correctly identify used empty applicators as used, and unused empty applicators as unused, and may be more accurate as an adherence measurement method than the VIREA assessment technique. Results showed that after three months of storage the accuracy of the techniques seemed similar, with the sensitivity and NPV increasing and the specificity decreasing for both techniques. This is in contrast to findings of a study that assessed the effect of storage after 30 days on assessment outcomes, where both the specificity of visual inspection and the sensitivity of UV light inspection showed a statistically significant increase (17). In our study, we would expect the specificity of the techniques, particularly VIREA, to decrease

with time due to possible contamination resulting in more false positives, especially since the same sample was assessed at each time point. The increased sensitivity may be attributed to the experience gained by the assessor who assessed the bulk of the applicators (n=1316) between the 3- and 7-month sensitivity and specificity assessments.

Previous findings in a substudy of CAPRISA 004, where 22.5% of applicators assessed by VIREA appeared not to have any visual evidence of vaginal insertion, lead authors to conclude that estimations of product adherence based solely on counts of returned empty applicators without VIREA may be over-estimates (12). Similarly we found that independent assessments by VIREA and UV light concurred that 22% of all returned empty applicators assessed appeared unused. In addition, UV light assessment identified another 18% of all returned empty applicators assessed, that were assessed as used by VIREA, which may not have been vaginally inserted. Given the higher specificity and NPV of UV light assessment found initially in our pilot sample, this points to the subjective and forgiving nature of the VIREA technique and suggests that VIREA may also overestimate actual product usage as compared to UV light inspection.

A study of DNA/protein biomarkers found these assays to be highly sensitive and specific in detecting vaginal insertion; however, these assays are both more costly and labour intensive than UV light assessment (17). Combining UV light assessment of all returned empty applicators, followed by further evaluation of those applicators appearing unused and possibly used using DNA and protein-based methods may increase the accuracy of adherence measurement whilst minimizing costs.

Assessment of returned empty applicators by UV light inspection is a relatively quick and simple method of measuring adherence to microbicide gel applicators. It can easily be incorporated into clinic flow to allow for real-time feedback of product use adherence that could be utilised to direct targeted adherence interventions. UV light inspection may not be as accurate as measured drug level concentrations, but offers the benefit of real-time understanding of product use. In cases where fluorescent properties of the candidate microbicide gel and placebo gel are similar, the method also lacks the risk of unblinding during follow-up especially if performed by pharmacy staff who are often unblinded to product assignment.

There was no difference in the time taken to assess applicators by either method. However, the apparatus used for UV light assessments allowed for only one applicator to be viewed at a time. This is in stark contrast to work by Moench et al, where readers were able to read a set of applicators by UV light in half the time taken to read the set by ambient light; however, apparatus used by Moench et al allowed for rapid viewing of all portions of multiple applicators at a time (16).

Several limitations make extrapolating the finding of this study challenging. The sample size in this pilot assessment of sensitivity and specificity is small and limits it interpretation. Due to the limited number of positive controls collected (n=12), the same sample of applicators had to be utilised for sensitivity and specificity analysis at 4-months and at 7-months. We cannot be sure that potential accidental contamination of the sample at initial assessment did not influence the outcome of assessment after an additional 3 months of storage. None of the currently used adherence measurement methods in microbicide trials are able to accurately measure actual product use (timing of product use in relation to sexual activity and HIV exposure nor the amount of product actually used) and assessment by VIREA and UV light are no exceptions. Unbiased and acceptable methods of measuring timing of gel use relative to sexual activity in microbicide trials are lacking and warrant further investigation. In addition, the practice of washing vaginal secretions off the used empty applicators may limit the accuracy of both methods of assessment, but adequate counselling against this practice may overcome this challenge. Moreover this was a substudy within an existing implementation trial nearing study close, and due to time constraints was not designed to capture all the data required to fully appreciate the value of the technique in assessing returned empty TFV gel applicators.

Although UV light assessment has been shown to be a feasible surrogate marker of product adherence, it would be pertinent for future studies planning to utilise UV light inspection to validate the measure against an available biomarker, and to investigate the fluorescent properties of the microbicide product under investigation so as to fully understand its potential to quench cervicovaginal fluid (CVF) fluorescence or lead to unblinding in a blinded placebo-controlled study. A larger study to

assess accuracy of the method, the impact of gel dosing on the technique and validation of the technique using biomarkers is warranted.

It is recommended that UV light assessment be used as part of a triangulated approach to measuring product adherence, possibly in conjunction with a DNA/protein biomarker test to verify assessment outcomes for applicators assessed as unused or possibly used, both to improve the accuracy of adherence data collected and to overcome the limitations associated with any single adherence measurement method.

In conclusion, assessment by UV light identified 40% of returned empty applicators as appearing not to be used, translating to 28% less product used as compared to that returned as used by this subset of women. The technique, used as a rapid on-site assessment of microbicide gel adherence, will aid in identifying those participants in need of focused adherence support during trial conduct.

FIGURES AND TABLES

Figure 1a

Figure 1b

Figure 1c

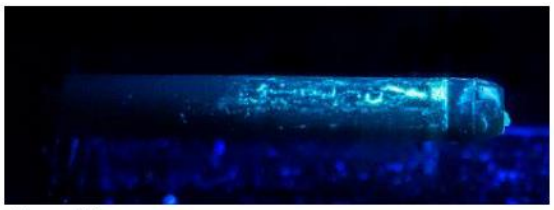

Figure 1d

Figure 1e

Figure 1f

Figure 1: Applicators as viewed under ambient light (a-c) and under 365nm UV light (d-f).

By VIREA, Figures 1a-c were assessed as appears used. By UV light, Figure 1d was assessed as "used"; Figure 1e was assessed as "appears possibly used", and Figure 1f was assessed as "appears unused.

Table I: Criteria for assessing empty applicators by the techniques of VIREA and UV Light inspection

	Appears used	Appears possibly used	Appears unused
VIREA	Any visible residue, mucus, gel, secretions or hair on barrel Any discolouration or residue that was not gel on tip under cap	N/A	No residue of any kind on barrel, clean tip or only clean gel visible on tip
UV Light	Streaked pattern of blue-green fluorescence on barrel	Generalised fluorescence on barrel, with or without fluorescent speckles	No streaked or generalised fluorescence on barrel, with or without fluorescent specks

Table II: Baseline and behavioural data, January 2015, n = 115

Baseline data	
Age, median (IQR)	**28** (24-31)
Total sex partners since sexual debut, median (IQR)	2 (1-3)
Sex acts last 30 days, median (IQR)	4 (2-6)
Relationship status (n, %)	
Married	**15** (13.0)
Stable	**100** (87.0)
Follow-up data, January 2015	
Sex acts since last visit, median(IQR)*	5 (3-7)
Sex acts last 30 days, median(IQR)*	4 (2-5)
Gel use last sex act (n, %)*	
No gels used	5 (4.4)
BAT 24	**108** (95.6)

IQR: Interquartile range, BAT24: One gel up to 12 hours **B**efore sex, one gel as soon as possible but with 12 hours **A**fter sex, and not more than **T**wo gels in **24** hours *2 had missing data

Table III: Sensitivity and Specificity of VIREA and UV light assessments

	VIREA 4 months		VIREA 7 months		UV Light 4 months		UV Light 7 months	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Sensitivity								
Positive controls (n =12)	75.0	42.8-94.5	100	73.5-100	83.3	51.6-97.9	100	73.5-100
Specificity								
Negative controls (n =12)	66.7	34.9-90.1	58.3	27.7-84.8	91.7	61.5-99.8	66.7	34.9-90.1
PPV	69.2	38.6-90.9	70.6	44.0-89.7	90.9	58.7-99.8	75.0	47.6-92.7
NPV	72.7	39.0-94.0	100	59.0-100	84.6	54.6-98.1	100	63.1-100

CI: Confidence Interval

Sensitivity (%) = 100 x true positive / (true positive + false negative), Specificity (%) = 100 x true negative / (true negative + false positive)

PPV: Positive predictive value, (%) = 100 x true positive / (true positive + false positive), NPV: Negative predictive value, (%) = 100 x true negative / (true negative + false negative)

Table IV: Analysis of Inter-assessor agreement, n = 1316

	VIREA	UV Light Inspection
Agreement, %	**85.0**	**78.6**
Kappa Measure of agreement	**0.627**	**0.676**
95% Confidence Interval	(0.580, 0.674)	(0.643, 0.709)
p-value	<0.001	<0.001

Table V: Comparison of assessment outcomes, n = 1316

		UV Light % (n)			
	Assessment outcome	**Appears used**	**Appears possibly used***	**Appears unused**	**Total**
VIREA % (n)	**Appears used**	46.3 (455)	29.7 (292)	**24.0** (236)	74.7 (983)
	Appears possibly used	-	-	-	-
	Appears unused	0.6 (2)	12.3 (41)	87.1 (**290**)	25.3 (333)
	Total	34.7 (457)	25.3 (333)	40.0 (526)	100 (1316)

*For comparison with VIREA assessment outcomes, those applicators assessed as appears possibly used by UVL were considered to be appears used

ACKNOWLEDGEMENTS

We acknowledge the women who participated in the CAPRISA 008 trial and their commitment to finding an HIV prevention method for women. The CAPRISA 008 trial was supported by the Centre for the AIDS program of Research in South Africa (CAPRISA), the United States Agency for International Development (USAID) through CONRAD (PPA-12-143) (Trial Sponsor), the South African Department of Science and Technology through the Technology Innovation Agency and the MACAIDS Fund through the Tides Foundation (Grant # TFR11-01545). Support from CONRAD for the product manufacturing and packaging as well as support from Gilead Sciences for the TFV used in the production of gel is gratefully acknowledged. We acknowledge Londeka Lucia Zondi for training our assessors in the technique of VIREA, Chanelle Smith for conducting gel assessments, and Peter Upfold for photographing applicators.

CONFLICT OF INTEREST

All authors report no conflicts of interest

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3.2 Masters candidates' contribution to the manuscript

Student name: Michele Upfold

Student number: 205525637

Title of the manuscript: *Measurement of vaginal microbicide adherence using visual inspection as compared to ultra violet light assessment of returned empty gel applicators*
Authors: Michele Upfold, Anneke Grobler, Fatima Suleman, Leila E Mansoor

Journal: *AIDS and Behavior*

Masters student's contribution to the manuscript:

1. Formulation of the hypothesis
   I formulated the study research question, with the guidance of my supervisor.
2. Study design
   Under the guidance of my supervisor, I designed the study with regards to methodology, with advice from CAPRISA statisticians regarding sample sizes and methodology. I reviewed available literature.
3. Work involved in the study
   I was involved in the dispensing and collection of returned empty and unused applicators from study participants enrolled in CAPRISA 008 TFV Gel Implementation trial. I was trained in assessing returned empty applicators by VIREA and UV light; and, for the purpose of verifying reliability of the assessment criteria, independently performed assessments of the applicators returned during the month of January 2015.
4. Data analysis
   I set up the Excel database for data analysis and performed the sensitivity and specificity calculations by standard methods, and agreement calculations using IBM SPSS Statistics Version 23. These calculations were verified by a CAPRISA statistician.
5. Write up
   I took overall responsibility for writing the manuscript, with regular review of drafts and feedback from my supervisor. After completion of the first full draft of the manuscript, I submitted the manuscript to co-authors for review. All co-authors read and approved the final version of the manuscript. I submitted the manuscript on-line for consideration for publication in the journal *AIDS and Behavior*.

I declare this to be a true reflection of my contributions to this manuscript.

Signature:  Date: 19 November 2015

CHAPTER FOUR: Overall Discussion

CHAPTER 4: OVERALL DISCUSSION

4.1 Discussion of major findings

The purpose of this study was to investigate and compare the techniques of VIREA and UV light inspection of empty TFV gel applicators to better understand the utility of these techniques as adherence measurement methods. Utility was assessed in terms of accuracy (sensitivity and specificity), reliability and concordance (verifying the assessment criteria, and investigating agreement), and practicality (time and feasibility). This research project was driven by the question as to whether UV light assessment of empty TFV gel applicators was comparable, or perhaps even less subjective, than assessment by VIREA.

The manuscript (chapter three) meets the four objectives as listed in the introduction chapter (chapter one) of this dissertation.

4.1.1 Objective 1: *To undertake a pilot study to estimate the accuracy of both VIREA and UV light assessments of empty TFV gel applicators by calculating the sensitivity and specificity of the techniques at time point one (within four months of applicator receipt).*

Time point one assessment found sensitivity and specificity of VIREA to be 75.0% and 66.7% respectively, and sensitivity and specificity of UV light to be 83.3% and 91.7% respectively.

Both the sensitivity (83.3% vs. 75.0%) and specificity (91.7% vs. 66.7%) of assessment by UV light was considerably higher as compared to that of the technique of VIREA. The higher sensitivity and specificity of UV light assessment at the time point one assessment is similar to overall findings of other studies that compared these measurement methods by assessing empty HEC placebo gel applicators (12, 13). Moench *et al*. found the sensitivity and specificity of UV light inspection (84% and 83% respectively) to be higher than visual inspection (76% and 63% respectively) (12). Thurman *et al.* found higher sensitivity of UV light as compared to visual inspection at 7-day assessments (74.2% vs. 54.4% respectively) and at 30-day assessments (92.2% vs. 51.9% respectively) (13). Although these assessments were performed at different time points as compared to our study, collectively these findings suggest that the UV light assessment technique is more likely to correctly identify used empty applicators as used, and unused empty applicators as unused, and may be more accurate as an adherence measurement method than the VIREA assessment technique.

4.1.2 Objective 2: *To assess the effect of storage on the sensitivity and specificity of the two techniques performed on the same set of applicators at time point one and two (after an additional three months of storage).*

Time point two assessment found sensitivity and specificity of VIREA to be 100% and 58.3% respectively, and UV light to be 100% and 66.7% respectively.

After storage at controlled room temperature for three months, the time point two assessments showed increased sensitivity for both methods, with decreased specificity. The increased sensitivity may be attributed to the experience gained by the primary assessor who assessed the bulk of the applicators between the 3-and 7-month sensitivity and specificity assessments. Our time point two analysis, at 7-months post receipt of applicators, suggests the techniques are not very different in terms of accuracy. These findings are in contrast to findings of a study that assessed the effect of storage after 30 days on assessment outcomes, where both the specificity of visual inspection and the sensitivity of UV light inspection showed a statistically significant increase (13). However, this study used two different sets of applicators for assessment at 7- and 30-days, whereas we used the same set of applicators that were assessed at time point one for the time point two assessment. We suspect that possible contamination of the sample applicators as well as experience gained by the primary assessor between the two assessment time points may have influenced our results.

4.1.3 Objective 3: *To assess the reliability and concordance of UV light assessment of a subset of returned used / empty TFV gel applicators in a select one-month period for agreement, with the VIREA assessment of the same applicators, in terms of evidence of vaginal insertion.*

Results of assessment outcomes of the 1316 returned empty applicators by the two assessors (appendix K), showed substantial inter-assessor agreement. This is evidenced in 85% agreement for the VIREA technique (Kappa statistic calculated as 0.627 ($p<0.001$), 95% CI (0.580, 0.674), and 78.6% agreement for the UV light technique (Kappa statistic calculated as 0.676 ($p<0.001$), 95% CI (0.643, 0.709). These results confirm the reliability of the assessment criteria for each technique.

Our assessment criteria for VIREA technique were based on those defined in the previous study of VIREA of empty TFV gel and placebo gel applicators, since this study was able to validate the technique by linking assessment outcomes with detectable TFV levels in a subset of women randomized to the TFV gel arm in CAPRISA 004 TFV Gel Efficacy trial. The assessment criteria for UV light assessment of empty TFV gel applicators was based on those used by Moench *et al.* with the added category of "appears possibly used" to accommodate for the possible wiping of applicators after use. It is possible that applicators assessed as "appears possibly used" by UV light may have been pre-sex applicators which have been shown to have a lower signal intensity, especially when viewed under 385nm UV light source (12). For the purpose of this study and for more accurate comparison with VIREA technique, applicators assessed as "appears possibly used" by UV light were considered to be "appears used".

Although we do not actually know how many of the empty applicators returned as used were actually used, concordance of the techniques, as per assessment outcomes of the primary assessor, was demonstrated by 78.8% agreement between the two assessment techniques (Kappa statistic calculated

as 0.529 ($p<0.001$), 95% CI (0.484, 0.574). Of the 983 applicators assessed as used by VIREA, 56.8% were assessed as used or possibly used by UV light inspection. Assessments by VIREA and UV light concurred that 22.0% of the 1316 empty applicators returned as used appeared not to have been used. Similarly, the previous study of VIREA in CAPRISA 004 TFV gel efficacy trial found 22.5% of applicators assessed appeared not to have been inserted vaginally (10). These findings suggest that measurement of product adherence should include a physical verification of returned empty applicators to obtain a more reliable estimate of adherence than self-report or product count alone.

Amongst the 21.2% disagreement between the techniques are two interesting points. Firstly, only 12.9% of the 333 applicators assessed as unused by VIREA were assessed as used / possibly used by UV light. This suggests that applicators assessed as unused by VIREA are most likely to be unused. Secondly, 24.0% of 983 applicators assessed as "appears used" by VIREA did not have any evidence of vaginal insertion by UV light inspection, and were classified as "appears unused". This is potentially another 18.0% of applicators that may not have been used, over and above the 22.0% that assessment by both methods agreed were unused.

Overall assessment by UV light identified 526 of the 1316 returned empty applicators as having no evidence of vaginal insertion. Adding this to the 30.0% (564/1880) unopened applicators returned as unused in January 2015, potentially increases the proportion of unused applicators to 58.0%, suggesting 28.0% less product used as compared to that returned as used by this subset of women.

Reliability and concordance of the techniques were assessed on average about seven months after receipt of the returned empty applicators. This informed the choice of the time point two assessment period for sensitivity and specificity.

4.1.4 Objective 4: *To measure the time taken to assess a set of returned used / empty applicators for each technique in order to evaluate the practicality and efficiency of the measurement methods.*

On average it took the primary assessor about 30 minutes to assess 100 empty applicators, irrespective of the measurement method employed (30 minutes 29 seconds for VIREA and 29 minutes 30 seconds for UV light). This is in sharp contrast to research by Moench *et al.* which also compared reading times for the methods assessed. Moench *et al.* found the mean reading time to read the full set of 250 applicators was only 15 minutes for UV light assessment and 32 minutes for ambient light assessment. However, this rapid assessment rate was possible because the UV viewing box utilised by Moench *et al.* allowed for loading of 36 applicators onto a tray which was slid over rails into the viewing box allowing for multiple applicators to be viewed at a time (12). This discrepancy between our findings and those of Moench *et al.* is expected since the visual inspection technique and the

viewing box to view applicators under UV light utilised in our research project allowed for viewing of only one applicator at a time.

This research project found that with training, assessments using VIREA and UV light were easy to perform. They could easily be incorporated into the clinic flow, and since counts of returned applicators are an integral part of study product accountability, applicator assessments are best performed by trained pharmacy staff.

4.2 Study limitations

This was a substudy within an existing implementation trial nearing study close, and due to time constraints was not designed to capture all the data required to fully appreciate the value of the UV light technique in assessing returned empty microbicide gel applicators. For optimal benefit of UV light assessment, these should be performed in real-time at the clinic visit so as to be able to inform targeted adherence interventions in those women identified as having difficulties with adherence. We were unfortunately not able to perform the assessment of reliability and concordance in real-time.

Several limitations should be considered when interpreting the results of this study. Due to the limited number of positive (known to be vaginally inserted) controls collected, the sample size in the pilot assessment of sensitivity and specificity is small, and the same set of applicators had to be utilised for sensitivity and specificity analysis at time point one and after storage for three months at controlled room temperature at time point two. We cannot be sure that accidental contamination of the sample at initial assessment did not influence the outcome of time point two assessments, and suspect this may have been the reason for decreased specificity of the methods after storage (more false positives).

As is the case with many of the other currently used adherence measurement methods, applicator tests are not able to accurately measure actual product use (timing of product use in relation to sexual activity and HIV exposure, nor the amount of product actually used or whether it was expelled ex-vivo prior to vaginal insertion of the applicator).

In an attempt to accommodate the practice of wiping applicators after use and before return to the clinic, we included an “appears possibly used” category for UV light assessment; however, the practice of washing vaginal secretions off the used empty applicators may limit the accuracy of both methods of assessment. Participants need to be adequately counselled to discourage these practices.

Validation of the techniques against biomarkers or detectable TFV in CVF may have strengthened the findings of this research project, however was not possible due to time and funding constraints. A

larger study to assess accuracy of UV light assessment, the impact of gel dosing on the technique and validation of the technique using biomarkers is warranted.

4.3 Recommendations for future research

UV light assessment should be considered in conjunction with other adherence measurement methods as part of a triangulated approach in future clinical trials of vaginal microbicide gels. However, it is essential to fully investigate the fluorescent properties of the microbicide product under investigation so as to fully understand the potential to quench CVF fluorescence or lead to unblinding in a blinded placebo-controlled study.

The challenge of low adherence in phase II trials assessing efficacy of a candidate microbicide needs to be addressed. Optimising adherence during trial conduct is one possible solution. Despite reported high gel acceptability and intensive adherence support strategies based on motivational interviewing techniques, about 40% of the women enrolled in CAPRISA 004 TFV Gel Efficacy trial were only able to use the gel for less than 50% of their sex acts (3). Careful consideration of the social and behavioural factors, such as risk perception, partner influence on use and effect of unknown efficacy on use-adherence, that impact adherence are critical (2). In addition, early identification of women with potential adherence challenges during the trial may enable targeted adherence interventions to encourage correct and consistent product use. An adherence measurement method that can serve the dual purpose of assessing product adherence in real-time whilst contributing to overall estimation of product adherence is an important consideration in microbicide clinical trials. A study comparing two applicator tests (DSA and UV light assessment) with Wisebag and self-reported adherence, found 14% and 16% disagreement between readers for DSA and UV assessment respectively, especially for negative control applicators. This unexpectedly high number of indeterminate results led the authors to suggest UV assessment of all applicators, followed by DSA only on those applicators deemed indeterminate by UV assessment (14). Another study has found DNA/protein biomarkers to have high sensitivity and specificity (98.3% and 100%) at both 7- and 30-day assessments (13). However, these biomarker tests are expensive. Combining UV light assessment of all returned empty applicators, followed by further evaluation of those applicators deemed “appears unused” and “appears possibly used” using DNA and protein-based methods to both increase the accuracy of real-time adherence measurement whilst minimizing costs, will allow for immediate feedback to direct adherence support interventions. This approach is recommended as a rapid on-site assessment of product adherence to identify women with challenges to adherence at their clinic visit, inform interventions to optimize adherence during the trial, contribute to accurate assessment of overall study product adherence, and ultimately enable better understanding of study product efficacy.

The utility of UV light assessment of other vaginally inserted microbicide formulations, such as vaginal rings, should also be explored further.

4.4 Concluding statements

Assessment by UV light identified 40% of returned empty applicators did not appear to be used, translating to potentially 28% less product used as compared to that returned as used by women. The preliminary sensitivity and specificity of the technique in assessing empty applicators returned as used suggests UV light assessment is both a more accurate and less subjective measure of adherence as compared to VIREA.

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APPENDICES

APPENDICES

APPENDIX A: Literature review summaries

Literature review: Methods of Measurement

Studies comparing multiple methods of measurement							
Authors, year	**Study design**	**Findings**					**Comments / Conclusions / Recommendations**
		Visual Inspection	**Ultraviolet light**	**DSA**	**MEMS-type**	**Other**	
Keller JM et al, 2013 (11)	Assessment of 95 reported pre-sex and 95 reported post-sex HEC placebo applicators by 3 readers Applicator: LDPE Microlax®-type; Dosing: pre- and post-sex twice a week for 3-4 weeks	Not assessed	**Streaked or speckled pattern of blue-green fluorescence** (single spotlight 60 x 385nm UV wide angle LED bulb) Sensitivity: Pre-sex 87% Post-sex 97% Specificity: Pre-sex and Post-sex 96%	**RSID™-Semen test, grainy/streaked pattern:** Sensitivity: Pre-sex 100% Post-sex 90% Specificity: Pre-sex and Post-sex 100%	Not assessed	Not assessed	Small study; both DSA & UV: reliable biomarker with pericoital BAT-24 dosing; non-invasive, require little time, inexpensive, easy to perform, easily incorporated, real-time feedback to address behavioural challenges; Inter-reader agreement higher on pre-sex for DSA, and lower for post-sex as compared to UV; larger studies & high-risk cohorts needed
Moench T et al, 2012 (12)	Evaluated 4 methods of assessment of 250 (170 known inserted / 80 sham-inserted) HEC placebo applicators: inspection under ambient light, UVL, and DSA with Alcian Blue and iodine: 2 readings each by 3 readers Applicator: HTI Polypropylene; Dosing: daily over 8 consecutive days;	**Ambient light: Visible adherent material (secretions and / or gel)** Sensitivity: 76% Specificity: 63% **Reading time:** 32 mins for 250 apps	**Streaked pattern of blue-green fluorescence** (two 385nm 60 LED array lamps) Sensitivity: 84% Specificity: 83% Sensitivity 65% without prior gel applications, increasing to 95% in applicators inserted after previous gel use (statistically significant) **Reading time:** 15 mins for 250 apps	**Alcian blue:** Sensitivity: 79% Specificity: 83% **Reading time:** 26 mins for 250 apps **Iodine:** Sensitivity: 65% Specificity: 80% **Reading time:** 63 mins for 250 apps	MEMS cap on container for storing +/- 60 applicators: data matched written records to within 2 minutes	Not assessed	Concern that without prior gel applications accuracy of all 4 methods in coital dosing may be limited; increased accuracy after prior insertions possibly due to accumulated gel from prior doses, allowing for retention of more gel, mucous and cells on applicator; MEMS and UV inspection should provide fast, reliable, quantitative adherence assessment in daily dosing regimens
Van der Straten A et al, 2013 (14)	Compared 872 returned empty applicators (plus 39 known inserted and 43 emptied ex-vivo applicators for sensitivity and specificity) HEC placebo applicators by DSA, UV light assay, Wisebag® and self-report: 2 readers	Not assessed	**Streaked pattern of blue-green fluorescence** (single 60 x 385nm UV wide-angle LED bulb) Sensitivity: 95% Specificity: 79%	**Food dye, grainy/streaked pattern:** Sensitivity: 97% Specificity: 79% 5% disagreement between readers	Wisebag® used as passive monitoring; 15% reported "pocket dosing", 10% reported additional openings; 59% reported Wisebag® useful as visual cue to remind of gel	**Self-report at study exit:** Retrospective assessment: days with missed doses, 6-point Likert scale assessing frequency of gel insertion;	DSA and UVA simple & inexpensive, UVA faster as no dye-drying time required; UVA provides immediate adherence feedback during study visits; neither test is "anatomically specific" i.e. unable to determine actual vaginal insertion; consider UVA on all applicators followed by DSA on indeterminate applicators; techniques require further evaluation: for pericoital and daily dosing regimens; with polypropylene apps; also for rectal dosing regimens; Wisebag acceptable but

Studies comparing multiple methods of measurement							
Authors, year	**Study design**	**Findings**					**Comments / Conclusions / Recommendations**
		Visual Inspection	**Ultraviolet light**	**DSA**	**MEMS-type**	**Other**	
	Applicator: LDPE Microlax®-type; Dosing: daily over 30 days;		8% disagreement between readers		dosing, 18% said impractical or lacked discretion;		underestimated adherence; Reasons for not opening every day: forgetting, traveling, not returning home / returning late; MEMS adapted devices useful as adherence-enhancing tools especially if integrated SMS reminders;
Thurman AR et al, 2014 (13)	Compared 240 (and additional 240 at 30 days) Visual and UV light inspection of HEC placebo filled applicators with DNA/protein biomarkers at 2 time points (7 days and 30 days): 3 readers Applicators: HTI polypropylene; Dosing: all 12 applicators per participant received in one visit;	**VIRA:** Day 7 Sensitivity: 54% Specificity: 49% Day 30 Sensitivity: 52% Specificity: 78%	(365nm light) Day 7 Sensitivity: 74% Specificity: 73% Day 30 Sensitivity: 92% Specificity: 66%	Not assessed	Not assessed	**DNA & cytokeratin analyses using multiplex PCR** Day 7 Sensitivity: 98.3% Specificity: 100% Day 30 Sensitivity: 98.3% Specificity: 100%	Analysed sensitivity per subgroup: all, all except wiped, no gel present, gel present, wiped; overall higher sensitivity of UVL at 30 days attributed to increased sensitivity of detecting wiped applicators; DNA/protein biomarkers more sensitive and specific in all subsets as compared to VIRA at days 7 and 30 (statistically significant); DNA and protein biomarkers overall higher sensitivity and specificity than UVL at days 7 and 30 (statistically significant), but expensive and labour intensive; both visual applicator tests easily performed on-site, inexpensive, need little ancillary equipment; but are still subjective assessments!, prone to Inter-reader variability; UVL assessment is low cost, with improved sensitivity and specificity after storage, but found significant IRV at both time points

Studies assessing a single method of measurement			
Authors, year	**Study design**	**Methods & Findings**	**Comments / Conclusions / Recommendations**
Gengiah TN et al, 2014 (10)	Visual Inspection of 59 800 returned empty applicators (1% tenofovir gel and HEC placebo) within CAPRISA 004; Applicators: HTI polypropylene; Dosing: pericoital	VIREA visual assessment under ambient light according to predefined criteria: "appears used" or "appears unused" Appears used:77.5% Appears unused: 22.5% **Identified about 16% more applicators with no visual evidence of vaginal insertion than that reported used by women** Validated using tenofovir detected in CVF at 375 matching study visits: TFV detected in CVF in only 13.5% of women who had ≤50% of empty applicators categorised as "used" by VIREA in contrast to 58.3% of the women who had >50% of empty applicators categorised as "used" by VIREA (Limitation: TFV only detected in vagina for short time after use – hence expect lower correlation between proportion of appearing used with vaginal TFV concentrations)	Estimating adherence by physical count of returned empty applicators without visual inspection may over-estimate; real-time identification of applicator mechanics issues; Technique is subjective despite standardised process Physical verification aids understanding of adherence and identifies participants with potential adherence challenges
van der Straten A et al, 2013 (22)	Feasibility, performance and acceptability of Wisebag® in a 3-armed double-blind pilot study (n=50)	Wisebag® - opened daily for 14 days, take study sticker from bag and place sticker on to diary card; day one at clinic under direct staff observation, day 14 Wisebag returned and data downloaded; quantitative: behavioural and acceptability questionnaire; qualitative: interview using semi structured guide to assess experience and attitude towards Wisebag and study procedures Randomised 2:2:1 to Online (events transmitted real-time via cellular signal); Offline (events stored in device memory); or Inactive ("dummy" devices) **By electronically recorded events: 26% adherent to once-day opening instructions (in contrast to 46% per diary card and 48% by self-report)** 22% opened Wisebag >1x per day ("curiosity events") 94% liked using the Wisebag, and almost 94% liked overall appearance	Short duration, no study product, lacks other objective measures to validate event data; Wisebag seemed feasible, acceptable technology with potential for as adherence measure for microbicide trials; data should be validated using actual products, with and without SMS notifications, and with biomarkers

Studies assessing a single method of measurement			
Authors, year	**Study design**	**Methods & Findings**	**Comments / Conclusions / Recommendations**
Gengiah TN et al, 2014 (23)	Monitoring microbicide gel use with Wisebag® in CAPRISA 004 (n=10)	Wisebag® - Pilot study of microbicide use within CAPRISA 004 with up to 4 months follow up; included SMS reminders of dosing regimen; opening events compared with self-reported sexual activity & applicator returns; over 33 monthly follow-up visits 47.8% of recorded opening events matched number of returned empty applicators; discrepancies postulated to be due to "pocket dosing"	Small study; challenges with interpreting event-data to self-reported gel use; concerns for possible social harms with SMS's proved unfounded
Austin NA et al, 2009 (24)	DSA of 169 participant inserted VivaGel (SPL7013) applicators; Applicators: HTI polypropylene	0.05% FD&C Blue Dye No. 1 solution, 3 evaluators Sensitivity for known single-use applicators: 81-95% Specificity for unused applicators: 86-93% Sensitivity by DSA for applicators inserted twice daily x 14 days: 47-77%, but 168/169 had vaginal epithelial cells by Gram stain	Sensitivity varied widely depending on evaluator and prior gel exposure; Sensitivity decreased with multiple gel applications; authors **cannot recommend DSA of HTI polypropylene applicators** as adherence monitoring tool
Katzen L et al, 2011 (26)	Validation of DSA of HEC placebo Applicators: LDPE Microlax®-type (n=1848)	Applicators stained with 0.05% FD&C Blue No. 1 Granular Food Dye for 5 seconds; assessed by 4-6 readers Experiment 1 (validate DSA): 132 applicators read by 5 readers - Sens = 92.4% / Spec = 93.9% Experiment 2 (validate DSA with applicators after 4 months storage): 132 applicators read by 4 readers – Sens 91.7% / Spec 95.1% Experiment 3 (validate reproducibility of DSA): 132 applicators read by 5 readers: Sens = 87.6% / Spec = 95.2%	Suggest further studies to validate DSA in after-sex applicators; DSA low-cost and relatively easy; recommend as tool to identify low adherence

Literature review: Vaginal Microbicide trials

Authors, year	Study / design / Country / target population	Findings	Comments
Abdool Karim Q et al, 2010 (3)	CAPRISA 004 RCT, double-blind, placebo controlled, pericoital 1% TFV gel, South Africa, HIV uninfected women aged 18-40 years, n=889	Overall, 1% TFV gel reduced HIV acquisition by 39% BUT BY 54% in women with high (>80%) gel adherence 38% in women with intermediate adherence (50-80%) 28% in women with low adherence (<50%) Gel adherence defined as "estimated proportion of reported sex acts covered by 2 doses of gel"; calculated: 1/2 the number of returned used apps each month / number of reported sex acts in that month	Accurate estimate of product efficacy difficult in face of inadequate adherence. Future trials: greater emphasis on optimizing & objectively measuring adherence
Rees H et al, Abstract as presented at CROI 2015 (5)	FACTS 001 (confirmatory trial of CAPRISA 004) RCT, double-blind, placebo controlled, pericoital 1% TFV gel, South Africa, HIV uninfected women aged 18-30 years, n=2059	Overall, pericoital TFV gel not effective in preventing HIV acquisition In case-cohort sub study, high TFV in CVL associated with a 52% reduction in HIV acquisition (statistically significant) TFV gel effectiveness highest in women who reported use of product in >72% of sex acts (consistently as prescribed in all or most of sex acts), but this subgroup only represented about 20% of participants	Association between adherence based on returned applicators and HIV effectiveness
Conrad press release: FACTS 001 Results presented at CROI 2015 (6)	As above	As above	High adherence in the overall study was too low to show TFV gel effectiveness Gel appeared acceptable and easy to use; most enrolled participants were young, unmarried, majority still living with parents and found consistent use of gel challenging
Marrazzo JM et al, *2015* (16)	VOICE RCT, placebo-controlled, daily oral TDF, daily oral TDF-FTC, or daily 1% TFV vaginal gel, Uganda, SA and Zimbabwe, HIV uninfected women aged 18-45 years, n=5029	Study commenced Sept 2009; Sept 2011 DSMB recommends oral TDF arm be stopped due to futility; Nov 2011 DSMB recommends TFV gel arm be stopped due to futility; only TDF-FTC and oral placebo groups continued to study end in August 2012 Mean adherence by returned-product counts =86%, by self-report in FTFI 90%, by ACASI 88%; however, subset analysis of 488 participants showed TFV detected in quarterly plasma samples in only 29% from TDF-FTC group, 30% in oral TDF group and 25% in TFV gel group For most women where TFV was not detected at first quarterly visit, none was detected at subsequent visits Detectable TFV levels at quarterly visit in TFV gel group had a significantly lower likelihood of HIV acquisition as compared to those with no TFV detected	Predominantly young unmarried women in SSA with low adherence to daily oral or vaginal TFV based products, showed no protective effect. Single women under 25 least likely to adhere to study product and most likely to acquire HIV as compared to older married women Study highlighted "the need to better understand behavioural barriers in the setting of strong social stigma"

Literature review: Adherence and Measurement in Microbicide trials

Authors, year	Brief summary of article and recommendations
Mansoor LE et al, 2014 (20)	Descriptive of CAPRISA 004: Adherence interventions and rates Adherence measurement by 3 methods 1. Returned used applicator (median # of applicators returned per study visit) 2. Self-report (proportion of adherent sex acts over all sex acts for each woman) 3. Applicator-based (primary measure) (half the number of returned used applicators / number of reported sex acts that month) Adherence calculated as 72.2% by applicator counts in relation to all reported sex acts cf. 82% self-reported during last sex act - suggesting a "white coat" effect: higher adherence in last sex act prior to scheduled study visit cf. to rest of the month. Returned used applicator counts are user-dependant, cannot account for frequency of sex, discarding gel, or forgetting to return applicators; self-report data in conjunction with objective applicator count gives a better applicator-based measure of adherence over 30 days prior to visit
Mansoor LE et al, 2014 (15)	Descriptive of CAPRISA 008 which plans to assess implementation effectiveness and safety of coital TFV gel provision through existing Family Planning services, whilst providing post-trial access to TFV gel for those eligible women who participated in CAPRISA 004. Aims to address the question of future scale-up within SA public health system, should TFV gel reach licensure Adherence assessment via brief interviewer-administered instruments; counts of returned empty and unused applicators; genital specimens at quarterly and exit visits to be archived for analysis of markers of product adherence to aid interpretation of study results Results forthcoming
Muchomba FM et al, 2012 (8)	Systematic review of oral PrEP & microbicide trials looking at adherence in terms of definitions and measures used, risk for non-adherence, adherence promotion strategies, effects on trial outcome: 19 trials published between 1987 – 2012 with 47 157 participants Self-report as an adherence measure upwardly biased in studies that employed multiple measures of adherence; adherence rate in microbicide studies ranged from 69-89%; av. adherence higher when dosing was before every sex act as compared to once / twice daily application For oral PrEP: High adherers by pill count, self-report and refill data achieved 41% higher efficacy cf. with low adherers; in a microbicide trial reporting statistically significant treatment effect, high adherers by comparing gel returns and self-report of sexual frequency, had 26% higher efficacy Few risks for non-adherence reported, most often cited risk was decreased motivation over duration of study, sex with primary partner, running out of treatment Average of 3.3 adherence support strategies identified in each study reviewed Recommendations for future Bio-behavioural Prevention trials: 1) pre-trial assessment of potential barriers esp. in vulnerable populations, consider stigma towards use of B-BPSs, develop a risk-screen; 2) include evidence-based and culturally appropriate interventions to promote adherence, including option of partner involvement; 3) protocols must include plans to accurately monitor adherence during the trial, measure that lacks reporting bias should be included as one of several measures used for triangulation; improve validity and reliability of self-report by considering optimal recall time-frame, normalising nonadherence, assessment of fidelity (the extent to which interventions are implemented as intended by protocols); 4) development of B-BPS that does not adversely impact pregnancy; 5) analysis of efficacy must include investigation of adherence as key effect moderator
Van der Straten et al, 2013 (9)	Evaluates methods of measuring adherence in recently completed microbicide trials, reviews strengths and limitations of methods, provides recommendations for future trial design Defines 4 dimensions of adherence (use as instructed): initiation (first use), execution (how actual dosing corresponds to dosing regimen), discontinuation (product use is stopped), persistence (time between initiation and discontinuation) To understand effectiveness of product, need to measure timing and frequency in relation to infection risk, and sexual behaviours / practices that affect risk Discusses role of study population in assessing product efficacy: engaging partners could increase adherence Need to pay attention to trial communities in context of social and cultural issues; effect of site-specific cultural / economic factors on reporting of sensitive behaviours Bo-behavioural prevention: standardised measures to assess the dimensions of adherence and other behaviours needed for interpretation and comparison of results Need for respondent-independent measures; in interim use measures with complementary strengths; tools to measure adherence will depend on goal of researcher: to understand, quantify, influence, or all of these
Tolley EE et al, 2010 (18)	Reviews lessons learned from completed microbicide trials, with recommendations for future trials Describes 4 purposes of adherence data collection: determining effectiveness, adjuntive evidence to support results, understanding safety, monitoring acceptability and optimizing adherence

Authors, year	Brief summary of article and recommendations
	Discusses practical experiences with adherence measures and strengths and weaknesses: direct measures (biomarkers of semen exposure, applicator tests, drug level assays and other biologic measures); indirect objective measures (applicator / pill counts); indirect self-reported measures; discusses strategies for improving measurement: mixed-method including triangulation, composite measures and baseline identification of predictors of adherence Describes approaches for optimizing adherence: at point of trial design; during study start; and during the trial as dictated by monitoring Recommendations: clarity regarding purpose of adherence data; consideration of adherence assessment in trial design and analysis; need to develop, test and validate new measurement approaches and improve indirect and self-report approaches; optimizing adherence in the trial setting (motivational, personal diaries, adherence buddies, individualised feedback on observed adherence patterns); cross-trial data collection and sharing; developing guidelines for reporting and analysing adherence
Woodsong C et al, 2013 (2)	Based on data and experience from completed microbicide efficacy trials, describes 6 adherence lessons learned: adherence measurement in clinical trials; understanding of instructions for use; unknown efficacy and effect on adherence; partner influence on product use; retention and continuation; generalisability of adherence behaviour. Provides recommendations for future work
Amico KR et al, 2013 (27)	Describes the adherence support experiences from 4 PrEP trials: CAPRISA 004 (pericoital 1% TFV gel in women); FEM-PrEP ((daily oral TDF/TFC in women); iPrEX (daily oral TDF/FTC in MSM and Transgendered women); and VOICE (daily 1% TFV gel, oral TDF, and oral TDF/FTC in women) Estimated product use across the trials, sites and participants varied considerably, mostly lower than desired; most likely due to diversity of participant populations, culture and community; risk-perceptions; promoters and inhibitors of product use, and culture of sites Moving from biomedical to a bio-behavioural / bio-psycho-social framework will contribute to evidence for effective PrEP adherence interventions
Agakos SW, Gable AR, Eds Methodological Challenges in Biomedical HIV Prevention Trials, Chapter 5. Design Considerations: Adherence *National Academy of Sciences*, 2008	For trials showing overall benefit: interpretation of results should to be able to relate protective effect to adherence; For trials failing to show protective effect: need to distinguish whether non-effect is due to lack of product efficacy, lack of adherence, or increase in risky behaviour due to perception of protection; Understanding the predictors of non-adherence provides guidance into potential future HIV prevention interventions; Defining adherence by a single percentage may limit insights into adherence challenges, product acceptability, potential areas for intervention; Product adherence is complex – involves acceptance, execution and discontinuation of prescribed regimen; Compliance is persistence and quality of execution
Spilker B, 1992 (4)	Undetected poor compliance in clinical trials may result in inability to show protective effect and an effective medicine being labelled as ineffective; Direct methods to measure / evaluate compliance: observation, biological markers, levels in biological fluids, spot checks Indirect measures: pill counts, electronic counters, pharmacy refills, questioning the patient during treatment, medication monitors, physiological markers, patient diaries, assessment through a school nurse Elaborates on reasons for poor compliance and methods to improve compliance Ideal method of measuring compliance not yet available, but use of multiple methods will yield reasonable data for most clinical trials
Farmer KC, 1999 (7)	Choice of adherence measurement method based on usefulness, reliability considering researchers goal, and resources available Adherence assessment in clinical trial pertinent to assess dose-response relationship and valid analysis of product efficacy Elaborates on direct & indirect methods for measuring adherence and compares methods; Ideal adherence monitoring method: inexpensive, reliable & objective, providing continuous record of compliance history, unobtrusive, easy to use and analyse Efficacy studies (phase IIa and IIb) require accurate measure of adherence Phase III studies: choice may depend on length, number of participants and sites – where feasible, combination of methods most effective Phase IV: consider longitudinal methods (electronic review of prescription records) combined with self-report Clinical practice: adapted self-report
Mauck CK et al, 2012 (21)	A review of DSA & other techniques to assess adherence; Discusses findings of various studies of methods of measuring adherence: Dye-Stain Assay of Microlax, HTI and Low-density Polyethylene applicators using Tryptan blue and FD&C Blue No. 1; Gram staining; Ultraviolet light assessment; DNA multiplex polymerase chain reaction tests; MEMS, using Wisebag; Drug levels Discusses concern regarding whether informing participants of adherence measurement using these techniques will influence their adherence: references research amongst sex workers in India where prior knowledge of assessment by DSA did not improve self-report nor encourage gel use; suggesting in some settings applicator tests may be incorporated without changing behaviour Reiterates applicator tests cannot verify expulsion of gel into vagina; who used the applicator; use in relation to sex; can calculate maximum number of applicators used, but cannot account for applicators lost, discarded or shared Concludes that support to develop an objective marker of adherence is needed

Literature review: Microbicides

Authors, year	Brief summary of article and recommendations
Abdool Karim, Q et al, 2013 (25)	Discusses importance of Microbicides; history of efficacy trials; what's new in microbicide development, next steps in product development, bio-behavioural challenges and multipurpose technologies; and gaps in microbicide research Women-initiated ARV-based prevention methods including microbicides important for young women, especially in Sub-Saharan Africa Better assessment and measurement of HIV exposure needed, better understanding of vaginal HIV acquisition needed
Abdool Karim, S. et al, 2011 (17)	Proposed possible explanation for FEM-PrEP (daily oral TDF/FCT in women) trial results based on publicly available information and other PrEP studies: threshold concentrations for protection effect not yet been established; in CAPRISA 004, HIV incidence rate in women with TFV concentrations >1000 ng/mL was significantly lower as compared to placebo arm; tenofovir diphosphate concentrations are approximately 100-fold higher in rectal than vaginal tissues with TDF/FTC orally, and approximately 1000-fold higher in vaginal tissues with gel formulation than with TDF/FTC orally Effectiveness trials are crucial to provide information about effectiveness of TDF alone or in combination with FTC in preventing HIV in different populations, formulations, and routes of transmission; future clinical trials of tenofovir should aim for the highest tolerable drug concentration in vaginal tissues (until threshold required for protective effect is established); optimizing adherence in PrEP trials is critical; new PrEP formulations need to accurately assess required threshold concentrations of drug at site of HIV infection to confer protection
Kashuba, ADM et al, 2015 (19)	Investigated the pharmacokinetic–pharmacodynamic relationship of 1% TFV gel in topical HIV prevention Assayed cervicovaginal fluid, plasma, and paired tissue samples from vagina and cervix of 34 women who seroconverted with random sample of 302 women who maintained HIV negative status in CAPRISA 004 TFV concentration of >100 ng/mL in CVF associated with 65% protective effect, whereas a >1000 ng/mL concentration correlated with 76% protective effect

APPENDIX B: Competency Test Results: Primary assessor (assessor 1)

VIREA COMPETENCY TEST

Name: Chanelle Smith Date: 1 July 2015

APPLICATOR #	APPEARS USED	APPEARS UNUSED	COMMENT
1	✓		
2	✓		
3	✓		
4	✓		
5	✓		
6		✓	
7		✓	
8	✓		
9	✓		
10	✓		

TEST Score: 100%

Scored by: M. UPFOLD Date: 01 JULY 15

UV Light COMPETENCY TEST

Name: Chanelle Smith Date: 06 Jul 15

APPLICATOR #	APPEARS USED	APPEARS POSSIBLY USED	APPEARS UNUSED	COMMENT
1			✓	
2	✓			
3			✓	
4	✓			
5	✓			
6	✓			
7	✓			
8		✓		
9			✓	
10	✓			

TEST Score: 100%

Scored by: [illegible] Date: 06 Jul 15

APPENDIX C: Competency Test Results: Assessor 2

VIREA COMPETENCY TEST

Name: Michele Uptold Date: 1 JULY 2015

APPLICATOR #	APPEARS USED	APPEARS UNUSED	COMMENT
1	X		
2	X		
3	X		
4	X		
5	X		
6		X	applicator is clean, bp has gel residue only
7		X	applicator is clean, bp has gel residue only
8	X		
9	X		
10	X		

TEST Score: 100%

Scored by: M.UPFOLD Date: 01 JULY 15

UV Light COMPETENCY TEST

Name: Michele Uptold Date: 06 JUL 15

APPLICATOR #	APPEARS USED	APPEARS POSSIBLY USED	APPEARS UNUSED	COMMENT
1			X	spectles only
2	X			
3			X	" "
4	X			
5	X			
6	X			
7	X			
8	X			
9			X	" "
10	X			

TEST Score: 100%

Scored by: [illegible] Date: 06 JUL 15

APPENDIX D: VIREA Assessment log_Sensitivity & Specificity

VIREA Assessment log: Sensitivity and Specificity

Date assessed by VIREA		Initials of VIREA Assessor	
Start time hh:mm		End time hh:mm	

APPLICATOR #	APPEARS USED	APPEARS UNUSED	COMMENT
1			
2			
3			
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APPENDIX E: UV Light Assessment log_Sensitivity & Specificity

UV Light Assessment log _Sensitivity and Specificity

Date assessed by UV light		Initials of UV light Assessor	
Start time hh:mm		End time hh:mm	

APPLICATOR #	APPEARS USED	APPEARS POSSIBLY USED	APPEARS UNUSED	COMMENT
1				
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APPENDIX F: VIREA Assessment log

VIREA Assessment log

Participant ID	008 - -		
Empty applicators returned from Visit code		# Empty applicators returned	
Date issued / dispensed		Date returned by PPT	
Date assessed by VIREA		Initials of VIREA Assessor	
Start time hh:mm		End time hh:mm	

APPLICATOR #	APPEARS USED	APPEARS UNUSED	COMMENT
1			
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QC DATA Check (initials)		Excel data entry (initials)	

APPENDIX G: UV Light Assessment log

UV Light Assessment log

Participant ID	**008 - -**		
Empty applicators returned from Visit code		**# Empty applicators returned**	
Date issued / dispensed		**Date returned by PPT**	
Date assessed by UV light		**Initials of UV light Assessor**	
Start time hh:mm		**End time hh:mm**	

APPLICATOR #	APPEARS USED	APPEARS POSSIBLY USED	APPEARS UNUSED	COMMENT
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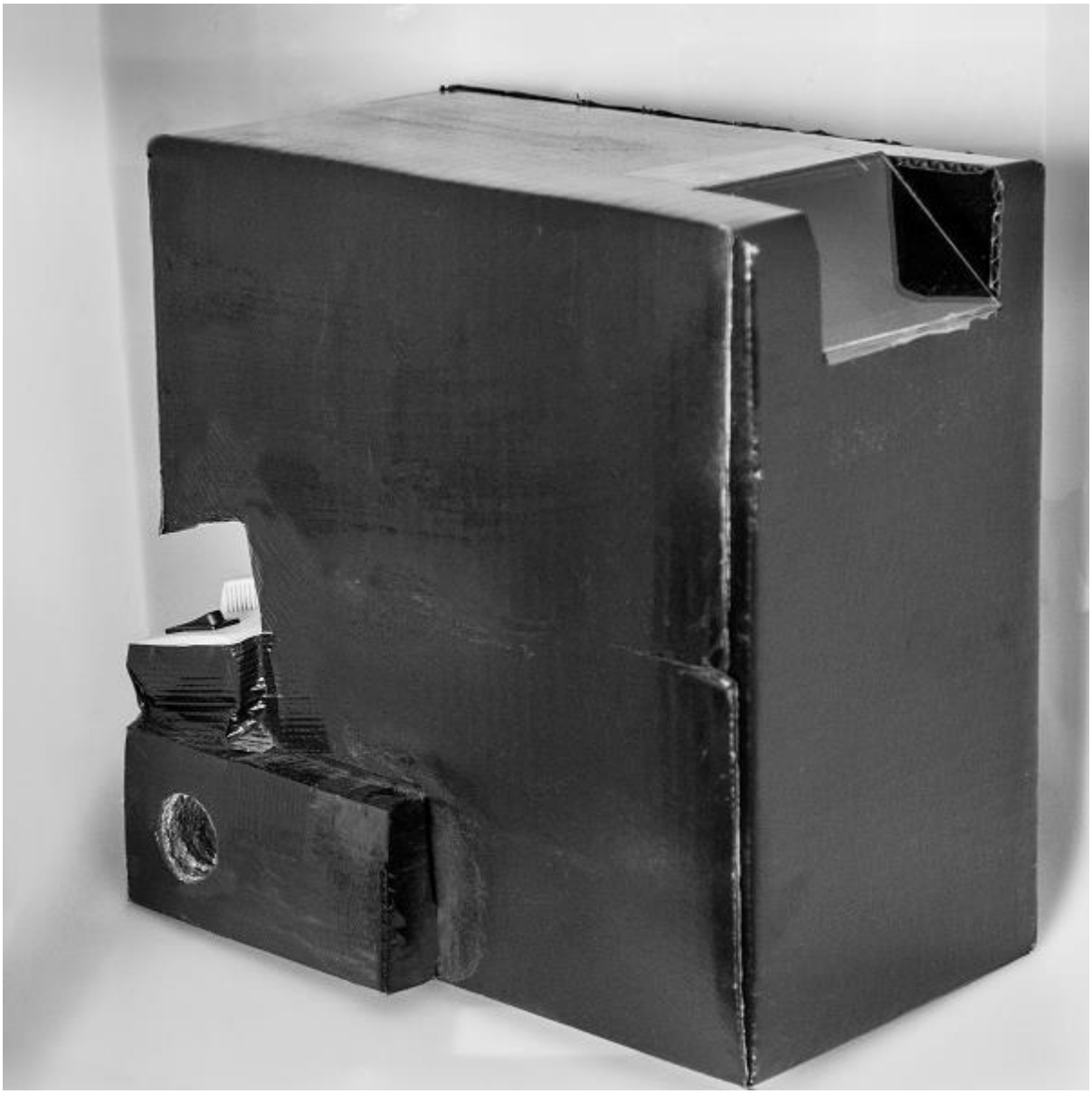
QC data check (initials)		**Excel Data entry (initials)**	

APPENDIX H: UV light viewing box



APPENDIX I: UKZN BREC Approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

29 May 2015

Mrs M Upfold (205525637)
Pharmaceutical Sciences
Health Sciences
Michele.upfold@caprisa.org

Protocol: Measurement of vaginal microbicide adherence using visual inspection as compared to ultra violet light assessment of returned empty gel applicators.
Degree: MSc
BREC reference number: BE083/15

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 24 March 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 20 May 2015 to queries raised on 17 May 2015 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from **29 May 2015.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **14 July 2015.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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APPENDIX J: Response from Journal

Michele Upfold

From: em.aibe.0.46ed32.bdb5eea8@editorialmanager.com on behalf of AIDS and Behavior (AIBE) <em@editorialmanager.com>
Sent: 03 November 2015 03:13 PM
To: Michele Upfold
Subject: Acknowledgement of Receipt

Dear Mrs Upfold:

Thank you for submitting your manuscript, "Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators", to AIDS and Behavior.

During the review process, you can keep track of the status of your manuscript by accessing the following web site:

http://aibe.edmgr.com/

Your username is: Michele Upfold
Your password is: available at this link
http://aibe.edmgr.com/Default.aspx?pg=accountFinder.aspx&firstname=Michele&lastname=Upfold&email_address=michele.upfold@caprisa.org

With kind regards,

The Editorial Office
AIDS and Behavior

AIDS and Behavior

Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators

--Manuscript Draft--

Manuscript Number:	AIBE-D-15-00802
Full Title:	Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators
Article Type:	Original Research
Keywords:	Adherence; Visual Inspection; Ultraviolet Light; Returned applicators; Microbicides
Corresponding Author:	Michele Upfold, BScPharm CAPRISA Durban, KwaZulu-Natal SOUTH AFRICA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	CAPRISA
Corresponding Author's Secondary Institution:	
First Author:	Michele Upfold, BScPharm
First Author Secondary Information:	
Order of Authors:	Michele Upfold, BScPharm
	Anneke Grobler, MSc, PhD
	Fatima Suleman, B. Pharm, M.Pharm, PhD
	Leila Essop Mansoor, B.Pharm, PhD
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Accurate and objective measurement of adherence is critical in microbicide trials. We compared 2 applicator tests: visual inspection of returned empty applicators (VIREA) and ultraviolet light (UVL) assessment in terms of sensitivity and specificity, and for concordance. Sensitivity and specificity analysis of 24 control applicators at 4-months after receipt was 75.0% and 66.7% for VIREA and 83.3% and 91.7% for UVL, respectively. After an additional 3 months of storage sensitivity and specificity was 100% and 58.3% for VIREA and 100% and 66.7% for UVL, respectively. In January 2015, 1316 empty applicators were returned as used by 115 participants enrolled at one site in a randomized controlled trial. Assessment outcomes showed 78.8% agreement between the techniques. Methods concurred that 22% of the returned empty applicators did not appear to be used. By UVL assessment, 40% of returned empty applicators had no evidence of vaginal insertion, translating to 28% less product used as compared to that returned as used by women. UVL assessment may be considered a more accurate and less subjective measure of adherence as compared to VIREA.
Suggested Reviewers:	Sarah Cohen scohen@wrhi.ac.za
	Andrea Thurman thurmaar@evms.edu

Cover Letter

CAPRISA
CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA

UNAIDS
CAPRISA is the UNAIDS Collaborating Centre for HIV Research and Policy

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Editors in Chief
Dr. Seth C. Kalichman, Ph.D
Center for HIV Prevention & Intervention
2006 Hillside Road, Unit 1248
University of Connecticut
Storrs, CT 06269

Email: aidsandbehavior@yahoo.com

03 November 2015

Dear Dr. Kalichman

Re: Original article for publication

We wish to submit an original article titled "Measurement of Vaginal Microbicide Adherence using Visual Inspection as Compared to Ultra Violet Light Assessment of Returned Empty Gel Applicators" for review and publication.

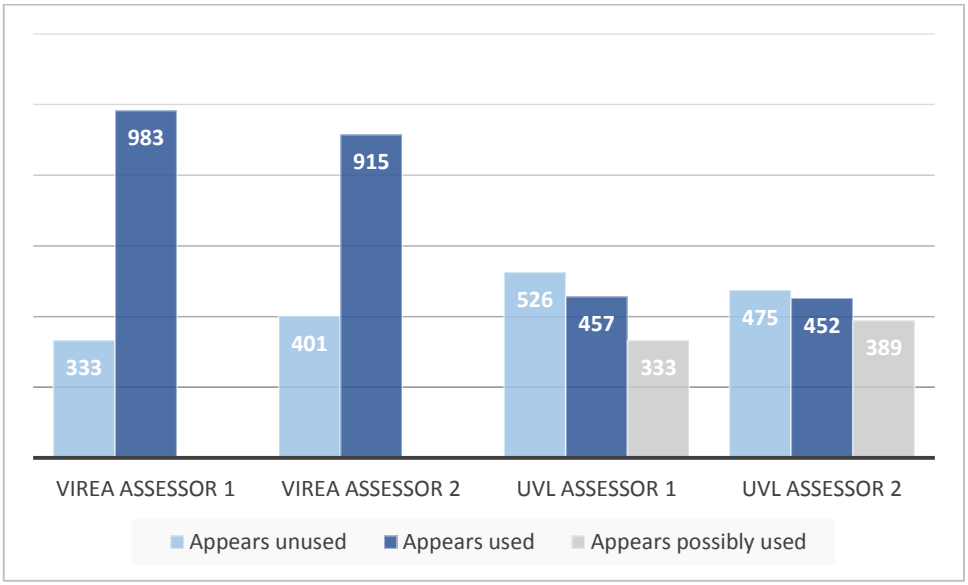
This manuscript has not been published in this or a substantially similar form (in print or electronically, including on a web site), nor accepted for publication elsewhere, nor is it under consideration by another publication. All authors have read and approved the paper, and have met the criteria for authorship as established by the International Committee of Medical Journal Editors.

Michele Upfold (Corresponding Author)

michele.upfold@caprisa.org

Tel: +27 33 260 6896

APPENDIX K: Outcomes of assessment for inter-assessor agreement



Outcomes of assessment by VIREA and UVL for assessor 1 and 2 (n = 1316)

APPENDIX L: Supervisor-Student Memorandum of Understanding

CHS Supervisor-Student Memorandum of Understanding

This memorandum states the responsibilities of the supervisor(s) and postgraduate student and requires both parties to accept the responsibilities by signing.

Details of Student, Supervisors, and Project

Student Name: MICHELE UPFOLD
Student Number: 205525637
School: PHARMACEUTICAL SCIENCES
Degree: MASTERS IN PHARMACY (PHARMACY PRACTICE)
Supervisor(s): DR. L.E. MANSOOR, PROF. F SULEMAN
Research Topic: MEASUREMENT OF VAGINAL MICROBICIDE ADHERENCE
Date 18 MAY 2015

Responsibilities of the Postgraduate Student

While there are many responsibilities carried by a student in pursuing postgraduate studies the following are the minimum expected.

1. Student should identify a research topic acceptable to the supervisor in order to register
2. Student must show commitment to the degree programme and undertake to produce a full proposal within 3 month of registering
3. Student must produce written work that is their best effortt for comments by the supervisor
4. Student should meet at least once per month (in person or through skype) with the supervisor and have the courage to request for such meetings. In all such meetings the student should provide a brief report of their work and take minutes of the discussions and retain such records until the degree has been awarded
5. Students must keep a laboratory manual where all experimental procedures and data are recorded. This laboratory manual remains the property of the university
6. Student must demonstrate the highest level of scientific honesty at all *stages (proposal writing, seeking ethical approval, collecting data, analyzing data and writing thesis or manuscripts)* of the degree programme.
7. Students must familiarize themselves with the university's policy on Plagiarism
8. Students should follow the advice provided by the supervisor and if they choose not to they should discuss the matter with the supervisor immediately
9. Student must always inform the supervisor of their whereabouts
10. Student should keep up to date with literature in their field of study and share any new literature they come across with the supervisor
11. Student must agree to complete studies within the time specified in the CHS handbook for the specific degree programme
12. Student should allow the supervisor to publish their work if they do not do so or show interest one year after graduating on the understanding that the student will be co-author

Responsibilities of the Supervisor

1. Supervisor must support student at all stages of the degree programme *(settling down, proposal writing, ethical applications, data collection, data analysis and write up of thesis or manuscripts)*
2. Supervisor must be sensitive to the overall well-being of the student
3. Supervisor must have good knowledge of the research area of the student
4. Supervisor must be available to the student and should have regular meetings (face to face or by skype) with the student. If the supervisor must be away for an extended period they should identify a co-supervisor to assist the student during that period
5. Supervisor must read work submitted by student for comments and give feedback within 3 weeks depending on the nature of the work submitted

1

6. Supervisor must be constructively critical to the student's work
7. Supervisor must have sufficient interest in the work of the student
8. In instances of co-supervision the supervisors must avoid confusing the student by giving conflicting opinions/comments. If there are differences in opinion those should be discussed among the supervisors and the student given the agreed opinion.
9. Supervisor should, where funds permit, facilitate arrangements for masters and doctoral students to present a paper or a poster at an international conference as part of training
10. Supervisor must provide an annual progress report on the research and progression of the student to the discipline
11. Supervisor must protect the work of the student by not pre-maturely publishing it or assigning another student to similar work
12. Student must always be the first author of their work and any co-authorship with other people not on the supervision team should be clarified at an early stage of the project

Conflict Resolution

Should there be a conflict or disagreement between supervisor and student which cannot be resolved by the parties involved, then either party can approach the Academic Leader Research or Dean and Head of School (or the College Dean of Research if the Dean and Head of School is one of the conflicting parties) about the conflict. The Dean and Head of School (or College Dean of Research) will then either arbitrate or choose a senior academic of the School not involved in the conflict to arbitrate. The arbitrator's decision is final and cannot be appealed.

Signatures:

Student.....

Supervisor.....

Co-Supervisor(s).....

Academic Leader Research or D&HoS.....