Urine tenofovir point-of-care test to identify patients in need of ART adherence support (UTRA study): **Aim 3**

CORE STUDY TEAM:

Gert van Zyl: Co-principal Investigator

Division of Medical Virology**,** Faculty of Medicine and Health Sciences, Stellenbosch University

guvz@sun.ac.za

Catherine Orrell: Co-principal Investigator

Desmond Tutu Health Foundation, University of Cape Town

Catherine.Orrell@hiv-research.org.za

Monica Gandhi: Co-principal Investigator

Center for AIDS Research (CFAR), University of California San Francisco (UCSF)

Monica.Gandhi@ucsf.edu

David Glidden: Biostatistician

University of California San Francisco (UCSF)

David.Glidden@ucsf.edu

Graeme Hoddinott: Socio-behavioural Science Lead

Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University

graemeh@sun.ac.za

Eric Decloedt: Clinical pharmacologist

Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University

ericdecloedt@sun.ac.za

Tracy Kellermann: Research pharmacologist

Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University

tkellermann@sun.ac.za

Marije van Schalkwyk: Clinician

Division of Infectious Diseases, Department of Medicine, Faculty of Medicine and Health Sciences

Stellenbosch University

marije@sun.ac.za

SITE RESEARCHERS:

Yashna Singh:

Desmond Tutu Health Foundation, University of Cape Town

yashna.singh@hiv-research.org.za

Lauren Jennings:

Desmond Tutu Health Foundation, University of Cape Town

lauren.jennings@hiv-research.org.za

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BACKGROUND AND RATIONALE

Viral load (VL) monitoring is essential for individual management and for treatment as prevention but is costly: Achieving a 90% suppression rate in patients receiving antiretroviral therapy (ART) is the third UNAIDS 90-90-90 target and is essential for optimizing the individual health benefits of ART. Viremic patients are also a source of ongoing transmission and achieving virological suppression in all patients with HIV is a cornerstone of treatment as prevention (TasP).[1–4] Preventing transmission and improving individual benefit requires timely identification of patients with virologic failure (VF) in order to support adherence to achieve resuppression of viral load and, in cases where adequate adherence does not achieve resuppression, trigger second-line ART (preceded by HIV drug resistance testing in resource rich settings). Therefore, the World Health Organization (WHO) recommends routine VL monitoring every 6-12 months.[5] Despite evidence of the cost effectiveness of VL, this testing increases the financial burden of HIV monitoring in high-prevalence limited-resource settings. For instance, South Africa currently has more than 7.52 million individuals living with HIV, all requiring life-long treatment and monitoring.[6] Although point-of-care (POC) VL tests may in future provide immediate feedback regarding virologic failure, current commercially available assays remain costly, require trained operators and have limited throughput.[7] Therefore, treatment programs in high-burden settings continue to rely on laboratory-based VL testing but use them less frequently than is ideal. An alternative low-cost, low complexity assay that would reduce the need or frequency of VL testing without increasing the risk for failure would therefore help to ensure sustainability of HIV treatment programs worldwide.

A low-cost point-of-care test to monitor adherence to antiretroviral therapy (ART) has recently been developed: Adherence is critical for maintaining the efficacy of ART. Self-reported adherence is subject to both social desirability and recall biases, so pharmacologic adherence measures, where drug concentrations are measured in a biological matrix, provide objective and more accurate predictors of virologic suppression.[8-19] However, no current real-time metric of adherence to antiretrovirals (ARVs) at the point of care has existed until now. The current techniques to measure ARVs in plasma,[20] urine,[21] dried blood spots (DBS),[22] or hair,[23] all require spectrometry machines and laboratory-based personnel, making them impractical to incorporate into routine clinical care. Antibody-based tests, when converted into lateral flow assays, are low-cost, enable drug detection to occur within minutes, and are easy to perform by non-trained personnel.[24,25] The most well-known lateral flow immunoassay is the urine-based pregnancy test.

Tenofovir disoproxil fumarate (TDF)/ lamivudine (3TC) or emtricitabine (FTC) are the backbone of ART regimens used worldwide. South Africa is rolling out dolutegravir (DTG)/TDF/3TC (TLD) as first-line treatment across the country; and exploring the use of TLD in second-line for those failing a first-line non-nucleoside reverse transcriptase-based (NNRTI) regimen.[26,27] Therefore, a low-cost adherence metric that could be deployed in resource-limited settings should target tenofovir (TFV, the breakdown product of TDF in plasma and urine); as this drug is likely to be utilized in both first- and second-line ART. The UCSF Hair Analytical Laboratory and Alere/Abbott™ Rapid Diagnostics recently developed a

Figure 1: Urine-based lateral flow assay for tenofovir

urine-based immunoassay for tenofovir (**UTRA: urine tenofovir rapid assay**).[21,28] Using samples from a directly-observed therapy (DOT) study of HIV-noninfected volunteers provided TDF/FTC,[29] the specificity and sensitivity of the immunoassay to quantitate tenofovir in urine compared to the gold standard method of liquid chromatography-tandem mass spectrometry (LC-MS/MS) is 99% and 94%, respectively.[28] Moreover, the DOT study allowed us to determine the appropriate adherence cut-off for the point-of-care (POC) lateral flow assay. An immunoassay cut-off of 1500ng/mL accurately classified 98% of patients who took a dose within the past 24 hours as adherent.[28] Urine collection is noninvasive[30] and preferred over blood sampling.[31,32] The lateral flow assay to detect tenofovir in urine has now been developed (**Figure 1**) and further validated against LC-MS/MS (preliminary data section). The test is low cost (<$2 per assay), provides results in ~2 minutes, and has been used in the first part of this study.

Low-cost urine tenofovir rapid assays (UTRA) could be used more frequently than VL testing to predict impending failure: Low urine concentrations of tenofovir in a large pre-exposure prophylaxis (PrEP) demonstration project heralded future seroconversion events.[33] In the setting of routine clinical care, this test could be performed at every visit to provide patients with 'real-time' feedback on their practices.

There is limited information on the pattern of adherence resulting in failure on new regimens: Interruption of treatment for periods longer than 48 hours has been associated with an increased risk of failure of non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens,[34] whereas average adherence on protease inhibitor (PI)-based regimens is predictive of failure.[35] The patterns of adherence associated with failure of modern integrase inhibitor regimens are not yet known.

Beyond pharmacologic adherence testing, other biomedical assays used to unravel the etiology of failure include HIV VL and HIV drug resistance testing (HIVDRT). When combined, these biomedical assays can assess which patterns of adherence are predictive of failure (**Table 1**). The utility and feasibility of a particular biomedical assay or combination of assays to unravel the etiology of virologic failure is dependent on assay availability, performance, practicality, and cost. Tenofovir diphosphate (TFV-DP) concentrations in dried blood spots (DBS) are currently one of the most commonly used metrics of long-term adherence in research settings.[36–38] However, their cost, complexity, and turn-around time make them less feasible to employ as routine metrics of monitoring adherence in resource-limited settings.

In contrast to the longer-term measures, drug concentrations in both plasma and urine provide information on adherence over short time frames (1-7 days). Therefore, adherence monitoring via these matrices may be susceptible to "white coat adherence", where adherence improves transiently before a visit (**Table 1**). Despite this theoretical concern, this phenomenon has not yet been observed; indeed, plasma concentrations of TFV/FTC served as the "gold standard" adherence metric in every one of the placebo-controlled trials of TDF/FTC-based PrEP.[20,39–46] Across these trials, plasma PrEP concentrations provided robust and critical information for interpreting trial results. However, further study, including Aim 1 of this study, which collected both short-term and long-term adherence metrics, will determine the susceptibility of the short-term urine assay to "white coat" patterns in more real-world settings.

Table 1: Pros and cons of biomedical assays to measure adherence and therapy success					
	UTRA	Hair TFV	TFV-DP in DBS	Plasma VL	HIVDRT
True point of care	✓	x	x	x *	x*
Low cost	✓	x	x	x	x
Not susceptible to white coat adherence	x	✓	✓	✓	✓
Cumulative measure of adherence	x	✓	✓	✓	✓
Differentiates poor adherence from drug resistance	x#	✓	✓	x	✓
Non-invasive	✓	✓	x	x	x

**POC assays in development – current near patient assays are relative high complexity, costly, have limited throughput and require trained staff, making their current iterations impractical for scaling in RLS;*
#In conjunction with VL could identify cases of failure with current poor adherence.

In our setting, undetectable urine tenofovir was strongly associated with drug resistance in those on NNRTI-based ART. This study is the second part of a proposal with three aims. In **Aim 1**, a pilot cross-sectional study, we enrolled patients from the Gugulethu Community Health Centre ART service who were taking tenofovir-containing ART, and were at risk of virologic failure, as evidenced by a recent raised VL and/or gap in treatment (confirmed by pharmacy refill data). In an interim analysis we found that only 2 of 48 (4%) participants to date have had undetectable UTRA tests (despite 38% experiencing virologic failure). Both cases with negative UTRA tests had either very low or undetectable TFV-DP concentrations in DBS (showing high specificity for poor adherence). The combination of an undetectable UTRA test and VF was 100% sensitive in predicting resistance on a low genetic barrier efavirenz-based regimen. Therefore, the combination of UTRA and VL testing could be used to target follow-up resistance testing or to prioritize transition to second-line regimens.

The utility of adherence reinforcement may, however, be dependent on the genetic barrier of the ART regimen and whether episodes of reduced adherence are detected and managed early. Periods of treatment interruption are strongly predictive of treatment failure and drug resistance on NNRTI-based regimens.[34,47] Indeed, many of these patients may already have drug resistance when identified with virologic failure[48,49] and may not achieve VL suppression despite high levels of adherence currently.[50] In contrast, drug resistance may take much longer to develop in patients treated with antiretrovirals with a high genetic barrier to resistance, such as protease inhibitor (PI) and second-generation integrase strand transfer inhibitor (INSTI), with adherence support allowing subsequent VL re-suppression.[51,52]

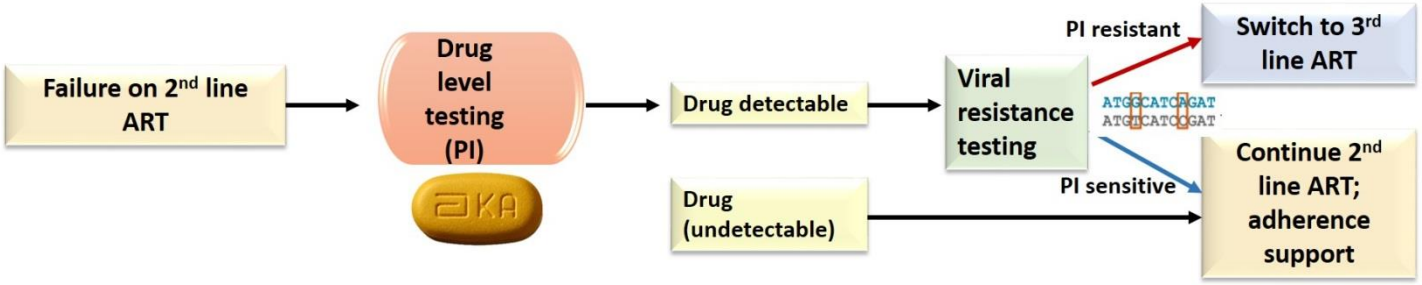
UTRA may help health care workers to elicit reasons for poor adherence: Aim 2 of this study assessed patients' and healthcare workers' perceptions about integrating the UTRA into adherence support. We conducted a series of once-off in-depth qualitative interviews with people living with HIV (PLHIV) (n=25) and health workers (n=5) from the Gugulethu CHC. The Aim 1 cohort was purposely sampled to maintain diversity in age and gender. Health workers were purposively sampled for 'rich-cases' (i.e., people with extensive experience in delivering ART services to patients in the setting) and diversity by profession.

Many PLHIV were willing to use UTRA because they were interested to know their adherence. Waiting for lab-based test results means that informing people about adherence is often long-delayed and typically only occurs in the context of a remedial intervention for poor adherence. Contrary to expectations that UTRA may be perceived as unwanted additional monitoring, the PLHIV in this evaluation were enthused by the opportunity to receive their results in real-time which they believed could lead to constructive discussions with their health workers. Participants were comfortable providing the urine, as it is a harmless, non-invasive procedure. In addition, PLHIV participants reported that the UTRA test would encourage them to take their treatment regularly because they could then show off this good adherence at their next clinic visit. Health workers reported that UTRA may offer earlier and more accurate assessment of patients' adherence, so enabling them to prepare earlier intervention on patients at risk of virologic failure.

Low failure rate and low rates of resistance in patients receiving high genetic barrier regimens warrant novel approaches to monitoring therapy success: Integrase strand transfer inhibitor (INSTIs) containing regimens, have been associated with rapid rates of VL suppression,[53–55] and favorable side effect profiles. With the use of second generation INSTIs, specifically dolutegravir (DTG), the development of INSTI-associated mutations in the face of virologic failure when used as first-line therapy in INSTI-naïve patients is rare.[56] Therefore, the planned rollout of TDF, lamivudine and DTG (TLD) fixed-dose-combination therapy as first-line, or possibly second-line, therapy worldwide will require a new approach to therapeutic monitoring, as drug resistance will be rare and switching to a second-line regimen infrequently warranted.

Drug concentration monitoring, when applied to drugs with a high genetic barrier to resistance, has proven useful: Protease inhibitors have a high genetic barrier to resistance. In a large study of protease inhibitor-based 2nd line ART failure from South Africa (the ITREMA study),[57] protease inhibitor (PI) drug concentrations were measured in plasma at the time of failure to evaluate whether they were predictive of viral resistance to the PI (**Figure 2)** PI viral resistance was shown to be infrequent (26.7%) and negative PI concentrations excluded PI-associated mutations with a high degree of certainty (negative predictive value 95%). This **argues for drug concentration testing when drugs with a higher genetic barrier to resistance are used to assess the subsequent necessity of costly resistance testing**. A similar relationship is expected for DTG-based regimens, as we assess our assay during the roll-out of TLD as first- and second-line, given that DTG has a high genetic barrier to resistance like PIs.

Figure 2: Algorithm proposed by ITREMA trial findings and how POC adherence testing could be useful for 2nd line failure



In the third part of this study, AIM 3, we will conduct a randomised study to determine the utility, feasibility and acceptability of UTRA as an adherence support tool in people on antiretroviral therapy regimens containing high-genetic barrier antiretrovirals e.g. dolutegravir and boosted lopinavir.

AIM:

- To determine the impact of feedback from the UTRA in an adherence-challenged population taking ART with a high genetic barrier to resistance, as compared to standard-of–care (SoC) adherence support.
- To assess the acceptability and feasibility of integrating the UTRA into adherence support amongst patients and healthcare workers.

HYPOTHESES

- Providing real-time information from the UTRA will allow extra opportunities to address poor adherence in the intervention arm and will result in improved retention and virological outcomes compared to the SoC group.
- UTRA will be acceptable and feasible to participants and health care providers.

STUDY DESIGN

This is a randomised study, recruiting 200 people taking TDF-based ART. Participants will be randomised 1:1 to intervention versus standard enhanced adherence counselling. Intervention participants will receive an adherence support package informed by feedback from the urine-based test. The study will assess the impact of the adherence test on VL suppression rates in each arm (without necessitating a regimen switch) at 12 months after enrolment. Enrolling participants with adherence challenges increases efficiency and mirrors the population requiring adherence support in our setting.

STUDY SITE

The study will take place at the Hannan Crusaid Treatment Centre (HCTC), a primary health care facility within the Gugulethu Community Health Centre (CHC) that is broadly representative of the spectrum of primary health care services across South Africa. Gugulethu (population 400 000) is located 15 kilometres (km) outside Cape Town. Residents are predominantly of low socioeconomic status and the majority use free, local public sector health services. HCTC is one of the Western Cape's largest ART clinics with more than 18000 patients initiated on ART in the past decade and more than 8500 currently enrolled in care (January 2021).

RECRUITMENT AND ENROLMENT:

The study will recruit 200 participants attending the HCTC for ART who are at current increased risk of virologic failure, due to having a previously recorded viral breakthrough (≥50 copies/ml).

Individuals will be identified at routine clinic appointments through a folder review conducted by study staff. These patients will be approached by study staff and the study will be explained to them. Should they be interested, they will be screened for eligibility. Full written informed consent in their chosen local language will be obtained prior to any study procedures. If eligible they will be invited to participate in the study. The informed consent document will explain the study in detail and briefly outline all procedures.

Participant Inclusion criteria:

- ≥18 years old
- Willing and able to provide written informed consent
- HIV-infected, receiving or (re-)starting a tenofovir-based ART regimen
- Current ART regimen includes at least one drug with a high genetic barrier to resistance e.g. dolutegravir, atazanavir/ritonavir, darunavir/ritonavir or lopinavir/ritonavir.
- Any previous raised viral load >50 copies/ml (after ART initiation).
- Willing and able to comply with laboratory tests and other study procedures

Participant Exclusion criteria:

- Not willing or able to provide informed consent in any of the languages provided
- Not receiving a tenofovir-based ART regimen
- Any other clinical condition that in the opinion of an investigator puts the patient at increased risk if participating in the study.

We will recruit a sub-sample of ~20 patient participants (2:1 receiving intervention to controls) from the above cohort for in-depth interviews. Participants will be selected by sampling for diversity (according to levels of adherence), rich-case sampling, and sampling to saturation.

In addition, we will recruit ~10 health care workers from the study health facility to participate in in-depth interviews, ideally including the 5 health care workers involved in Aim 2 (see introduction above). The health care workers must be willing and able to provide written informed consent; and be experienced in delivering adherence support at the study health facility, or knowledgeable about DoH ART programmes.

STUDY PROCEDURES

SCHEDULE OF EVENTS:

Table 2: Schedule of events

Study procedures	**Visit 1: Recruit-ment and screening**	**Visit 2: Enrolment (with 42 days of screen)**	**Visit 3: Month 3**	**Visit 4: Month 6**	**Visit 5: Month 9**	**Visit 6: Month 12**
Informed consent*	X					
Locator information	X	X	X	X	X	X
Demographic and disease data	X					
Confirm ART regimen	X	X	X	X	X	X
Eligibility check	X	X				
Randomisation		X				
Stored blood sample (for viral load (VL), plasma tenofovir, DBS TFV-DP, drug resistance genotype)[#]		X		X		X

Stored blood sample for future analysis[$]		X		X		X
Haematocrit		X				
Height		X				
Weight, hip circumference, waist circumference		X		X		X
Standard of care viral load	X					
Adherence support (SoC or Intervention)	X					
Urine sample for UTRA ** with adherence discussion - **intervention arm only**		X	X	X	X	X
Self-reported adherence assessment	X	X	X	X	X	X
Pharmacy refill data assessment	X	X	X	X	X	X
In-depth interview***			X	X	X	
Acceptability questionnaire		X		X		X

* Informed consent will include permission for the study team to review prescribed medication in their folder or from the Provincial Health Data Centre (PHDC) database and to access laboratory results via NHLS.

Plasma will be stored for study viral loads at enrolment, 6- and 12 months, which will be performed at the study endpoint. Cases with VL> 400 copies/mL will have genotypic drug resistance testing. DBS will be stored at enrolment, 6 and 12 months and plasma at the same time points; in cases with virologic failure and controls with suppression a nested case design will compare TFV plasma levels and TFV-DP in DBS.

$ Future assays related to the study objectives will only focus on this study question which include investigating the factors associated with antiretroviral treatment failure (e.g. markers of cerebral small vessel disease, antiretroviral therapy adverse events) and antiretroviral drug exposure in plasma.

** UTRA completed for all participants in the intervention arm at visits 2-5. Urine stored for future tenofovir concentration analysis.

***Qualitative interviews completed in a subset of participants.

VISIT PROCEDURES

1. **Demographic and disease-status data:** age, gender, WHO stage and CD4 cell count as well as current disease status and ART history will be collected from the patient and through folder review.

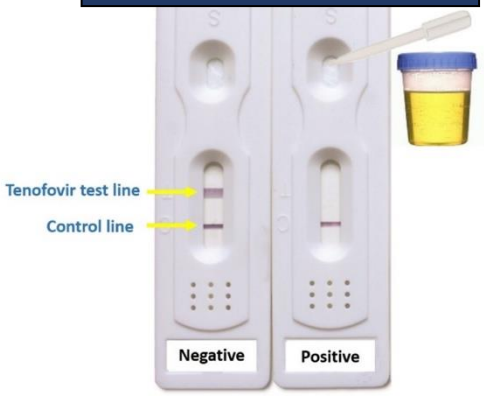
2. **Blood sample for viral load:** Plasma will be stored for viral load analysis at the end of the study.

3. **HIV drug resistance testing:** Plasma will be stored for HIV drug resistance testing. HIV drug resistance testing will be performed in participants with VF (VL<400 copies/mL) only. HIV drug resistance testing after first-line VF is not standard of care in resource-limited settings. In this study, HIV drug resistance results will be reported back to the caregivers.

4. **TFV-DP testing in DBS:** 50 µl of whole blood collected by venipuncture into EDTA tubes without centrifugation will be accurately pipetted onto Whatman 903 protein saver cards (or equivalent) (five 50 µl spots per card). The pipetting onto the protein saver cards should be done within 1 hour of collection of whole blood into the EDTA. The protein saver card will be air dried at room temperature for at least two hours prior to storing in a sealed bag with desiccant sachets and humidity indicators. The sealed bags will be stored in cryoboxes at -20°C. At the laboratory, the TFV-DP concentration will be determined using a validated assay.

5. **Urine sample for UTRA:** Urine will be collected into a cup at each study visit for those in the intervention arm. The urine will be analysed by the LFA illustrated in Figure 3 to provide a yes/no value for adherence to tenofovir over the past 4 days. A counselor will explain and interpret the result of the POC urine tenofovir assay at the end of the visit. The urine will be stored to assess the appropriateness of UTRA thresholds for quality assurance and future assessment of the performance characteristics of the UTRA assay in detecting tenofovir in urine in this population.

Figure 3: Negative and positive UTRA test



6. **Self-reported adherence:** Participants will be asked for the date and time of their last ART dose taken (to correspond with urine-based TFV measure). They will also be asked to provide a self-report of their adherence over the past 30 days using a three-item scale (to correspond with the TFV-DP in DBS measure).[58]

7. **Pharmacy refill adherence:** Pharmacy refill data over the past calendar year will be collected from folder review and from the central pharmacy database associated with the Gugulethu Community Health Centre.

8. **Covariates of antiretroviral pharmacokinetic variability:** ARV dosing time, DBS sampling time, weight, height, waist circumference, hip circumference, most recent creatinine (extracted from routine laboratory results) and haematocrit (extracted from DBS sample).

9. **Inclusion and exclusion criteria:** will be checked at both screening and enrolment visits.

10. **Sample storage for future analyses:** We will store blood for additional markers that may become of interest during the study. Future assays related to the study objectives will only focus on this study question which includes investigating the factors associated with antiretroviral treatment failure such as markers of cerebral small vessel disease, antiretroviral therapy adverse events antiretroviral drug exposure in plasma.

RANDOMISATION:

100 random numbers between 1 and 200 will be generated in excel and assigned as intervention by the data team. Study arm assignments will be placed in sequential sealed envelopes and delivered to site. Envelopes will be opened at the time of randomization by the Medical Officer/study coordinator.

STANDARD OF CARE ADHERENCE SUPPORT:

If a participant's VL test shows elevated virus (>50copies/ml), he/she is informed of this result at the subsequent clinic visit and referred to adherence support. This support is given via a) a VL driven counselling session with a clinician and b) a peer counselling session (as per the MINIMUM PACKAGE OF INTERVENTIONS TO SUPPORT LINKAGE TO CARE, ADHERENCE AND RETENTION IN CARE, Standard Operation Procedures, published by the SA Department of Health, March 2020).

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Table 3: Standard of Care Adherence Support

Clinician's role	Counsellor's role	Patient's role:
a. Screen patients as recommended in the clinical guidelines to monitor adherence to treatment including review results from previous assessment. b. Explain abnormal result to the patient. c. Careful review of patient's adherence history. d. Determine if abnormal result is likely to be adherence related and if so, refer for EAC. e. Assess and manage side effects swiftly. f. Screen and provide treatment based on clinical guidelines. g. Consider switching to alternate regimen as per clinical guidelines. h. Emphasize importance of treatment continuation. i. Ensure communication between facilities when the patient is referred to another facility.	a. Educate on abnormal result and that adherence challenges are the common cause. b. Check that patient is taking treatment regimen correctly (no misunderstandings). c. Assess and address barriers to adherence. d. Assess misconceptions and beliefs about treatment. e. Provide support strategies to overcome barriers such as taking treatment even if drinking alcohol. f. Set new goals for next assessment such as having undetectable VL (<50 copies/ml), BP <140/90, HbA1c ≤7% or negative sputum (or revised thresholds in updated clinical guidelines). g. Encourage excellent adherence to influence next result.	a. Express barriers to adherence (if any) and potential reason for treatment failure. b. Review and adapt adherence plan with counsellor. c. Set new treatment goals. d. Adhere to treatment. e. Come for next appointment and inform the staff of any changes of contact number or address or if travelling.

Intervention Enhanced Adherence Support:

A study counsellor will explain and interpret the result of the POC UTRA and conduct an adherence discussion at the end of each visit. Participants with no tenofovir in their urine will be referred for SoC adherence support. Participants in the intervention arm will therefore have additional engagement with health care providers and an increased chance of receiving adherence support.

Visit Windows:

Enrolment must take place within 6 weeks of the screening visit. If this period is exceeded, the screening visit can be repeated.

Follow up visits will occur in 12-week increments post-enrolment: at month 3, month 6, month 9 and month 12; and will be synchronized wherever possible with routine clinic visits. Visit windows will be continuous, with each window extending to 6 weeks before and 6 weeks after the visit target date.

A visit will be considered missed if the participant does not attend during the 12-week visit window.

Retention

Participants will be called the day before a scheduled visit as a reminder. If the participant is unable to attend a scheduled visit, the visit will be rescheduled to a new date within the visit window.

If a participant does not attend a scheduled visit, study staff will attempt to contact them telephonically. If they are unable to contact the participant after three attempts at different times of day, they will conduct a home visit if it is feasible to do so.

Feasibility:

We will track retention in the study among participants in each arm, missed visits, and the number of urine assessments performed in the intervention arm.

Acceptability:

All participants enrolled will also complete a standardised questionnaire about the acceptability of the adherence support they received. This measure of acceptability will be assessed as a secondary outcome by arm, along with socio-demographics profiles associated with low/high acceptability described within arm.

Qualitative procedures:

1. **Participant in-depth interviews**: Qualitative data will include in-depth interviews of participants (n~20; ~3-4 interviews with each participant over 6-9 months of their treatment) using semi-structured guides conducted by experienced socio-behavioural scientists. The semi-structured interview guide will elicit feelings about the urine adherence metric and counseling messages, concerns regarding privacy, advantages/disadvantages of receiving such results, and the likely impact of this monitoring test on sustained adherence to ART or just on short-term adherence. Interviews will be conducted in participants' preferred language.
2. **Health care worker interviews:** We will additionally assess the feasibility of the intervention by interviewing health care providers, who will be administering the UTRA test at the clinical point of

care in the future. We will examine provider perceptions of the assay at the end of the study using in-depth interviews (n~10), assessing perceived usefulness, complexity to use, stigma/social harm, and benefits. These interviews will also elicit barriers and facilitators to delivery of the urine assay-informed counseling messages.

Interviews will be approximately 45-60 minutes in duration, audio-recorded, transcribed, and (where necessary) translated into English. Names and other identifying information will be removed/replaced with pseudonyms in all public presentations of data to protect participant confidentiality.

LAB MANAGEMENT

1. **Viral load assays:** Plasma will be stored an enrolment, month 6 and month 12 for viral load assay completion after the study. All participants will continue to have viral loads performed as part of routine viral load monitoring as per National Health Laboratory Service (NHLS) National Tender during the study. Samples are assayed either with the Alinity m HIV-1 assay (Abbott Laboratories. Abbott Park, Illinois, U.S.A.); lower limit of detection (LLD) 10 copies/mL or the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Diagnostics, Basel, Switzerland); LLD 20 copies/mL. Whole blood will be centrifuged and plasma then tested on the automated analyzer according to national standard operating procedures.
2. **HIV drug resistance testing:** Plasma from 5 mL EDTA blood taken at baseline, month 6 and month 12, will be stored at -80°C until HIV drug resistance testing. HIV drug resistance testing will only be performed in participants with VF (VL>400 copies/mL). Resistance testing is performed by Sanger Sequencing of HIV protease, reverse transcriptase and integrase using previously published and validated in-house methods.[59,60]
3. **Plasma for tenofovir quantification:** Plasma will be collected for tenofovir quantification at enrolment, month 6 and month 12 and stored on site at -20°C. Samples will be shipped to Tygerberg weekly on dry ice. This will be determined in cases with virologic failure and controls without and compared to urine UTRA and quantitative tenofovir determination.
4. **DBS for TFV-DP:** DBS will be collected at enrolment, month 6 and month 12 and tested in nested case control study to compare TFV-DP levels between cases with virologic failure and controls without.

DATA MANAGEMENT

Each participant will receive a unique participant identification number, which will be used for identification for the duration of the study on all source, case report forms (CRFs) and transcript documents. The exception is the locator information form that contains their address and contact information, which will be kept separately in a locked cupboard at the research office.

Data will be collected for this study on an open access electronic data capture system using a password protected platform, with a limited number of paper-based documents. The study visit CRFs will be completed as soon as possible after the study visit. The principal investigators have overall responsibility for ensuring the data collected are complete, accurate, and recorded in a timely manner. Confidentiality of records will be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements.

Once the electronic case report form (eCRF) is completed data staff will monitor the data for completeness and accuracy. Any discrepancies either manual or automatic will be corrected by site staff. Corrections to eCRFs will only be possible by study personnel with sufficient authorisation to make changes. All changes will have an electronic date stamp. Corrections to paper-based forms can only be done by study staff and must be signed and dated. All hard copies of source and CRF's documents will be kept in a locked cupboard at the research office or secure long-term storage facility for five years.

Electronic data will be stored in a password-protected folder on the secure company server. A descriptive analysis of participant characteristics and acceptability of the UTRA and will be conducted.

Qualitative interviews will be digitally recorded, and these data (including any field notes and transcripts) managed in ATLAS.ti or similar software. Qualitative data will be kept on a password-protected server at the Desmond Tutu TB Centre, Stellenbosch University with only the study investigators and socio-behavioural team having access.

STUDY WITHDRAWAL:

Participants reserve the right to withdraw from the study at any stage, without an obligation to provide reasons for their withdrawal. Should a participant voluntarily withdraw their consent, the study team will attempt to ascertain the reason for withdrawal, while respecting the participant's decision.

If a participant requires a regimen switch for adverse effects, they will remain in the study, provided they are still using a tenofovir-based regimen. If the clinician decides that VF is due to resistance and the regimen must be switched, that switch will be adjudicated as an outcome and data collection for that participant censored at that point.

If, in the opinion of the investigator, a participant's continued participation in the study poses unacceptable risks to themselves, the participant may be withdrawn from the study.

Data from participants who do not complete the study will be used for secondary endpoint analyses.

ADVERSE EVENT MANAGEMENT

Not applicable

STATISTICAL ANALYSIS:

SAMPLE SIZE:

We assessed sample size based on the primary effectiveness outcome of this pilot RCT e.g. sustained virologic suppression with real-time monitoring via the urine assay and adherence counselling in the intervention arm compared to the standard-of-care adherence counselling arm. We expect 80% suppression (VL<50 copies/mL) in the control group; a sample size of 200 (intervention and control) will enable us to detect an increase in suppression to 93% with 80% power. Our actual precision will likely be better because of multiple observations per person and the contribution of within-person changes to the overall estimate.

PRIMARY OUTCOME

The primary outcome is the proportion of participants in each arm achieving viral suppression to <50 copies/ml by month 12.

SECONDARY OUTCOME

Secondary outcomes include retention in care at month 12; viral suppression (<50 copies/mL) at month 6; and feasibility and acceptability measured at enrollment, and months 6 and 12 with standardized questionnaires.

ANALYSIS PLAN

We will assess the suppression rates (VL< 50 copies/mL) in both study arms using an intention-to-treat analysis. Missing values for suppression will be imputed using chained equations which will include the history of previous suppression and dispensation and will also include baseline characteristics such as sex and age. The suppression and retention analysis will be analyzed by logistic regression extension of generalized estimating equations with a robust variance estimate.

In-depth interviews:

As this is a qualitative component no statistical analysis applies to this component.

STUDY MONITORING

Quality assurance and quality control (QA/QC) systems with procedures to maximise patient safety and assure the quality of every aspect of the study will be implemented.

On-site monitoring will be performed by independent study monitors on a regular basis. The monitors will:

- verify completeness of the investigator site file
- confirm adherence to protocol
- review all informed consent documents
- review eligibility verification and consent procedures
- verify completeness, consistency, and accuracy of data being entered on CRFs
- verify completeness, consistency, and accuracy of study samples
- provide additional training as needed
- document findings in a formal feedback letter (monitoring report) to the site

ETHICAL CONSIDERATIONS AND INFORMED CONSENT

ETHICAL CONDUCT

This study will be conducted in accordance with the ethical principles laid out in the National Statement on Ethical Conduct in Research Involving Humans, the Declaration of Helsinki (most current version issued, available at www.wma.net), and will be consistent with GCP and applicable regulatory requirements.

The rights, safety, and wellbeing of the study participants are the most important consideration and should prevail over the interests of science and society. All personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s).

Institutional review board/independent ethics committee (IRB/IEC) and local regulatory approval will be documented and kept in the investigator site file, specifying the version number of the protocol and informed consent as well as the date of approval. Any amendments will require IRB/IEC and regulatory approval.

The principal investigator will comply with all IRB/EC and regulatory authorities, reporting requirements for all safety reporting, annual updates, safety updates, end of study reports and any other important information relevant to the conduct of the study.

COMPLIANCE WITH THE PROTOCOL

The study will be conducted as described in this protocol. The principal investigator will not implement any deviation or change to the protocol without prior review and documented approval/favourable opinion from the IRB/IEC and regulatory authorities of an amendment, except where necessary to eliminate an immediate hazard(s) to study participants. Any significant deviation will be reported to sponsor, IRB/IEC, and local regulatory authority.

PROTOCOL AMENDMENTS

When revisions to the protocol are made by the sponsor, if the revision is an administrative letter, the principal investigator will submit this for the information of their IRB/IEC. Study documents will be updated in line with the changes required in the protocol amendment.

INFORMED CONSENT AND PROCEDURES

A study specific informed consent will include all elements required by GCP as well as all local ethics and regulatory requirements.

The principal investigator will ensure that participants are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they participate and that their participation is voluntary. A copy of the informed consent will be given to the participant in a language of their choice. Informed consent process will be conducted as per the trials site standard operating procedures. A copy of the signed informed consent will be given to the participant.

If the patient is illiterate, an impartial witness will be present during the entire consent discussion. A thumb print may be used as a signature.

The informed consent document will be updated with any pertinent information that becomes available during the study.

POTENTIAL RISKS

Participants enrolled in the study may experience the following risks and discomforts. They will be informed of these potential risks prior to providing their informed consent.

The venipuncture procedures to obtain blood have some minor risks. The participant may experience a small amount of pain at the needle-prick site, but this should be minimal. A small amount of bleeding under the skin may produce a hematoma (bruising), which should resolve in a few days. Risks will be minimized by the use of sterile technique by trained, qualified study staff.

We will take steps to minimize inadvertent disclosure of HIV status through participation in this study and will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study, or information collected by the study, could become known to

others, and that participants may experience stigmatization or discrimination as a result. We will take the following steps to protect against the risk of a confidentiality breach.

1) All data, including blood samples, assessments, and participant files will be labeled only with study identification numbers, and no participant names will be attached to study data.

2) Study documents linking study identification numbers with participant names (e.g. locator forms, screening logs) will be kept by the SCO in locked cupboards at the DTHF that can only be accessed by study staff. Personal information including participant's name, address and phone number will be stored separately from research data.

3) Electronic data files will be password protected and stored on a secure study server, which requires authentication to access.

4) All paper research data will be kept in locked cupboards and will be available only to research staff directly involved in this project and institutional personnel for the purpose of routine audits.

5) All study staff will receive training on procedures to protect participant confidentiality. Participants will be told that all data are confidential within the limits of the law.

POTENTIAL BENEFITS

Some participants may avoid virologic failure as a result of receiving adherence counselling and UTRA results during study participation.

The knowledge gained from the study may be of benefit to both the participants and the wider population in the future, should the UTRA test become part of routine care.

CONFIDENTIALITY OF DATA

The site principal investigator agrees that the Desmond Tutu Health Foundation, University of Cape Town and funder, IRB/EC or regulatory authorities may consult and/or copy study documents to verify information in the CRF. By signing the consent form the participant agrees to these processes.

Participant confidentiality will be maintained at all times and no documents containing the participant's name or other identifying information will be collected by the funding organisation. It may be necessary for the funder's representatives, the IRB/EC and regulatory authority representatives to have direct access to the participant's medical records. If study documents need to be photocopied during the process of verifying CRF data, the participant will be identified by a unique code only; full names and other identifying information will be masked.

The principal investigator also agrees to maintain confidentiality with all study information and only divulge necessary information to the staff, ethics committee and regulatory authorities. The data generated by this study will be considered confidential, except where it is included in a publication as agreed in the publication policy of this protocol.

APPENDIX A: TIMELINE AND GANTT CHART (AIMS 1-3)

Current GANTT – started 1 July 20

Activity	Aim	Responsible	2020				2021				2022				2023				2024	
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
Training of site staff	1	CO, GVZ			X															
Qualitative experience of UTRA	2,**3**	GH			X	X	X	X	X	X	X	X	X	X	X	X	X			
Sensitivity of UTRA for VF; association with adherence measures	1	GVZ, CO			X	X	X	X	X											
Training of site staff	**3**	CO, GvZ							X	X										
RCT: Utility and feasibility of UTRA	**3**	CO, GvZ, MG, DG								X	X	X	X	X	X	X	X			
Laboratory tests: TFV-DP in DBS and Drug Resistance	1	GvZ, ED					X	X	X	X	X	X	X	X	X	X	X	X		
Final Data Analysis, write up, Finding Disseminatio n	1,2, **3**	MG, GvZ, DG,CO																	X	X

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