Comparison of Pharmacokinetics of Tenofovir Alafenamide (TAF) with Tenofovir Disoproxil (TDF) in Pregnant and Postpartum Women and their Infants in PrEP-PP study

PrEP-PP

Pre-exposure Prophylaxis in Pregnancy & Postpartum Period

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Protocol 5

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1. INTRODUCTION

1.1 Background and rationale:

Pregnant and postpartum adolescent girls and women in sub-Saharan Africa are one of the populations at highest risk of HIV acquisition.[1,2] Among women living with HIV, acute maternal HIV infection during pregnancy and breastfeeding substantially increases risk of vertical transmission. In sub-Saharan Africa, acute maternal HIV infections account for an estimated 26% of all vertical HIV transmissions.[3,4] While services to prevent vertical HIV transmission have expanded rapidly in the region, few prevention interventions exist for the majority of pregnant women who initially test HIV-negative.[5,6] Although oral pre-exposure prophylaxis (PrEP) with tenofovir-based regimens has been shown to be effective across a variety of settings, effectiveness is highly dependent on adherence to the regimen, and may differ when pregnant or postpartum. [7-10]

Although considerable evidence has been gathered about the safety of antiretroviral drug use in HIV-infected pregnant women, evidence supporting the safety, feasibility, and acceptability of PrEP use during the antenatal and postpartum periods among HIV-uninfected women is needed. [11] In a recent systematic review done by our group, we found that there is no safety-related rationale for prohibiting TDF-based PrEP during pregnancy and/or breast feeding.[5] Additionally, there is minimal penetration of tenofovir (the metabolite of TDF) into breastmilk.[12] While safety data are reassuring, more data on what is the most effective drug for pregnant and postpartum women are needed.

Pharmacokinetic (PK) data for PrEP remain limited. Intracellular tenofovir-diphosphate (TFV-DP) concentration in red blood cells, measured via dried blood spots (DBS) has been used to monitor adherence in many settings. In the IMPAACT 2009 study in Malawi, South Africa, Uganda and Zimbabwe found that TFV-DP in n=40 (n=20 pregnant and n=20 postpartum) African adolescent girls and young women (AGYW) was 31-37% lower in pregnancy than postpartum – indicating that strict adherence to PrEP is needed in pregnancy.

Our study will establish benchmarks of TFV-DP concentrations as measures of adherence following daily dosing with Tenofovir Alafenamide (TAF) compared with Tenofovir Disoproxil Fumarate (TDF) during pregnancy and postpartum. We will recruit from an ongoing observational cohort study in Cape Town, South Africa, PrEP-PP (recruitment ongoing through July, 2021; NIMH R01MH116771; PI Coates & Myer). Findings form this PK sub-study will be used to inform future PrEP in pregnancy and postpartum studies and develop benchmarks of the relative PK between TDF and TAF.

2. STUDY

Study aims. (1) To establish benchmarks of TFV-DP concentrations as measures of adherence following daily dosing with TAF vs TDF in pregnancy and again in postpartum; (2) To compare the difference of TFV-DP within TDF and TAF for pregnancy vs. postpartum, and to establish adherence benchmarks of levels of TFV-DP in breastmilk in postpartum women comparing the TAF and TDF samples.

Setting. The study will take place in urban townships in Cape Town (Gugulethu, NY1 and Vuyani) with high HIV incidence that spans the different socioeconomic, cultural, and ethnic groups in South Africa. We selected this community because of the high HIV prevalence there in pregnant and breastfeeding women, and because of the high number of mothers visiting every month for ANC and labour/delivery. Further, our team has several years' experience implementing research studies in this clinic which will facilitate the integration of the study there. Our study on PrEP in pregnancy ("PrEP-PP"; NIMH R01MH11677, NCT03826199) is one of the first studies to integrate PrEP into maternal child health care in South Africa and will provide the platform from which to recruit for the PK sub-study.

In 2019, the Gugulethu Midwife Obstetric Unit (MOU) saw over 3300 new ANC consultations per month, and 72% (n=2300) are HIV-negative. Considering our experience and patient flow, we are confident that at least 60 women/month per facility can be enrolled. Further, Drs. Joseph Davey and Myer have conducted research in the Gugulethu community for more than a decade and have substantial experience partnering with local health services. Building on this platform, we will use existing infrastructure in each clinic, including capacitating existing clinical providers, pharmacists and counsellors, to

provide PrEP care to pregnant and breastfeeding mothers. We will hire study staff to assist in the coordination, data management, and overall quality assurance of the drug provision, phlebotomy, and follow up.

COVID-19 and study implications. Our PrEP-PP study has continued during the COVID-19 lockdown in South Africa because it is considered to be an essential service. We continue to recruit over 15 women/week and have over 20 study retention visits in pregnant and postpartum women (telephonic and in person) during the COVID lockdown. We observe all University of Cape Town and Department of Health COVID-19 infection prevention and control methods.

Study population. Study counsellors will enroll consecutive eligible, consenting pregnant adolescent girls and women (16-40 years old) in antenatal care with singleton pregnancies, without recent PrEP exposure.

- We will enroll a separate cohort of women during singleton pregnancy at 24-30 weeks' gestation followed through postpartum until 6-12 weeks after delivery
- Women will be randomized to TAF vs. TDF and provided with directly observed oral PrEP for 8 weeks with weekly clinic visits and phone video directly observed therapy while pregnant and another 8 weeks of directly observed PrEP in postpartum period
- Women will serve as their own controls to compare their drug levels in pregnancy vs. postpartum periods
- The study will also allow for women who are enrolled and then lost to follow up to be re-enrolled into the study at a latwr time if they still comply with all inclusion criteria.

Sample Size: A target sample size of at least 15 women per group is consistent with similar studies of directly observed PrEP that estimated steady-state concentrations of TFV-DP, providing adequate precision for outcome estimates.[13] We increased the sample size to 35 participants per group to account for potential attrition (total N=70 women). This study is not powered to assess safety or efficacy.

Inclusion Criteria:

1. ≥18 years old
2. confirmed HIV-negative (confirmed with a 4th generation antigen HIV test) at time of study entry
3. Attending ANC visits at Gugulethu MOU, NY1 Clinic or Vuyani Clinic
4. confirmed to be 20-30 weeks pregnant
5. without psychiatric or medical contraindications to PrEP
6. estimated creatinine clearance (CrCl) >60mL/min
7. resides close to clinic (<10km)
8. has a smart phone that can take video footage (with data bundle from study)
9. agrees to provide video phone footage of taking a pill a day for 8 weeks during pregnancy and again for 8 weeks in postpartum period
10. Previously lost to follow up participants who wish to be re-enrolled in the study so long as they still meet all of the above inclusion criteria.

Exclusion criteria:

Individuals not meeting the above criteria or meeting any of the following criteria will be excluded:

1. Concurrent enrolment in another HIV-1 vaccine or prevention trial
2. Currently taking PrEP or taken in last 90 days (except in cases of re-enrollment of previously lost to follow up participants)
3. History of renal disease
4. Current clinical diagnosis of hypertension
5. Exhibiting psychotic symptoms
6. Currently or history of taking an anti-psychotic medication

7. Positive Hepatitis B surface antigen (HBsAg) test on screening
8. History of bone fracture not related to trauma
9. Any other medical, psychiatric or social condition which in the opinion of the investigators would affect the ability to consent and/or participate in the study
10. Any maternal or fetal complication, obstetric or medical, detected during routine care or study procedures that requires referral of pregnant or postpartum women/infants to secondary or tertiary obstetric or medical care.

Study censorship will include:

1. Seroconversion
2. Moves away
3. Transfers out of care
4. Lost to follow up (e.g. does not return for study visit for >1 week and study staff are unable to track the participant, or she does not want to continue in the study).

Table 1. PK Maternal Screening Visit Procedures	
Administrative and regulatory	- Obtain written informed consent - Assign participant identification number (PID)
Behavioral and counselling	- HIV testing and counselling and condom provision
Clinical	- Obtain medical records and medical history - Complete maternal physical exam - Weight, BMI and weight gain (monthly)
Laboratory - Blood	- Baseline and Monthly: Abbott 4th generation ab/ag test (negative test required prior to enrolment) - Baseline and postpartum: ALT, creatinine, CrCl, renal function panel - Baseline: Hepatitis B surface antigen (rapid test) (negative test required prior to enrolment) - Complete blood count (CBC) with differential and platelets
Laboratory- Breastmilk	- Collect breastmilk in postpartum women (see SOP for dried milk samples in appendix)
Study drug	- Prescribe and dispense study drug (TDF vs TAF depending on group) - Directly observe participant taking drug (day 1-7)
Rapid urine tests	- Done as needed to verify PrEP dosing prior to drawing blood samples.
Exit Interview	- Done at last study visit to ask questions around perceptions and experience of video observed PrEP dosing

Enrolment procedures:

Eligible, consenting women will receive up to R250 (~$15) in grocery vouchers, transportation and refreshments per weekly visit for their time and effort in the study as well as transportation to the facility. All participants will also receive data bundles of 1GB at enrolment and 10GB every month thereafter for the duration thri follow up to send videos of them taking daily PrEP. During weekly visits, participants will receive minor refreshments (e.g. sandwich and cool drink).

Procedures for the informed consent process are outlined below. Throughout, trained study staff will ensure that women are aware of their right to refuse and/or withdraw from the study at any time. In addition, study staff will emphasize that all study activities are entirely separate from routine ANC and postnatal care services received and that refusal or withdrawal from the study will have no impact on their ability to access any services provided at any public-sector health facility.

Trained study counsellors will recruit n=70 women that are 20-30 weeks pregnant and NOT part of the ongoing PrEP-PP study for the PK sub-study. Participants will be screened for HIV infection; only those who test negative will be eligible to initiate once-daily FTC/TDF or FTC/TAF for PrEP. Participants must be willing to initiate and take PrEP and to take videos and send to the study for daily adherence for 8 weeks in pregnancy and again for 8 weeks postpartum. Women will be invited to participate in the study through ~2-3 months postpartum (depending on when they start the 8 week postpartum DOT). Women will be randomized into the TDF vs. TAF arm. Women will provide their own controls, providing pregnant and postpartum samples.

- **TDF Arm :** Women will receive a fixed dose combination of 200 mg emtricitabine (FTC) and 300 mg tenofovir disoproxil fumarate (TDF) administered once daily under direct observation for 8 weeks during pregnancy and again for 8 weeks in postpartum period
- **TAF Arm:** Fixed dose combination of 200 mg emtricitabine (FTC) and 25 mg tenofovir alafenamide (TAF) administered once daily under direct observation for 8 weeks during pregnancy and again for 8 weeks in postpartum period
- **At study end**: Participants in both arms will be given a 3 months supply of Tenemine (FTC200mg/TDF300mg).

Study Duration: Approximately 12 months total for this component. Analysis of PrEP (TAF vs TDF) drug levels will be completed within approximately three months after the last participant follow-up visit. The duration is shorter because we have ongoing cohort recruitment ongoing in Gugulethu, NY1 and Vuyani and can recruit 70 women who reside close to the clinic within 2-3 months.

Study procedures: Weekly (see **Table 2** for scheduled events), following PrEP initiation a maternal blood specimen will be collected via venepuncture (at home or in clinic, depending on participant's preference). This specimen will be used to create a DBS specimen to be tested at the laboratory at the University of Colorado. DBS cards will be prepared and stored following standard protocols and stored at -80°C for shipment and analysis at the end of the study with the clinical pharmacoanalytic laboratory of Dr. Pete Anderson at University of Colorado, Denver (funding proposal under separate cover).

Enrolled maternal participants can continue to take PrEP through delivery and postpartum period to prevent HIV during this time. Pregnancy and postpartum periods are associated with elevated risk for HIV acquisition. As such, the study aims to evaluate the different levels of TAF vs TDF associated with once-daily FTC/TDF regimen vs FTD/TAF regimen. Study staff will monitor and document maternal participants' daily administration of the study drug.

Only doses directly observed in-person or via video call by study staff or sent video recordings will be considered adequate documentation of the PrEP doses taken for the purposes of defining an evaluable participant (see Analytics).

These data will be used to determine the drug thresholds for understanding of TAF vs TDF adherence measures. The drug threshold corresponding to adequate adherence will be defined from the distribution of steady-state TFV-DP concentrations at antenatal vs postnatal visits.

If up to 3 PrEP doses were not directly observed, a urine test will be done to detect the presence of tenofovir metabolite in the participant's system prior to the drawing of blood samples.

We will evaluate the 'tail' (plasma and intracellular) of TFV and TFV-DP in the PrEP setting, comparing TAF to TDF and TAF and TDF levels in pregnant to postpartum women. See SOPs for dried milk spots (DMS) and dried blood spot (DBS) collection in Appendix.

- Plasma PK (TFV) – 8 samples in pregnancy, and 8 samples in postpartum (1 per week)
- PBMC (plasma)- 2 samples in pregnancy and 2 in postpartum at weeks 4 and 8 (4 PBMC samples per participant=100 samples)
- Breastmilk PK for TAF and TDF arms – Given there are no data on TAF in breastmilk, we propose to conduct a single sample of DMS matched with plasma samples and compare to TDF samples.
  1 sample x 40 participants (TAF and TDF arms) =40 tests at 4 hours after peak (week 8)
- Urine tests- Tenofovir rapid tests will be done as needed to verify the presence of tenofovir metabolites in those participants who have stated that they have taken their PrEP but were not observed doing so.

Video DOT : We will provide the option of sharing videos of taking PrEP daily (live) instead of coming to the clinic, which has been used in previous studies led by Dr. Anderson and team, including IMPAACT 2009. Participants will also be asked a few questions around their experience and perceptions of taking video observed PrEP doses during an exit interview at their final study visit.

Homebased collection of specimens : In light of COVID-19 lockdown and complexity of plasma collection, we will offer homebased collection of DBS and DMS to women by a nurse who will travel to their homes for collection of samples as required.

The PK Component will establish PK parameters for TFV-DP under TFV vs TAF under adequate adherence during pregnancy and postpartum. This PK study is necessary because it will allow investigators to compare levels of TFV-DP in TAF vs TDF and to compare levels needed for optimal adherence in pregnancy vs postpartum periods.

Table 2: Schedule of events of PK study

	During third trimester									During postpartum period							
Weeks	B	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Informed consent process	X																
Medical history	X																
Concomitant medication	X																
Gestational age	X																
Safety bloods*	X												X				
Complete blood count (CBC) with differential and platelets, HCT	X												X				
HIV test (4th gen)	X				X				X				X				X
Randomise to TAF vs TDF	X																
Dispense PrEP	X			X				X					X				X
Observed daily PrEP dosing via video call	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Plasma PK sampling (TFV)	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
DBS	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
PBMC					X				X				X				X
Breastmilk Sampling^																	X

*Creatinine, HepBSag, ALT, renal function panel (baseline and postpartum, 2 time points),

^ For both TAF and TDF arms

ANALYTICS

Primary Objective: To establish benchmarks of TFV-DP concentrations following daily dosing with TAF vs TDF in pregnancy and again in postpartum

Secondary Objectives: To compare the difference with TFV-DP with TDF and TAF for pregnancy against postpartum and to establish benchmarks of TFV-DP in breastmilk in postpartum women and compare with TDF arm.

Prior to conducting analyses, the study team, including the study Pharmacologists, will review the pattern of completeness of each participant's directly observed PrEP therapy (via video call) to ascertain if all 8 weeks were taken daily in antenatal and postnatal care to determine suitability for inclusion in the analysis. In each of the groups (TDF vs TAF in antenatal and postpartum groups), the distributions of steady state concentration (Css) defined as the TFV-DP concentration observed will be summarized.

For each group, we will plot median and interquartile range (IQR) TFV-DP concentrations for each week and fit a Loess curve and 95% confidence interval (CI) to the concentration-time data. The plateau in the concentration-time curve was assumed to represent steady-state. Tenofovir diphosphate at week 8 (steady-state) will be analyzed as median, IQR, mean, standard deviation (SD), and 95% CI in the pregnancy and postpartum groups by study arm. The Wilcoxon rank-sum test will be used to evaluate differences in the distribution of 8-week concentrations between groups. Additionally, a nonlinear mixed-effects population PK model will be fit to the TFV-DP data using a 1-compartment constant input model using Phoenix (Certara, Princeton, NJ). The model will be used to summarize PK parameters for both groups and between TDF and TAF arms.

Differences between the antenatal and postpartum group intra-individual comparison of plasma TDF concentrations will be evaluated using two sample t-tests. We will evaluate bio-equivalence in plasma TFV-DP between TAF and TDF in pregnant and postpartum women.

The PK testing laboratory will provide drug level results by study arm, comparing TAF to TDF in pregnancy and again in postpartum periods in the same women. The secondary objective is to compare the difference in TFV-DP in pregnancy against postpartum samples in the TAF vs TDF group. We will compare with reference ranges as it relates to the categorized level of adherence to use during adherence counselling sessions. The reference ranges will include approximately three adherence categories, aligned with other similar studies in the field. Finally, we will evaluate the level of TFV-DP in breastmilk in postpartum women and compare with TFV-DP in the TDF arm.

Qualitative Data:

- At the final study visit, participants will be asked to answer a few questions about their experience and perceptions of taking video observed PrEP doses.
- This will be done during a 20 minute exit interview.
- Participants will be asked to select a rating, from a scale, that best reflects their perceptions of the convenience, privacy, and ease of use the video observation process
- Participants will also be asked open ended questions about what they perceive to be barriers and facilitators of the video observation process.
- All answers will be anonymized by assigned ID numbers (PIDs)

Safety:

There are no indications that metabolism of TAF, TDF and TFV is sensitive to genetic polymorphisms. Population-based PK analyses indicated that race is not a clinically relevant covariate. Exposure of TAF/TFV was negligible in breastmilk.

3. ETHICAL CONSIDERATIONS

Ethical review

The study protocol, informed consent form, all data collection tools, and other requested documents will be reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (UCT-HREC). After the initial review and approval, UCT-HREC will review progress of the study at least annually. Following the approval, we will submit the protocol and approval to rely on UCT's HREC to the University of California Los Angeles (UCLA) for secondary review and approval. UCLA IRB will also review progress of the study annually and we will report all adverse events or amendments to both Ethics Committees.

Insurance

Currently we propose that this protocol is covered by UCT's no-fault insurance policy as this is non-commercially sponsored interventional research.

Ethics approach for including adolescent girls (16+ years old) in study

Our study has approval to collect assent from the minor without the parental consent as they are deemed to be emancipated minors after 16. The risk is justified by the anticipated benefit to the subjects: the risks associated with the sub-study procedures are minor and largely non-invasive (except for phlebotomy) and are justified by the anticipated benefit that PrEP would have on prevention of HIV acquisition in this vulnerable group. Thus, there is the potential for direct benefit associated with participation.

Informed consent

Our study will have two consent forms.

1. The first would be for screening and would include the consenting for the screening survey (inclusion criteria questionnaire) and consenting for the HIV testing (using antigen/antibody testing to screen for acute HIV infection) and hepatitis B surface antigen testing. The study consent form will be for enrolled participants' consent.
2. The second is the main study ICF. The study informed consent for the study and questionnaire are modelled after that used in previous studies and will be delivered in participants' home language (isiXhosa or Afrikaans) by trained interviewers. This study ICF details the purpose of the study, study procedures, and the risks and benefits to mothers that participants may encounter at the additional study measurement visit.

Here, study staff will emphasize to participants that:

- Participation is entirely voluntary, and their choice regarding participation will in no way influence the quality of routine medical care for mothers or their infants
- Women may exit the study at any time for any reason without compromising the quality of health care received.

English versions of the informed consent documents are provided in the Appendix to this document. Translated isiXhosa versions (as well as a certification of their translation and back-translation) will be submitted to the HREC before the start of the study.

Risks

The potential risks to participants in the study include:

- Risks due to loss of confidentiality due to study procedures—for instance, in the process of data collection
- Risk associated with asking participants to disclose their status, and potential for interpersonal violence by the partner resultant from the disclosure.

The potential risks to participants who take PrEP include:

- Risks associated with PrEP side effects (headaches, gastro-intestinal) for women on PrEP
- Risks associated with PrEP adverse events for women on PrEP
- Risks associated with collection of dried blood spots and blood

All participants will be informed of these risks, and the strategies to minimize these, as part of the informed consent process. These strategies draw directly from prior experiences conducting research on HIV prevention and treatment in Gugulethu and similar communities across Cape Town.

Benefits

Direct benefit

The major potential direct benefit from participating in this study is that pregnant women will receive PrEP to prevent HIV along with Hepatitis B screening. Their participation may help us answer our research questions and help us inform PrEP interventions for pregnant women in the future. The benefit of participating in the study and preventing HIV acquisition and onward infant transmission is likely to outweigh the risk of PrEP exposure or other risks.

Indirect benefit

By identifying the optimal strategy for delivering PrEP to pregnant women, this study has the potential to lead to improved HIV prevention interventions to protect against HIV acquisition and vertical transmission in HIV-uninfected women and their infants in Cape Town, the Western Cape Province, and across South Africa.

Compensation

Participants will be given up to R250 in grocery vouchers for their time and effort in every study visit as well as additional money to cover the cost of transport for each study visit. Women will also receive air time to support video DOT (10-15GB per month)

This is in line with ongoing research in Gugulethu. All participants will also receive refreshment on the day of their visit.

Confidentiality

The following steps will be taken to minimize the risk of any loss of confidentiality throughout study design and conduct.

- All personnel involved in data collection and management will undergo specific training for the study in confidentiality and related patient protection issues.
- Following standard practice, all patient- and study-related information will be kept in locked cabinets at either the study office in Gugulethu or at UCT.
- Anonymous participant identification numbers will be used on all study documents. Collection of participant names and other identifiers will be restricted to informed consent documents, patient tracing materials, and a study identification key, all of which will be kept in a locked cabinet in the study office at Gugulethu and at UCT separate from other study documentation and accessible only by the project coordinator and local PI. No CRF will include participant name, including CRF that may reflect HIV status of women or their children (including STI test results or treatment).
- All electronic records will be kept in password-protected files. All electronic communications of study data will be through password-protected, encrypted files. All data storage at the University of Cape Town will be within a firewall-protected server.

While efforts will be made to minimize the loss of confidentiality, in the event that staff learn that the participant is a threat to themselves or to others or of possible abuse by partners, the proper authorities will be notified. This exception will be included in all study informed consent forms.

Internal monitoring

The purpose of monitoring is to verify the rights and well-being of human subjects are protected; that trial data is accurate, complete and verifiable with source data; that the trial is conducted in compliance with the protocol, GCP and the applicable regulatory requirements. Throughout the conduct of the study, internal study monitoring will be led by the study PIs. Throughout the duration of the study, study PIs, co-investigators and the study coordinator will participate in weekly conference calls to monitor the rate of participant enrolment and the integrity of protocol implementation (including the completion of informed consent and quality of study measures). In addition, participant retention and safety endpoints will be discussed as well. We will report all serious maternal and/or infant adverse events, including any laboratory or other measures that require women to stop PrEP, to the study's DSMB and Ethics Committee.

Appendix A: Emergency trolley contents

Here is the list of emergency trolley contents that we have on site that are specific to infants and adults:

1. Laryngoscope set (with paediatric blades)
2. Tracheal tubes (for adults & infants)
3. Tape or equivalent to tie tube in place
4. Oropharyngeal airways (for adults & infants)
5. Pulse Oximeter (for adults & infants)
6. Introducers for ET tubes (for adults & infants)
7. Magill's forceps (for adults & infants)
8. Laryngeal masks (for adults & infants)
9. Bag valve ventilation devices (for adults & infants)
10. Oxygen delivery devices (for adults & infants)
11. Oxygen supply
12. Defibrillator or Automated external defibrillator (for adults & infants)
13. IV cannulae (various), needles and syringes, sharps containers and IV administration sets (for adults & infants)
14. Stethoscope (for adults & infants)
15. Non-invasive blood pressure monitoring device (for adults & infants)
16. Thermometer (for adults & infants)
17. Glucometer
18. Drip stand or equivalent hanging device
19. Suction devices and suction catheters (for adults & infants)
20. Universal precautions (gloves, masks, PPEs)
21. Essential medicines and solutions (adrenaline, antihistamine, aspirin, atropine, dextrose, diazepam, hydrocortisone, lignocaine, ringer's lactate, sodium chloride) (for adults & infants)

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APPEDNICES: STANDARD OPERATING PROCEDURES FOR DBS AND BREASTMILK COLLECTION

SOP for DBS collection

Preparation

- Blood specimens for a research study will only be obtained once the study participant has signed informed consent.
- When a study participant presents for the obtaining of blood specimens, the study nurse:
    1) introduces him/herself,
    2) asks for the study participant's name
    3) offers the study participant a seat
    4) checks the study participant's name against the source document to confirm that blood is being drawn from the correct participant
    5) shows the study participant all the equipment s/he will use to draw the blood and explains the tests required at this study visit.
- The RN prepares the laboratory items specific for each study visit i.e. the required blood tubes, needles / lancets / specimen containers, requisition form, etc.
- The RN labels each specimen container/card with the participants study identification number.

Dried Blood Spot

- Dried blood spots (DBS) can be obtained from venous blood or by finger-prick depending on the protocol and visit.
- For venous blood DBS, the study RN can obtain blood as described above and use a disposable pipette to spot 50µL of whole blood onto DBS cards which are properly labelled with participant study identification number, visit number, etc. The number of spots collected will be outlined in the protocol.
- For finger-prick DBS:
    - The RN washes his/her hands and draws on gloves if wanted. The SCO/RN asks the study participant which finger they prefer to give blood from and the study participant exposes the finger.
    - The RN cleans the appropriate site with an alcohol.
    - The RN uses a lancet to prick the indicated finger of the participant. The lancet is disposed of in the sharps container.
    - The RN will spot blood from the pricked finger onto a properly labeled DBS card (the number of spots collected will be outlined in the protocol). The RN will avoid "milking" the finger to avoid pain and hematoma. A second finger prick may be required.
    - When blood spots collection is complete, the RN will apply a bandage to the participant's finger to stop bleeding and help prevent infection.
    - The SRN removes the gloves and disinfects his/her hands. Then s/he records the correct time of taking the specimens on the laboratory requisition forms or on the app, as appropriate.
- The DBS cards are allowed to dry. To achieve this:
    - The DBS circle should not be touched once blood is applied.
    - Spots should be air dried overnight (min. is 4 hours) without the flap over the spots and placed in a clean and dry location, which is protected from rodents, insects and direct sunlight.
    - When the blood has dried, the flap of the DBS should be tucked as indicated on the card.
- DBS cards can be stored in envelopes in the freezer. Refer to the protocol for study specific storage guidelines. Study freezers have a temperature monitoring log to ensure the freezer(s) maintain appropriate temperature (Appendix A). DBS cards ready for analysis will be packaged and transported as per the procedures outlined

SOP for Breast Milk Collection and Storage for PK Studies

The methodology for dried milk spots (DMS) has been developed and validated at the University of Liverpool.

Materials required

1) Clean, universal container
2) 200µl pipette tips
3) 20-200µl pipette
4) Whatman 903 Protein Saver Cards
5) Dessicant sachets
6) Zip locked gas-impermeable plastic bags
7) Drying racks
8) 2ml cryovials

Manual expression of breast milk

1) Label the container with the ID before handing the container to the mother
2) Ask the mother to wash her hands
3) Encourage mother to manually express a small amount of breast milk
4) Collect into clean container – ensure the cup is tightly closed (aim for about 3-5ml)
5) Send sample to the laboratory

Preparation of Dried Milk Spots on Filter Paper

1) DMS should be prepared within 24 hours of sample collection
2) Lipid and aqueous fractions of milk will separate upon standing; prior to spotting, invert the tube 10 times to mix the milk thoroughly
3) Using a micro-pipettor with disposable tip, aspirate 30µl whole milk
4) Without touching the tip to the paper, dispense milk to the centre of one pre-printed circle to fully saturate the circle
5) Prepare five spots (one complete card) for each patient sample
6) Leave the DMS cards on a drying rack for >2 hours or overnight
7) Place remaining sample into refrigerator
8) Ensure that DMS are completely dry before packing
9) Individually package DMS cards in zip-locked gas-impermeable plastic bags with 2-3 dessicant sachets
10) Ensure that the same ID and collection date are clearly written on both the DMS card and the plastic bag.
11) Store at -80ºC temperature until analysis
12) DMS can be shipped as non-hazardous materials using regular mail or courier services. Shipment can be at ambient temperature.

Storage of Milk Aliquots

1) After preparation of the DMS, freeze 2 aliquots of approx. 1.5 mL of whole milk in 2 mL cryovials.