



MTN 034 HIGHLIGHTS

Wayne Hall

Summary of lab tests:

		Vis1 SCR	Vis 2 ENR	Vis 4 & 5	Vis 6	Vis 7 & 8	Vis 9	Vis 11-12	Vis 13	Vis 14 & 15	Vis 16	Vis 18 & 19	Vis 20	Vis 21 & 22	Vis 23
		70 days to ENR	Day 0	Wks 4 & 8	Wk 12	Wks 16 & 20	Wk 24	Wks 28 & 32	Wk 36	Wks 40 & 44	Wk 48	Wks 52 & 56	Wk 60	Wks 64 & 68	Wk 72
Pelvic Examination (PE)		X	X	*	X	*	X	*	X	*	X	*	X	*	X
LABORATORY															
URINE	hCG	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Urine dipstick and/or culture	*	*	*	*	*	*	*	*	*	*	*	*	*	*
BLOOD	HIV-1 testing	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	HSV-2 antibody		X	*	X	*	X	*	X		X	*	X	*	X
	HBsAG	X													
	Plasma archive		X												
	Plasma storage				X		X		X		X		X		X
	Blood creatinine and creatinine clearance	X	*	*	*	*	X	*		*	X	*		*	X
	CBC with platelets	X	*	*	*	*	X	*		*	X	*		*	X
	Syphilis serology	X	*	*	X	*	X	*	X	*	X	*	X	*	X
	Dry Blood Spot (when on Truvada)			X	X	X	X	X	X	X	X	X	X	X	X
PELVIC	Rapid Trichomonas test	X	*	*	X	*	X	*	X	*	X	*	X	*	X
	Saline wet mount for BV	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	KOH wet mount for yeast	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Vaginal pH	*	X	*	X	*	X	*	X	*	X	*	X	*	*
	Vaginal swabs (3) for qPCR (microbiota)		X		X		X		X		X		X		X
	NAAT for GC/CT	X	*	*	X	*	X	*	X	*	X	*	X	*	X
	Vaginal Gram Stain		X		X		X		X		X		X		X
	Vaginal swab for biomarkers¹		X		X		X		X		X		X		X
Cervico-vaginal	CVL for biomarkers		X		X		X		X		X		X		X
	Cervical swab for biomarkers		X		X		X		X		X		X		X
	Cervical Cytobrush for Flow Cytometry (designated site only)		X		X		X		X		X		X		X
STUDY PRODUCT / SUPPLIES															
Collection of study VR(s) or tablets				X	X	X	X	X	X	X	X	X	X	X	X

Great resource to determine what tests go with what visits!

X= required, *= if indicated and/or per local standard of care,
¹= If there is a Pelvic Exam, then the clinician will collect this sample. If there is not a PE, then the participant will self-collect the sample.

Some differences MTN-034 from other MTN protocols:

Schwartz Creatinine Clearance Calculation:

- We are using the Revised Schwartz Estimate formula (also called the Bedside IDMS-traceable Schwartz GFR) instead of the Cockcroft-Gault Equation.
- Estimated Glomerular Filtration Rate (eGFR) = Calculated Creatinine Clearance
- The Schwartz formula will be used to calculate CCr for both adolescents and adults
- Standardization of creatinine calibration to be traceable to IDMS (Isotope Dilution Mass Spectrometry) is required for Enzyme and Jaffé reaction testing.

The Creatinine Clearance Calculator is located on the MTN-034 webpage in the [Study Implementation Materials](#) section.

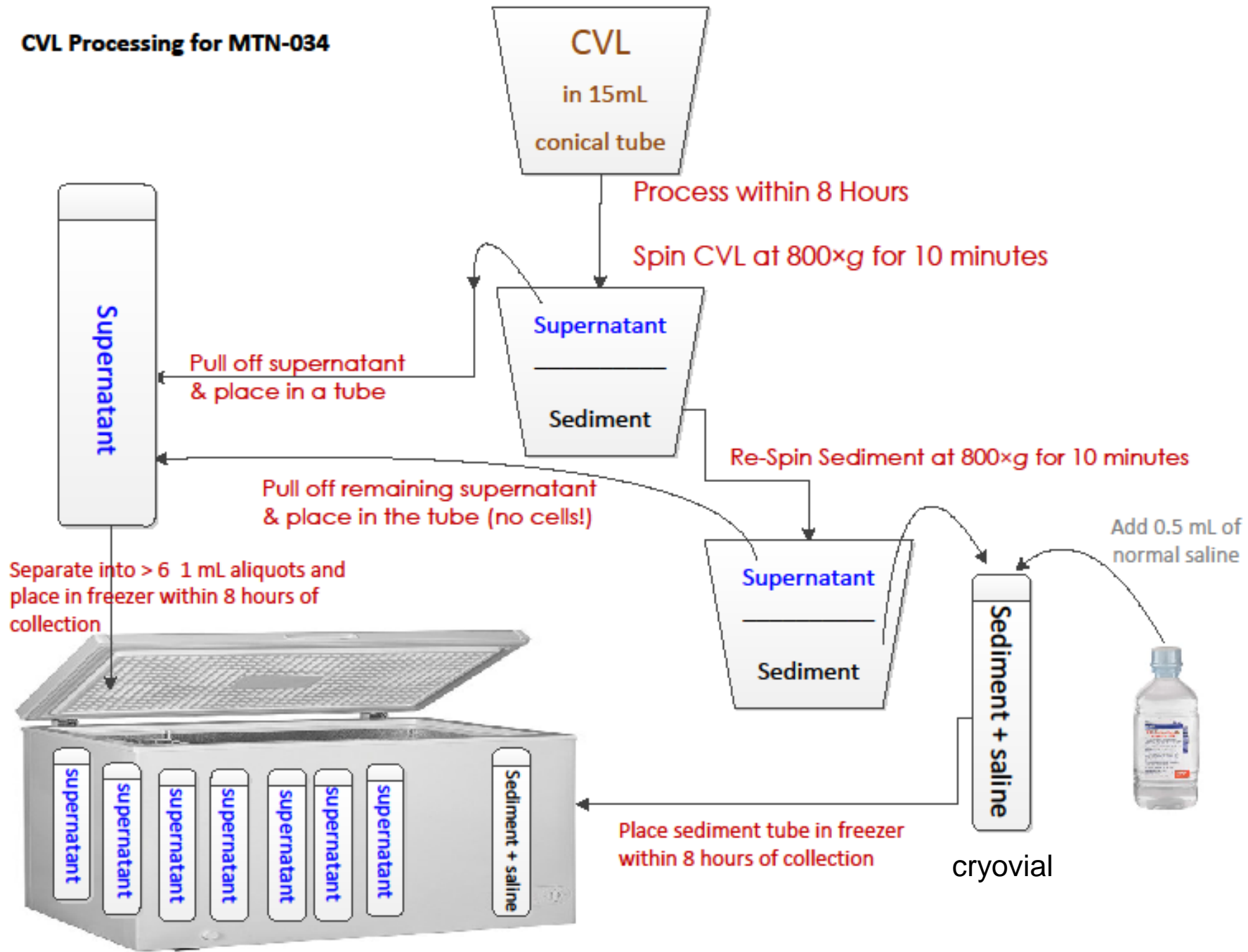
- Blood Creatinine and CCr calculation are entered onto the CRF
 - AE grade ranges are based on the March 2017 v2.1 DAIDS Grading Table

Dry Blood Spot (DBS) in MTN-034

DBS will be used when participant is orally taking the pill Truvada to determining their PK concentration.

DBS is used to determine the phosphorylated tenofovir (tenofovir diphosphate) in red blood cells. TFV (tenofovir) and FTC (emtricitabine) are broken down into their intracellular nucleotide forms and concentrated inside cells.

CVL Processing for MTN-034



Cryovials are frozen

Test Order:

Example of 034 quarterly visit pelvic exam samples

Vaginal swabs:

- 1: Swab for Rapid OSOM *Trichomonas* testing
- 2, 3, 4: Three flocked swabs for microbiota
- 5: Swab to rub onto pH paper
 - If a wet mount/KOH prep is 'if indicated', the swab will be collected after pH.
- 6: Swab for Gram stain, 2 slides
- 7: Swab for Vag Biomarker

Cervical swab:

- 8: Swab for GC/CT NAAT*
- 9: Swab for Cervical Biomarker
- 10: Cytobrush Endocervical cytobrush for flow cytometry (Zim only)
- 11: CVL: Collect 8-10 ml fluid

Note: The GC/CT NAAT swab will not be from the vagina now, but rather the cervix. This will help eliminate how many samples are taken out of the vagina (decreasing a 'dilution' effect).



Summary of more recent changes, reminders, and some additional information:

- Microbiota: now 3 flocked swabs
 - Also, no longer taking this sample at visits 20 & 23.
- Wet mount/ KOH prep will be only 'if indicated' for all visits
- GC/CT NAAT swab will be from the Cervix, not the Vagina.
- Originally the Revised Schwartz Estimate formula could only be used for <18 year olds, but newer studies shows that it can now be used for adults (Dr. Schwartz himself vouched for this).
- Swab type consistency: Other than the flocked swab, use the same type of swab for all collections (example: the biomarker swab for the vagina should be the *same type* as the biomarker swab for the cervix).

More on DBS & it's Advantages:

- Dried blood spots provide an excellent matrix for TFV-DP testing
- The presence of TFV-DP in RBCs, in which there are millions in a DBS, along with an extended drug half life compared to plasma testing.
- Simple processing, minimal blood volume, easy transportation and storage.
- DBS has many applications: newborn screening, therapeutic drug monitoring, antibody testing and nucleic acid testing.



DBS tips...

When pipetting dry blood spots, pre-wet the tip by aspirating liquid into the tip once and then dispensing all liquid out first, do this two to three times before dispensing into the circles

Pipette tips with filters may work better for making spots than the un-filtered tips.

DBS cards should dry for at least 2 hours....but longer or over night if there is high humidity in your lab!

If there is difficulty obtaining DBS supplies, a good contact person at Lasec is Megan van der Poel (megan.vd.poel@lasec.co.za).

DBS Shipping Highlights:

- Plastic Sample Ziplock Bags will contain DBS (once properly dried), desiccant, and humidity cards can be stored at 2-8 °C.
 - Exception: Cape Town does not have to store in the refrigerator as long as they can transport to BARC within 5 days of collection.
- Ship (overnight or express) using a thermal box, 2 gel cold packs, and a single use temperature monitor. Can use local transporters or Biocair. (I may update this closer to study activation)
- Bags with samples will be shipped weekly to the Division of Clinical Pharmacology, University of Cape Town (LDMS 499)
- Notes:
 - Stability times for DBS as of now: RT=5 days, Ref= 90 days , <-70 °C = ~Year
 - Prior to spotting: Make spots within 4 hours of blood collection (we prefer keeping the blood tube on ice)
 - Inspect DBS specimens for contamination or humidity change, and replace or add additional desiccants prior to shipping; document any changes in the humidity indicator.



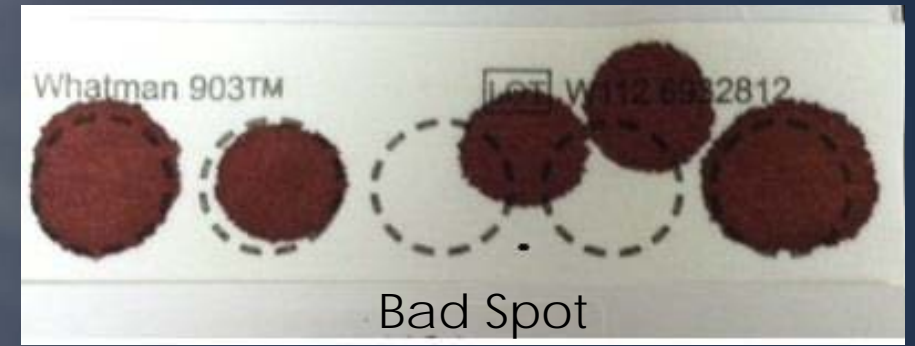
Questions?

DBS Supplies:

- 4 mL (smaller volume tubes acceptable) EDTA tube Blood Collection
- *Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific #05-715-121)
- Gas-impermeable plastic sealable storage bag (LasecSA) or Whatman Plastic Zipper Seal Sample Bags (Whatman 10548232 or Fisher Scientific#50-853-570)
- Desiccant pack (Gel Silica Sachets – 1 gm) (LasecSA or Whatman WB100003 or Fisher Scientific#09-923-360)
- Humidity indicator cards (Multisorb Des Manufacture # MS200032, DESCO Industries #13870 or Fisher Scientific # NC0281067 or NC9511648)
- Whatman card drying rack (VWR catalogue # 89015-592)
- Power free (preferable) Latex or nitrile gloves
- Water proof marker
- 10-100 μ L or 20-200 μ L micropipette and appropriate tips with filters. Sites should check with local suppliers for appropriate tips for their micropipette

DBS Processing continued:

1. Gently invert the EDTA tube (8 to 10 times) to mix the blood thoroughly.
2. Within 4 hours (and preferably keeping the blood collection tube on ice), blood must be pipetted onto the filter paper spots in the card.
3. Pipette exactly 50 μ L of the whole blood into each single spot on a PTID labeled Whatman Protein Saver 903 Card.
 - Performed with a calibrated 50 μ L pipette and a disposable pipette tip using the wet tip technique (pre-wet the tip by aspirating liquid into the tip once and then dispensing all liquid out first, do this two to three times before dispensing into the circles).
 - Do not touch the filter paper with your hands.
 - Do not touch the card with the pipette tip.
 - Slowly expel blood from the tip and touch the drop to the paper, allowing the blood to absorb. Care must be taken when applying larger volumes of blood to ensure the spots do not run outside of the circle.
 - A single tip may be used to load the card.
 - Do not touch the DBS circle once blood is applied
4. All five spots on the card should be filled (each spot 50 μ L).
 - If a difficult (short volume) blood draw was encountered, a minimum number of 50 μ L spots is 3.



Sample Drying:

Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (i.e. biosafety cabinet, drying racks) that is protected from rodents, insects and direct sunlight for at least 2 hours (drying overnight may be necessary in areas with higher humidity).

Do not heat, stack or allow DBS to touch other surfaces during the drying process.

Once confirmed to be completely dry, tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card to protect the samples from contamination.

Store the card in a PTID labelled sealed plastic bag with a desiccant pack (sachet of desiccant) and humidity indicator.

Do not store more than one card per bag.



Have fun with the demo and don't worry...we'll keep an eye on you!



You have
a
question?

Please ask any question, anytime, and we'll do our best to answer,
even if we look like a deer in headlights!