VOICE Laboratory Considerations

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Goals For this Meeting

- Look at VOICE Lab Testing Menu
- Focus on Specimen Management
- Review Laboratory Activation Requirements
- Generate questions and identify areas that require attention

VOICE Sites

- HPTN 035/MTN 001/MTN 015 Sites:
  - Lusaka
  - Blantyre
  - Lilongwe
  - Harare
  - Durban
  - Kampala
- New sites-Johannesburg-experience in HPTN 039 and MDP 301

VOICE Blood Testing

- HIV testing
  - Rapid Tests
  - Western Blot
  - RNA Viral Load
- Hematology-CBC
- Chemistry
  - ALT, AST, Creatinine, Phosphate

VOICE Blood Testing

- Hepatitis B
  - Surface Antigen
  - Surface Antibody
- Syphilis serology
  - RPR
  - Treponemal Confirmation (TPHA or other)
- Seroconverters
  - Resistance Testing
  - CD4 Count
  - HIV RNA Viral Load

VOICE Plasma Storage

- PK analyses
- HIV testing
  - Confirmatory (QA) testing at the MTN NL
  - Resolution of ambiguous endpoints
  - Resistance Testing among seroconverters
- Future research

VOICE Urine and Pelvic tests

- Urine hCG
- Dipstick
- SDA for GC/CT
- Vaginal pH
- KOH Wet Mount for Fungal elements (Candidiasis)
- Vaginal Gram stain
- Pap Smear
- Vaginal and Endocervical Swabs for storage

VOICE Laboratory Testing

- Some key areas of importance for laboratory testing
  - Products can cause Liver toxicity
    - Screening for Hepatitis B
    - AST, ALT
  - Products can cause Hypophosphatemia
  - Products can cause Kidney Toxicity
    - Urine Dipstick Screening
    - Creatinine

VOICE Laboratory Testing

- Some key areas of importance for laboratory testing
  - HIV Testing
    - Drug resistance
  - Pregnancy
    - Products not yet approved for use in pregnancy

Specimen Collection and Transport

- Urine Specimens
  - Urine Dipstick
    - Glucose
    - Protein
    - Nitrite
    - Leukocyte Esterase
  - hCG/Pregnancy Test
  - Strand Displacement Amplification (SDA)
    - *Neisseria gonorrhoeae* (GC)
    - *Chlamydia trachomatis* (CT)

Specimen Collection and Transport (cont.)

- SDA
  - DNA Amplification method
    - Instrument is the BD Probetec
    - Validated for use with Tenofovir Gel
  - Cross Sample Contamination can occur
    - Very small amounts of GC/CT organism from one sample can contaminate another sample causing a false positive
    - Proper handling crucial

Specimen Collection and Transport (cont.)

- To prevent Contamination
  - Change gloves between specimens
  - Open one specimen at a time
  - Use sterile screw top containers
  - Do not introduce non sterile items (such as pipettes) into the sample

Specimen Collection and Transport (cont.)

- Urine
  - Collect urine specimens before performing any pelvic tests
  - Collect first specimen-not mid stream
  - 15-60 mls of urine
  - If performing SDA, hCG and Dipstick
    - Separate urine first for hCG and dipstick
    - Refrigerate urine for SDA

Specimen Collection and Transport (cont.)

- Urine
  - hCG and Dipstick may be done Point of Care (POC) or transported to a laboratory
  - The site laboratory is responsible to oversee any testing done at the site including non-lab personnel

Testing Considerations-measurements for dipsticks

Leucocytes					
Negative	Trace	+	++	+++	
Nitrites					
Negative	Positive				
Protein (mg/dl)					
Negative	Trace	+ (30)	++ (100)	+++ (300)	++++ (≥ 2000)
Glucose (mg/dl)					
Negative	Trace (100)	+ (250)	++ (500)	+++ (1000)	++++ (≥ 2000)

Testing Considerations

- CRF's will either match this scoring system or give instructions to grade a result as positive or negative
- If sites can obtain 2 test sticks made by Bayer/Siemens, these can be used for follow up of 1+ results.
  - Protein/Glucose (Cat#2184)
  - Nitrite/Leukocyte (Cat#2166)

Specimen Collection and Transport (cont.)

- Specific Kits for hCG and Dipstick have been validated for use with gels
- Urine Dipsticks
  - Bayer/Siemens
  - Validation to be done for Protein and Glucose
- Urine hCG
  - Quidel

Specimen Collection and Transport (cont.)

- Specimen Stability
- SDA-refrigerate urines before transport to lab
  - 2-30ºC: 30 hours
  - 2-8ºC: 7 Days
  - ≤ -20º: 2 months
- hCG
  - Room Temp: 8 hours
  - Refrigerated: 72 Hours
- Dipstick
  - Room Temp: Analyze within 2 hours of Collection

Specimen Collection and Transport (cont.)

- Pelvic Specimens
  - Vaginal pH
  - Vaginal Gram Stain (read at MTN NL)
  - Trichomonas Rapid Test
  - BV Rapid Test
  - KOH wet mount for Fungal Elements
  - Vaginal Swab For Storage
  - Endocervical Swab For Storage
  - Pap Smear (done per local guidelines)

Specimen Collection and Transport (cont.)

- Vaginal pH
  - Two methods available:
    - placing pH paper directly on the vagina
    - swabbing vagina and application to pH paper
  - pH Indicator Strips (pH range 3.6 to 6.1)
  - These Brands are all acceptable-sites will be asked to purchase directly. Contact NL if this is not possible.
    - Machery-Nagel
    - Baker
    - SP

Specimen Collection and Transport (cont.)

- KOH wet Prep
  - Swab lateral vaginal wall
    - Smear vaginal fluid directly on slide OR
    - Place swab in glass or plastic tube with 6 drops of saline for transport to testing area; then swab slide
  - Add one drop of 10% KOH to slide
  - Coverslip and observe for fungal elements

Specimen Collection and Transport (cont.)

- KOH wet Prep
  - Used to identify yeast and fungal elements
  - Cannot identify clue cells or *Trichomonas*
  - Turn Around Time
    - Does not need to be read within 30 minutes like saline wet mount-this is only relevant to observe *Trichomonas* motility (movement)
    - Yeast will stay stable in saline for several days-should be read during visit. This test is only done if clinically indicated so will be needed for treatment

OSOM BV Blue

Specimen Collection and Transport (cont.)

- OSOM BV Blue
  - Tests for high levels of sialidase produced by organisms associated with BV.
    - *G. vaginalis*, *Prevotella* sp., and *Mobiluncus* sp.
  - Approximately 12 minutes to perform
  - Swabs can be held at room temperature for 48 hours prior to testing; refrigerated up to 7 days
  - Testing should be done during visit for enrollment or treatment considerations
  - Refer to handouts for examples of testing logs

BV Blue Test Procedure

- Collect swab with vaginal fluid from lateral wall
- May be placed in a tube with no media and capped or tested immediately
- Put swab in BV test vessel, swirl and let stand for 10 minutes
- Add one drop of developer
- Results
  - Positive: Blue or Green
  - Negative: Yellow

osom BV BLUE® Test

This is a CLIA Waived Test

QUICK REFERENCE INSTRUCTIONS

WARNING: Read the Directional Insert prior to performing the test.

Collect a vaginal fluid sample with a swab. Contact the swab with the lower one-third of the vaginal wall. Collect as much fluid as possible. Put the swab into the BV Test Vessel. Gently swirl the mixture.

Let the BV Test Vessel containing the swab stand for 10 minutes between 17° and 37°C.

Add one drop of Developer Solution to the BV Test Vessel containing the swab.

Gently swirl the mixture. Read the results immediately.

CAUTION: The Developer Solution is a dilute alkaline solution. This may cause skin and eye irritation. If the solution comes in contact with the skin or eyes, flush with large volumes of water.

a) **Positive Result:** A blue or green color in the BV Test Vessel or on the head of the swab.

b) **Negative Result:** A yellow color in the BV Test Vessel.

POSITIVE NEGATIVE

INTERPRETATION OF TEST RESULTS:
There are two possible results:
(a) positive result or (b) negative result

NOTE: You may need to remove the swab to read the test results.

A Positive Result shows a high level of sialidase activity. A Negative Result shows a normal level of sialidase activity.

OSOM Rapid Trichomonas

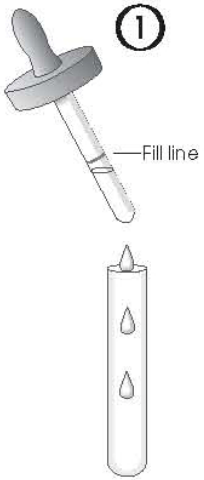
Specimen Collection and Transport (cont.)

- OSOM Trichomonas Test
  - Detection of *Trichomonas* protein antigen
  - A capillary flow dipstick
  - Takes approximately 12 minutes to perform
  - Collect vaginal swab and place in a clean tube with no additives until testing can be performed.
  - Swabs can be held at room temperature for 24 hours; refrigerated for 36 hours before testing.
  - Testing should be done during visit for enrollment and treatment considerations

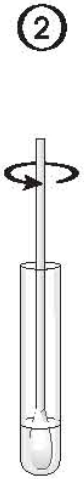
Rapid *Trichomonas* Test Procedure

- Fill dropper with buffer and add to OSOM test tube
- Transfer swab from collection tube to tube with buffer and mix vigorously
- Allow to soak for 1 minute
- Remove swab and expel as much fluid as possible
- Add test stick, wait ten minutes
- Results
  - Positive: Blue test line, red control line
  - Negative: No blue test line, red control line
  - Invalid: no red control line

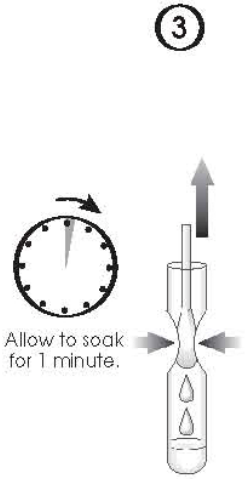
osom Trichomonas Rapid Test



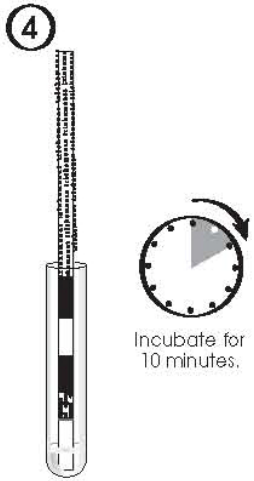
Add Sample Buffer
Fill the dropper to the line indicated on the barrel and expel entire contents into tube.



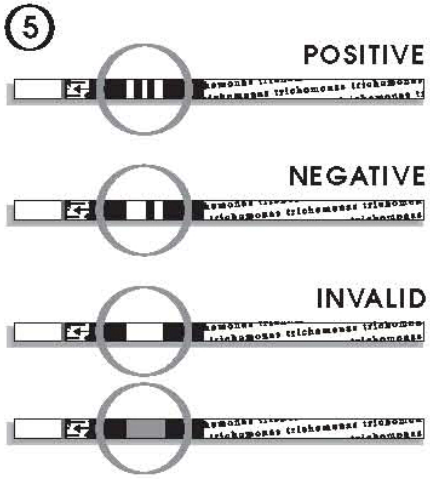
Mix Swab in Buffer
Add swab to tube and mix vigorously (approx. 10 times.)



Squeeze Liquid from Swab
Squeeze side of tube to express as much liquid from swab as possible.



Add Test Stick and Incubate Place absorbent end of test stick into the solution.



- A blue Test Line and a red Control Line is a positive result
- A red Control Line but no blue Test Line is a negative result.
- If no red Control Line appears or background color makes reading the red Control Line impossible, the result is invalid.

QUALITY CONTROL (QC)
Internal Procedural Controls
1. The appearance of the control line in the results window is an internal positive procedural control.
2. The clearing of the background in the results area may be documented as an internal negative procedural control.

External Controls
Genzyme recommends that positive and negative external controls be run with each new lot and with each new untrained operator. One positive control swab (pink shaft) is included with each kit. For a negative control, run one of the sterile swabs supplied with the kit. Run controls in the same manner as patient swabs.

genzyme Diagnostics

Rev. 3733-3, 03/06

Specimen Collection and Transport (cont.)

- CAP Panel available for the Rapid Trichomonas Test (VS1 panel-already added for confirmed sites for 2009)
- No Panel available for Rapid BV test
- Validation of these kits in progress at the Network Laboratory

Specimen Collection and Transport (cont.)

- Vaginal and Endocervical Swabs
  - One swab from posterior fornix of vagina
  - One swab from endocervix
  - Collect and put into separate PBS cryovials
  - Freeze within 8 hours of collections
    - If specimens come in late, will need to be processed the same day
  - Store on site until notification from study team or Network Laboratory

Specimen Collection and Transport (cont.)

- Vaginal Gram Stain
  - Make two slides, allow to air dry (no heat fixing)
  - Do not stain
  - One slide shipped to Network Lab, one retained onsite in case of problem
  - Periodic shipments during the study will be made

Specimen Collection and Transport (cont.)

- Blood Specimens
  - Chemistries
  - Hematology
  - Hepatitis B
  - HIV Testing
  - Plasma Archive
  - Viral Load (as needed for HIV Testing Algorithm)
  - Seroconversion
    - Viral Load
    - CD4 Count
    - Resistance Testing

Specimen Collection and Transport (cont.)

- Most testing of blood specimens done onsite for VOICE
  - Are not specified methods-sites choose and validate methods. In these cases, there will be review of SOP's and oversight of the testing by the Network Laboratories and SMILE
  - Specimen Handling requirements are locally defined
- Most specimens that are shipped will have mandated handling criteria

Blood Draw Volumes

- Refer to Blood Draw Volume Tables-these are approximate volumes
- Sites must determine tubes to be drawn that will satisfy local testing requirements and yield adequate volumes for testing done at Network Laboratories
- Volumes must be consistent with Informed Consent Process

Specimen Collection and Transport (cont.)

- Chemistry (Serum)
  - Liver Function: AST+ALT
  - Kidney Function: Creatinine (Calculate Creatinine Clearance each time performed)
    - Cockroft-Gault Formula in mL/min = (140 - age in years) x (weight in kg) x 0.85/ 72 x (serum creatinine in mg/dL)
  - Phosphate
  - Performed per local SOP
- Testing done same day or as allowable per site SOP

Specimen Collection and Transport (cont.)

- Hematology (FBC or CBC) (EDTA Whole Blood)
  - Hemoglobin
  - Hematocrit
  - Mean Corpuscular Volume
  - Platelets
  - White blood cell count with differential
    - Absolute neutrophil count
    - Percent neutrophils
    - Absolute lymphocyte count
    - Absolute monocyte count
    - Absolute eosinophil count
    - Absolute basophil count
- Per Site SOP
- Testing done same day of collection or as acceptable by site SOP

Specimen Collection and Transport (cont.)

- Specimen Quality is key
  - Hemolysed Serum
    - Affects many chemistry tests
    - Can cause false elevation of Phosphate, AST, ALT
  - Clotted EDTA Tubes
    - Affect numerous hematology parameters
  - Underfilled EDTA Tubes
    - May dilute specimens for hematology

Specimen Collection and Transport (cont.)

- Specimen Quality is key
  - Phlebotomy technique and handling will affect specimen quality
  - Proper training (and retraining when problems are noted) is key.
  - Some issues
    - Trauma caused by technique-too much needle movement during draw, etc…
    - Proper needle gauge
    - Allow alcohol to dry
    - Properly filled tubes (use appropriate size)
    - Properly connected phlebotomy equipment
    - Syringes-do not draw back to hard
    - Hemolysis may also occur during transport-handle specimens with care

Some Testing Considerations

- Normal Ranges
  - These must be validated or established for chemistry and hematology for local populations before VOICE activation
- Abnormal values
  - Sites need to have procedures in place for Urgent Value management and Adverse Event reporting

Some Testing Considerations

- White Blood Cell Differentials
  - Results will be required as absolute units on the CRF's
  - Needed for adverse event reporting
    - DAIDS Tox Tables do not list ranges for percent values

Specimen Collection and Transport (cont.)

- Syphilis Serology
  - RPR for screening
    - Very Non specific
  - Treponemal Confirmation
    - TPPA, TPHA or other
  - Testing done per local SOP on serum or plasma
  - These are batched per local SOP-usually at least weekly

Specimen Collection and Transport (cont.)

- Hepatitis B Surface Antigen and Antibody
- Most sites already have antigen test on site but must bring in antibody test
- Will be done per local SOP
- Samples will be batched
  - Turn around time will be defined by local SOP
    - Screening-frequent enough for enrollment
    - Post Vaccination-frequent for patient management

Testing Considerations

- Hepatitis B interpretation
  - For screening, only a positive or negative result is needed (Appendix IV)
  - HBsAG negative: eligible
    - HBsAB- Not HBV immune, counsel and offer vaccination
    - HBsAB+ HBV Immune
  - HBsAG Positive
    - Ineligible-counsel and referral

Testing Considerations

- Hepatitis B interpretation
  - Post vaccination:
    - Hepatitis B Surface Antibody only
    - A qualitative result is needed to determine response to vaccine
    - Specific cutoffs for interpretation to be determined but will likely follow WHO guidelines

Specimen Collection and Transport (cont.)

- HIV testing-rapid tests
  - Specimen type may be venous serum, EDTA plasma or whole blood as allowed by kit directions (validation required)
  - Fingerstick testing may be done as allowed by kit directions (validation required)
  - Results must be available during visit
    - Screening samples required for eligibility
    - Positive rapid tests at follow up will result in a product hold

Specimen Collection and Transport (cont.)

- HIV testing-rapid tests
- Two rapid tests (at least one FDA approved) required for screening
- One FDA approved rapid test done monthly for follow up visits
- (Algorithm to be covered separately)
- Kits selected by sites and approved by MTN before study activation

Testing Considerations

- HIV Rapid tests at Follow Up
  - The MTN Network Lab does not believe that using 2 rapid tests will detect HIV infection earlier at follow up.
  - Please refer questions on this to the MTN Network Laboratory
  - Sites may perform 2 rapid tests if required by local regulations

Testing Considerations

- Discussion-what kits do sites plan to use?
- Sites have previously validated
  - FDA Approved
    - Oraquick
    - Unigold
  - Non-FDA approved
    - Determine
- Other FDA Approved Kits (As of February 2008)
  - Reveal (Trinity)
  - Multispot (Biorad)
  - Clearview (Inverness)
  - http://www.cdc.gov/hiv/topics/testing/rapid/rt-comparison.htm

Specimen Collection and Transport (cont.)

- HIV Testing-Western Blot
  - Requires venous sample (EDTA Plasma)
  - Testing usually batched-must be done frequently enough for enrollment purposes and patient management
  - Western Blot results should routinely be available within 7 days; consideration for participants

Specimen Collection and Transport (cont.)

- HIV RNA Viral Load-performed in 2 situations
  - HIV testing Algorithm at follow up
  - Seroconverters

Specimen Collection and Transport (cont.)

- HIV RNA Viral Load (As part of Testing algorithm)
  - When Sample 1 Western Blot is negative or indeterminate (See algorithm)
  - As directed by the Network Lab
  - Batched per site SOP
    - Testing must be frequent enough for patient management
    - Patients will have questions about HIV status and will be waiting on results

Specimen Collection and Transport (cont.)

- HIV RNA Viral Load (Seroconversion)
  - EDTA double spun plasma
  - Batched per site SOP-should be frequent enough for patient management
  - Most sites will perform standard method per local SOP
    - In these cases, when results are below the level of detection (400 copies):
      - the sites can ship plasma to the Network lab for the ultra sensitive method
      - Requires 2 ml EDTA double spun plasma
      - Not required for patient management

Specimen Collection and Transport (cont.)

- Flow Cytometry (Seroconversion)
  - CD4 Positive T-Lymphocytes
  - EDTA Whole Blood
  - Testing done per site SOP's
  - Generally done within 48 hours or per site SOP

Specimen Collection and Transport (cont.)

- EDTA Plasma Archive
  - Kept at room temp: freeze within 4 hours
  - Refrigerated: freeze within 24 hours
    - **We recommend that sites routinely refrigerate these samples**
  - Resistance Testing (Seroconversion)
    - 5 mls preferred; 4 minimum
    - Batched and shipped to Network Lab
  - Routine Archive
    - Store all available (at least 3 mls)

Specimen Collection and Transport (cont.)

- Specimen Storage and Shipment
  - All sites will use LDMS to track specimen storage and shipments
  - Specimen shipment schedules to follow
  - Specimens stored in LDMS
    - Plasma
    - Vaginal Gram Stains
    - Vaginal Swabs
    - Endocervical Swabs

Laboratory Activation Requirements

- Refer to Handout

Thanks

- Questions?