

Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-007.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, rectal, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control and Prevention can be found at the following website:

- http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Some laboratory procedures will be performed in the study site clinic or laboratory and others in the MTN Network Laboratory (NL). Table 12-1 lists for each test, the testing location, specimen type, specimen container and kit/method (if specified). Table 12-2 specifies blood collection by visit type and suggested volumes.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

All site laboratories will be monitored by the MTN NL which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc.) to monitor and certify laboratories for testing. US sites that utilize Clinical Laboratory Improvement Amendment (CLIA) certified laboratories for testing will be able to substitute a valid CLIA certificate as documentation of the quality of the work at the laboratory.

**Table 12-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-007**

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urine pregnancy test	In Clinic	Urine	Plastic screw top cup	Quidel Quick Vue or Fisher Sure-Vue
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Urine Preservation Tube (UPT)	GenProbe Aptima or BD Probetec
Dipstick Urinalysis	In Clinic	Urine	Plastic screw top cup	Bayer Multistix® 10 SG or Bayer Uristix 4
Complete blood count w/differential and platelets	Local CLIA Lab	Whole Blood	EDTA tube	Not specified
Chemistries (BUN, Creatinine, ALT, AST)	Local CLIA Lab	Serum or plasma	Consult local lab requirements	Not specified
Syphilis RPR (confirmatory test as needed)	Local CLIA Lab	Serum or Plasma	Red or Purple top tube	Not specified
HIV antibody screen and Western Blot	Local CLIA Lab	Plasma or whole blood (serum acceptable)	EDTA or plain tube	FDA approved tests
HBsAg	Local CLIA Lab	Serum	SST or plain tube	Not specified
HSV-1 and HSV-2 IgG Serology	Local CLIA Lab or MTN NL	Serum	SST or plain tube	Not specified
Plasma archive	Site Lab	Plasma	EDTA tube	N/A
Rectal NAAT for Gonorrhea and Chlamydia	Local CLIA Lab or MTN NL	Anorectal swab	Transport tube	GenProbe Aptima or BD Probetec
Rectal microflora	MTN NL	Rectal swab	Port-a-cul Tube	Culture
Epithelial sloughing	MTN NL	Rectal effluent	Specimen pan	MTN NL protocol
Histology	MTN NL	Rectal biopsies	Vial with 10% formalin	N/A
Cytokine RT PCR	MTN NL	Rectal biopsies	Cryovial with <i>RNAlater</i>	MTN NL protocol
Mucosal T cell phenotyping	MTN NL	Rectal biopsies	Tube with cRPMI	MTN NL protocol
Cytokines	MTN NL	Rectal sponge	Cryovial	Luminex
Fecal Calprotectin	Genova Diagnostics	Stool	Genova container	Genova protocol
Mucosal gene expression array	MTN Immunology Core	Rectal biopsies	Cryovial with <i>RNAlater</i>	MTN Immunology core protocol

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN NL may request details of collection containers and volumes for this purpose.

**Table 12-2
Scheduled Blood Collection by Visit Type and Suggested Volumes**

Visit Type	Total Blood Volume (mL)	Volume By Tube Type (mL)	Purpose
Screening	18	Red Top: 12	BUN, Creatinine, ALT, AST RPR, HBsAg, HSV serology
		Purple Top: 6	CBC w/diff and platelets, HIV-1 serology
Enrollment	10	Red Top: 4	HSV serology, *RPR
		Purple Top: 6	*HIV-1 serology, Plasma archive
Final Clinic Visit	14	Red Top: 8	BUN, Creatinine, ALT, AST RPR
		Purple Top: 6	CBC w/ diff and platelets, HIV-1 serology
Early Termination Visit	14	Red Top: 8	BUN, Creatinine, ALT, AST RPR
		Purple Top: 6	CBC w/ diff and platelets, HIV-1 serology
Interim Contacts and Visits	14	Red Top: 8	BUN, Creatinine, ALT, AST RPR
		Purple Top: 6	CBC w/diff and platelets, HIV-1 serology

**Notes: Additional blood may be collected for any clinically indicated testing. Red top tubes contain no additive. Purple top tubes contain EDTA.*

Ideally, one method, one type of test kit, and/or a combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or test kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to implementing a change in methods. The MTN NL must be notified before implementing the change and the MTN NL can provide further guidance on validation requirements. Similarly, the MTN NL must be notified of changes to normal lab ranges.

Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities.

This section of the MTN-007 SSP manual gives basic guidance to the sites, but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with Standard Operating Procedures. The MTN NL is available to assist in the creation of any SOPs upon request. Essential SOPs include but are not limited to:

- SOPs created by the site
 - Specimen Collection and transport*
 - Chain of Custody *
 - Urine Dipstick *

*Must be approved by the MTN NL for study activation

12.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. The date of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen).

When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Specimens that are sent to the NL or are archived at the site will be entered into LDMS and labeled with LDMS-generated labels.

12.3 Procedures for Specimens that can not be Evaluated

When possible, specimens will be redrawn or recollected if it is found that they cannot be evaluated per site SOP's. The site will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected due to a laboratory error (lost or broken specimen or clerical error) or a clinic error (clerical error), a protocol deviation form may be required.

12.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used to track the collection, storage, and shipment of eight types of specimens in MTN-007: plasma for archive, rectal microflora, epithelial sloughing, biopsies for histology, cytokine RT PCR, mucosal T cell phenotyping, mucosal gene expression, and sponges for cytokines. See Table 12.4 for further information.

Detailed instructions for use of LDMS are provided at: <https://www.fstrf.org/ldms> (may require a password).

The site will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. The site will be responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Questions related to use of LDMS in MTN-007 may be directed to Pam Kunjara or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm (ET) on Monday and Fridays and 7:30 am - 8:00 pm (ET) on Tuesdays, Wednesdays, and Thursdays. During business hours, please contact LDMS User Support as follows:

Email: ldmshelp@fstrf.org

Phone: +716-834-0900, ext 7311

Fax: +716-898-7711

LDMS User Support can be paged via e-mail during off business hours if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1 (address shown in table below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)
- The full telephone number at which you can be reached, including the country code and city code if you are outside the United States
- A short description of the problem

If a response is not received within 15 minutes after emailing LDMS 1, try emailing LDMS 2, then finally, LDMS 3.

Table 12-3
LDMS User Support Paging Details

Pager	Email Address
LDMS 1	ldmspager1@fstrf.org
LDMS 2	ldmspager2@fstrf.org
LDMS 3	ldmspager3@fstrf.org

The site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for the site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN NL is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., blood needed for confirmatory HIV testing) that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Table 12-4
LDMS Specimen Management Guide to Logging in 007 Specimens

The table below should be used as a guide when logging in 007 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tests that are listed as local do not require that a sample be logged into the LDMS. See Appendix 12-1 for a copy of the LDMS tracking sheet.

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume	Units
Plasma Archive	BLD	EDT	PL1	N/A	3.0	1.5	ml
Rectal Microflora	REC	NON	SWB	N/A	Variable	0.1	ml
Rectal sponge for cytokines	REC	PBS	SPG	N/A	Variable	1	Ea
Epithelial Sloughing	REC	NOR	LAV	PFM	Variable	variable	ml
¹ Biopsies for cytokine Anoscope 9 cm	ARB	RNL	TIS	N/A	2	1	Ea
² Biopsies for Gene Expression Anoscope 9 cm	ARB	RNL	TIS	N/A	1	1	Ea
Biopsies for Mucosal T cell phenotyping Anoscope 9 cm	ARB	RPM	TIS	N/A	3	3	Ea
Biopsies for Histology Anoscope 9 cm	ARB	FOR	TIS	N/A	1	1	Ea
¹ Biopsies for cytokine Sigmoidoscopy 15 cm	FSR	RNL	TIS	N/A	2	1	Ea
² Biopsies for Gene Expression Sigmoidoscopy 15 cm	FSR	RNL	TIS	N/A	1	1	Ea
Biopsies for Mucosal T cell phenotyping Sigmoidoscopy 15 cm	FSR	RPM	TIS	N/A	3	3	Ea
Biopsies for Histology Sigmoidoscopy 15 cm	FSR	FOR	TIS	N/A	1	1	Ea

1, 2: Specify Test Setup in LMDS Specimen Management Module (this is only for biopsy cytokine and biopsy gene expression)

- After creating aliquots in LDMS, right click on an aliquot
- Select “Test Setup”; this will bring up a dialogue box.
- Select either Cytokines or Gene Expression depending on the aliquot.
- Click save. When LDMS prompts that the changes have been saved, click Done to exit the dialogue box.
- In the “Other Spec ID” field of the aliquot line (between “Cond” and “Group ID”) enter either CYK for Cytokines or GENE for Gene Expression. Click Save.

BLD: Whole Blood
EDT: EDTA
PL1: Single spun Plasma
REC: Rectal
NON: None
SWB: Swab

SPG: Sponge
PBS: Phosphate Buffered Saline
NOR: Normosol-R
LAV: Lavage
PFM: Paraformaldehyde
ARB: Rectal biopsy by anoscope

FSR: Rectal biopsy by Flexible sigmoidoscopy
TIS: Tissue
RPM: cRPMI media
RNL: RNAlater
FOR: Formalin

**Table 12-5
Specimen Shipping Summary**

Specimen	Use LDMS?	Ship to:	Shipping schedule
Rectal Microflora	Yes	MTN NL - Pittsburgh	Shipped daily and refrigerated on ice packs
Rectal effluent for Epithelial Sloughing	Yes	MTN NL - Pittsburgh	Shipped daily and refrigerated on ice packs
Biopsies for Histology	Yes	MTN NL - Pittsburgh	Shipped daily ambient or refrigerated on ice packs
Biopsies for Mucosal T cell Phenotyping	Yes	MTN NL - Pittsburgh	Shipped daily and refrigerated on ice packs
Rectal sponge for Cytokines	Yes	MTN NL - Pittsburgh	Batched and shipped frozen on dry ice
Biopsies for Cytokine RT PCR	Yes	MTN NL - Pittsburgh	Batched and shipped frozen on dry ice
Biopsies for Mucosal Gene Expression Array	Yes	MTN Immunology Core - Seattle	Batched and shipped frozen on dry ice
Rectal Stool for Fecal Calprotectin	No	Genova Diagnostics	Shipped daily at ambient temperature (Friday collected specimens are shipped on Monday)

12.5 Urine Testing for Urinalysis, Pregnancy, Chlamydia and Gonorrhea

The urine tests performed at the study visit will depend on the time point of the visit and the clinical presentation of the participant. In general at study visits when urine testing is required, a single specimen will be collected and aliquots will be made for each test when possible. When doing multiple tests from one specimen, the correct order is separation of urine for the Chlamydia and Gonorrhea first and then the urine dipstick last.

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only.

12.5.1 Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the female participant not to clean the labia prior to specimen collection. Male participants should withdraw foreskin if present.
- Collect the first 15-60 mL of voided urine in a sterile collection cup. (Not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5-10 mL for these tests and store the remaining urine at 2-8°C or introduce the urine immediately into the UPT for subsequent Chlamydia and Gonorrhea testing.

12.5.2 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine microscopy and a urine culture according to local SOP. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

12.5.3 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5-10 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the urine is too dark to read the pregnancy test, another urine sample will need to be collected.

Note: Protocol-specified pregnancy testing is not discontinued during pregnancy.

The Fisher Sure-Vue or Quidel QuickVue One-Step hCG urine pregnancy test must be used at all sites. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

12.5.4 Chlamydia and Gonorrhea Testing

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only.

This testing will be done using the Gen-Probe Aptima or Becton Dickinson ProbeTec NAAT Methods by the local laboratory.

Instructions for transferring urine into the UPT

- Collect urine as noted above.
- Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.
- Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
- The specimen can now remain at 2-30°C for 30 days.
- Results will be sent to the clinic for reporting on the CRF.

12.6 Blood Testing

The blood tests performed depend on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

12.6.1 Specimen Collection and Initial Processing

Label all required primary tubes with a SCHARP-provided PTID label at the time of collection.

After collection:

- Allow red top tubes (no additive) to clot, then centrifuge per site SOPs for, syphilis, liver function, and renal function testing.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for plasma archive.

Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.

12.6.2 HIV Testing

HIV testing must be validated at the study site per the CLIA standards. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status at screening will be assessed using an FDA-approved HIV test per the MTN-007 HIV testing algorithm (see appendix II in the current version of the MTN-007 protocol). If the test is non-reactive, the participant will be considered HIV-seronegative. If the test is reactive, an FDA-approved Western Blot (WB) or Immunofluorescent Antibody (IFA) test will be performed; if additional blood must be drawn for the WB or IFA, this is still considered sample 1 per the algorithm. If the WB or IFA is negative, the participant will be considered HIV-seronegative; this situation is not anticipated. Contact the MTN NL if this occurs. If the WB or IFA is positive, the participant will be considered HIV-seropositive. A second specimen will be drawn for confirmatory testing. If the WB or IFA is indeterminate, the site should contact the NL for further instructions.

Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

All test results must be documented on local laboratory log sheets or other laboratory source documents. In addition to initialing or signing the testing logs to document review and verification of the results, the second lab staff member must also record the time at which the results were reviewed and verified.

12.6.3 Syphilis Testing

Syphilis testing will be performed using a rapid plasma reagin (RPR) screening test followed by a confirmatory microhemagglutinin assay for *Treponema pallidum* (MHA-TP) or *Treponema pallidum* haemagglutination assay (TPHA). Any RPR, MHA-TP, and/TPHA test may be used; however titers must be obtained and reported for all positive RPR tests. RPR tests may be performed on either serum or plasma. MHA-TP and TPHA tests must be performed on serum. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

For reactive RPR tests observed during screening, a confirmatory test result must be received and appropriate clinical management action taken, prior to enrollment in the study. Clinical management should include repeat RPR tests at quarterly intervals following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease four-fold or revert to seronegative within three months after treatment, treatment should be repeated.

Please consult the MTN NL with any questions related to Syphilis testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation vis-à-vis eligibility and enrollment in the study should be directed to the MTN-007 Protocol Safety Review Team.

12.6.4 Hepatitis B Surface Antigen and HSV-1 and HSV-2 IgG Serology

This testing will be done on serum per local SOPs

12.6.5 HSV serology

Testing will be done for both HSV-1 and HSV-2 IgG antibody
Testing will be performed on serum per local SOPs

12.6.6 Hematology Testing

Complete blood counts (CBC) with five-part differentials will be performed at all sites. Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Platelets
- White blood cell count with differential
- Red blood cell count

These tests will be performed on EDTA whole blood per local site SOPs.

12.6.7 Liver and Renal Function Testing

The following tests will be performed to evaluate liver and renal function:

Liver Function

- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)

Renal Function

- BUN
- Creatinine

These chemistry tests will be collected and performed according to local laboratory SOPs.

12.6.8 Plasma Archive

For plasma archive, use EDTA. These will be stored at $\leq -70^{\circ}\text{C}$ and batched onsite until the MTN-007 study team requests shipping and/or testing. The Pittsburgh site will send whole blood EDTA to be processed at the NL on the day of collection.

- LDMS will be used to label and track the specimens.
- If at room temp, freeze within 4 hours. If refrigerated or on ice after collection, freeze within 24 hours.
- Prepare as many 1.5 mL aliquots as available to store. If less than 1.5 mL of plasma are available, store that plasma and inform the MTN NL for instruction.
- The MTN NL will send instructions to the site when shipping and/or testing is required.

12.7 Testing of Rectal Specimens

The tests performed on rectal specimens depend on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

Rectal samples should be collected in the following order.

1. Rectal swab for GC/CT
2. Rectal swab for microflora
3. Rectal sponges for cytokines
4. Digital rectal examination
5. Rectal lavage/effluent for epithelial sloughing
6. Stool sample for fecal calprotectin
7. *Flexible sigmoidoscopy and biopsies at 15cm for Histology, Cytokine RT PCR, Mucosal T cell phenotyping, and mucosal gene expression array
8. *Anoscopic biopsies at 9cm for Histology, Cytokine RT PCR, Mucosal T cell phenotyping, and mucosal gene expression array

Table 12-6 gives a brief summary of how these rectal samples should be handled.

**If at anytime the collection of biopsies is limited, submit for assays in order of importance - Histology, Mucosal Gene Expression Array, Cytokine RT PCR, then T Cell Phenotyping.*

**Table 12-6
Specimen Handling Guidelines**

Assay	Primary Specimen	Additive/Container	Minimum volume required	Handling Requirements
Rectal GC/CT	Rectal Swab	Transport tube	N/A	Store at 2-30°C for up to 30 days
Rectal Microflora	Rectal Swab	Port-A-Cul tube	N/A	Store at RT up to 4 hours, then refrigerate
Rectal Sponge for Cytokines	Rectal Sponge	5mL polystyrene tube	N/A	Freeze at -80°C within 4 hours of collection
Epithelial Sloughing	Lavage Effluent	2% paraformaldehyde	N/A	Process within 8 hours of collection
Fecal Calprotectin	Stool	White container from Genova Kit	20g stool (Lima bean sized)	Store at RT
Histology	Biopsy	10% formalin *(orange top vial)	1 biopsy per site	Store at RT
Cytokine RT PCR	Biopsy	RNAlater (green top tube)	1 biopsy x2 per site	Store at 4°C overnight (16-24 hours) then transfer to -80°C.
Mucosal T Cell Phenotyping	Biopsy	cRPMI in 15ml conical tube	3 biopsies per site	Keep refrigerated
Mucosal Gene Expression Array	Biopsy	RNAlater (blue top tube)	1 biopsy per site	Store at 4°C overnight (16-24 hours) then transfer to -80°C.

**The Magee site will be using cassettes and pre-filled formalin cups.*

12.7.1 Rectal NAAT for Gonorrhea and Chlamydia

Note: Testing for Chlamydia and Gonorrhea is done at screening and when clinically indicated only. Product gel may cause interference during testing. Please be sure gel has not been used within the past 24 hours.

This testing will be done by the local lab using either the BD Probe Tec or Gen-Probe Aptima Methods. Following are collection and transport instructions:

Instructions for collection and transport of rectal swabs for GC/CT testing

- For specimens to be tested with the ProbeTec instrument use the BD ProbeTec CT/GC Endocervical Specimen Collection and Dry Transport kit.
- For specimen to be tested with the Gen-Probe Aptima instrument use the Gen-Probe Aptima Unisex Swab (blue swab).
- Label the transport tube with the participants PTID number and date.
- Remove the swab from the plastic transport tube and insert into the rectum according to the procedure outlined in the SSP for Clinical Considerations (Section 10) and rotate gently through 360 degrees and remove.
- Immediately place the swab in the transport tube, break off shaft of swab and cap. The specimen can now remain at 2-30°C for 30 days.
- Place the transport tube in a biohazard zip-lock bag and transport to the local laboratory for testing.
- Specimens for GC/CT are not logged into LDMS. The results are sent to the clinic and are reported on a CRF.

12.7.2 Rectal microflora

Rectal swabs will be collected for semi-quantitative cultures and sent to the MTN NL. Shipping instructions follow.

- The following supplies will be provided by the NL: Sterile Dacron swabs, Port-a-cul transport tubes, and shipping containers. Sites will need to provide their own ice packs.
- Rectal swabs collection:
 - Insert the swab into the anal canal following the procedure described in the SSP for Clinical Considerations.
 - Rotate the swab through 360 degrees.
 - Slowly remove the swab and place into a Port-A-Cul transport tube (labeled with a SCHARP label), submerging the swab into the gel. Break off the shaft of the swab and cap.
- The specimen may be kept at controlled room temperature for up to 4 hours. It must be refrigerated after that and shipped with ice packs.
- Deliver the Port-A-Cul and the LDMS specimen tracking sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the culture into LDMS (specimen type =REC) and label the Port-A-Cul tube with an LDMS label.
- Use LDMS to generate a shipping manifest for the culture to be shipped.
- Ship the Port-A-Cul tube the same day of collection by overnight courier.
- Place the Port-A-Cul in a biohazard bag and secure in the leak-proof container with absorbent material. Place the container, ice packs, and a copy of the LDMS manifest in a cardboard box lined with Styrofoam.
- Use diagnostics packing code 650, UN3373.

- The Research Institute is not open for delivery on the weekend. Please ship overnight Monday thru Thursday.

Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room A530
Pittsburgh, PA 15213
Phone# 412-641-6042

Notify the MTN NL via email (lrabe@mwri.magee.edu, kstoner@mwri.magee.edu, pkunjara@mwri.magee.edu) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the LDMS batch to the e-mail notification, and include the shipment tracking number.

12.7.3 Rectal Sponge for Cytokines

- The clinician will collect specimen at 9 cm in the rectum according to the procedures outlined in the SSP for Clinical Considerations.
- After collection the sponge will be placed in a 5.0ml polystyrene tube (Nalgene Cryogenic vials cat. No. 5000-0050) containing 50µl PBS and labeled with the PTID, visit #, and date.
- Complete the LDMS tracking sheet and submit to lab for LDMS entry.
- Log into LDMS and label specimen with LDMS label.
- Freeze at -80°C within 4 hours of collection until ready to ship.
- Specimens may be batched and shipped on dry ice. Once the NL notifies the site to ship, use LDMS to create a shipping manifest.
- Ship specimens to MTN NL Pittsburgh Monday through Wednesday for overnight delivery. See section 12.7.2 for shipping address.

12.7.4 Fecal Calprotectin

Genova Diagnostics kits provide the collection container, transport container, shipping box and FedEx labels. The instructions are printed on the outside of each kit and in Appendix 12-2.

- 20 grams of stool collected after the Normasol enema are transferred into the white container provided in the Genova kit and sent to Genova overnight.
- For specimens collected on Friday, store the specimen at ambient temperature (room temperature) and ship on Monday. The specimens must arrive at Genova within 5 days of collection.
- Results will be sent to Dr. Ian McGowan in Pittsburgh.

12.7.5 Epithelial sloughing

The clinic will transport the effluent in a container with a SCHARP label and date to the local laboratory for initial processing. The specimen should be processed within 8 hours of collection. After processing, the specimen will be shipped to the MTN NL the day of collection.

Procedure for processing specimen at the site prior to shipping:

***If the effluent is clear:**

1. Label a 50 cc conical tube and a cryovial with the PTID and date of collection.
2. Transfer the effluent to a 50 cc conical tube.
3. Record the volume of fluid on the LDMS tracking sheet.
4. Centrifuge the fluid at 1000 rpm for 5 minutes.
5. Remove as much supernatant as possible without disturbing the cell pellet and discard.
6. Add 1 mL of 2% paraformaldehyde (See Section Appendix 12-3 for ordering and preparing solution).
7. Resuspend the pellet gently by swirling and transfer to the cryovial.
8. Fill out the LDMS Tracking sheet and submit to lab for LDMS entry.
9. Log the specimen into LDMS, label specimen with LDMS label, and create a shipping manifest.
10. The specimens must be shipped the day of collection with ice packs to the MTN NL. (Send in the same box as the biopsies for mucosal T cell Phenotyping. See section 12.7.2 above for shipping

****If the effluent is muddy with excessive fecal material:**

1. Label **two** 50 cc conical tubes and **two** cryovials with the PTID and date of collection.
2. Transfer the effluent to **one** of the 50 cc conical tubes.
3. Record the volume of fluid on the LDMS tracking sheet.
4. Centrifuge the fluid at 1000 rpm for 5 minutes.
5. Transfer as much supernatant as possible without disturbing the cell pellet into the second 50 cc conical tube and centrifuge as described above.
6. Remove the supernatant from the second conical tube without disturbing the cell pellet and discard. **Note:** After the second centrifugation, you will have two 50 cc conical tubes with a pellet.
7. Add 1 mL of 2% paraformaldehyde to both pellets.
8. Follow steps 7-10 above.

12.7.6 Biopsy for Histology

- There will be biopsies from the 9 cm and the 15 cm sites. Label 2 containers with the PTID, visit #, visit date, and site location (9 cm and 15 cm).
- The clinic staff will place 1 biopsy from each site into the designated container with 10% formalin. The Pitt site will place biopsies into histology cassettes and pre-filled containers of 10% formalin which will be delivered to MWRI. UAB and Fenway sites will place biopsies into microtubes filled with 10% formalin for shipping. These can be kept at room temperature.
- Complete the LDMS tracking sheet and submit to lab for LDMS entry.
- The tissue processing, staining and evaluation will be performed at the MTN NL.
- Log specimens into LDMS, label specimen with LDMS label, and create a shipping manifest.
- Ship the specimens to the MTN NL at room temperature or on ice with the other samples within 24 hours. See section 12.7.2 above for shipping instructions.

12.7.7 Biopsies for Cytokine RT PCR

- There will be 2 biopsies from the 9 cm site and 2 biopsies from the 15 cm site. Label 4 green topped (Nalgene 5045-0004) cryovials (Nalgene 5000-0020) with the PTID, visit #, visit date, and biopsy site (9 cm or 15 cm).
- Submerge 1 tissue biopsy from each site into the appropriate cryovial containing 1.5 mL of RNAlater Solution (Ambion, Invitrogen Cat #AM7020) See Section Appendix 12-3 below.
- Store each vial containing one rectal biopsy in RNAlater at 4°C overnight (16-24 hours).
- Complete the LDMS tracking sheet and submit to lab for LDMS entry.
- Log the specimens into LDMS and label specimens with LDMS label.
- Transfer vials from 4°C to -80°C. Each biopsy must be stored a minimum of 24 hours at -80°C prior to shipping.
- Batch and send to MTN NL. You will be notified when to send the specimens. See section 12.7.2 for shipping address and e-mail notification.

12.7.8 Biopsies for Mucosal T Cell Phenotyping

- There will be biopsies from the 9 cm and 15 cm sites. Label two 15 cc conical tubes with the PTID, visit #, visit date, and site location (9 cm or 15 cm).
- Submerge 3 biopsies from each site into the site designated tube containing 12-15 mL of cRPMI media (See Appendix 12-3 for ordering and preparing media).
- Keep refrigerated.
- Complete the LDMS tracking sheet and submit to lab for LDMS entry.
- Log the specimen into LDMS, label specimen with LDMS label, and create a shipping manifest.
- Send overnight the day of collection to MTN NL with ice pack. See section 12.7.2 for shipping and e-mail notification.

12.7.9 Mucosal Gene Expression Array

- Take two blue topped (Nalgene 5045-0003) cryovials (Nalgene 5000-0020) each containing 1.5 mL of RNAlater (Ambion, Invitrogen Cat #AM7020) and label them with PID, study visit, biopsy site, and date.
- Place one biopsy from each sampling site (anoscopy at 9 cm and Flex. Sigmoidoscopy at 15 cm) into each cryovial and submerge the tissue in the RNAlater solution.
- Store each vial containing one rectal biopsy in RNAlater at 4°C overnight (16-24 hours).
- Complete the LDMS tracking sheet and submit to lab for LDMS entry.
- Log the specimen into LDMS and label specimen with LDMS label.
- Transfer vials from 4°C to -80°C. Each biopsy must be stored a minimum of 24 hours at -80°C prior to shipping.
- When a batch of 20-30 vials has been collected, ship samples on dry ice Monday thru Wednesday to:

MTN Immunology Core
Attn: Florian Hladik, MD, PhD
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North, D3-355
Seattle, Washington 98109-1024

- Generate a manifest (excel, preferably) to include shipper's address & phone number, recipient's address & phone number, and identification of each sample in the shipment. This can be generated using the LDMS Shipment Module. When creating the batch, select Excel as the shipment type located in the top right corner of the View Shipment screen.
- Send a pre-notification email to the McElrath Lab Repository:
 - Jen Leo (jleo@fhcrc.org)
 - Leo French (lfrench@fhcrc.org)
 - Kristina Robinson (ksrobins@fhcrc.org).
- The pre-notification email must include the manifest as an attachment and the FedEx tracking number for the shipment. The Repository will send an email to the shipper the day the shipment arrives, indicating receipt of shipment.
- Also, shipments are only sent Monday thru Wednesday. Exceptions should be cleared with the lab on a case-by-case basis to make sure someone is available to receive the shipment.

Appendix 12-1

MTN 007

LDMS Specimen Tracking Sheet

For login of MTN 007 stored specimens into LDMS

Page 1 of 2

Participant ID <input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/> <input type="text"/> <p style="font-size: small; margin-top: 5px;">Site Number Participant Number Chk</p>			Visit Code <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>		Specimen Collection Date <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <p style="font-size: small; margin-top: 5px; text-align: center;"><i>dd MMM yy</i></p>		
# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	INSTRUCTIONS FOR PROCESSING LAB		
<input type="checkbox"/>	Blood – Plasma (BLD)	EDT (purple top)	PL1	N/A	Store in aliquots of 1-2 ml. If held at room temperature, plasma must be frozen within 4 hours of collection. If refrigerated or on ice, plasma must be frozen within 24 hours of collection.		
<input type="checkbox"/>	Rectal Swab – Microflora (REC)	NON (no additive)	SWB	N/A	Ship to NL on ice the day of collection		
<input type="checkbox"/>	Rectal Sponge – Cytokines (REC)	PBS (phosphate buffered saline)	SPG	N/A	Store @ <-70°C within 4 hours of collection.		
<input type="checkbox"/>	Rectal Effluent – Epithelial Sloughing (REC)	NOR	LAV	PFM	Process and ship to NL the day of collection. Ship on ice. Volume collected: _____ mL		
<input type="checkbox"/>	Anoscopy Biopsies - Histology (ARB)	FOR	TIS	N/A	Ship to NL at room temperature the day of collection.		
<input type="checkbox"/>	Anoscopy Biopsies - Cytokines (ARB)	RNL	TIS	N/A	Store @ 4°C overnight then transfer to -80°C. Must be stored at -80°C for a minimum of 24 hours prior to shipping. Specify cytokine test in specimen management. (See lab SSP section Table 12-4)		

Comments: _____

Initials: _____ LDMS Data Entry Date: / / _____

Sending Staff Receiving Staff dd MMM yy LDMS Staff

Version 2.0, 01-NOV-10

Purpose: This non-DataFax form is used to document collection and entry of MTN 007 specimens into the Laboratory Data Management System (LDMS).

General Information/Instructions: A copy of this form accompanies specimens for storage (in their original specimen collection containers) to the LDMS entry laboratory. Once the specimens have been entered into LDMS, this form is kept on file at the LDMS entry laboratory. If the site chooses, a copy of this completed form may be made once the specimens have been entered into LDMS and the copy kept in the participant's study notebook. This is not required, however. Because this form is a non-DataFax form, this form should NOT be faxed to SCHARP DataFax.

Item-specific Instructions:

- **Visit Code:** Record the visit code of the visit at which the LDMS specimens were collected.
- **# of TUBES or SPECIMENS COLLECTED:** In the box provided, record the total number of tubes or specimens collected for that primary specimen type. If no LDMS specimens of the primary specimen type were collected, record "0".
- **Initials – Sending Staff:** The clinic staff person who completed the form and/or who is sending the LDMS form and specimens to the LDMS entry lab, records his/her initials here.
- **Initials – Receiving Staff:** The laboratory staff person who received this form (and the LDMS specimens accompanying the form), records his/her initials here.
- **LDMS Data Entry Date:** Record the date the LDMS specimens listed on this form were entered into LDMS.
- **LDMS Data Entry Date – LDMS Staff:** The LDMS laboratory staff person who entered the specimens into LDMS, records his/her initials here.

Version 2.0, 01-NOV-10

MTN 007

LDMS Specimen Tracking Sheet

For login of MTN 007 stored specimens into LDMS

Page 2 of 2

Participant ID <input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> - <input type="text"/> <small>Site Number Participant Number Chk</small>			Visit Code <input type="text"/> <input type="text"/>		Specimen Collection Date <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <small>dd MMM yy</small>		
# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADDITIVE/ DERIVATIVE	INSTRUCTIONS FOR PROCESSING LAB		
<input type="checkbox"/>	Anoscopy Biopsies - Phenotyping (ARB)	RPM	TIS	N/A	Ship to NL on ice the day of collection		
<input type="checkbox"/>	Anoscopy Biopsies - Gene expression microarrays (ARB)	RNL	TIS	N/A	Store @ 4°C overnight then transfer to -80°C. Must be stored at -80°C for a minimum of 24 hours prior to shipping. Specify Gene expression test in specimen management. (See lab SSP section Table 12-4)		
<input type="checkbox"/>	Sigmoidoscopy Biopsies - Histology (FSR)	FOR	TIS	N/A	Ship to NL the day of collection.		
<input type="checkbox"/>	Sigmoidoscopy Biopsies - Cytokines (FSR)	RNL	TIS	N/A	Store @ 4°C overnight then transfer to -80°C. Must be stored at -80°C for a minimum of 24 hours prior to shipping. Specify cytokine test in specimen management. (See lab SSP section Table 12-4)		
<input type="checkbox"/>	Sigmoidoscopy Biopsies - Phenotyping (FSR)	RPM	TIS	N/A	Ship to NL on ice the day of collection		
<input type="checkbox"/>	Sigmoidoscopy Biopsies - Gene expression microarrays (FSR)	RNL	TIS	N/A	Store @ 4°C overnight then transfer to -80°C. Must be stored at -80°C for a minimum of 24 hours prior to shipping. Specify Gene expression test in specimen management. (See lab SSP section Table 12-4)		

Comments: _____

Initials: _____ LDMS Data Entry Date: / / _____
Sending Staff Receiving Staff dd MMM yy LDMS Staff

Version 2.0, 01-NOV-10

Item-specific Instructions:

- **Visit Code:** Check to make sure the Visit Code recorded on page 1 and page 2 match.
- **NUMBER OF TUBES or SPECIMENS COLLECTED:** In the box provided, record the total number of tubes or specimens collected for that primary specimen type. If no LDMS specimens of the primary specimen type were collected, record "0."
- **Initials – Sending Staff:** The clinic staff person who completed the form and/or who is sending the LDMS form and specimens to the LDMS entry lab, records his/her initials here.
- **Initials – Receiving Staff:** The laboratory staff person who received this form (and the LDMS specimens accompanying the form), records his/her initials here.
- **LDMS Data Entry Date:** Record the date the LDMS specimens listed on this form were entered into LDMS.
- **LDMS Data Entry Date – LDMS Staff:** The LDMS laboratory staff person who entered the specimens into LDMS, records his/her initials here.

Appendix 12-2

Collection and Transport of Rectal Lavage Specimens for Fecal Calprotectin Test

Background

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils; it constitutes approximately 60% of their cytosolic protein. Elevated fecal calprotectin levels have been observed in patients with inflammatory bowel disease (IBD) and GI tract infections. Fecal calprotectin correlates strongly with ¹¹¹indium-labeled granulocytes, as well as IBD activity determined by histological and endoscopic evaluation. Elevated levels have been observed to precede clinical relapse in patients with quiescent IBD. Fecal calprotectin is also elevated in patients with non-steroidal anti-inflammatory drug (NSAID)-induced enteropathy.

Rectal Lavage is a procedure which involves instilling a solution to wash the rectum. The effluent is collected for analyses.

Specimen

Patient Preparation: See procedure steps below

Type: Random stool

Optimum/Minimum Specimen volume: 20 grams of stool, in stool cup.

Handling Instructions:

Samples may be stored at ambient temperature for up to 5 days or stored frozen at -20⁰C for up to one year.

Ship the specimen overnight in the pre-paid mailing envelope supplied by the Genova kit. (See SOP for shipping biological specimens) The specimen should be packaged according to IATA regulations regarding shipment of biological material.

Unacceptable Specimens: Sample ambient longer than 5 days

Materials

Pre-packaged enema to be used for lavage (Abbott Labs, Normosol-R, 500ml #00409796703)

Enema bottle, 120 mL (MediDose, #EPS212316)

Genova Diagnostics Calprotectin kit (#64) including specimen pan

60ml catheter tip syringe (BD #309-620)

50ml conical tubes (Corning #430828)

PRE Personal lubricant (provided by MTN)

Procedure –Stepwise

Clinical Staff:

1. Place specimen pan into available toilet.
2. Fill enema bottle with 120 mL of appropriate solution, if not pre-packaged.
3. Have subject rotate onto his/her left-hand side with right knee bent.
4. If enema bottle is not pre-lubricated, apply a dime size amount of water-based lubricant (PRE Personal lubricant provided by MTN. DO NOT USE Surgilube and other chlorhexidine containing lubricants!)
5. Gently insert the tip of the enema bottle into the rectum.
6. Slowly instill the solution into the rectum.
7. After holding the fluid in the rectum for approximately 5 minutes ask the subject to use the restroom, taking care to relieve themselves into the specimen pan.
8. Transfer stool with the flat wooden stick provided in the kit into the white-top vial that includes the participant I.D.# and collection date on it.
9. Add a sufficient stool to fill to the 20 mL line, ~ ½ inch.
10. Recap white-top vial securely.
11. Place white-top vial (with specimen) in the biohazard bag and seal.
12. Fill out the requisition to include the fields listed below. See example.
Diagnosis code and physician signature are not required.
 - a. Collection Date
 - b. Test Ordered: Calprotectin
 - c. Last name: Visit Code
 - d. First name: PID
 - e. Payment/Billing Option: Bill Practitioner Account
13. Transport the white-top vial in Biohazard bag to the laboratory for immediate shipping.

Laboratory Staff:

1. Place biohazard bag that contains specimen, into small cardboard box provided in kit. ***Specimen MUST be returned in this bag & box.***
2. Write your name and address on the “From” portion of the FedEx mailer. Keep shipping and tracking numbers for tracking purposes.
3. Place these items in the pre-paid mailing envelope:
 - Cardboard box containing Biohazard bag with the specimen in the white-top vial.
 - Completed Requisition, refolded with personal information hidden.
4. Schedule a pickup:
 - Call 1-800-GoFedEx (1-800-463-3339)
 - Say, “Return a package.”
 - When connected with a FedEx customer service representative, please tell them:
 - ❖ We are using FedEx Express Billable Stamps.
 - ❖ Your name, phone number, address, and zip code.

- FedEx will ask for the number of packages and then provide you with your pickup time.

**** For specimens received on a Friday, ship out Monday morning****

Methodology: Enzyme-linked Immunosorbent Assay (ELISA)

Reference Range*: < 50 µg Calprotectin/g stool Normal
50-100 µg Calprotectin/g stool Moderate GI inflammation
> 100 µg Calprotectin/g stool Significant GI inflammation
> 250 µg Calprotectin/g stool Mild to moderate IBD activity
> 500 µg Calprotectin/g stool Severe IBD activity

*Elevated calprotectin levels have been reported in healthy infant's ≤ 10 weeks of age.



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www.GenovaDX.n

COLLECTION
DATE



Date Final Sample Collected:

Mo.	Day	Year
-----	-----	------

Requisition

Check all requested tests below and provide Diagnosis Codes for each test.
Individual Diagnosis Codes are required for Medicare and insurance billing.

*This form must be completed
(including responsible party signature)
and returned with the specimen in
order to process this test.*

Test Ordered: **Pancreatic Elastase (PE)**

	<u>CPT</u>	<u>Diagnosis Code (Required)</u>
Pancreatic Elastase	82656	_____

Physician Information

Address change? Check here and indicate new address on reverse side.

ID# X00RB
Ian McGowan, MD
MTN Lab- Lab B530 F/G
204 Craft Ave
Pittsburgh, PA 15213
412-641-8905

And/Or

Test Ordered: X **Calprotectin**

	<u>CPT</u>	<u>Diagnosis Code (Required)</u>
Calprotectin	83993	<u>NOT REQUIRED</u>

**Physician Signature
(Required)**

Print _____

Sign _____

NPI# _____

• Medicare and Medicaid will not reimburse for tests which are ordered for screening purposes only. Physicians are legally responsible for limiting requests for tests to only those that are medically necessary for the diagnosis and treatment of the patient. Your ordering of the test(s) means that you believe the test(s) is medically necessary

• If the ordering physician is different from the preprinted physician above, please ensure your name, address, and NPI# or Tax ID# is provided.

} NOT REQUIRED

THIS SPACE FOR LAB USE ONLY



Patient Information

Social Security #: _____ *← VISIT CODE*
 Name (last): VST 2 Gender: M F *← PID*
 (first): 1 2 3 4 5 6 7 8 9 (middle): _____
 Date of Birth: _____ Age: _____ Phone: _____
 Mailing Address: _____
 City: _____ State: _____ Zip: _____
 Country: _____ Email: _____

Responsible Party Information: (if different from patient)

Name (last): _____ (first): _____ (middle): _____
 Mailing Address: _____
 City: _____ State: _____ Zip: _____
 Phone: _____ Social Security #: _____

Payment/Billing Options

(select one):

- Bill Practitioner Account
If nothing is indicated, practitioner account will be billed.
- Bill Insurance
- Bill Medicare
- Payment Enclosed
Contact your health care practitioner for pricing.

Payment provided:

\$ _____

Check or Credit Card Information

Medicare, Medicaid patients: see reverse side for Important Information. Please do not send cash. Contact your health care practitioner for pricing. A receipt will be provided which can be used to file your own insurance claim.

Credit Card from: Patient Physician
Select one: MasterCard Visa Discover AMEX
 Credit Card #: _____
 Expiration Date: ____/____
 Cardholder Signature: _____
 Printed Name: _____
 Billing Address: _____
 City: _____ State: _____ Zip: _____

Check from: Patient Physician Check #: _____
(make checks payable in US dollars to Genova Diagnostics)

Insurance Information

(Print clearly) Medicare, Medicaid patients: see reverse side for Important Information. Fill out this section only if you intend for Genova Diagnostics to file a claim on your behalf. Contact your health care practitioner for pricing. Genova Diagnostics does not participate in any HMO or other managed care contracts, so out-of-network benefits may apply. It is your responsibility to verify insurance coverage; Genova Diagnostics does not guarantee insurance coverage. Please call your insurance company for preauthorization, referral, and/or benefit verification, referring to the CPT code(s) listed by profile ordered on the requisition. Attach a copy of both sides of your insurance card to this form.

Primary

Secondary

Insurance Company: _____
 Claims Address: _____
 City/State/Zip: _____
 Phone #: (____) _____
 Subscriber Name: _____
 Subscriber ID #/Medicare #: _____
 Group #: _____
 Subscriber Date of Birth: ____/____/____
 Relation to Patient: Self Spouse Other _____ Self Spouse Other _____

Except in the case of prepayment, I authorize payment of all medical benefits to be paid directly to Genova Diagnostics and authorize the release of any medical information necessary for this insurance claim. I permit a copy of this requisition to be used in place of the original. The patient/responsible party is personally & fully responsible for payment of any balance not paid by their insurance within 45 days of billing. This excludes government carriers such as Medicare, Tricare.

Patient/Responsible Party Signature (required) X _____

Appendix 12-3
Procedure for preparing reagents for MTN-007

Equipment:

1. 2-8°C Refrigerator
2. -20°C freezer
3. Biological laminar flow hood
4. Pipette Aid

Disposables:

1. Corning 15 cc conical tubes (#430766)
2. 10 mL serological pipettes
3. 25 mL serological pipettes

Reagents:

1. RPMI (1x) 1640 w/HEPES w/L-glutamine Invitrogen #22400-089 (500 mL)
Freeze until needed.
2. Heat inactivated, certified Fetal Bovine Serum (FBS), Invitrogen #10082-147 (500 mL) or 10082-139 (100 mL). Aliquot into 50 cc tubes and freeze at -20°C
3. Antibiotic/antimycotic (100x)Invitrogen #15240-104 (100 mL) aliquot 5 mL quantities into 10 mL size tubes and freeze at -20°C

Procedure:

1. Complete RPMI media (for transporting biopsies for T cell phenotyping)

Ingredients	Quantities for 500 mL	100 mL
RPMI	445 mL	94 mL
FBS (f.c. 10%)	50 mL	10 mL
Antibiotics (f.c. 1%)	5 mL	1 mL

1. Take precautions to maintain the sterility of the media and use sterile techniques with transferring.
2. Thaw the RPMI, FBS, and antibiotic/antimycotics.
3. Combine the above ingredients and mix
4. Dispense 10-12 mL aliquots into 15 cc tubes labeled cRPMI and expiration date of 1 month from preparation.
5. Store at 4°C

2. Paraformaldehyde solution, 2% in PBS (for transporting cells for epithelial sloughing)

Order from USB, Paraformaldehyde solution 4%, item #19943, 1 liter

1. Dilute the 4% solution to 2% in PBS.
2. Dispense 1 mL aliquots into cryovials.
3. Store at 4°C until used.

3. RNAlater (to be used for collecting biopsies for cytokine RT-PCR and biopsies for Mucosal Gene Expression Array)

Order RNAlater Solution (Ambion, Invitrogen Cat #AM7020) 100 mL

1. Dispense 1.5 mL aliquots of the RNAlater into cryovials.
2. Store at room temperature until used.