**BD MAX Enteric Parasite Panel Procedure**

1. **PRINCIPLE**
	1. The BD MAX Enteric Parasite Panel performed on the BD MAX System is an automated in vitro diagnostic test for the direct qualitative detection of enteric parasitic pathogens. The BD MAX Enteric Parasite Panel detects nucleic acids from:
		1. Giardia lamblia
		2. Cryptosporidium (C. hominis and C. parvum only)
		3. Entamoeba histolytica
	2. Testing is performed on unpreserved or 10% formalin-fixed stool specimens from symptomatic patients with suspected gastroenteritis, enteritis or colitis. Stool specimens collected in Total Fix preservative have been validated for use with this assay by the laboratory.
	3. The BD MAX System automates sample preparation, including target organism lysis, DNA extraction and concentration, reagent rehydration, target nucleic acid sequence amplification and detection using real-time polymerase chain reaction (PCR). The test utilizes fluorogenic gene-specific hybridization probes for detection of the amplified DNA.
	4. Following enzymatic cell lysis at an elevated temperature, the released nucleic acids are captured on magnetic affinity beads.
	5. The beads, with the bound nucleic acids, are washed and the nucleic acids are eluted by heat and high pH in Elution Buffer. Eluted DNA is neutralized and transferred to the Master Mix Tube to rehydrate the PCR reagents.
	6. After rehydration, the BD MAX dispenses a fixed volume of PCR- ready solution into the BD MAX Cartridge. Microvalves in the BD MAX PCR Cartridge are sealed by the system prior to initializing PCR to contain the amplified mixture thus preventing evaporation and contamination.
	7. The amplified DNA targets are detected using hydrolysis probes, labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety.
	8. Probes labeled with different fluorophores are used to detect amplicons for enteric parasite targets and Sample Processing Control in four different optical channels of the BD MAX System. (Giardia/FAM, Crypto/ROX, E. histo/VICc, SPC/Cy5.5).
	9. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity of the quencher.
	10. However, in the presence of Target DNA, the probes hybridize to their complimentary sequences and are hydrolyzed by the 3’-5’ exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template.
	11. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The BD MAX System monitors these signals at each cycle and interprets the data at the end of the program to report the results.
2. **AVAILABLILITY**
	1. Test will be performed once per day, Monday-Friday
3. **SPECIMEN COLLECTION AND PREPARATION**
	1. INPATIENT SPECIMENS
		1. Unpreserved specimens: Must be received in a clean, dry container devoid of urine, toilet paper, soap, or water.
		2. Any specimen received in a clean dry container must be transferred to Total-Fix in the planting hood at the time of receipt.
	2. OUTPATIENT SPECIMENS
		1. Total-Fix or Formalin preserved specimens devoid of urine, toilet paper, soap, or water.
4. **MATERIALS AND EQUIPMENT**
	1. Materials
		1. 0.01 disposable inoculating loops (blue)
		2. BD MAX™ PCR Cartridges (BD Cat. No. 437519)
		3. BD MAX™ EPP Assay Kit (BD Cat. No. 442960)
		4. Total-Fix, stool fixative and transport media (15 mL)
		5. Dry, clean containers for specimen collection
	2. Equipment
		1. BD MAX™ Instrument
		2. BD Pre-warm Heater (BD Cat. No. 443159)
		3. VWR multi-tube vortexer (VWR Cat No. 58816-115)
		4. Vortex Genie
5. **STORAGE AND HANDLING**
	1. Specimen storage and handling
		1. Collected specimens should be transported at 2-8°C.
		2. Keep collection kits from freezing or extreme heat.
		3. Once in the lab, the specimen should be stored at 2-8°C.
		4. Alternatively, fresh specimens can be stored at 2-8°C for up to 5 days or at RT (25 + 2°C) for a maximum of 48 hours.
		5. Specimens will be shipped to Coro with ice on the regularly scheduled courier runs.
	2. Special handling instructions
		1. BD MAX ™ EPP test kits can be stored at 2-25°C through stated expiration date.
		2. Do not use the kit if the label that seals the outer box is broken on arrival.
		3. BD MAX™ EPP Master Mix and Extraction Tubes should be kept in their foil pouches until they are used.
			1. Once opened, the tubes must be used within 14 days. Always write a 2 week outdate on the foil pouch when opened for the first time.
			2. Always keep the pouches closed with air removed when not in use.
			3. Do not use reagents if protective pouch is broken/open on arrival or if there is no desiccant present.
	3. Inoculated BD MAX™ EPP Sample Buffer Tubes can be stored at 2-8°C for a maximum of 120 hours (5 days) OR at 25 + 2°C for a maximum of 48 hours after sample has been added to the sample buffer tube.
		1. The Pre-warm step does not change these storage requirements.
	4. The Microbiologics Gastrointestinal Panel for BD MAX Enteric Parasite Panel positive control should be stored at 2-25 °C and is stabile until the expiration date.
6. **QUALITY CONTROL**
	1. External Controls:
		1. Positive and negative external processing controls are to be run monthly to monitor sample preparation and to QC new lots/shipments of kits and after system repairs and software upgrades to monitor sample preparation.
		2. Positive external controls, purchased from Microbiologics, are intended to monitor for substantial reagent failure.
			1. DO NOT OPEN FOIL POUCHES UNTIL READY TO USE
			2. Take out 3 Parasite buffer tubes from your kit. Label 1 Pool A, label a 2nd tube as Pool B, and label your 3rd tube as Negative.
			3. Open the foil pouch and place the pellet into the appropriate QC buffer tube.
			4. Program the specimens onto the BD MAX instrument using the BD MAX ENT PARA code and select the appropriate kit lot associated with the buffer tube.
			5. Run Sample buffer tube as usual.
		3. Negative external control is intended to detect reagent or environmental contamination by target nucleic acids.
			1. A previously run negative patient will be used as the negative external control.
		4. For New Lot/New Shipment and monthly QC, all three analytes must be run along with a negative control.
		5. No patient results will be reported unless all control results are as expected. Bring any unexpected control results to the attention of the Senior or Lead Medical Technologist, Director, Assistant Director, or Manager.
		6. If a repeat of an External Control is warranted, a new buffer tube must be inoculated.
	2. Internal Control:
		1. Each Extraction tube contains a Sample Processing Control (IC) which is a plasmid containing a synthetic target DNA sequence.
		2. The Sample Processing Control (IC) is extracted, eluted, and amplified along with any DNA present in the processed specimen, ensuring the predictivity of the assay.
		3. The Sample Processing Control (IC) monitors the efficiency of DNA capture, washing and elution during the sample processing step, as well as the efficiency of DNA amplification and detection during PCR analysis.
			1. If the Sample Processing Control (IC) fails to meet the acceptance criteria, the result of the specimen will be reported as UNR and should be rerun from the buffer tube.
	3. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer to Monthly BD MAX™ QC sheet for directions.
	4. Positivity Rate is monitored monthly.
	5. The laboratory’s Individualized Quality Control Plan (IQCP) for BD Max Enteric Parasite Panel contains complete details of the QC data and QA plan approved by the Director. Refer to this document for complete details.
7. **TEST PROCEDURE**
	1. Use bleach, DI water and 70% ethanol to clean the BD MAX™, racks, hood, and surrounding bench area.
		1. DO NOT clean the mirror within the BD MAX™. Lightly dust with clean gauze only if needed.
	2. Run a pending report using test code PARSC, dating back at least 1 week. Account for all pending specimens.
	3. A MAXIMUM OF 24 STOOLS CAN BE RUN AT A TIME
	4. BD MAX™ operation:
		1. Put on clean gloves then log into BD MAX™ using your personal “user name” and “password”.
		2. Place one sample buffer tube for each specimen to be run into an UNWIRE red or white test tube rack (small). Number tubes according to numbered stools without marking the tubes’ barcodes.
		3. Click the RUN tab at the bottom of the screen and fill in the appropriate fields.
		4. Test- choose BD MAX™ Ent Para 53 from the drop-down list.
		5. Lot number- use the drop-down list and choose the lot number from the in use EPP kit.
		6. External Control- this should be left blank while entering patient specimen information.
		7. With the curser in the Sample Tube window, scan the 2-D barcode on the side of the tube.
		8. The curser will automatically move to the Accession window. Scan the accession number from the patient sample. Continue in this manner until all specimens are logged into BD MAX™.
	5. Specimen preparation:
		1. In a clean hood, place labeled sample buffer tubes, a stack of 4X4 gauze, blue loops, and enough blue septum caps to cover each specimen.
		2. Specimens should be brought into the hood and processed one at a time.
		3. Stools in Total-Fix, Formalin, or clean containers can be used for this test. Total-Fix is the preferred container.
		4. Vortex specimen for 15 seconds.
		5. Remove cap of specimen using a gauze to protect gloves from contamination.
		6. Remove and discard the clear cap from the corresponding sample buffer tube.
		7. Dip only the loop portion of a 0.01µl blue loop into the specimen
		8. Insert the loop into the Sample Buffer Tube and twirl the stem of the loop to dispense the sample. Specimen should be tea colored. See below:



* + 1. Place a blue septum cap on the sample buffer tube without touching the top area to avoid carry-over contamination. Place the tube back in the UNWIRE rack, recap specimen and remove it from the hood.
		2. Continue in this manner until all Sample Buffer Tubes are inoculated.
		3. CHANGE GLOVES AND CLEAN HOOD BEFORE MOVING TO NEXT STOOL ANYTIME THEY BECOME CONTAMINATED WITH SPECIMEN
	1. Pre-warm Heater operation:
		1. Go to the worklist that was just created on BD MAX.
		2. Check the boxes under the Schedule column for each stool set up for EPP.
		3. Transfer the Sample Buffer tubes to the pre-warm heater and close the lid.
		4. Select “Start Pre-warm” on the worklist panel to the left.
		5. Confirm the number of tubes placed in the heater corresponds to the number in the pop-up box.
		6. Select yes to start pre-warm.
		7. The countdown to completion of the Pre-warm will be shown on the Status Screen.
		8. Pre-warm showing “failed” on the status screen must be repeated.
			1. In this case the Sample Buffer Tube must cool for 30 minutes before Pre-warm can be re-attempted.
		9. Pre-warm showing “complete” can be moved to a red rack for vortexing.
			1. Specimens requiring repeat on the BD MAX do not need to go through the pre-warm step again.
	2. Utilizing the Run Wizard to assist in loading the BD MAX System Racks
		1. The run wizard has the ability to assist in proper placement of the Reagent Strips for maximum usage and minimal waste of the PCR card.
		2. Select the Run Wizard box to the left of the worklist screen.
		3. Choose the Scan Cartridge tab to the right of the window.
		4. Scan the barcode from the front of the cartridge.
			1. The spaces crossed out on the graphic indicate a used spot.
			2. If five spots are used, you can begin setting up reagent strips in the sixth position, etc.
	3. Setting up the EPP Reagent Strips:
		1. Remove the required number of BD MAX™ EPP Reagent Strips from the BD MAX™ EPP kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and place in the metal BD MAX™ system rack. Push strip in and down to lock into place.
		2. Remove the required number of EPP Extraction Tube(s) and EPP Master Mix tube(s) from their protective pouches. This should be done 30 minutes prior to loading your samples. Remove excess air, and close pouches with the zip seal. Once opened, bags expire after 14 days. Write an outdate of 14 days on the bag to ensure integrity of Extraction Tubes.
			1. Place one (1) BD MAX™ EPP Extraction Tube (white foil seal) into Position 1 of each of the BD MAX™ EPP Strips as shown in Figure 1 below.
			2. Place one (1) BD MAX™ EPP Master Mix Tube (green foil seal) into Position 2 of each of the BD MAX™ EPP Strips as shown in Figure 1 below.
		3. Using the cap from the orange EXPO marker in the drawer below the BD MAX™ computer, snap all the tubes into the strips.

Figure 1: Snap BD MAX™ EBP Extraction tubes and Master Mix tubes into reagent strips.



* 1. Vortex the sample buffer tubes.
		1. Remove the tubes from the heater and place in red rack keeping them in order.
		2. Cover the septum caps with parafilm. Place the UNWIRE rack with the sample buffer tubes between the plates of the multi-tube vortexer; tighten the knobs on the vortexer without pressing down on the top plate. Press the button labeled timer.
		3. The vortex will shut down after 1 minute. At that point, remove the rack with the sample buffer tubes.
		4. NEXT STEP MUST BE DONE IMMEDIATELY AFTER VORTEXING SAMPLE BUFFER TUBES. IF THERE IS A DELAY THE TUBES MUST BE VORTEXED ONCE AGAIN
	2. Place each tube in its corresponding spot on the BD MAX™ System Rack
	3. Place System rack(s) into BD MAX™, ensuring that the placement of the racks corresponds to the order in which the specimens were numbered. Close the lid of the BD MAX™ until it clicks indicating it is locked.
	4. At the BD MAX™ computer, select Start icon at the bottom of the screen. Enter through run name. Cataloguing will begin.
1. **POST ANALYSIS**
	1. IMMEDIATELY REVIEW ALL RESULTS
	2. Any patient positive for two or more targets must be repeated from the original stool.
	3. Any UNR, INC or IND results must be repeated using the sample buffer tube.
	4. Any patient specimen that still has not yielded a result after the second attempt will be reported as Invalid for some or all non-resulted analytes.
	5. Any specimen positive for Entamoeba histolytica should be repeated from the buffer tube and brought up on rounds.
		1. If results repeat, the specimen will be sent to a reference lab for verification.
	6. Setting up repeat buffer tubes on the BD MAX computer
		1. Under the RUN tab, scan the sample buffer tube to be repeated.
		2. Select OK in the popup window.
		3. Highlight the specimen from the list.
		4. Add an “R” to the end of the accession number, select ENTER.
		5. Select SAVE
	7. Setting up new reagent strips
		1. Set up a new reagent strips using new Extraction and Master Mix Tubes for each sample to be repeated.
		2. Vortex the buffer tubes and place in the System Rack.
		3. Load System rack and start run immediately after vortex.
	8. Resulting
		1. All positive specimens must be called according to the Critical Results Notification Procedure
		2. All results will be uploaded to the instrument menu.
			1. From SoftLab, go to “interfaces”, and “Instrument Menu”.
			2. Select “RBDMX1/RBDMX2” “BD MAX™”
			3. Select “Loadlist and todays results”, “Not Posted”, “By Sequence”.
			4. Each order will be highlighted individually. Verify the result against the instrument printout.
			5. To add result comments, i.e., Phone reports
				1. Highlight the order number on left of screen.
				2. At bottom of screen click on Lab Results
				3. Open “Comment” box and add comment/phone report using @CALM.
				4. Click back to Instrument tab and save when asked.
				5. Click Post All to verify the report.
				6. Order number should disappear from list on left.
		3. Invalids
			1. Invalid specimens must be manually resulted in result entry.
				1. Go to result entry.
				2. In the Order: box, enter the Order number, select Next.
				3. Select “invalid” from the GPP window for all three targets.
				4. Select Verify All
		4. Soft Comment:
			1. @PARI- The comment “The presence or absence of pathogens included in the parasite screen cannot be determined. Repeat specimen collection if clinically indicated.” will be used when a specimen is run on the BD MAX twice and still results as UNR or IND.
2. **INTERPRETATION**
	1. Unresolved (UNR) results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or IC amplification. If the IC does not amplify, the sample will be reported as UNR; however, any positive (POS) assay results will be reported, and no targets will be called NEG. Refer to Post-analysis section above for repeat test procedure. Alternatively, the sample can be set up again.
	2. Indeterminate (IND) results may be obtained in the event that a System failure occurs. Refer to post-analysis section above for repeat test procedure. Alternatively, the sample can be set up again.
	3. Incomplete (INC) results may be obtained in the event that the Specimen Preparation or the PCR failed to complete. Refer to Post-analysis section above for repeat test procedure. Alternatively, the sample can be set up again.
	4. Pre-warm Failure In the event of a pre-warm failure, affected Sample Buffer Tubes must be left in the pre-warm station for a full 30 minutes for a cool down. Once the cooling cycle has completed, restart the pre-warm.
	5. A complete copy of the report from the BD MAX™ will be printed for every run.
		1. To print a report from the BD MAX™, go to results tab in the results window on the BD MAX™ monitor. Select the run from the list. Uncheck graphs box then select print.
3. **LIMITATIONS**
	1. Stool specimens received in Total-Fix preservative have been validated by the RIH Microbiology Lab for this assay.
	2. This product is intended for use only with unpreserved or 10% formalin-fixed human stool specimens. Stool specimens from rectal swabs and stools contaminated with barium have not been validated with the BD MAX™ Enteric Parasite Panel.
	3. Erroneous results may occur from improper collection, handling, storage, technical error, sample mix-up, or if the number of organisms present in the sample is below the analytical sensitivity of the test.
	4. A BD MAX™ Enteric Parasite Panel positive result does not necessarily indicate the presence of viable organisms. It does, however, indicate the presence of DNA from Giardia lamblia, Cryptosporidium parvum, Cryptosporidium hominis or Entamoeba histolytica, allowing for identification of the BD MAX™ Enteric Parasite Panel organisms.
	5. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of the target organisms, resulting in a false negative result with the BD MAX™ Enteric Parasite Panel.
	6. As with all PCR-based in vitro diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
	7. False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate organism lysis. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Sample Processing Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or whether cells have been inadequately lysed.
	8. Results from the BD MAX™ Enteric Parasite Panel should be used as an adjunct to clinical observations and other information available to the physician.
	9. As with all in vitro diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD MAX™ Enteric Parasite Panel performance may vary depending on the prevalence and population tested.
	10. BD MAX™ Sample Buffer Tubes have not been designed to support organism morphology examination. Morphologic examination and/or staining must be performed from the original specimen.
	11. The performance of this test has not been established for monitoring treatment of Giardia lamblia, Cryptosporidium parvum, Cryptosporidium hominis or Entamoeba histolytica.
	12. The BD MAX™ Enteric Parasite Panel is intended to detect DNA from C. hominis and C. parvum without distinguishing between these two species. This test is not intended to detect DNA from other species of Cryptosporidium.
	13. This test is a qualitative test and does not provide quantitative values nor indicate the quantity of organisms present.
	14. The performance of this test has not been evaluated for immunocompromised individuals or for patients without symptoms of gastrointestinal infection.
	15. The effect of interfering substances has only been evaluated for those substances listed in the Interfering Substances section of the package inset. Potential interference has not been evaluated for substances other than those described in the Interfering Substances section.
	16. Cross-reactivity with organisms other than those listed in the Analytical Specificity section in the package insert have not been evaluated.
4. **NOTES**
	1. Unitized Reagent Strips must be checked for proper liquid fills and to ensure all pipette tips are present.
	2. Sample Buffer Tubes Pre-warmed on one BD MAX cannot be run on a different BD MAX.
	3. Always check that there are sufficient tests remaining on the PCR cards before starting the run. If the BD MAX™ is unable to start the PCR step, an internal clock will begin, and the run will abort if the issue is not resolved in time.
	4. Caution should be used during cleaning with chemicals. Splashing onto barcodes of kit components will render them unreadable.
	5. The following conditions may cause erroneous results. Do Not:
		1. Use any part of the BD MAX™ EPP kit after the stated expiration date.
		2. Use a kit where the outer seal has been broken at time of delivery.
		3. Use reagents if the protective pouched are opened or broken upon arrival.
		4. Use reagents if desiccant is not present or is broken in pouch.
		5. Remove desiccant from pouch.
		6. Use reagents if the protective foil cover on the tube is broken or damaged.
		7. Mix reagents across different lots or mix them between different pouches.
	6. Kits should be kept free from excessive heat and humidity. Prolonged exposure to increased humidity may affect product performance.
	7. Do not interchange or reuse buffer tube clear caps or septum caps to avoid contamination.
	8. Performing the BD MAX™ EPP outside the recommended time or temperature ranges for specimen transport and storage may produce invalid results. Assays not performed within specific time frames should be repeated.
	9. Results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions.
	10. Positive results do not rule out co-infection with other organisms not detected by this test and may not be the sole or definitive cause of patient illness.
	11. Clean gloves should be worn whenever handling kit components to avoid contamination from handling stool specimens. Gloves should be changed as soon as they become visibly contaminated.
	12. In cases where other PCR tests are conducted in the same area, care must be taken to ensure the assay and its reagents are not contaminated by microbial DNA or DNase. Gloves must ALWAYS be changed before handling reagents and cartridges.
5. **TECHNICAL SUPPORT**
	1. Technical Support- 800-638-8663
	2. technical\_services@bd.com
6. **REFERENCES**
	1. BD MAX™ Enteric Parasite Panel PI ver. P0199(05) 2023 06
	2. BD MAX™ Enteric Parasite Panel Validation Report
	3. Medical Chemical Corp. (MCC) Total-Fix Procedure PI rev. 06-07-2016
	4. BD Pre-Warm Heater Manual (2014-06)
	5. Microbiologics Synthetic Helix Elite Molecular Standards for use with the BD MAX Enteric Parasite Panel PI. 2015.OCT.05 Rev A
7. **REVISIONS**
	1. 10/18/2023: Updated after instruments moved to Coro lab.