**BD MAX Enteric Bacterial Panel Procedure**

1. **PRINCIPLE**
	1. The BD MAX™ Enteric Bacterial Panel (EBP) performed on the BD MAX™ is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. The BD MAX™ EBP detects nucleic acids from:
		1. Salmonella spp.
		2. Campylobacter spp. (jejuni and coli)
		3. Shigella spp./Enteroinvasive E. coli (EIEC)
		4. Shiga toxin 1 (stx1)/Shiga toxin 2 (stx2) genes (found in Shiga toxin producing E. coli [STEC]) as well as Shigella dysenteriae, which can possess a Shiga toxin gene (stx) that is identical to the stx1 gene of STEC.
	2. Following enzymatic cell lysis at an elevated temperature, the released nucleic acids are captured on magnetic affinity beads.
	3. The beads, with the bound nucleic acids, are washed and the nucleic acids are eluted. Eluted nuclei are neutralized and transferred to the Master Mix Tube to rehydrate the PCR reagents.
	4. After rehydration, the BD MAX™ System dispenses a fixed volume of PCR-ready solution into the BD MAX™ PCR cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to contain the amplified mixture thus preventing evaporation and contamination.
	5. The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety.
	6. Probes labeled with different fluorophores are used to detect amplicons for enteric bacterial targets (Campylobacter specific tuf gene sequence variants, the SpaO gene for specific detection of Salmonella spp., the ipaH gene for specific detection of Shigella spp. or Enteroinvasive E. coli (EIEC), the stx1 & stx2 genes associated with production of Shiga toxins in STEC and S. dysenteriae) and the SPC in five different optical channels of the BD MAX™ System.
	7. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher.
	8. However, in the presence of target DNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5’-3’ exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template.
	9. 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 run once per day, Monday-Friday
3. **SPECIMEN COLLECTION AND PREPARATION**
	1. Liquid or soft stool can be collected in the following ways.
		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 Cary-Blair in the planting hood at the time of receipt.
		2. OUTPATIENT SPECIMENS
			1. Cary-Blair preserved specimens devoid of urine, toilet paper, soap, or water.
	2. Rectal swabs are acceptable when received in a Copan FecalSwab™.
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™ EBP Assay Kit (BD Cat. No. 442963)
		4. Cary-Blair transport media (15 mL)
		5. Dry, clean containers for specimen collection
		6. Copan FecalSwab™ collection kits (4C024S)
		7. Assorted media for cultivation of positive specimens
	2. Equipment
		1. BD MAX™ Instrument
		2. VWR multi-tube vortexer (VWR Cat No. 58816-115)
		3. Vortex Genie
5. **STORAGE AND HANDLING**
	1. Specimen storage and handling
		1. Collected specimens should be kept between 2-25°C.
		2. Keep specimens from freezing or extreme heat.
		3. Once in the lab, the specimen should be kept at 2-8°C.
			1. Specimens can be stored at 2-8°C for 5 days or at RT (25 + 2°C) for 24 hours before testing.
	2. Special handling instructions:
		1. BD MAX ™ EBP 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™ EBP 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™ EBP 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.
	4. Microbiologic Positive Controls for Salmonella, Shigella, Campylobacter, and STEC are to be kept at 2-8°C.
	5. Aliquots of negative control will be stored in -70°C freezer. Alternatively, the aliquot “in use” can be stored in the refrigerator at 2-8°C for up to 5 days.
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.
		3. Negative external control is intended to detect reagent or environmental contamination by target nucleic acids.
			1. The Microbiologics Molecular Standards for BD MAX Enteric Bacterial Panel positive control should be stored at 2-25°C and is stable until the expiration date.
			2. DO NOT OPEN FOIL POUCHES UNTIL READY TO USE
			3. For New Lot/New Shipment and monthly QC, all four analytes must be run along with a negative control.
			4. Take out 3 Enteric Pathogen buffer tubes from your kit. Label 1 Pool A, label a 2nd Pool B, and label your 3rd tube as negative.
			5. Open the foil pouch and place the pellet into the appropriate tube.
			6. Program the QC onto the BD MAX instrument using the BD MAX ENT PAR code and select the appropriate kit lot associated with the buffer tube.
			7. Run Sample buffer tube as usual.
			8. 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
			9. 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.
		4. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer to Monthly BD MAX™ Environmental Testing sheet for directions.
		5. Positivity Rate is monitored monthly.
		6. The laboratory’s Individualized Quality Control Plan (IQCP) for BD Max Enteric Bacterial 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™ 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 ENTB, back dating at least 1 week. Account for all pending specimens. Number each stool in Cary-Blair first then continue numbering the FecalSwab™ specimens.
	3. BD MAX™ operation:
		1. Put on clean gloves then log into BD MAX™ using your personal “username” and “password.”
		2. Remove the required number of BD MAX™ EBP Reagent Strips from the BD +MAX™ EBP 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.
		3. Remove the required number of EBP Extraction Tube(s) and EBP Master Mix tube(s) from their protective pouches. 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.
		4. For each sample to be tested, place one (1) BD MAX™ Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially with no open spaces.
		5. Place one (1) BD MAX™ EBP Extraction Tube (white foil seal) into Position 1 of each of the BD MAX™ EBP Strips as shown in Figure 1 below.
		6. Place one (1) BD MAX™ EBP Master Mix Tube (green foil seal) into Position 2 of each of the BD MAX™ EBP Strips as shown in Figure 1 below.
			1. Using the cap from the orange EXPO marker in the drawer below the BD MAX™ computer, snap all the tubes into the strips.
		7. 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.

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



* + 1. Click the RUN tab at the bottom of the screen and fill in the appropriate fields.
			1. Test- choose BD MAX™ Ent Bac 52 from the drop-down list.
			2. Lot number- use the drop-down list and choose the lot number from the in use EBP kit.
			3. External Control- this should be left blank while entering patient specimen information.
		2. With the curser in the Sample Tube window, scan the 2-D barcode on the side of the tube.
		3. 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™.
		4. Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 2, below)
			1. Each cartridge accommodates 2 runs of up to 12 samples for a total of 24 samples.
			2. BD MAX™ cartridges may be used multiple times until all lanes have been utilized.
			3. Use Run Wizard to utilize as many spaces as possible on the PCR card.

Figure 2: Insert PCR Cartridges into the instrument.



* 1. 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 Cary-Blair
			1. Vortex specimen for 15 seconds.
			2. Remove cap of specimen using a gauze to protect gloves from contamination.
			3. Remove and discard the cap from the corresponding sample buffer tube.
			4. Dip only the loop portion of a 0.01µl blue loop into the specimen and twirl to stem of the loop.
			5. Place a blue septum cap on the sample buffer tube without touching the top area to avoid carry-over contamination. Place tube back in UNWIRE rack, recap specimen and remove it from the hood.
			6. CHANGE GLOVES BEFORE MOVING TO NEXT STOOL ANYTIME THEY BECOME CONTAMINATED WITH SPECIMEN
		4. FecalSwab™ specimens
			1. Vortex specimen for 15 seconds.
			2. Remove cap from specimen pressing the swab on the inside of the tube to expel excess fluid.
			3. Place cap with swab attached upside-down in hood.
			4. Remove and discard the cap from the corresponding sample buffer tube.
			5. Pipette 500 µL of specimen into the sample buffer tube.
			6. Place a blue septum cap on the sample buffer tube without touching the top area to avoid carry-over contamination.
			7. Replace tube in UNWIRE rack, recap specimen and remove it from the hood.
		5. 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.
		6. The vortex will shut down after 1 minute. At that point, remove the rack with the sample buffer tubes.
		7. NEXT STEP MUST BE DONE IMMEDIATELY AFTER VORTEXING SAMPLE BUFFER TUBES. IF THERE IS A DELAY THE TUBES MUST BE VORTEXED ONCE AGAIN
		8. Place each tube in its corresponding spot on the BD MAX™ System Rack with the 1-D barcode facing out.
		9. 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.
		10. 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 sample buffer tube. There is enough specimen in each buffer tube for one repeat.
	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. 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.
	6. 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.
	7. Resulting
		1. All positive specimens must be called according to the Critical Results Notification Procedure
		2. All results will go to the instrument menu.
			1. From SoftLab, go to “interfaces”, and “Instrument Menu”.
			2. Select “RBDMX” “BD MAX™” (#59)
			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 GPPR window for the four targets.
				4. Select “GIIN” from the GIINV window.
				5. Select Verify All
	8. Sending to the RIDOH
		1. Complete DOH requisition form.
		2. Print a copy of the instant report.
		3. Track sample to RIH Micro.
		4. Parafilm media and place in a biohazard bag with tracking list.
		5. Ship to micro with the next courier run.
2. **INTERPRETATION**

|  |  |
| --- | --- |
| Assay Result Reported | Interpretation of Result |
| Shig POS | *Shigella* spp. / EIEC DNA Detected |
| Shig NEG | No *Shigella* spp. / EIEC DNA Detected |
| Shig UNR | Unresolved- inhibitory sample or reagent failure; no target or Sample Processing Control (IC) |
| STX POS | Shiga toxin-producing gene(s) detected |
| STX NEG | No Shiga toxin-producing gene(s) detected |
| STX UNR | Unresolved- inhibitory sample or reagent failure; no target or Sample Processing Control (IC) |
| Campy POS | *Campylobacter* spp. (*jejuni* and *coli*) DNA Detected |
| Campy NEG | No *Campylobacter* spp. (*jejuni* and *coli*) DNA Detected |
| Campy UNR | Unresolved- inhibitory sample or reagent failure; no target or Sample Processing Control (IC) |
| Salm POS | *Salmonella* spp. DNA Detected |
| Salm NEG | No *Salmonella* spp. DNA Detected |
| Salm UNR | Unresolved- inhibitory sample or reagent failure; no target or Sample Processing Control (IC) |
| IND | Indeterminate result due to BD MAX™ System failure (with Warning or Error Codes) |
| INC | Incomplete run (with Warning or Error Codes) |

* 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. 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. Select print. Be sure to print all graphs as well as reports.
1. **LIMITATIONS**
	1. This product can only be used on the BD MAX™ System by trained laboratory personnel.
	2. This product is intended for use only with unpreserved and Cary-Blair preserved human stool specimens. Stool specimens from rectal swabs or fixed stools have not been validated with the BD MAX™ Enteric Bacterial Panel.
	3. Erroneous results may occur from improper specimen collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test.
	4. If the BD MAX™ Enteric Bacterial Panel result is IND, INC, or UNR (for one or more targets) then the test should be repeated.
	5. A BD MAX™ Enteric Bacterial Panel positive result does not necessarily indicate the presence of viable organisms. It does, however, indicate the presence of the Campylobacter specific tuf gene sequence variants, SpaO, ipaH and stx1/stx2 genes and allows for identification of the Enteric Bacterial Panel organisms.
	6. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of the genera Salmonella and Campylobacter (jejuni and coli), Shigella spp., Enteroinvasive Escherichia coli (EIEC] as well as Shiga toxin-producing E. coli variants, resulting in a false negative result with the BD MAX™ Enteric Bacterial Panel.
	7. The BD MAX™ Enteric Bacterial Panel does not distinguish which Shiga toxin gene (stx1/stx2) is present in a specimen.
	8. In rare instances, Shiga toxin genes can be found in Enterobacteriaceae other than STEC or Shigella dysenterieae
	9. The BD MAX™ Enteric Bacterial Panel detects only Campylobacter jejuni and Campylobacter coli and does not differentiate between the species. Other Campylobacter species are not detected by the assay.
	10. In silico analysis predicts that variant stx2f will not be detected by the BD MAX™ Enteric Bacterial Panel.
	11. The BD MAX™ Enteric Bacterial Panel does not differentiate between Shigella spp. And Enteroinvasive Escherichia coli (EIEC).
	12. Not all serotypes of Salmonella were evaluated in analytical studies; however all but one (Salmonella enterica serotype Mississippi) of the most prevalent serotypes recently circulating in the U.S. were evaluated.18 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.
	13. False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate bacterial cell 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 bacterial cells have been inadequately lysed.
	14. Results from the BD MAX™ Enteric Bacterial Panel should be used as an adjunct to clinical observations and other information available to the physician.
	15. As with all in vitro diagnostic tests, positive and negative predictive values are highly dependent on prevalence. The BD MAX™ Enteric Bacterial Panel performance may vary depending on the prevalence and population tested.
	16. BD MAX™ Enteric Bacterial Panel results may or may not be affected by concurrent antimicrobial therapy, which may reduce the amount of target present.
	17. The Sample Buffer Tube has not been designed to support organism viability. If culture is necessary, it must be performed from the original specimen.
	18. The performance of this test has not been established for monitoring treatment of Salmonella spp., Shigella spp., Campylobacter jejuni, Campylobacter coli or STEC infections.
	19. This test is a qualitative test and does not provide quantitative values nor indicate the quantity of organisms present.
	20. The performance of this test has not been evaluated for immunocompromised individuals or for patients without symptoms of gastrointestinal infection.
	21. The effect of interfering substances has only been evaluated for those listed in the package insert. Potential interference has not been evaluated for substances other than those described in the Interference section in the package insert.
2. **NOTES**
	1. Unitized Reagent Strips must be checked for proper liquid fills and to ensure all pipette tips are present.
	2. 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.
	3. The following conditions may cause erroneous results. **Do Not:**
		1. Use any part of the BD MAX™ EBP 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.
	4. Kits should be kept free from excessive heat and humidity. Prolonged exposure to increased humidity may affect product performance.
	5. Do not interchange or reuse buffer tube clear caps or septum caps to avoid contamination.
	6. Performing the BD MAX™ EBP 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.
	7. 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.
	8. 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.
3. **TECHNICAL SUPPORT**
	1. Technical Support- 800-638-8663
	2. technical\_services@bd.com
4. **REFERENCES**
	1. BD MAX™ Enteric Bacterial Panel PI ver. P0014(06) 2023 07
	2. Copan FecalSwab™ PI
	3. BD MAX™ Enteric Bacterial Panel Validation Report
5. **REVISIONS**
	1. 10/18/2023: Updated with workflow changes after moving to Coro Lab