PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

BD MAX™ Enteric Bacterial Panel Procedure

Department of Microbiology

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1.0 Clinical Significance

Gastroenteritis is most commonly caused by viral etiologies in both children and adults. However, bacterial enteric pathogens represent a significant portion of gastrointestinal infections. Enteric pathogens are often associated with food-borne outbreaks and pose a risk to public health. Illness associated with bacterial gastroenteritis can range from mild to severe and usually manifests with symptoms of vomiting, diarrhea, and abdominal discomfort. The most common complication is dehydration. Infections are usually self-limited, but improper management may lead to prolonged symptoms or may even be associated with serious complications.

2.0 Principle

The BD MAX™ Enteric Bacterial Panel performed on the BD MAX™ System is an automated *in vitro* diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. The BD MAX Enteric Bacterial Panel detects nucleic acids from:

- *Salmonella* spp.
- *Campylobacter* spp. (*jejuni* and *coli*)
- *Shigella* spp. / Enteroinvasive *E. coli* (EIEC)
- Shiga toxin 1 (*stx*1) / Shiga toxin 2 (*stx*2) 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 *stx*1 gene of STEC.

Testing is performed on unpreserved soft to diarrheal stool specimens or Cary-Blair preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of *Salmonella* specific *SpaO* gene, a *Campylobacter* specific *tuf* gene sequence, an *ipaH* gene specific for *Shigella* or Enteroinvasive *E. coli*, and the *stx*1/*stx*2 genes associated with the production of Shiga toxins in STEC. The test utilizes fluorogenic sequence-specific hybridization probes for detection of the amplified DNA.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BD MAX™ testing platform. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

The reagent(s) and/or chemical(s) that are used in this procedure may be hazardous to your health if handled incorrectly. Information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Enteric pathogens
- Bloodborne pathogens

- Slightly hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- Biological safety cabinet (for specimen processing)

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.
- Ethyl Alcohol (70%)

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

Collect liquid or soft stool in a clean, dry container. Avoid contamination with water or urine. Avoid mixing toilet paper, water or soap with the sample. Stool may be transferred to a Cary-Blair transport media vial according to the manufacturer's instructions. Label the container and transport to the laboratory. Either unpreserved stool or stool in Cary-Blair can be stored for up to 5 days at 2-8°C or up to 24 h at room temperature.

6.0 Materials

6.1 Equipment and/or Testing System

- BD MAX™ System
- Multi-vial vortex

6.2 Consumables

- BD MAX™ PCR Cartridges REF 441770. Store at 2-25°C

6.3 Reagents and Media

- BD MAX™ Enteric Bacterial Panel Kit (BD catalog no. 443378), 24 tests. Store at 2-25°C.
  - BD MAX™ Enteric Bacterial Panel Master Mix (B5) and Extraction Tubes (B2) are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening. Reagent tubes are stable for up to 14 d at 2-25°C after initial opening and re-sealing.
  - BD MAX™ Enteric Bacterial Panel Sample Buffer Tubes
  - Septum caps
  - BD MAX™ Enteric Bacterial Panel Reagent Strips

6.4 Control Materials

Suspensions of the control strains should be prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of ~1.0 X 10^4 CFU/mL. Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** Pooled, diluted suspensions of *Campylobacter jejuni* ATCC 33291, *E. coli* O157:H7 ATCC 35150, *Salmonella enteritidis* ATCC 14028, and *Shigella sonnei* ATCC 9290.
- **Negative External Control:** Saline

7.0 Interfering Substances

The manufacturer performed studies with the BD MAX™ Enteric Bacterial Panel in the presence of potential biological and chemical interfering substances in order to characterize the ability of the assay to detect target DNA under these conditions. A complete description of the studies can

be found in the manufacture's package insert. Vagisil and Nystatin cream were identified as potential interfering substances.

The manufacturer also evaluated the assay for competitive interference by testing each target organism in low concentration (1.5 X their respective LOD) in combination with high concentrations of the other target organisms (> $1x10^6$ CFU/mL). All four low target organisms were detected when combined with their respective simulated high target concentration mixed infection preparations.

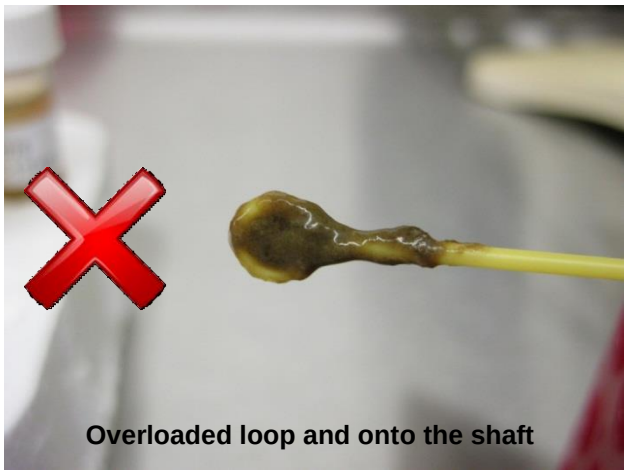
8.0 Warnings and Precautions

- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Check reagent strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Check reagent strips to ensure that all pipette tips are present.
- Do not remove desiccant from reagent pouches.
- Do not use reagents if desiccant is not present or is broken inside reagent pouches.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or reuse caps, as contamination may occur and compromise test results.

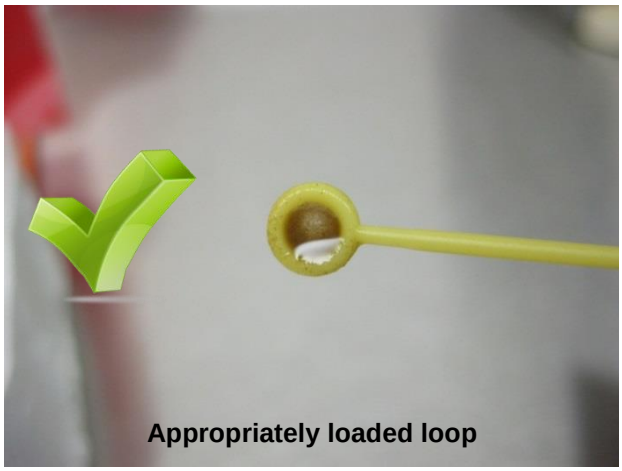
9.0 Procedure

9.1 Specimen Preparation

1. Obtain the number of Sample Buffer Tubes corresponding to the number of specimens and external controls to be run.
2. Label a bar-coded BD MAX™ SBT (clear cap) with the appropriate sample identification. Do not obscure, write or label over the 2D-barcode.
3. Vortex unpreserved or Cary-Blair preserved samples at high speed for 15 s.
4. Remove the clear cap from the SBT.
5. Insert a 10 µL disposable inoculation loop until the entire loop portion is submerged in the sample. Do not insert beyond the loop as any additional stool on the shaft can overload the PCR reaction. If the stool consistency of a fresh specimen is unsuitable for proper sampling, transfer a portion of the specimen to a 15-mL vial of Cary Blair. Vortex the mixture for 15 s and use a new 10 µL disposable inoculation loop to sample the specimen.



Overloaded loop and onto the shaft



Appropriately loaded loop

6. Insert the loaded loop into the SBT and express the sample using a swirling motion.

   NOTE: Removal of the entire sample from the loop is not necessary. The resultant SBT solution should be "tea-stained" in color.

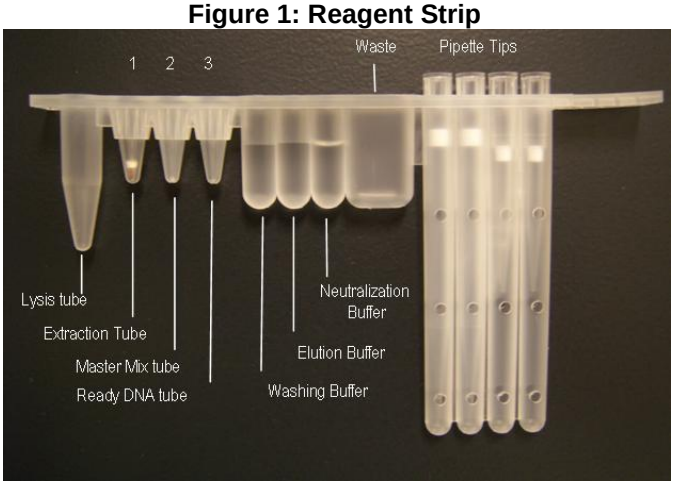
7. Recap the inoculated SBT using a Septum Cap.
8. Place the SBT in a rack compatible with a multi-tube vortex mixer.
9. Prepare any additional samples for testing by repeating Steps 1 through 5; ensuring gloves are clean prior to handling additional specimens.
10. Vortex all prepared samples simultaneously at maximum speed for 1 min with the multi-tube vortex mixer.
11. Proceed to BD MAX™ System Operation section to perform testing of the BD MAX™ Enteric Bacterial Panel on the BD MAX™ System.

9.2 BD MAX™ Operation

Refer to the BD MAX™ System User's Manual for detailed instructions (Operation section). Testing of the BD MAX™ Enteric Bacterial Panel must be performed immediately after the vortexing step above. If retesting is necessary, re-vortex sample(s).

1. After donning a new pair of gloves, remove the required number of BD MAX™ Enteric Bacterial Panel Reagent Strips from the kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes.

Figure 1: Reagent Strip



2. For each specimen to be tested, place one Enteric Bacterial Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially. Do not skip spaces.
3. Remove the required number of Enteric Bacterial Extraction Tubes and Enteric Bacterial Master Mix Tubes from their protective pouches. Remove excess air, and close pouches quickly with the zip seal.
4. Snap one BD MAX™ Enteric Bacterial Extraction Tube (white foil) into Position 1 of each BD MAX™ Enteric Bacterial Reagent Strip (see Figure 2).
5. Snap one BD MAX™ Enteric Bacterial Master Mix tube (green foil) into Position 2 of each BD MAX™ Enteric Bacterial Reagent Strip (see Figure 2).

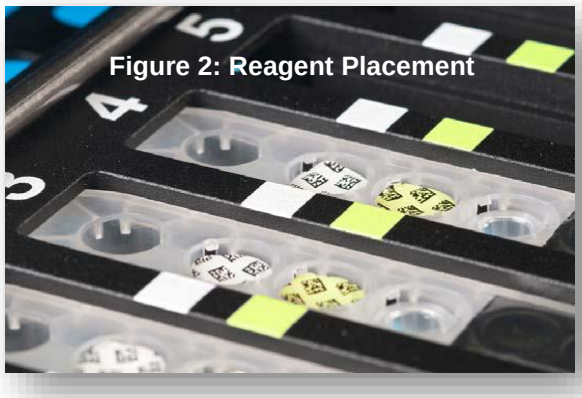
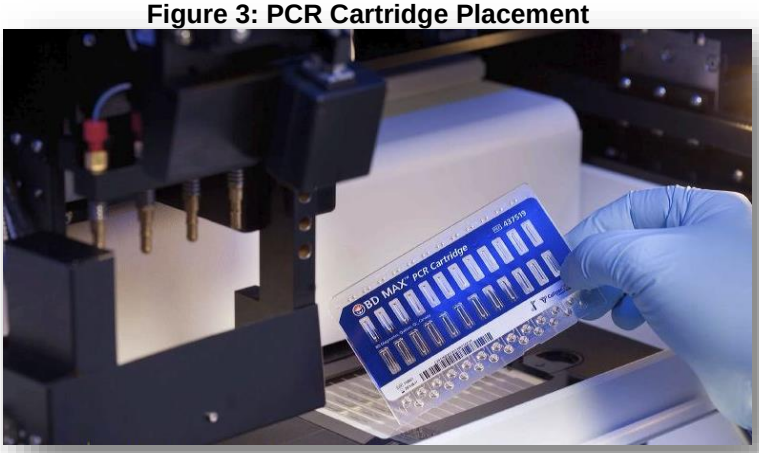


Figure 2: Reagent Placement

6. Place the Sample Buffer Tubes into the BD MAX™ System rack so that the number on the tube corresponds to the position on the rack.
7. Select the <Work List> tab, click on the <Assay> field and using the pull down menu, select <MAX Ent Bac>.
8. Enter the BD MAX™ Enteric Bacterial Sample Buffer Tube ID, and Patient ID or Accession information for Position 1 of Rack A using either the barcode scanner or manual entry.
9. Click on the <Lot Number> field and using the pull down menu, select the appropriate box lot number.
10. Enter information for Position 2 of Rack A, and continue until all Sample Buffer Tubes information is entered.
11. Place the number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 3). One cartridge is required per rack per work list. Each cartridge is sufficient for up to 24 specimens and up to 2 work lists. The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run.

Figure 3: PCR Cartridge Placement



12. Load Rack(s) into the BD MAX™ System. Ensure that the placement of Rack(s) (left to right) corresponds to the Work List created (top to bottom).
13. Close the BD MAX™ System lid, and click the <Start Run> button to begin processing.
14. At the end of the run, check results immediately.

10.0 Interpretation and Reporting of Results

Results are available on the **<Results>** tab in the **<Results>** window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets test results. Results are reported for each analyte and for the Sample Processing Control (SPC). A test result may be called NEG (Negative), POS (Positive) or UNR (Unresolved) based on the amplification status of the target and of the SPC. IND (Indeterminate) or INC (Incomplete) results are due to BD MAX™ System failure. In the case of a partial UNR, where one or more targets have a POS result and all other targets have a UNR result, no targets will be called NEG.

NOTE: Prepared BD MAX™ Enteric Bacterial Panel Sample Buffer Tubes can be stored at 2-8°C for a maximum of 5 d OR at 25 ± 2°C for a maximum of 48 h after the sample has been added to the Sample Buffer Tubes. When an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained, or when an External Control failure occurs, a repeat test from the prepared Sample Buffer Tube must be performed within this timeframe. If a septum cap was damaged during the run, replace it with a new one before storing the sample.

10.1 Positive Result(s)

A positive (POS) result indicates that target DNA was detected. Report the respective positive result(s) first, followed by the negative results. All positive results should be automatically appended with contact precaution and reportable disease comments. Samples positive for

Salmonella, Shigella, or Shiga toxin-producing *E. coli* should be subcultured to obtain the isolate to send to the state public health laboratory (see Culturing Positive Samples below).

10.1.1 *Campylobacter* spp. Reporting

#	Report:
1.	Positive for Campylobacter species by PCR. Enteric contact precautions required. This is a reportable disease. Please contact your County/State Health Department.
2.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
3.	Negative for Salmonella species by PCR.
4.	Negative for Shigella species and Enteroinvasive E. coli by PCR.
5.	Negative for Shiga Toxin-Producing E. coli by PCR.

10.1.2 *Salmonella* spp. Reporting

#	Report:
1.	Positive for Salmonella species by PCR. Enteric contact precautions required. This is a reportable disease. Please contact your County/State Health Department.
2.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
3.	Sent to state public health laboratory for additional testing.
4.	Negative for Campylobacter species by PCR.
5.	Negative for Shigella species and Enteroinvasive E. coli by PCR.
6.	Negative for Shiga Toxin-Producing E. coli by PCR.

10.1.3 *Shigella* spp. Reporting

#	Report:
1.	Positive for Shigella species or Enteroinvasive E. coli by PCR. Enteric contact precautions required. This is a reportable disease. Please contact your County/State Health Department.
2.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
3.	Sent to state public health laboratory for additional testing.
4.	Negative for Campylobacter species by PCR.
5.	Negative for Salmonella species.
6.	Negative for Shiga Toxin-Producing E. coli by PCR.

10.1.4 Shiga Toxin Reporting

#	Report:
1.	Positive for Shiga Toxin-Producing E. coli by PCR. Enteric contact precautions required. This is a reportable disease. Please contact your County/State Health Department.
2.	Antimicrobial therapy in patients infected with Shiga Toxin-producing E. coli is not recommended as it may increase the risk of serious complications such as hemolytic-uremic syndrome.
3.	Results called to: Lisa R on 9/25/13 at 1310 (inpatient example)
4.	Sent to state public health laboratory for additional testing.
5.	Negative for Campylobacter species by PCR.
6.	Negative for Salmonella species.
7.	Negative for Shigella species and Enteroinvasive E. coli by PCR.

10.2 Negative Result

A negative (NEG) result indicates that no target DNA was detected. A successful negative result is only reported when the Sample Processing Control was amplified and detected.

#	Report:
1.	Negative for Campylobacter species by PCR.
2.	Negative for Salmonella species by PCR.
3.	Negative for Shigella species and Enteroinvasive E. coli by PCR.
4.	Negative for Shiga Toxin-Producing E. coli by PCR.

10.3 Unresolved Result

Unresolved results may be obtained in the event that sample-associated inhibition or reagent failure prevents proper target or SPC amplification. If the SPC 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.

The BD MAX™ System reports results for each target individually, and a UNR result may be obtained for one or more BD MAX™ Enteric Bacterial Panel targets. In the case of a UNR result, it is necessary to repeat the test. In rare cases, discrepant results may be observed when a repeat test is run for those targets that were initially reported as POS. If this happens, subculture the specimen onto appropriate media, and consult with Rounds.

The remaining stool sample should be used for repeat testing with a new SBT. If the stool was fresh, it should be transferred to a 15-mL vial of Cary Blair before repeating the test. Vortex the mixture for 15 s prior to sampling. If the results are unresolved a second time, report: **Uninterpretable PCR results. Culture in progress.**

10.4 Indeterminate Result

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from their corresponding SBT(s) within the timeframe defined above. Vortex the sample(s) for 1 min, and repeat testing with a new reagent strip, extraction tube and master mix for each sample. The remaining stool sample may also be used for repeat testing with a new SBT. For the interpretation of warning or error code messages, refer to the BD MAX™ System User's Manual (Troubleshooting section).

10.5 Incomplete Result

Incomplete results may be obtained in the event that the Sample Preparation or the PCR failed to complete. Sample(s) can be repeated from their corresponding SBT(s) within the allowed timeframes defined above. Vortex the sample(s) for 1 min, and repeat testing with a new reagent strip, extraction tube and master mix for each sample. The remaining stool sample may also be used for repeat testing with a new SBT. For the interpretation of warning or error code messages, refer to the BD MAX™ System User's Manual (Troubleshooting section).

10.6 External Control Failure

External Controls should yield expected results when tested. If samples have to be repeated due to an incorrect External Control result, they should be repeated from their SBT along with freshly prepared External Controls within the allowed timeframes defined above. Vortex the samples for 1 min, and repeat testing with a new reagent strip, extraction tube and master mix for each sample.

10.7 Culturing Positive Samples

Samples that test positive for *Salmonella, Shigella,* or Shiga toxin-producing *E. coli* should be subcultured to obtain the isolate to send to the state public health laboratory. If the isolate cannot be recovered, the original specimen should be sent. Use the following media and conditions for subculture.

- *Salmonella* spp. – BBL™ CHROMagar™ Salmonella – ambient air at 35 ± 2 °C
- *Shigella* spp. – MacConkey II Agar – ambient air at 35 ± 2 °C
- Shiga toxin-producing *E. coli* – BBL™ CHROMagar™ O157 – ambient air at 35 ± 2 °C

Refer to the Stool Culture Procedure for protocols to confirm isolate identification.

11.0 Quality Control & Quality Assurance

11.1 External Controls

External control materials must be used to evaluate each new lot or shipment of BD MAX™ Enteric Bacterial Panel kits. External controls must be tested every 30 d while a lot is in use. Quality control results should be entered into the LIS. Notify technical specialist or supervisor if

results are not as expected. Do not report any patient results obtained from the failed run. Repeat testing using new external controls.

Suspensions of the control strains should be prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies. Dilute the *Salmonella, Shigella,* and *E. coli* organisms 1:10 and the *Campylobacter* 1:100. Dilute each suspension 2:5. Combine equal portions of each control suspension to obtain a final concentration of ~1.0 X 10^6 CFU/mL (for *Salmonella, Shigella*, and *E. coli*) and ~1.0 X 10^5 CFU/mL (for *Campylobacter*). When the control material is used for testing, the final concentration of organisms per Sample Buffer Tube is approximately 6.7 X 10^3 CFU/mL (for *Salmonella, Shigella*, and *E. coli*) and 6.7 X 10^2 CFU/mL (for *Campylobacter*). This is close to the manufacturer's published limit of detection for each organism. The weak positive control material helps to verify the lower limit of detection for each new lot/shipment. Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** Pooled, diluted suspensions of *Campylobacter jejuni* ATCC 33291, *E. coli* O157:H7 ATCC 35150, *Salmonella enteritidis* ATCC 14028, and *Shigella sonnei* ATCC 9290. An external positive control that yields a negative test result is indicative of a reagent or BD MAX™ System error. Repeat Quality Control testing with new controls. Check the BD MAX™ System monitor for any error messages. If the problem persists, use unopened reagents or a new BD MAX™ Enteric Bacterial Panel Kit.
- **Negative External Control:** Saline. An external negative control that yields a positive test result is indicative of a specimen handling and/or a contamination problem.

An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the "System Error Summary" section of the BD MAX™ System User's Manual for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new BD MAX™ Enteric Bacterial Panel kit.

Note: External Positive and Negative Controls are not used by the BD MAX™ System software for the purpose of sample test result interpretation.

11.2 Internal Control

Each BD MAX™ Enteric Bacterial Extraction Tube contains a Sample Processing Control (SPC) which is a plasmid containing a synthetic target DNA sequence. The SPC monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA amplification and detection during PCR analysis. If the SPC result fails to meet the acceptance criteria, the result of the sample will be reported as Unresolved; however, any positive (POS) assay results will be reported and no targets will be called NEG. An Unresolved result is indicative of sample-associated inhibition or reagent failure. Repeat any sample reported as Unresolved.

11.3 Report Review

All test results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Results should be compared to the printed results from the BD MAX™ computer. The review should be documented on the MRSA PCR Batch Log.

12.0 Maintenance

12.1 Daily Cleanup

Caution: Do not use any decontamination or cleaning agents that could cause a hazard as a result of a reaction with parts of the equipment. Do not use abrasive or corrosive cleaners on heater boards. Do not spray or pour liquid directly on surfaces.

At the end of each day, perform the following cleaning procedure:

1. Wipe down the following items and areas with disinfecting wipes containing 1% sodium hypochlorite.
    - sample racks (should be cleaned between each run)

- work surfaces
- ancillary items such as pipettes, tube racks, etc.
- all external and internal work surfaces of the BD MAX™ instrument, EXCEPT the monitor screen, the clear part of the instrument door, and the glass surface of the cartridge drawer. External instrument surfaces should be cleaned before internal surfaces.

2. Using a unidirectional motion, thoroughly wipe off all system parts that came into contact with sodium hypochlorite (a known PCR inhibitor) with a lint-free cloth dampened with deionized (DI) water, then with 70% alcohol.
3. Use a new, dampened lint-free cloth for each solution.
4. Dry the system with a lint-free cloth.

12.2 Weekly Cleaning

1. Perform routine Daily Cleanup as described above.
2. Inspect the cartridge drawer for foreign objects, dirt, or dust. If any are discovered in the tray, remove and clean the surface with a 70% alcohol solution on a lint-free cloth.
3. If necessary, wipe the monitor screen with an alcohol wipe, and then dry the screen with a soft cloth.
4. Use either an alcohol wipe or glass cleaner to clean both the transparent cover of the system and the mirror inside the instrument, using a lint-free cloth to dry.
5. Put on a clean pair of disposable gloves before beginning instrument operation.

13.0 Instrument Maintenance and Service

13.1 Preventative Maintenance

Preventative Maintenance is performed by a BD field service engineer every 6 months. The engineer checks all of the instrument calibrations and the thermocycler functionality. After the PM is complete, previously tested patient samples should be run to verify the instrument's performance. This should include 5 positive and 5 negative samples for each analyte.

13.2 Service Repairs

If the BD MAX™ instrument malfunctions or operates unusually in any way, initial attempts should be made to solve the problem by following the recommendations in the Troubleshooting section of the System User's Manual. All other servicing attempts will terminate the responsibility of the manufacturer under the terms of the warranty.

If instrument malfunction cannot be corrected, contact BD Technical Services. Technical Services is available Monday through Friday from 5:30 a.m. to 5:00 p.m. Pacific Time. Locate the instrument serial number located on the front of the instrument before placing the call.

Technical Service Information

Telephone Number: 800-638-8663

Email Address: technical_services@bd.com

After major repairs have been made to the instrument, previously tested patient samples should be retested to verify that the instrument is performing as expected.

14.0 Limitations

1. This product is intended for use only with unpreserved and Cary-Blair preserved human stool samples. Stool samples from rectal swabs or fixed stools have not been validated with the BD MAX™ Bacterial Panel.
2. Erroneous results may occur from improper sample collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the sample is below the analytical sensitivity of the test.
3. If the BD MAX™ Enteric Bacterial Panel result is IND, INC, or UNR (for one or more targets) then the test should be repeated.

4. 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 *stx*1/*stx*2 genes and allows for identification of the Enteric Bacterial Panel organisms.
5. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of the genera *Salmonella* and *Campylobacter* (*jejuni* and *coli*), *Shigella* spp., Enteroinvasive *E. coli* (EIEC] as well as Shiga toxin-producing *E. coli* variants, resulting in a false negative result with the BD MAX™ Enteric Bacterial Panel.
6. The BD MAX™ Enteric Bacterial Panel does not distinguish which Shiga toxin gene (*stx*1/*stx*2) is present in a specimen.
7. In rare instances, Shiga toxin genes can be found in *Enterobacteriaceae* other than STEC or *Shigella dysenteriae.*
8. 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.
9. *In silico* analysis predicts that variant stx2f will not be detected by the BD MAX™ Enteric Bacterial Panel.
10. The BD MAX™ Enteric Bacterial panel does not differentiate between *Shigella* spp. and Enteroinvasive *Escherichia coli* (EIEC).
11. 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.
12. 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 SPC has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The SPC 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. BD MAX™ Enteric Bacterial Panel results may or may not be affected by concurrent antimicrobial therapy, which may reduce the amount of target present.
15. The sample buffer tube has not been designed to support organism viability. If culture is necessary, it must be performed from the original specimen.
16. The performance of this test has not been established for monitoring treatment of *Salmonella* spp., *Shigella* spp., *C. jejuni*/*C. coli* or STEC infections.
17. This test is a qualitative test and does not provide quantitative values nor indicate the quantity of organisms present.
18. The performance of this test has not been evaluated for immunocompromised individuals or for patients without symptoms of gastrointestinal infection.
19. The effect of interfering substances has only been evaluated for those listed in the product package insert. Potential interference has not been evaluated for substances other than those described in the “Interference” section.
20. Cross-reactivity with organisms other than those listed in the “Analytical Specificity” section of the package insert has not been evaluated.

15.0 Verification Information

Performance Characteristics

The BD MAX™ Enteric Bacterial Panel has been cleared by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. In this evaluation, stool specimens that were previously tested by culture were subsequently tested using the BD MAX™ assay. The positive samples were comprised of archived clinical specimens stored at -70°C. Both fresh stool and stool in Cary-Blair were tested.

A total of 19 specimens (9 Cary-Blair preserved and 10 unpreserved) that were culture-positive for *Campylobacter* spp. were tested with the BD MAX™ Enteric Bacterial Panel. An additional 59

specimens that were culture-negative for *Campylobacter* spp. were also tested. There was 100% agreement between the BD MAX™ assay and the culture results. Table 1 summarizes the overall performance of the BD MAX™ Enteric Bacterial Panel for the detection of *Campylobacter* spp., compared to culture.

Table 1: *Campylobacter* spp.

	Negative Culture	Positive Culture	Total
Negative MAX	59	0	59
Positive MAX	0	20	20
Total	59	20	79

Overall Agreement	100% (95.4 to 100%)
Sensitivity	100% (83.9 to 100%)
Specificity	100% (93.9 to 100%)
Positive Agreement	100%
Negative Agreement	100%
Predicitve Value Positive	100%
Predicitve Value Negative	100%

A total of 20 specimens (9 Cary-Blair preserved and 11 unpreserved) that were culture-positive for *Salmonella* spp. were tested with the BD MAX™ Enteric Bacterial Panel. An additional 58 specimens that were culture-negative for *Salmonella* spp. were also tested. Table 2 summarizes the overall performance of the BD MAX™ Enteric Bacterial Panel for the detection of *Salmonella* spp., compared to culture. The assay failed to detect *Salmonella* spp. from one of the Cary-Blair samples. The culture produced low numbers of *Salmonella* (< 10 colonies) on CHROMagar™ Salmonella agar. The isolate was detected by the assay when tested using a 1×10^6 CFU/mL suspension. The isolate was likely missed in the original specimen because the concentration of the target organisms in the specimen was below the LoD of the assay.

Table 2: *Salmonella* spp.

	Negative Culture	Positive Culture	Total
Negative MAX	58	1	59
Positive MAX	0	19	19
Total	58	20	78

Overall Agreement	98.7% (93.1 to 99.8%)
Sensitivity	95.0% (76.4 to 99.1%)
Specificity	100% (93.8 to 100%)
Positive Agreement	95%
Negative Agreement	100%
Predicitve Value Positive	100%
Predicitve Value Negative	98.3%

A total of 5 specimens (2 Cary-Blair preserved and 3 unpreserved) that were culture-positive for *Shigella* spp. were tested with the BD MAX™ Enteric Bacterial Panel. Due to the low numbers of available specimens that were positive for *Shigella,* additional seeded samples were created using 11 clinical isolates and 1 ATCC strain of *Shigella* spp. A 0.5 McFarland suspension was prepared for each *Shigella* isolate. Each suspension was diluted and seeded into a target-negative stool matrix to achieve a final concentration of 1×10^5 CFU/mL of stool. An additional 62 specimens that were culture-negative for *Shigella* spp. were also tested. There was 100% agreement between the BD MAX™ assay and the culture results. Table 3 summarizes the overall performance of the BD MAX™ Enteric Bacterial Panel for the detection of *Shigella* spp., compared to culture.

Table 3: *Shigella* spp.

	Negative Culture	Positive Culture/Seed	Total
Negative MAX	62	0	62
Positive MAX	0	16	16
Total	62	16	78

Overall Agreement	100% (95.3 to 100%)
Sensitivity	100% (80.6 to 100%)
Specificity	100% (94.2 to 100%)
Positive Agreement	100%
Negative Agreement	100%
Predicitve Value Positive	100%
Predicitve Value Negative	100%

A total of 20 specimens (8 Cary-Blair preserved and 12 unpreserved) that were culture-positive for Shiga-toxin *E. coli* (STEC) were tested with the BD MAX™ Enteric Bacterial Panel. This included 10 specimens that produced *E. coli* O157 and 10 cultures that produced non-O157 strains of STEC, which were initially detected by Shiga toxin EIA. An additional 58 specimens that were culture-negative for *E. coli* O157, and Shiga toxin-negative by EIA, were also tested. Table 4 summarizes the overall performance of the BD MAX™ Enteric Bacterial Panel for the detection of STEC. The assay failed to detect STEC from one of the unpreserved samples. The culture produced low numbers of *E. coli* O157 (< 10 colonies) on CHROMagar™ O157 agar. The isolate was detected by the assay when tested using a 1×10^6 CFU/mL suspension. The isolate was likely missed in the original specimen because the concentration of the target organisms in the specimen was below the LoD of the assay.

Of the 20 specimens which were positive for Shiga toxin-producing *E. coli*, 7 (35%) of the cultures grew *E. coli* O157 but were negative for Shiga toxin by EIA using the Immuno*Card* STAT! EHEC produced by Meridian Bioscience, Inc. (Cincinnati, OH). The BD MAX™ Enteric Bacterial Panel detected Shiga toxin genes in all 7 of these samples. This demonstrates a significantly improved sensitivity for the detection of Shiga toxin-producing *E. coli*. While these *E. coli* O157 isolates were detected due to the highly selective and differential characteristics of CHROMagar™ O157, the detection of non-O157 STEC strains relies solely on the EIA for Shiga toxin detection. The BD MAX™ Enteric Bacterial Panel will likely provide greater sensitivity for the detection of non-O157 STEC.

Table 4: Shiga toxin-producing *E. coli*			
	Negative Culture	Positive Culture	Total
Negative MAX	58	1	59
Positive MAX	0	19	19
Total	58	20	78
Overall Agreement	98.7% (93.1 to 99.8%)		
Sensitivity	95.0% (76.4 to 99.1%)		
Specificity	100% (93.8 to 100%)		
Positive Agreement	95%		
Negative Agreement	100%		
Predicitve Value Positive	100%		
Predicitve Value Negative	98.3%		

Of the 80 clinical and seeded stool specimens, 2 (3%) produced unresolved results. Both of these specimens produced the expected results upon repeat testing. It should be noted that, the majority of the clinical specimens used for this verification study were archived, frozen samples. The freeze/thaw process is known to reduce substances which inhibit PCR. Unresolved rates during patient testing will be monitored on a monthly basis.

Analytical Sensitivity

The Limit of Detection (LoD) for each target, as determined by the manufacturer, is shown in Table 5 below.

Table 5: Limit of Detection	Unpreserved Stool LoD (CFU/mL in stool) [95% CI]	Cary-Blair Preserved Stool LoD (CFU/mL in stool) [95% CI]
Campylobacter coli ATCC 43134	14,250 [10,500 – 19,200]	8,250 [6,150 – 11,400]
Campylobacter jejuni ATCC 43429	6,300 [5,400 – 7,350]	1,500 [1,350 – 1,500]
Salmonella typhimurium ATCC 14028	44,400 [34,950 – 56,400]	28,950 [21,300 – 39,450]
Salmonella enteriditis ATCC 13076	93,000 [60,450 – 143,100]	75,300 [51,750 – 109,350]
Shigella flexneri ATCC 700930	56,100 [37,350 – 84,150]	34,350 [22,650 – 52,050]
Shigella sonnei ATCC 10523	12,600 [8,850 – 17,700]	18,600 [10,050 – 34,350]
E. coli stx1 ATCC 43890	38,202 [29,259 – 49,865]	33,495 [25,026 – 44,817]
E. coli stx1 / stx2 BD ENF 10513	136,500 [82,500 – 225,750]	97,950 [57,600 – 166,650]
E. coli stx2 ATCC 43889	108,300 [77,850 – 150,900]	89,850 [43,650 – 184,650]

The overall sensitivity of the assay in this verification study was 97%, with 75 of the 77 targets detected. The clinical specimens used included samples with varying concentrations of target organisms. Cultures of some samples produced growth of pathogens into the fourth quadrant and some only to the first. The two targets that were not detected grew in very low numbers on culture media and were likely below the LoD for the respective assays.

The external positive control material is prepared with concentrations of organisms close to the LoD to provide an ongoing assessment of the assay LoD.

Analytical specificity

During this verification study, no false-positive results were encountered. The manufacturer of the BD MAX™ Enteric Bacterial Panel evaluated the assay on samples containing phylogenetically related species and other organisms (bacteria, viruses, parasites and yeast) likely to be found in stool specimens.

- Nine (100%) of *Campylobacter* strains (*Campylobacter* species other than *C. jejuni* or *C. coli*) with undetectable *tuf* gene sequences, tested at a concentration ≥ 1 x 10^6 CFU/mL in the SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel.
- Six (100%) of *E. coli* strains other than Shiga toxin-producing strains, tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel.
- Ninety-eight (99%) of other bacterial strains (including 53 species and subspecies), tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel. *S. boydii* (ATCC 12028) produced 1 replicate out of 3 as positive for the presence of s*tx*.
- Fifteen (100%) of viruses, tested at a concentration ≥ 1 x 10^4 PFU/mL of SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel.
- Three (100%) of ova and parasites, tested at a concentration ≥ 1 x 10^5 cysts/mL of SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel.
- Two (100%) of *Candida* species tested at a concentration ≥ 1 x 10^5 organisms/mL of SBT, produced negative results with the BD MAX™ Enteric Bacterial Panel.
- Sixteen Enteric organisms representing each target of the BD MAX™ Enteric Bacterial Panel were tested, with results as follows:
    - Three (100%) of *Campylobacter* spp.; one *C. coli*, one *C. jejuni*, subsp. *doylei* and one *C. jejuni*, subsp. *jejuni* bearing the *tuf* gene tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced positive results for *Campylobacter* and negative results for all other targets.
    - Four (100%) of *E. coli*; two O157 and two non-O157 strains bearing the *stx* gene tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced positive results for *E. coli* and negative results for all other targets.
    - Five (100%) of *Salmonella* spp. bearing the *spaO* gene tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced positive results for *Salmonella* and negative results for all other targets.
    - Three (3) of 4 *Shigella* spp.; one *S. sonnei*, one *S. boydii*, one *S. flexneri* and one *S. dysenteriae* bearing the *ipaH* gene tested at a concentration ≥ 1 x 10^6 CFU/mL of SBT, produced positive results for *ipaH* and negative results for all other targets.
    - Initial testing of *S. boydii* (ATCC 12028) produced 1 replicate out of 3 as positive for the presence of s*tx*. Subsequent testing of this strain produced positive results with 8 out of 20 replicates for the presence of *stx*.

Precision

Precision testing was performed using the external control materials that were prepared as described under the Quality Control section. These controls provided low-level positive and negative samples for testing. Intra-assay precision testing was performed by testing 10 replicates of positive and 10 replicates of negative external controls. Inter-assay precision was established

by testing 1 positive and 1 negative external control daily for 20 d. All of the external controls tested during the precision verification produced the expected results.

16.0 References

1. Package insert: BD MAX™ Enteric Bacterial Panel Kit, 05-2014
2. BD MAX™ System User's Manual BD Diagnostics, Sparks, MD, USA.

17.0 Document Control History

Reviewed by director (AR) 12/03/2014

Reviewed by supervisor (JC) 12/04/2014, Jason Ammons 12/2015

Changes and updates:

09/14/2015 Instructions were added for transferring fresh specimens to Cary Blair if they are difficult to sample using an inoculation loop. Instruction were added for using Cary Blair for fresh specimens that produce an unresolved result during initial testing.