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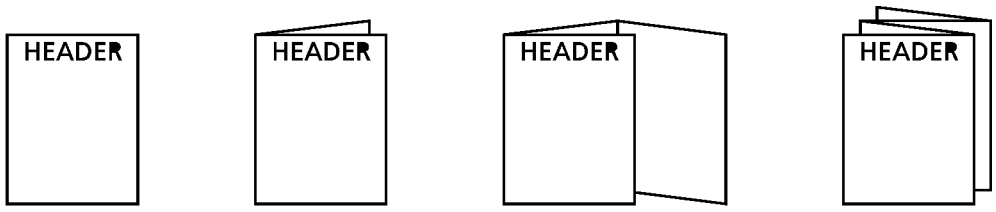

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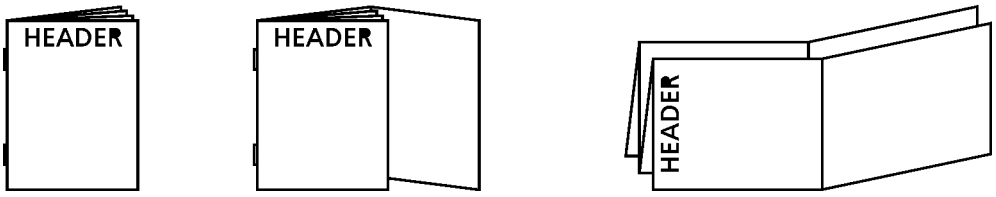

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4. See Specification Control Number N/A for Material Information
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Label Design Date Proofer Date Checked By Date Part Number: L0009181		BD Becton, Dickinson and Company 7 Loveton Circle Sparks, MD 21152 USA	
	Category and Description Package Insert, BACTEC MGIT Para TB Media and Supplement	Sheet: 1 of 9 Scale: N/A	A

BD BACTEC™ MGIT™ Para TB Medium
BACTEC™ MGIT™ Para TB Supplement

L0009181
2009/11

INTENDED USE

The **BACTEC™ MGIT™** Para TB Medium tube, supplemented with **BACTEC MGIT** Para TB Supplement, is intended for the detection and recovery of *Mycobacterium paratuberculosis* using the **BACTEC MGIT** System. Acceptable specimen types are decontaminated bovine fecal specimens.

SUMMARY AND EXPLANATION

M. paratuberculosis has been demonstrated to be the causative agent of Johne's disease, a chronic wasting disease of ruminants in general and dairy cattle in particular. *M. paratuberculosis* colonizes the lymphatic tissues of the animal's intestinal walls, causing chronic diarrhea and wasting, and eventually resulting in the animal's death.[1] Culture of the organism from fecal specimens is the most sensitive, definitive determination of the presence of *M. paratuberculosis* infestation within a herd of animals. The **BACTEC MGIT** Para TB Medium and **BACTEC MGIT** Para TB Supplement for *M. paratuberculosis* are designed to recover the organism from decontaminated bovine fecal specimens. The fully supplemented medium (including egg yolk) can detect as few as 1 to 10 viable organisms from processed specimens in < 49 days. The **BACTEC MGIT** Para TB Medium and **BACTEC MGIT** Para TB Supplement are designed to be a diagnostic tool to assist in Johne's monitoring and control programs. The **BACTEC MGIT** Para TB Medium tube contains 7 mL of modified Middlebrook 7H9 Broth base.[2,3] The medium has been modified to specifically stimulate the growth of *M. paratuberculosis* following extraction from decontaminated specimens. There are advantages and disadvantages to using liquid medium for this purpose. The advantage is that liquid medium is the best resuscitation and growth support medium for the quick recovery of mycobacteria from processed specimens. This is based on the utilization of the **BACTEC** 12B radiometric medium for the growth and recovery of *M. paratuberculosis.*[4] The disadvantage is that liquid culture is much more sensitive to the quality of the inoculum in terms of remaining toxins and contaminants from the decontamination procedure.

PRINCIPLES OF THE PROCEDURE

A fluorescent compound is embedded in silicone on the bottom of each **MGIT** Para TB Medium tube. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. Actively respiring microorganisms consume oxygen and cause the sensor to fluoresce. **BACTEC MGIT** Para TB Medium tubes entered into the **BACTEC MGIT** System are continuously incubated at 37°C and monitored every 60 minutes for increasing fluorescence. Analysis of the fluorescence is used to determine if the tube is instrument positive; i.e., the test sample contains viable organisms. An instrument positive tube contains approximately 10^5 to 10^6 colony-forming units per milliliter (CFU/mL). Culture vials which remain negative for 49 days (seven weeks) and which show no visible signs of positivity are removed from the instrument as negatives.

The **BACTEC MGIT** Para TB Supplement is added to each **BACTEC MGIT** Para TB Medium tube to provide substances essential for the rapid growth of mycobacteria. Oleic acid plays an important role in the metabolism of mycobacteria. Albumin acts as a protective agent by binding essential free fatty acids that may be toxic in the unbound form, thereby enhancing recovery. Casein is added as a carbon and energy source. Catalase destroys toxic peroxides that may be present in the medium.

Contamination is reduced when supplementing the complete **BACTEC MGIT** Para TB Medium with an antibiotic mixture prior to inoculation with a decontaminated, processed specimen.

REAGENTS

The **MGIT** Para TB Medium tube (capped with polypropylene caps) contains:

Fluorescent indicator (Indicator contains Tris 4, 7-diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate in a silicone rubber base.)	110 µL
Broth	7 mL

Approximate Formula* Per L of Purified Water:

Modified Middlebrook 7H9 Broth base (with Mycobactin J)	21.9 g

MGIT Para TB Supplement contains 15 mL of the following formula:

Approximate Formula* Per L of Purified Water:

Bovine albumin	50.0 g
Catalase	0.03 g
Casein	0.5 g
Oleic acid	0.1 g

*Adjusted and/or supplemented as required to meet performance criteria.

Storage of Reagents: MGIT Para TB Medium tubes - On receipt, store at 2-25°C. DO NOT FREEZE. Minimize exposure to light. Broth should appear milky and aqueous in consistency. **MGIT** Para TB Medium tubes stored as labeled prior to use may be inoculated up to the expiration date and incubated for up to 8 weeks.

MGIT Para TB Supplement: On receipt, store in the dark at 2-8°C. Avoid freezing or overheating. Do not open until ready to use. Minimize exposure to light.

Product Deterioration: Congealed medium or clumps in the medium is an indication that the tube has been contaminated. Do not use medium or supplement if it shows evidence of microbial contamination, discoloration, drying or other signs of deterioration.

Warnings and Precautions

For Veterinary Use.

This Product Contains Dry Natural Rubber.

Working with *Mycobacterium avium* subspecies *paratuberculosis* grown in culture requires Biosafety Level 2 (BSL-2) practices, containment equipment and facilities. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.[5]

Prior to use, each **MGIT** Para TB tube should be examined for evidence of contamination or damage. Discard any tubes if they appear damaged or unsuitable.

In the event of tube breakage: 1) Close the instrument drawers; 2) Turn off the instrument; 3) Consult your facility guidelines. An inoculated leaking or broken vial may produce an aerosol of mycobacteria; appropriate handling should be observed.

Autoclave all inoculated **MGIT** Para TB tubes prior to disposal.

SPECIMEN HANDLING

Refer to appropriate references for details of specimen collection and handling procedures.[6]

PROCEDURE

Materials Provided: MGIT Para TB Medium and **MGIT** Para TB Supplement (see "Availability").

Materials Required But Not Provided: BACTEC MGIT instrument, **BBL™** Brain Heart Infusion (BHI), **Difco™** Egg Yolk Enrichment, 50% and **Bacto™** Yeast Extract (see "Availability"), pyruvic acid sodium salt, malachite green oxalate salt (Sigma M9015 is specifically recommended), **Falcon™** brand 50 mL centrifuge tubes, hexadecylpyridinium chloride (HPC), vancomycin HCl, nalidixic acid sodium salt, amphotericin B (solubilized), mechanical tube rocker, magnetic stir plate and stir bars, pipettors, pipette tips, sterile cotton-tipped applicators, filters (0.2 micron membranes), microcentrifuge tubes, microscope and materials for staining slides, **Difco/BBL** TB Quick Stain Kit (see "Availability"), and sterile purified water (dH_2O).

Note: MGIT Para TB Medium tubes must be read with a **BACTEC MGIT** instrument.

Test Procedure: Observe aseptic techniques.

WARNING: The following table lists the two types of software available for the BACTEC MGIT system, their intended applications, and the culture medium tubes for each application. Culture medium tubes for one application cannot be used in a BACTEC MGIT instrument running software for the other application!

BACTEC MGIT Software	Intended Application	Culture Medium Tubes
Clinical software for mycobacteria & *M. bovis*	Clinical detection of mycobacteria (including *M. tuberculosis*) and veterinary detection of *M. bovis*	**BBL MGIT** Mycobacteria Growth Indicator Tubes (cat. no. 245122, with clear growth medium)
Para TB software	Veterinary detection of *M. avium* subsp. *paratuberculosis* from processed bovine feces	**BACTEC MGIT** Para TB Media (cat. no. 245154, with white growth medium)

Preparation of Stock Solutions[7]

1. **1/2X Brain Heart Infusion (BHI)- 0.9% HPC Solution:** This solution is used during Day 1 of the fecal sample processing procedure.
   a. Prepare a 1/2X solution of Brain Heart Infusion (BHI) per L of purified water. For example, if the dehydrated medium label states 37 g of powder/L (1X), a 1/2X concentration would be 18.5 g of powder/L. Follow manufacturer's label instructions for preparation and sterilization, and include a magnetic stir bar in the vessel.
   b. Allow prepared BHI to cool to room temperature completely (overnight).
   c. Aseptically, add 9 g/L HPC (Hexadecylpyridinium chloride) to prepared 1/2X BHI. Stir overnight to dissolve. Store at room temperature for up to one month. Stir overnight before each use.

   Note: The HPC will precipitate at 4°C. If precipitation occurs at room temperature, try to re-dissolve by stirring solution on a stir plate overnight. If overnight stirring does not re-dissolve the HPC, discard the solution and make fresh 1/2X BHI-0.9% HPC.
2. **Antimicrobial Stock Solutions (Vancomycin, Nalidixic Acid, Amphotericin B).** These stock solutions are used to prepare the Antibiotic Brew and Additive Cocktail. Store stocks at -20°C for up to 6 weeks or -70°C for up to 6 months.[8]

   Vancomycin HCl (VAN) Stock Solution

   a. Prepare a 2.5% solution (25,000 µg/mL) of vancomycin HCl in purified water and filter-sterilize. Use a VAN product that is certified for USP testing, cell culture or biotechnology applications.

   Nalidixic Acid Sodium Salt (NAL) Stock Solution

   b. Prepare a 2.5% solution (25,000 µg/mL) of nalidixic acid in purified water and filter-sterilize. Use a NAL product that is certified for USP testing, cell culture or biotechnology applications.

   Amphotericin B (AMB) Stock Solution

   c. Prepare a 1% solution (10,000 µg/mL) of amphotericin B in purified water. Amphotericin B is a sterile powder that forms an emulsion in water, and, therefore, cannot be filtered once in solution. Aseptically add sterile purified water to all of the amphotericin B powder in its original container. Adjust the volume of water added to the actual weight and potency of the powder in the container (provided by manufacturer) to produce the 1% solution. Solubilized amphotericin B contains emulsifiers and buffers so that the potency of the material is less than 45%, which must be taken into account with a potency-adjustment calculation when making a stock solution.

   For example: a lot of solubilized amphotericin B has a listed potency of 40.6%. Theoretically, 10 mL of sterile water should be added to 100 mg powder to make a 1% (10,000 µg/mL) stock solution. Potency adjustment calculation: 10 mL × 0.406 = 4.06 mL, so 4.06 mL of sterile water should be added to the 100 mg bottle of powder to make the 1% potency-adjusted stock solution.

   Some protocols list 50 µg/mL AMB for pellet resuspension on Day 2 of fecal processing, but this value is without potency-adjustment and is therefore actually 20-25 µg/mL. NAL and VAN powders are close to 100% potency, so potency-adjustment calculations are unnecessary.
3. **Antibiotic Brew:** This solution is used during Day 2 of the fecal sample processing procedure and contains 100 µg/mL each of vancomycin and nalidixic acid, and

25 µg/mL of potency-adjusted amphotericin B. The Antimicrobial Stock Solutions (see Step 2 above) are used in this preparation.

To prepare a 10 mL volume of Antibiotic Brew:

a. Add 10 mL sterile 1/2X BHI to a sterile container

b. Add 40 µL of vancomycin HCl stock solution

c. Add 40 µL of nalidixic acid stock solution

d. Add 25 µL of amphotericin B stock solution

Note: Antimicrobial Stock Solutions and Antimicrobial Brew may be stored at -70°C for up to 6 months or -20°C for up to 6 weeks.[8]

4. **Additive Cocktail:** each **BACTEC MGIT** Para TB Medium tube receives 1.5 mL of Additive Cocktail on Day 2 of fecal processing, containing 0.8 mL **BACTEC MGIT** Para TB Supplement, 0.5 mL Egg Yolk Enrichment, and antimicrobials. Determine the amount of Additive Cocktail to be prepared by multiplying the number of **BACTEC MGIT** Para TB Medium tubes to be inoculated by 1.5 mL, and add an extra 10–15 mL to this value. Add ingredients in this order:

   BACTEC MGIT Para TB Supplement, antimicrobials, Egg Yolk, and then bring to final volume with sterile dH_2O. AMB is an emulsion and can settle out of solution over time. Swirl Additive Cocktail periodically in order to keep the AMB dispersed. Tubes of **Difco** Egg Yolk Enrichment may also show settling and should always be vortexed well before use. Make Additive Cocktail on the day of use, or make the Additive Cocktail ahead of time without egg yolk and store this at -20°C for up to 6 weeks. Thaw at room temperature or in a 35-37°C incubator, mix in the appropriate amount of egg yolk, and add 1.5 mL per **BACTEC MGIT** Para TB Medium tube on Day 2 of fecal processing. Remember that a different Additive Cocktail formulation, with a higher concentration of nalidixic acid, is used for re-culturing with frozen inocula.

   Below is a table listing the formulations of convenient quantities of standard and high nalidixic acid Additive Cocktails. Some volumes have been rounded off, however those for antimicrobials have not due to the high concentrations of the antimicrobial stock solutions. Standard Additive Cocktail is 53.3% **BACTEC MGIT** Para TB Supplement, VAN and NAL stocks are each added to 101.19 µg/mL, AMB stock is added to 38.41 µg/mL, and 33.3% is Egg Yolk Enrichment. Samples re-cultured with frozen inocula require a cocktail with 1146.7 µg/mL NAL stock and correspondingly less sterile dH_2O for **BACTEC MGIT** Para TB Medium with 200 µg/mL nalidixic acid.

Standard Additive Cocktail Component	Amount of Standard Additive Cocktail				
	10 mL	30 mL	60 mL	90 mL	120 mL
Para TB Supplement	5.3 mL	16 mL	32 mL	48 mL	64 mL
2.5% Vancomycin	40 µL	121 µL	243 µL	364 µL	486 µL
2.5% Nalidixic Acid*	40 µL	121 µL	243 µL	364 µL	486 µL
1% Amphotericin B	38 µL	115 µL	230 µL	346 µL	461 µL
Egg Yolk Enrichment	3.3 mL	10 mL	20 mL	30 mL	40 mL
sterile dH_2O	1.3 mL	3.6 mL	7.3 mL	10.9 mL	14.6 mL

*Additive Cocktail Changes for 200 µg/mL Nalidixic Acid for Frozen Inocula:

NAL_{200} Additive Cocktail Component	Amount of NAL_{200} Additive Cocktail				
	10 mL	30 mL	60 mL	90 mL	120 mL
2.5% Nalidixic Acid	459 µL	1.376 mL	2.752 mL	4.128 mL	5.5 mL
sterile dH_2O	0.9 mL	2.3 mL	4.8 mL	7.1 mL	9.6 mL

5. **Bacto Yeast Extract Solution:** Prepare a 15% w/v solution by dissolving powder in 80% final volume of dH_2O, with stirring, and heat until steaming but not boiling. After cooling, bring to full volume with dH_2O, filter-sterilize, and store at 4°C for up to 6 weeks. If precipitation or turbidity occurs during storage, discard and make a fresh 15% stock. **Note:** it is important to use **Bacto** Yeast Extract, as other yeast extract products will be much less effective.
6. **Pyruvic Acid Sodium Salt Solution:** Prepare a 10% w/v solution by dissolving powder in 80% final volume of dH_2O, then bring to full volume with dH_2O, and, filter-sterilize. Store at 4°C in the dark up to 6 months.
7. **Malachite Green Solution:** Prepare a 5% w/v solution by adding powder to 90% final volume of dH_2O in a 50 mL disposable centrifuge tube, then bring to full volume with dH_2O. Dissolve powder by rocking on a tube rocker at room temperature for 2.5 h, then filter-sterilize, and store at room temperature for up to 6 months.

   Note: only use malachite green oxalate salt products with Chemical Abstracts Service (CAS) number 2437-29-8, but not those listed as technical dye or technical grade, or pre-made malachite green solutions.

Fecal Processing

WARNING: All quality control testing, fecal processing, smear preparations, subculturing, etc., of fecal samples or presumptive positive tubes must be performed using bio-safety level 2 (BSL-2) practices and containment facilities. This includes aerosol containment measures for centrifugation, and use of a phenolic-based disinfectant validated for use against mycobacteria. Nalidixic acid is carcinogenic and HPC is neurotoxic; follow manufacturer's instructions for safe handling of these materials.

WARNING: Below are listed the major changes from previous fecal processing protocol instructions. Adherence to these and other instructions is required for successful fecal sample decontamination and *M. paratuberculosis* detection. Failure to comply with processing protocol instructions outlined in this insert will result in contamination issues and decreased *M. paratuberculosis* detection.

1. **Suspend 2.0 g of feces in 17.5 mL, not 35 mL sterile dH_2O.**
2. **Draw off 2.5 mL feces/water suspension and add to 2.5 mL 15% Bacto Yeast Extract and 0.2 mL 10% sodium pyruvate for a 90 min incubation.**
3. **Addition of 0.3 mL 5% malachite green to 25 mL 1/2X BHI-0.9% HPC.**
4. **Supplement MGIT Para TB Medium tubes with a single, 1.5 mL addition of Additive Cocktail on Day 2 of fecal processing.**
5. **Inoculate ONLY 0.1 mL processed sample per MGIT Para TB Medium tube!**
6. **Use the sodium salt of nalidixic acid, not the free acid form that requires NaOH in order to dissolve in water.**
7. **Perform positive culture tube removal, vortexing and re-entry, and follow other workflow instructions listed below. Never re-enter a culture tube into the BACTEC MGIT instrument more than once.**
8. **Stain smears for acid-fast bacilli (AFB) according to instructions below. Do not mix, invert or vortex removed BACTEC MGIT Para TB Medium tubes prior to making a smear for AFB staining.**

Fecal Sample Processing, Day 1[9-14]

1. In a BSL-2 safety cabinet, weigh out 2.0 g of feces (if feces are frozen, thaw for 30–60 min at room temperature first) and add this to 17.5 mL sterile purified water in a 50 mL disposable centrifuge tube.
2. Vortex well and allow the tube to stand at room temperature for 30 min so particulate matter settles to the bottom.
3. Aseptically transfer 2.5 mL from the top of the suspension to a fresh 50 mL tube that contains 2.5 mL of room temperature 15% **Bacto** Yeast Extract and 0.2 mL of 10% sodium pyruvate. Do not add sodium pyruvate to the **Bacto** Yeast Extract more than 2 h before the addition of fecal suspension.
4. Vortex tube briefly and incubate 90 min at 35–37°C to promote the germination of bacterial endospores.
5. For each fecal sample preparation, add 0.3 mL of sterile 5% malachite green solution to 25 mL sterile 1/2X BHI-0.9% HPC solution. Do not add malachite green to 1/2X BHI-0.9% HPC more than 2 h before the addition of fecal suspension.
6. Add the entire 5.2 mL feces/germination mix to the 25 mL 1/2X BHI-0.9% HPC/malachite green solution to produce a 30 mL decontamination suspension of 0.75% HPC and 0.05% malachite green.
7. Vortex the tube briefly and incubate overnight (18-24 h) at 35-37°C for sample decontamination.

Fecal Sample Processing, Day 2

1. Vortex the tube from Day 1 briefly and centrifuge for 30 min at 900 x *g*.
2. Quickly but gently pour off the supernatant, leaving the black-brown pellet in the bottom of the tube. *If desired, pellet can be stored at -70°C at this stage. Thaw frozen pellet at room temperature before proceeding.*
3. Add 1.0 mL of the Antibiotic Brew (*See Step 3 of Preparation of Stock Solutions*) to the pellet. To prevent foaming, resuspend the pellet by swirling the contents of the tube and/or drawing it in and out of a pipette or pipetter tip.
4. Incubate the suspension overnight (18–24 h) at 35–37°C for additional decontamination.
5. Aseptically prepare a sufficient volume of Additive Cocktail (*See Step 4 of Preparation of Stock Solutions*) and supplement **BACTEC MGIT** Para TB Medium tubes with 1.5 mL Additive Cocktail each.
6. Leave supplemented **BACTEC MGIT** Para TB Medium tubes in the BSL-2 safety cabinet overnight (18–24 h) at room temperature to allow the egg yolk to equlibrate with the culture medium.

Fecal Sample Processing, Day 3

1. Mix the concentrated specimen suspension prepared on Day 2 by swirling and inoculate 0.1 mL per **BACTEC MGIT** Para TB Medium tube.
2. Tightly recap tubes and vortex well.
3. Enter **BACTEC MGIT** Para TB Medium tubes into the **BACTEC MGIT** instrument with the barcode scanner. If the malachite green does not appear evenly dispersed, invert tubes several times. Make sure tubes are inserted all the way into the **BACTEC MGIT** stations, or the instrument will not be able to read them. Users adding their own labels to the tubes should be especially vigilant about inserting the tubes completely.
4. Inoculate solid medium if desired, also with 0.1 mL inoculum per culture tube.
5. Store the remaining inoculum at -70°C in a cryo-vial, or for temporary storage (2 months or or less), at -20°C in the disposable centrifuge tube.
6. The cultures will be automatically tested by the **BACTEC MGIT** instrument for the duration of the recommended 49-day (7 week) testing protocol. Follow the instrument workflow and AFB staining procedures listed below.

BACTEC MGIT INSTRUMENT WORKFLOW AND REPORTING OF CULTURE RESULTS

1. **BACTEC MGIT Para TB Medium tube is initially flagged as positive by the BACTEC MGIT instrument before 42 days (6 weeks) of incubation in the instrument:** remove the tube, vortex it well, and re-enter it into the **BACTEC MGIT** instrument without AFB staining or performing PCR. Use the barcode scanner to remove and re-enter tubes, and always re-enter a tube within 5 h of removal or the instrument will not associate the data from the first station with the same tube in the second station.
   a. **If the BACTEC MGIT Para TB Medium tube is not flagged positive in the second station and is declared negative at the end of protocol:** the initial positive signal was a false positive, report the sample negative for *M. paratuberculosis* detection by culture.
   b. **If the BACTEC MGIT Para TB Medium tube is flagged positive in the second station (after re-entry):** permanently remove the tube from the instrument and perform PCR and/or AFB staining.
      i. If the culture is confirmed positive for *M. paratuberculosis*, report the sample positive for *M. paratuberculosis*.
      ii. If *M. paratuberculosis* cannot be confirmed, then the positive signals were due to the growth of contaminants and the **BACTEC MGIT** Para TB Medium tube should be discarded as lost to contamination. Re-culture the sample by thawing frozen inoculum and inoculating 0.1 mL into a fresh **BACTEC MGIT** Para TB Medium tube supplemented with Additive Cocktail providing 200 µg/mL nalidixic acid (*See Step 4 of Preparation of Stock Solutions*). As usual, supplement **BACTEC MGIT** Para TB Medium tubes with Additive Cocktail the day before thawing frozen inoculum and let them sit overnight at room temperature. If frozen inoculum is unavailable, re-process the fecal sample and inoculate **BACTEC MGIT** Para TB Medium with 200 µg/mL nalidixic acid.
2. **BACTEC MGIT Para TB Medium tube is initially flagged as positive by the BACTEC MGIT instrument at 42-49 days of incubation in the instrument:** permanently remove the culture tube from the instrument, vortex it well, incubate offline in a 35–37°C incubator at least 72 h, then perform PCR and/or AFB staining.
   a. If the culture is confirmed positive for *M. paratuberculosis*, report the sample positive for *M. paratuberculosis*.

b. If *M. paratuberculosis* cannot be confirmed, the positive signal was a false positive or contaminant growth and *M. paratuberculosis* did not grow in the medium. Report the sample negative for *M. paratuberculosis* detection by culture.

3. **BACTEC MGIT Para TB Medium tube is never flagged positive in the initial station and is declared negative at the end of protocol:** report the sample negative for *M. paratuberculosis* detection by culture.

Preparing Stained Smears for Observation of Acid-Fast Bacilli (AFB)

1. **Do not mix, invert or vortex BACTEC MGIT Para TB Medium tubes when permanently removing them from the BACTEC MGIT instrument!**
2. Aseptically remove 0.1 mL of the egg yolk mass resting on the sensor at the bottom of the tube and transfer it to a microcentrifuge tube.
3. Insert a sterile cotton-tipped applicator into the microcentrifuge tube. Absorb the entire sample into the cotton tip of the applicator. Do not use rayon-tipped applicators because rayon fibers will obscure the smear.
4. Add 0.15 mL of 0.85% saline to a second microcentrifuge tube.
5. Without allowing the cotton tip to contact the saline, press the cotton-tipped applicator against the wall of the second tube to squeeze liquid out so it runs down into the saline.
6. Briefly vortex the second microcentrifuge tube to bring liquid squeezed out of the applicator down from the sides of the tube into the saline.
7. Transfer 0.05 mL of this suspension onto a glass slide and heat-fix on a slide warmer for a minimum of 2 h. The remaining 0.1 mL of suspension is available for PCR.
8. Acid-fast stain the smear and examine it microscopically for AFB.

QUALITY CONTROL

Upon receipt of a shipment or lot of **BACTEC MGIT** Para TB Medium tubes, it is suggested that a suspension of the ATCC™ control organism shown in Table 1 be prepared in Middlebrook 7H9 Broth or Saline.

1. From solid media cultures less than 15 days old, prepare a suspension in Middlebrook 7H9 Broth or Saline.
2. Adjust the suspension to a turbidity equivalent to a McFarland No. 0.5 standard.
3. Dilute the control organism suspensions following the dilution scheme outlined in Table 1.
4. Unscrew the cap and aseptically add 800 µL of **BACTEC MGIT** Para TB supplement. Inoculate the supplemented tube with 500 µL of *M. paratuberculosis* ATCC 19698 (1:50,000 dilution). Tightly recap the tube and mix well. Enter the tube into the **BACTEC MGIT** instrument.

The **BACTEC MGIT** Para TB Medium tubes should be detected as instrument positive within the time frame shown in Table 1. If the **BACTEC MGIT** Para TB Medium Quality Control tubes do not give the expected results, do not use the remaining tubes until you have contacted Technical Services at 800-638-8663 (United States only).

Table 1

Species	ATCC Number	Dilution of 0.5 McFarland Suspension in Saline	Days to Instrument Positivity
M. paratuberculosis	19698	1:50000	7-20

LIMITATIONS OF THE PROCEDURE

Recovery of mycobacteria in the **BACTEC MGIT** Para TB Medium tube is dependent on the number of organisms present in the specimen, specimen collection methods, prior treatment and the methods of processing.

BACTEC MGIT Para TB Medium tubes which are instrument-positive may contain one or more species of mycobacteria. Faster growing mycobacteria may be detected prior to slower growing mycobacteria. Although acid-fast bacilli are expected in stained smears from *M. paratuberculosis*-positive liquid cultures, AFB from all positive Para TB Medium cultures must be confirmed as *M. avium* subsp. *paratuberculosis* by PCR or mycobactin J dependency.

Due to the richness and nonselective nature of the **BACTEC MGIT** Para TB Medium broth, it is important to follow the stated decontamination procedure to reduce the possibility of contamination.

The use of HPC, malachite green, and antimicrobials, although necessary for decontamination of fecal samples, may have inhibitory effects on some mycobacteria.

PERFORMANCE CHARACTERISTICS

Twenty-five frozen fecal samples from 25 different animals were evaluated in a blind study. The study contained a mixture of 16 known positives and 9 known negatives for *M. paratuberculosis*. Specimens represented animals characterized as high (> 50 CFU/slant), moderate (10–50) and low (< 10) shedders. Samples were processed according to directions described in the Procedure section of this insert. A 0.1 mL aliquot of the concentrated specimen was added to the **BACTEC MGIT** Para TB Medium with supplements (as described in Procedure Section).

The **BACTEC MGIT** Para TB tubes were placed in the **BACTEC MGIT** 960 instrument for a 42-day protocol or until flagged positive by the **BACTEC MGIT** 960 instrument. The **BACTEC MGIT** 960 instrument recovered all of the 16 known positives. The TTD (Time-to Detection) ranged from 8–32 days, with an average TTD of 18.5 days.

The results of this study indicate that the recovery of *M. paratuberculosis* has an acceptable recovery rate using known bovine fecal specimens.

RECOMMENDATIONS, INFORMATION AND FREQUENTLY ASKED QUESTIONS

1. Controlling contamination.

a. **Fecal sample contaminants.** Bovine feces are contaminated with high levels of non-mycobacterial bacteria, primarily gram-positive bacteria, and often fungal cells as well. Bacterial endospores from the genus *Bacillus* are the most common cause of contamination from processed bovine feces. Endospores are dormant cells that are resistant to heat, drying, starvation and antimicrobials, in contrast to vegetative cells with active metabolic processes. The most common fungi that survive fecal decontamination are *Mucor* species and *Aspergillus fumigatus*.[15] Contamination is often worse in fecal samples from dairy rather than beef cattle, due to the practice of feeding silage to dairy cattle.

b. **Decontaminants.** HPC, a quaternary ammonium disinfectant, generally reduces growth recovery of bacterial endospore populations by at least 99%, and is even more effective against non-mycobacterial vegetative bacterial cells and fungal cells. Malachite green is less effective than HPC against fungi and is primarily bacteriostatic toward endospores, but rates of **BACTEC MGIT** TB Medium contamination are dramatically reduced when HPC and malachite green are employed together. Organic material in feces protects cells from the effects of decontaminants, and so decontamination is never 100% effective. HPC and malachite green are carried over into the **BACTEC MGIT** Para TB Medium in the inoculum, and along with the antimicrobials in the Additive Cocktail, suppress contaminant growth during culture incubation.

c. **Endospore germination.** Traditional fecal processing protocols do not effectively control endospore contamination from many fecal samples. For this reason an endospore germination step is included in the processing protocol. Initiation of the conversion of endospores into vegetative cells is termed germination, and occurs when endospores sense environments hospitable to growth, notably from the presence of specific nutrients (germinants). Endospores from different *Bacillus* species respond to different germinants, and bovine fecal samples contain endospores from numerous *Bacillus* species.[16] Therefore, the growth medium component yeast extract (YE) is used as a source of germinants as it contains a wide variety of organic molecules and can trigger the germination of many endospores.[17] A germination step consisting of a 90 min exposure to **Bacto** Yeast Extract on Day 1 of fecal processing protocol stimulates endospore germination into normal, vegetative *Bacillus* cells that are killed much more effectively during subsequent decontamination with HPC, malachite green, and antimicrobials. The 15% **Bacto** Yeast Extract is stored at 4°C but must warm to room temperature before use as low temperature is inhospitable to *Bacillus* growth and inhibits germination. Sodium pyruvate serves as an antioxidant that neutralizes reactive chemical compounds in feces and autoclaved growth media and thereby provides an environment more hospitable to *Bacillus* growth. In order to accommodate the added volume from the yeast extract treatment without altering the 5 mL volume added to the 25 mL of 1/2X BHI-0.9% HPC solution, feces are suspended in one-half the volume of dH_2O versus the standard processing protocol (17.5 mL instead of 35 mL).

d. **Re-culturing samples with frozen inocula.** Despite the use of an endospore germination step and malachite green, a small number of **BACTEC MGIT** Para TB Medium cultures will still be lost to contamination with very highly contaminated samples. Such samples must be re-cultured, and this is done most expediently by thawing frozen inocula and inoculating this into fresh **BACTEC MGIT** Para TB Medium tubes containing nalidixic acid at 200 µg/mL instead of the standard 18–19 µg/mL. Many *Bacillus* species are inhibited by high nalidixic acid concentrations[18], and the additional freeze-thaw itself often reduces the levels of contaminant organisms. The higher concentration of nalidixic acid may lengthen detection times for some strains of *M. paratuberculosis*, but has no effect on recovery rates. However, the additional freeze-thaw may also add several days to detection times and can reduce recovery with samples from low-shedder animals. Low-shedder detection may be better from inocula frozen at -20°C rather than -70°C. Laboratories that routinely process very highly contaminated fecal samples can supplement all **BACTEC MGIT** Para TB Medium tubes at 200 µg/mL nalidixic acid, instead of reserving this option for frozen inocula. Likewise, if samples from specific customers had contamination issues in previous years, 200 µg/mL nalidixic acid can be used in the initial cultures. Workflow with respect to supplementation of **BACTEC MGIT** Para TB Medium the day before thawing and inoculation, and re-entry of initial positives, is the same for frozen as for fresh inocula.

e. **High contamination due to improper sample handling.** Samples can arrive after improper storage or shipping, i.e., at room temperature, such that contaminant growth has occurred. Sample collectors must be informed that samples should be frozen immediately after collection and shipped frozen, or at the very least, refrigerated. If a sample arrives with fungal growth on the surface, *M. paratuberculosis* culture may be attempted by scraping the top portion of the sample off with a sterile implement and processing the interior material that has had less exposure to oxygen. If *Bacillus* overgrowth is suspected due to improper sample storage, *M. paratuberculosis* culture may be attempted by supplementing **BACTEC MGIT** Para TB Medium tubes with 200 µg/mL of nalidixic acid for the fresh inoculum instead of the standard 18–19 µg/mL, although this approach is ineffective against fungi, may add several days to *M. paratuberculosis* TTDs, and is not uniformly effective against all *Bacillus* species. Note that the higher concentration of nalidixic acid is never in the Antibiotic Brew used to resuspend pellets on Day 2 of fecal processing. If there is any possibility that fecal samples will be processed more than once, they should be weighed out as soon as thawing allows and leftover feces immediately re-frozen to preclude contaminant growth.

2. Controlling false positives.

a. **False positive definition.** False positive detections are distinct from contamination, in which growth of undesirable microorganisms is accurately detected by the system. False positives are due to an infrequent, poorly understood, oxygen-consuming interaction between egg yolk and feces that usually occurs early in the protocol. Combinations of certain fecal samples and egg yolk are more prone to this than others. Supplementation of **BACTEC MGIT** Para TB Medium tubes with Additive Cocktail on Day 2 of fecal processing, and allowing the egg yolk to equilibrate with the growth medium overnight prior to inoculation, greatly reduces the frequency of false positives.

b. **Positive tube re-entry. BACTEC MGIT** Para TB Medium tubes that flag positive before 42 days (six weeks) of incubation in the **BACTEC MGIT** should be removed, vortexed, and re-entered into the instrument using the barcode scanner. This serves two purposes: firstly, it "re-sets" incubation to circumvent occasional false positives; and secondly, the vortexing disperses clumped *M. paratuberculosis* and oxygenates the medium to invigorate growth so that the organism is much more likely to be observed after AFB staining when the culture is flagged positive the second time a few days later. A "+T" in the Tube Status column on an Unloaded Positives Report from the **BACTEC MGIT** instrument indicates a threshold positive, caused by a signal increase too rapid to be generated by *M. paratuberculosis* growth. A threshold positive suggests contamination, but is not conclusive for contaminant growth. Likewise, the absence of a "+T" for a positive **BACTEC MGIT** Para TB Medium culture tube is not conclusive for *M. paratuberculosis* growth.

3. Instrument workflow recommendations and overview.

a. It is recommended that the **BACTEC MGIT** instrument be set to a 49-day (7 week) protocol.

b. As long as a **BACTEC MGIT** 960 instrument has fewer than 640 samples at a given time, the bottom drawer may be reserved for re-entered tubes only. It may be helpful to write the initial station, such as A/J04 (drawer A, row J, station 4) on positive tubes as they are removed from the top 2 drawers.

c. A typical laboratory schedule may call for the **BACTEC MGIT** instrument to be checked for positive cultures 2 or 3 times a week (Tues/Fri or Mon/Wed/Fri), depending on sample number, PCR and/or AFB staining schedules, etc. This would include the following tasks:

i. If positive cultures are present, they should be wanded out of the instrument with the barcode scanner without mixing, inverting or vortexing. Print out an Unloaded Positives Report.

ii. **BACTEC MGIT** Para TB Medium tubes that have gone positive for the second time are ready for PCR and/or AFB staining and will not be re-entered. These are the tubes that must not be mixed, inverted or vortexed.

iii. **BACTEC MGIT** Para TB Medium tubes that have gone positive the first time, but less than 42 days have elapsed since the start of instrument protocol should be vortexed well and re-entered with the barcode scanner into the bottom drawer. Start of protocol dates are listed on Unloaded Positives Reports. If re-entry occurs within 5 h of removal, the data from the first station carries over to the second station, where the culture tube status is now ongoing instead of positive.

iv. **BACTEC MGIT** Para TB Medium tubes that have gone positive the first time, but 42 or more days have elapsed since entry into the instrument, should be incubated at least 72 h offline in a 35–37°C incubator and never re-entered. Re-entry within a week of the end of protocol is not done because the slower growing among *M. paratuberculosis* strains may not re-detect before the end of the 49-day protocol and the instrument would declare these positive cultures as negative for growth. Vortexing with offline incubation will still result in the desired burst of growth. **BACTEC MGIT** Para TB Medium tubes can incubate beyond 72 h offline in order to batch them if it is convenient to perform PCR or AFB staining on groups of cultures.

v. **BACTEC MGIT** Para TB Medium tubes that have incubated for 49 days total in the initial stations without ever going positive are negative for *M. paratuberculosis* detection by culture. This is also true for tubes that were re-entered after an initial positive but never went positive in the second station, as the initial detection was a false positive. PCR and/or AFB staining from **BACTEC MGIT** Para TB Medium tubes that are declared negative for growth by the **BACTEC MGIT** instrument at the end of protocol is not required and is at the discretion of the user.

vi. If detection times are of interest to the user, the time-to-detection (TTD) for a **BACTEC MGIT** Para TB Medium tube is that obtained from the initial station, prior to a re-entry, after the culture has later been confirmed as positive for *M. paratuberculosis*. Detection in the second station, after re-entry, is a confirmation of growth. The second station TTD will depend on how soon after the initial detection a tube is re-entered.

4. Workflow for large numbers of samples.

a. **Sample weighing.** Thawed feces can be weighed out directly into a **Falcon** tube containing 17.5 mL sterile purified water if the tube is in a tube holder on the balance in the BSL-2 safety cabinet.

b. **Reagent preparation.** The 1/2X BHI-0.9% HPC solution can be distributed in 25 mL aliquots into 50 mL **Falcon** tubes days or weeks ahead of fecal processing and stored at room temperature. Likewise, Antibiotic Brew, and Additive Cocktail *without egg yolk*, can be prepared in advance and stored at -20°C for up to 6 weeks or -70°C for up to 6 months. Frozen solutions should be mixed after thawing because amphotericin B tends to settle out of solution.

c. **Reagent distribution.** A 10 mL pipette holds enough Additive Cocktail to supplement 8 **BACTEC MGIT** Para TB tubes at a time, but a repeat pipetter with a 50 mL tip can do 33–34 tubes at a time. Distribution of 2.5 mL 15% **Bacto** Yeast Extract, and addition of 0.2 mL 10% sodium pyruvate to the yeast extract within 2 h of use, can be quickly carried out with a repeat pipetter. Antibiotic Brew can likewise quickly be added to numerous pellets as long as care is taken to keep the repeat pipetter tip well above the centrifuge tubes to insure against cross-contamination.

d. **Fecal processing in 15 mL tubes.** Process feces in 15 mL rather than 50 mL **Falcon** tubes by reducing sample and reagent volumes to 40% of those normally used. Add 0.8 g of feces to 7 mL dH_2O, after settling 30 min transfer 1.0 mL from the top to a fresh 15 mL tube containing 1.0 mL sterile, room temperature 15% **Bacto** Yeast Extract and 80 µL sterile 10% sodium pyruvate. After this has incubated for 90 min at 35–37ºC, add it to 10 mL 1/2X BHI-0.9% HPC to which 120 µL of sterile 5% malachite green has been added, and incubate overnight at 35–37°C. Centrifuge on Day 2 as usual and resuspend the pellet in 0.4 mL of Antibiotic Brew, then finish processing. However, inoculate 0.1 mL per **BACTEC MGIT** Para TB Medium tube as usual and freeze excess inocula. Fecal processing in 15 mL tubes may allow more tubes per centrifugation on Day 2 of the processing protocol, and processing samples of less than 2 g of feces. However, fecal processing in 15 mL tubes only yields 0.5–0.6 mL inoculum per sample, not enough to inoculate a **BACTEC MGIT** Para TB medium tube, 4 or 5 tubes of solid medium, and freeze inoculum in case of culture loss to contamination.

Frequently Asked Questions

a. Is it acceptable to use materials that have only slight irregularities, if they are used right away?

Answer: Absolutely not. Turbidity, clumping or odors can indicate contamination of materials like egg yolk enrichment, **BACTEC MGIT** Para TB Supplement, or even antimicrobial solutions. Also, if nalidixic acid sodium salt will not completely dissolve in water at 2.5% prior to filtration, it has gone bad and should be discarded or it will interfere with *M. paratuberculosis* growth. Similarly, **BACTEC MGIT** Para TB Medium tubes with irregularities should not be used. When an irregular material is discovered, the manufacturer should be alerted.

b. Why can't sodium pyruvate be added to **Bacto** Yeast Extract, or malachite green added to 1/2X BHI-0.9% HPC, more than 2 h before use?

Answer: The antioxidant properties of the pyruvate will dissipate over time and not be available to neutralize reactive chemical compounds present in feces, and the HPC will bleach the malachite green.

c. Can changing the formulation of the Antibiotic Brew or Additive Cocktail improve **BACTEC MGIT** Para TB System performance in some situations?

Answer: No, these formulations are optimized and the most likely outcomes from implementing changes are either overwhelming contaminant growth or a reduced likelihood of *M. paratuberculosis* detection.

d. What has gone wrong when sticky, difficult to resuspend pellets are obtained after centrifugation on Day 2 of fecal processing?

Answer: Nothing has gone wrong, some fecal samples yield very sticky pellets. Excessive vortexing is usually not helpful, as HPC carried over from Day 1 of fecal processing converts much of the Antibiotic Brew to foam. The best approach is to break up the pellet with the narrow handle of a sterile, disposable inoculating loop. Smear the sticky pellet material along the bottom of the **Falcon** tube and wash it off with the Antibiotic Brew and a pipetter with a 1.0 mL tip, until most of the pellet is resuspended. Some of the sticky pellet material may never resuspend completely; do not inoculate with these remaining clumps. Some fecal processing protocols specify centrifugation at 2000 × *g* for 20 min. Centrifugation at 900 × *g* for 30 min, as specified for fecal processing with the **BACTEC MGIT** Para TB System, will yield pellets that resuspend with less time and effort, whether the pellets are unusually sticky or not.

e. On Day 3 of fecal processing, resuspended pellets appear as a thin fluid above particles that have settled out during the overnight incubation, is this a problem?

Answer: No, but the *M. paratuberculosis* cells will have settled during the night with the particulate material, so swirl the suspensions before inoculating **BACTEC MGIT** Para TB Medium tubes. Likewise, if malachite green is not evenly dispersed in **BACTEC MGIT** Para TB Medium tubes after inoculation, invert these tubes several times as they are entered into the **BACTEC MGIT** instrument to insure proper mixing.

f. Will inoculating **MGIT** Para TB Medium tubes with more than 0.1 mL of inoculum lead to more rapid detection of *M. paratuberculosis* growth?

Answer: No, an excess of the decontaminants carried over into the inoculum inhibit the growth of *M. paratuberculosis* and may cause instrument errors such that the **BACTEC MGIT** Para TB Medium tube will have to be discarded and the sample re-cultured with the recommended 0.1 mL inoculum per tube. This is also true for solid medium, which should only be inoculated with 0.1 mL per culture tube as well.

g. Freezing excess inoculum sounds inconvenient and freezer space is in short supply, so how important is this step?

Answer: Use of frozen inocula is the fastest way to re-culture a sample in the event that a **BACTEC MGIT** Para TB Medium culture tube is lost to contamination. Excess inocula can be frozen in 1.0 mL cryo-vials to conserve space. Or, to save time, tightly cap the disposable centrifuge tubes from which inoculations are made on Day 3 of fecal processing, label the tubes and set them in a -20°C freezer. At the end of the 49-day protocol, they can all just be discarded unless any are needed for the rare samples lost to contamination. **Note:** the 50 mL and 15 mL **Falcon** tubes are polypropylene, and may crack if stored at -70°C, so cryo-vials are recommended for longer-term, -70°C storage of excess inocula and antimicrobial solutions.

h. Can **BACTEC MGIT** Para TB Medium tubes determined to be lost to contamination somehow be treated or re-processed and *M. paratuberculosis* detected if the tubes are then re-entered into the **BACTEC MGIT** instrument?

Answer: No. Uncontrolled contaminant growth that is detected by the **BACTEC MGIT** instrument quickly saturates the growth medium. Such high microbial populations require powerful decontamination treatments that would also kill *M. paratuberculosis* cells that may be present. Discard the occasional **BACTEC MGIT** Para TB Medium tube lost to contamination and re-culture frozen inoculum into a fresh **BACTEC MGIT** Para TB Medium tube supplemented with the 200 µg/mL nalidixic acid Additive Cocktail formulation.

i. If a **BACTEC MGIT** Para TB Medium tube is lost to contamination, can the contaminant organism be identified by plating out the culture medium?

Answer: Usually not. Culture medium always contains ungerminated endospores and the predominant species growing on antimicrobial-free plated medium may not be the species that detected in the culture tube. Simply plating out medium from a contaminated culture is usually not informative unless a large number of non-*Bacillus* bacteria are recovered. Liquid culture overgrowth by gram-positive cocci may result from allowing feces to sit at room temperature after collection by the customer, or after thawing for processing in the laboratory. Fungi tend not to produce conidia (asexual spores) in liquid culture but grow as compact, filamentous clumps that are unlikely to be picked up by sampling. Cultures overgrown with *Aspergillus fumigatus*, however, can often be identified by the blue-grey color of the growth medium. To isolate the fungus may require plating out the entire liquid culture on selective medium (see below). To assess fecal contamination levels, dilute and plate out the feces/dH_2O suspension on Day 1 of fecal processing. Plate dilutions on **BBL** Chocolate II Agar for optimum recovery of non-mycobacterial bacteria (cat. no. 221169 or 221267 for 20 or 100 plates, respectively), and **BBL** BHIA with 10% Sheep Blood, Gentamicin and Chloramphenicol for selective recovery of fungi (cat. no. 221841 for 20 plates). Incubate plates at 35–37°C and count colonies periodically for several weeks (some endospores and fungi take a long time to produce colonies). Seal the edges of plates with stretchable film to prevent the medium from drying out or fungal spores from contaminating the incubator. Colony counts from plating diluted feces/dH_2O suspensions are better predictors of contamination problems than colony counts from plating dilutions of inocula on Day 3 of fecal processing. Be aware that repeated freezing and thawing of fecal samples reduces contaminant populations.

j. Is it unusual for a greater percentage of fecal samples to test positive for *M. paratuberculosis* after a lab switches from testing with solid medium to the **BACTEC MGIT** Para TB liquid culture system?

Answer: No, liquid culture is more sensitive than culture with solid medium, so *M. paratuberculosis* is detected from samples from very low shedding animals that are missed by solid culture. It is not unusual to obtain positive liquid cultures with inocula that yield no colonies on solid medium. Acid-fast bacilli should be present in all *M. paratuberculosis*-positive liquid culture tubes, but these organisms must be confirmed as *M. partuberculosis* by PCR or mycobactin J dependency testing because culture media that support the growth of *M. paratuberculosis* will also support the growth of other mycobacteria.

k. Why can't **BACTEC MGIT** Para TB Medium tubes be mixed, inverted or vortexed prior to sampling them for AFB staining, and why is the cotton-tipped applicator wrung out against the dry, inside wall of a microfuge tube?

Answer: *M. paratuberculosis* cells grow preferentially in the egg yolk mass at the bottom of the tube; keeping this mass intact concentrates the cells and improves the likelihood of detection after staining. Egg yolk remains in the cotton swab and *M. paratuberculosis* exits the swab when the swab is wrung out against the inside wall of a microfuge tube, so the resulting smear contains less egg yolk and is easier to read.

AVAILABILITY

Cat. No.	Description
245154	**BACTEC™ MGIT™** Para TB Medium Tube, Pkg. of 100 Tubes
245156	**BACTEC™ MGIT™** Para TB Supplement, Pkg. of 6 x 15 mL
445870	**BACTEC™ MGIT™** Instrument System
440979	**BACTEC™ MGIT™** Para TB Starter Kit (includes **BACTEC™ MGIT™** Para TB User's Manual Addendum and **BACTEC™ MGIT™** Para TB Application Software)
211059	**BBL™** Brain Heart Infusion, 500 g
221060	**BBL™** Brain Heart Infusion, 5 lb.
212750	**Bacto™** Yeast Extract, 500 g
233471	**BBL™** Egg Yolk Enrichment 50%, 12 x 10 mL
233472	**BBL™** Egg Yolk Enrichment 50%, 6 x 100 mL
212315	**BBL™** 2-Step AFB Stain Kit
231391	**BBL™** AFB Slide

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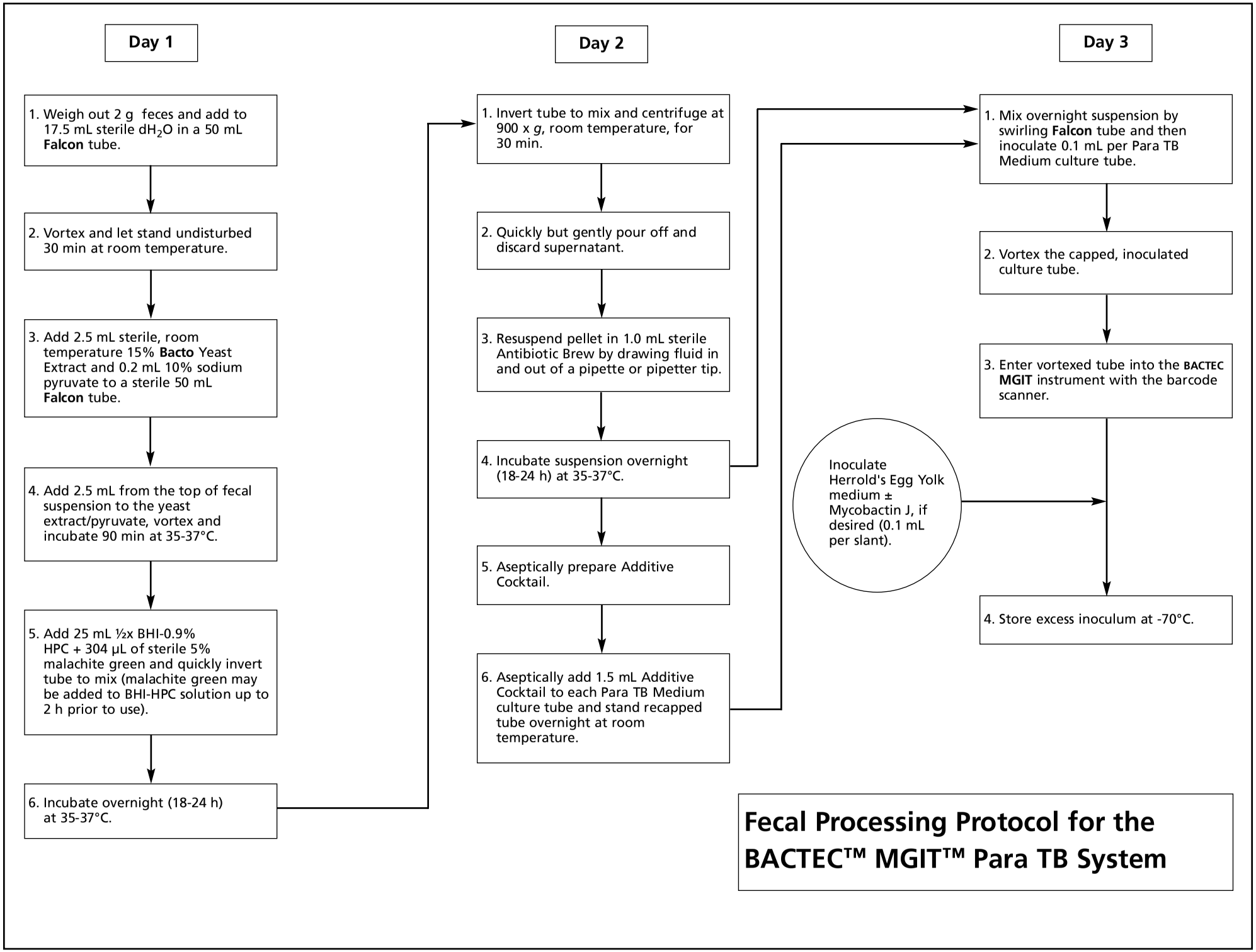
Day 1
1. Weigh out 2 g feces and add to 17.5 mL sterile dH2O in a 50 mL Falcon tube.
2. Vortex and let stand undisturbed 30 min at room temperature.
3. Add 2.5 mL sterile, room temperature 15% Bacto Yeast Extract and 0.2 mL 10% sodium pyruvate to a sterile 50 mL Falcon tube.
4. Add 2.5 mL from the top of fecal suspension to the yeast extract/pyruvate, vortex and incubate 90 min at 35-37°C.
5. Add 25 mL ½x BHI-0.9% HPC + 304 µL of sterile 5% malachite green and quickly invert tube to mix (malachite green may be added to BHI-HPC solution up to 2 h prior to use).
6. Incubate overnight (18-24 h) at 35-37°C.
Day 2
1. Invert tube to mix and centrifuge at 900 x g, room temperature, for 30 min.
2. Quickly but gently pour off and discard supernatant.
3. Resuspend pellet in 1.0 mL sterile Antibiotic Brew by drawing fluid in and out of a pipette or pipetter tip.
4. Incubate suspension overnight (18-24 h) at 35-37°C.
5. Aseptically prepare Additive Cocktail.
6. Aseptically add 1.5 mL Additive Cocktail to each Para TB Medium culture tube and stand recapped tube overnight at room temperature.
Day 3
1. Mix overnight suspension by swirling Falcon tube and then inoculate 0.1 mL per Para TB Medium culture tube.
2. Vortex the capped, inoculated culture tube.
3. Enter vortexed tube into the BACTEC MGIT instrument with the barcode scanner.
Inoculate Herrold's Egg Yolk medium ± Mycobactin J, if desired (0.1 mL per slant).
4. Store excess inoculum at -70°C.
Fecal Processing Protocol for the BACTEC™ MGIT™ Para TB System

Instrument Workflow for the BACTEC™ MGIT™ Para TB System