BBL ™ MGIT™

Mycobacteria Growth Indicator Tube, OADC Enrichment, PANTA™ Antibiotic Mixture

I. INTENDED USE

The **BBL™ MGIT™** Mycobacteria Growth Indicator Tube supplemented with **BBL™ MGIT™** OADC enrichment and **BBL™ MGIT™ PANTA™** antibiotic mixture, when appropriate, is intended for the detection and recovery of mycobacteria. Acceptable specimen types are digested and decontaminated clinical specimens (except urine) and sterile body fluids (except blood).

II. SUMMARY AND EXPLANATION

From 1985 to 1992, the number of MTB cases reported increased 18%. Tuberculosis still kills an estimated 3 million persons a year worldwide, making it the leading infectious disease cause of death.[1] Between 1981 and 1987, AIDS case surveillances indicated that 5.5% of the patients with AIDS had disseminated nontuberculous mycobacterial infections, e.g., MAC. By 1990, the increased cases of disseminated nontuberculous mycobacterial infections had resulted in a cumulative incidence of 7.6%.[2] In addition to the resurgence of MTB, multidrug-resistant MTB (MDR-TB) has become an increasing concern. Laboratory delays in the growth, identification, and reporting of these MDR-TB cases contributed at least in part to the spread of the disease.[3]

The U.S. Centers for Disease Control and Prevention (CDC) have recommended that every effort must be made by laboratories to use the most rapid methods available for diagnostic mycobacteria testing. These recommendations include the use of both a liquid and a solid medium for mycobacterial culture.[3]

The **MGIT** Mycobacteria Growth Indicator Tube contains 4 ml of modified Middlebrook 7H9 Broth Base.[4, 5] The complete medium, with 0.5 ml OADC enrichment and 0.1 ml of **PANTA** antibiotic mixture, is one of the most commonly used liquid media for the cultivation of mycobacteria.

All types of clinical specimens, pulmonary as well as extra-pulmonary (except blood and urine), can be processed for primary isolation in the **MGIT** tube using conventional methods.[6] The processed specimen is inoculated into a **MGIT** tube, incubated, and read daily from the second day of incubation using a longwave UV light. At the time of tube positivity, there are approximately 10^4 to 10^7 CFU/ml of mycobacteria present.

III. PRINCIPLES OF THE PROCEDURE

A fluorescent compound is embedded in silicone on the bottom of 16 x 100 mm round-bottom tubes. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. Initially, the large amount of dissolved oxygen quenches emissions from the compound and little fluorescence can be detected. Later, actively respiring microorganisms consume the oxygen and allow the fluorescence to be observed using a 365 nm UV transilluminator or longwave UV light (Wood's lamp). Growth can also be detected by the presence of a non-homogeneous turbidity or small grains or flakes in the culture medium.

The medium components are substances essential for the rapid growth of mycobacteria. Oleic acid is utilized by tubercle bacilli and plays an important role in the metabolism of mycobacteria. Albumin acts as a protective agent by binding free fatty acids, which may be toxic to *Mycobacterium* species, thereby enhancing their recovery. Dextrose is an energy source. Catalase destroys toxic peroxides that may be present in the medium.

Contamination may be reduced by supplementing the combined **BBL MGIT** base and **BBL MGIT** OADC enrichment with the **BBL MGIT PANTA** antibiotic mixture prior to inoculation with a clinical specimen.

IV. REAGENTS

The **BBL MGIT** Mycobacteria Growth Indicator Tube contains: 110 µl of fluorescent indicator and 4 ml of broth. The indicator contains Tris 4, 7 - diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate in a silicone rubber base. The tubes are flushed with 10% CO_2 and capped with polypropylene caps.

Approximate Formula* Per L Purified Water

Modified Middlebrook 7H9 Broth base .... 5.9 g
Casein peptone .... 1.25 g

BBL MGIT OADC contains 15 ml Middlebrook OADC enrichment.

Approximate Formula* Per L Purified Water

Bovine albumin .... 50.0 g
Dextrose .... 20.0 g
Catalase .... 0.03 g
Oleic acid .... 0.6 g

The **BBL MGIT PANTA** vial contains a lyophilized mixture of antimicrobial agents.

Approximate Formula* Per Vial Lyophilized **PANTA**

Polymyxin B .... 6,000 units
Amphotericin B .... 600 µg
Nalidixic acid .... 2,400 µg
Trimethoprim ... 600 µg
Azlocillin .... 600 µg

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

Directions for Use: Reconstitute a lyophilized vial of **BBL MGIT PANTA** antibiotic mixture with 3 ml of sterile distilled or deionized water.

Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus may be present in specimens. "Universal Precautions"[6,7] should be followed in handling all items contaminated with blood or other body fluids.

Precautions: For *in vitro* Diagnostic Use.

Working with *Mycobacterium tuberculosis* grown in culture requires Biosafety Level 3 practices, containment equipment and facilities.[6]

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

Dropped tubes should be examined carefully. If damage is seen, the tube should be discarded.

Wear UV protective glasses when observing fluorescence and use only longwave illumination (365 nm). DO NOT USE SHORTWAVE UV LIGHT FOR READING TUBES.

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

Storage of Reagents: BBL MGIT Mycobacteria Growth Indicator Tubes - On receipt, store at 2 to 25°C (35 to 77°F). DO NOT FREEZE. Minimize exposure to light. Broth should appear clear and colorless. Do not use if turbid. **MGIT** tubes stored as labeled prior to use may be inoculated up to the expiration date and incubated for up to eight weeks.

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

BBL MGIT PANTA Antibiotic Mixture - On receipt, store lyophilized vials at 2 to 8°C. Once reconstituted, the **PANTA** mixture may be used within 72 h, provided it is stored at 2 to 8°C, or up to 6 months if stored at -20°C or colder. Once thawed, the **PANTA** mixture must be used immediately. Discard unused portion.

V. SPECIMEN COLLECTION AND HANDLING

All specimens should be collected and transported as recommended by the CDC, the *Clinical Microbiology Procedures Handbook* or your laboratory procedure manual.[6, 8]

VI. DIGESTION, DECONTAMINATION AND CONCENTRATION

Specimens from different body sites should be processed for inoculation of **MGIT** tubes as follows:

SPUTUM: Specimens should be processed using the NALC-NaOH method as recommended by the CDC's *Public Health Mycobacteriology: A Guide for the Level III Laboratory.*[6] Alternatively, use the **BBL™ MycoPrep™** kit for processing mycobacterial specimens (see "Availability").

GASTRIC ASPIRATES: Specimens should be decontaminated as for sputum. If the volume of the specimen is more than 10 ml, concentrate by centrifugation. Resuspend the sediment in about 5 ml of sterile water and then decontaminate. Add a small amount of NALC powder (50 to 100 mg) if the specimen is thick or mucoid. After decontamination, concentrate again prior to inoculation into **MGIT** tube.

BODY FLUIDS (CSF, synovial fluid, pleural fluid, etc.): Specimens which are collected aseptically and are expected to have no other bacteria can be inoculated without decontamination. If the specimen volume is more than 10 ml, concentrate by centrifugation at 3,000 x *g* for 15 min. Pour off supernatant fluid. Inoculate **MGIT** tube with sediment. Specimens that are expected to contain other bacteria must be decontaminated.

TISSUE: Tissue specimens should be processed as recommended by the CDC's *Public Health Mycobacteriology: A Guide for the Level III Laboratory*.[6]

STOOL: Suspend 1 g of feces in 5 ml of Middlebrook Broth. Agitate the suspension on a vortex mixer for 5 sec. Proceed to the NALC-NaOH procedure as recommended by the CDC's *Public Health Mycobacteriology: A Guide for the Level III Laboratory.*[6]

VII. PROCEDURE

Materials Provided: BBL™ MGIT™ Mycobacteria Growth Indicator Tubes, 4 ml, package of 25 and 100 Tubes, or **BBL™ MGIT™** OADC, 6 vials, 15 ml, or **BBL™ MGIT™ PANTA™** antibiotic mixture, 6 lyophilized vials (see "Availability").

Materials Not Provided: Falcon™ brand 50 ml centrifuge tubes, 4% sodium hydroxide, 2.9% sodium citrate solution, N-acetyl-L-cysteine powder, phosphate buffer pH 6.8, vortex mixer, 37°C incubator, 1 ml sterile pipettes, sterile transfer pipettes, UV transilluminator (365 nm) or Wood's lamp with longwave bulb or blacklight, 0.4% sodium sulfite solution (procedure below), **BBL™** Middlebrook and Cohn 7H10 Agar, **BBL™ MycoPrep™**, **BBL™** Middlebrook 7H9 Broth (see "Availability") or other mycobacterial agar or egg-based medium, tissue homogenizer or sterile swab, **BBL™** Normal Saline (see "Availability"), ATCC® strains #27294, 12478, 6841, microscope and materials for staining slides, pipettes 100 µl and 500 µl, corresponding pipette tips, 5% sheep blood agar plate, Eye Guard Spectacles (UVP #UVC-303, San Gabriel, CA) and tuberculocidal disinfectant.

Inoculation of MGIT Tubes:

1. Label the **MGIT** tube with specimen number.
2. Unscrew the cap and aseptically add 0.5 ml of **MGIT** OADC.
3. Aseptically add 0.1 ml of reconstituted **MGIT PANTA** antibiotic mixture. For best results, the addition of OADC enrichment and **PANTA** antibiotic mixture should be made just prior to specimen inoculation.
4. Add 0.5 ml of the concentrated specimen suspension prepared above. Also add a drop (0.1ml) of specimen to a 7H10 agar plate or other mycobacterial solid agar or egg-based medium. *NOTE: Specimen volumes greater than 0.5 ml can increase contamination or otherwise adversely affect the performance of the tubes.*
5. Tightly recap the tube and mix well.
6. Tubes should be incubated at 37°C.
   - For specimens in which mycobacteria with different incubation requirements are suspected, a duplicate **MGIT** tube can be set up and incubated at the appropriate temperature; e.g. 30°C or 42°C. Inoculate and incubate at the required temperature.
   - For specimens suspected of containing *Mycobacterium haemophilum*, a source of hemin must be introduced into the tube at the time of inoculation and the tube incubated at 30°C. Aseptically place one strip of **BBL™ Taxo™ X** Factor Strip into each **MGIT** tube requiring the addition of hemin prior to inoculation of specimen (see "Availability").
7. Read tubes daily starting on the second day of incubation following the procedure "Reading the Tubes" below.

Preparation of Interpretive Negative and Positive Control Tubes: Use of the Positive and Negative Control tubes is only for the interpretation of fluorescence and is not intended as a control for the performance of the media.

Positive Control Tube:

1. Empty broth from an uninoculated **MGIT** tube.
2. Label tube as a Positive Control and record the date.
3. Prepare 0.4% sodium sulfite solution (0.4 g in 100 ml sterile distilled or deionized water). Discard unused portion.
4. Add 5 ml of sodium sulfite solution to the tube, replace the cap, tighten and allow the tube to stand for a minimum of 1 h at room temperature before use.
5. Positive Control tubes can be used many times. Each Positive Control tube can be used for up to four weeks when stored at room temperature.

Negative Control Tube: An unopened, uninoculated **MGIT** tube is used as a control.

Reading the Tubes:

1. A Positive Control and a Negative Control are important for correctly interpreting results.
2. Remove tubes from the incubator. Place tubes on the UV light next to a Positive Control tube and an uninoculated tube (Negative Control). It is recommended that one rack at a time of tubes (4 by 10 tubes) be placed on the UV light. *NOTE: Wear UV protective glasses when observing fluorescence. Normal room light is preferred. Avoid reading tubes in a sunlit room or in a darkened room.*
3. Visually locate **MGIT** tubes that show bright fluorescence. Fluorescence is detected as a bright orange color in the bottom of the tube and also an orange reflection on the meniscus. The **MGIT** tube should then be taken out of the rack and compared to Positive Control and Negative Control tubes. The Positive Control should show a high amount of fluorescence (very bright orange color). The Negative Control should have very little or no fluorescence. If fluorescence in the **MGIT** tube looks more like the Positive Control, it is a positive tube. If it looks more like the Negative Control, it is a negative tube. Growth can also be detected by the presence of a non-homogeneous turbidity, small grains or flakes in the culture medium.
4. Positive tubes should be stained for acid-fast bacilli. Smear-negative tubes should be checked for bacterial contamination. Subcultures for identification and drug susceptibility testing may be performed using fluid from the **BBL MGIT** tube.
5. Negative tubes should continue to be read daily for eight weeks or longer depending on the type of specimen and the past experience of the laboratory. Alternative reading schedules may be established. Failure to read the tubes for several days, such as during weekends or holidays, may delay the detection of positive tubes, but will not otherwise adversely affect the performance of the media. Tubes should be visually checked for the presence of turbidity and small grains or granules before discarding. Negative **MGIT** tubes cannot be reused. If mycobacterial growth is suspected, follow the "Processing a Positive **MGIT** Tube" procedure as stated below.

Reprocessing Contaminated MGIT Tubes: Contaminated **MGIT** tubes may be re-decontaminated and re-concentrated using the same procedure used to process the specimen initially.

1. Add the contents of the contaminated **MGIT** tube to a 50 ml plastic centrifuge tube.
2. Add 5 ml NALC-NaOH solution to the centrifuge tube. With the cap tightened, vortex the tube for 5 to 20 sec.
3. Allow the tube to stand for 15 to 20 min. Do not treat for more than 20 min.
4. Add 35 ml sterile phosphate buffer pH 6.8. Replace the cap and mix the contents.
5. Concentrate the specimen in a centrifuge at a speed of 3,000 x *g* for 15 min.
6. Carefully decant the supernatant fluid from the pellet. Resuspend the pellet using a sterile Pasteur pipette with phosphate buffer pH 6.8.
7. Inoculate 0.5 ml of the suspension to a new **MGIT** tube.

User Quality Control: Upon receipt of a new shipment or lot number of **MGIT** tubes, it is suggested that suspensions of the ATCC control organisms be prepared in Middlebrook 7H9 Broth.

1. From solid media cultures less than 15 days old, prepare a suspension in Middlebrook 7H9 Broth.
2. Allow the suspension to sit for 20 min.
3. Transfer the supernatant to an empty, sterile tube and allow to sit for an additional 15 min.
4. Transfer the supernatant to another empty, sterile tube.
5. Adjust the suspension to a turbidity comparable to a 0.5 McFarland standard.
6. Dilute the control organism suspensions following the dilution scheme outlined in Table 1.
7. Inoculate the **MGIT** tubes following the "Inoculation of **MGIT** Tubes" procedure.

The **MGIT** tubes should show fluorescence within the time frame shown in Table 1.

Table 1

Species	ATCC® Numbers	Dilution of 0.5 McFarland in Saline	Days to Positive
M. tuberculosis	27294	1:50	6 - 10
M. kansasii	12478	1:5000	7 - 11
M. fortuitum	6841	1:5000	2 - 3

At the time of tube positivity, there are approximately 10^4 to 10^7 CFU/ml of mycobacteria present. If the QC **MGIT** 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).

VIII. RESULTS

A culture-positive sample is identified by the observation of fluorescence or non-homogenous turbidity, small grains or flakes in an inoculated **MGIT** tube. Positive tubes should be subcultured and an acid-fast smear prepared. A positive acid-fast smear result indicates the presumptive presence of viable microorganisms in the tube.

Processing a Positive MGIT Tube:
NOTE: All steps should be performed in a biological safety cabinet.

1. Remove **MGIT** tube from test rack.
2. Using a sterile transfer pipet, remove an aliquot from the bottom of the tube (approx. 0.1 ml) for stain preparations (AFB and Gram stains).
3. Inspect smear and preparations. Report preliminary results only after acid-fast stain evaluation.

If AFB positive, subculture to solid media and report as: Growth positive, AFB smear positive, ID pending.

If microorganisms other than AFB are present, report as: Growth positive, AFB smear negative, Contaminated.

If no microorganisms are present, no reportable result. Subculture broth to blood agar plate and mycobacterial culture medium; repeat smear using the addition of protein to ensure the inoculum has been adequately fixed to the slide.

IX. LIMITATIONS OF THE PROCEDURE

Recovery of mycobacteria in the **MGIT** tube is dependent on the number of organisms present in the specimen, specimen collection methods, patient factors such as presence of symptoms, prior treatment and the method of processing.

Decontamination with the N-acetyl-L-cysteine Sodium hydroxide (NALC-NaOH) or Oxalic acid methods is recommended. Other decontamination methods have not been tested in conjunction with the **BBL™ MGIT™** medium. Digestant-decontaminant solutions may have harmful effects on mycobacteria.

Colony morphology and pigmentation can only be determined on solid media. Mycobacteria may vary in acid-fastness depending on strain, age of culture and other variables. The consistency of microscopic morphology in **BBL™ MGIT™** medium has not been established.

An AFB smear-positive **MGIT** tube can be subcultured, to both selective and nonselective mycobacterial media, for isolation to perform identification and susceptibility testing.

MGIT tubes which appear positive may contain other non-mycobacterial species. Non-mycobacterial species may overgrow mycobacteria present. Such **MGIT** tubes should be re-decontaminated and re-cultured.

MGIT tubes which appear positive may contain one or more species of mycobacteria. Faster growing mycobacteria may develop positive fluorescence prior to slower growing mycobacteria; therefore, it is important to subculture positive **MGIT** tubes to ensure proper identification of all mycobacteria present in the sample.

Specimen volumes greater than 0.5 ml can increase contamination or otherwise adversely affect the performance of the **MGIT** tubes.

Due to the richness of the **MGIT** broth and to the non-selective nature of the **MGIT** indicator, it is important to follow the stated digestion/decontamination procedure to reduce the possibility of contamination. Adherence to procedural instructions is critical for optimum recovery of mycobacteria.

The use of **PANTA** antibiotic mixture, although necessary for all non-sterile specimens, may have inhibitory effects on some mycobacteria.

Terminal subcultures were not routinely performed during clinical studies. Therefore, an actual false negative rate (defined as a **MGIT** tube that remained negative throughout the eight-week incubation period, was subcultured and grew a mycobacterial organism) cannot be determined at this time.

Seeded culture studies were performed with twenty-three species (ATCC and wild strains) of mycobacteria using inoculum levels ranging from 10^3 and 10^5 CFU/ml. The following species were detected as positive in the **MGIT** tube:

M. africanum	*M. intracellulare*	*M. smegmatis*
M. avium Complex*	*M. kansasii**	*M. szulgai*
*M. chelonae**	*M. malmoense*	*M. terrae*
*M. flavescens**	*M. marinum*	*M. triviale*
*M. fortuitum**	*M. nonchromogenicum*	*M. tuberculosis**
M. gastri	*M. phlei*	*M. vaccae*
*M. gordonae**	*M. scrofulaceum*	*M. xenopi**
M. haemophilum	*M. simiae**	

*Species recovered during clinical evaluation of the **MGIT** tube.

Clinical studies have demonstrated recovery of mycobacteria from respiratory specimens, gastric aspirates, tissue, stool and sterile body fluids except blood; recovery of mycobacteria from other body fluids has not been established for this product.

X. PERFORMANCE CHARACTERISTICS

The **BBL MGIT** Mycobacteria Growth Indicator Tube was evaluated at six clinical sites, which included public health laboratories as well as large acute care hospitals in geographicallydiverse areas. The site population included patients infected with HIV, immunocompromised patients and transplant patients. The **BBL MGIT** tubes were compared to the **BACTEC™** 460TB radiometric system, the **BBL™ SEPTI-**

CHEK™ AFB Mycobacteria Culture System and conventional solid growth media for the detection and recovery of mycobacteria from clinical specimens (except blood and urine). A total of 2801 specimens were tested during the study. The distribution of specimens tested by source was: respiratory (78%), gastric (0.4%), body fluid (9.8%), tissue (7.0%), stool (2.5%) and other (2.4%). A total of 318 specimens were positive which represented 330 isolates recovered during the study. Of these 330 isolates, 253 (77%) were recovered by the **BBL MGIT** tubes, 260 (79%) were recovered by the **BACTEC** 460TB and the **BBL SEPTI-CHEK** AFB and 219 (66%) were recovered by conventional solid media. The **BBL MGIT** tubes demonstrated a 0.5% false positive rate (**MGIT** fluorescent, no AFB present). The **BBL MGIT** tubes failed to recover 3.7% of the isolates which were recovered in one or more of the reference systems (**BACTEC** 460TB, **BBL SEPTI-CHEK** AFB or conventional solid media). While this percentage represents a potential loss of recovery, it is not indicative of an actual false negative determination (refer to "Limitations of the Procedure" section). Use of a second medium, as recommended, will increase the probability of recovery of mycobacterial organisms. The average breakthrough contamination rate for the **BBL MGIT** tubes was 9.7%.

BACTEC SITES

Table 2-Detection of Mycobacteria Positive Isolates in Clinical Evaluations

Isolate	Total Isolates	Total MGIT	MGIT Only	Total BACTEC	BACTEC Only	Total CONV	CONV Only
MTB	113	91	2	98	7	92	6
MAC	99	76	9	86	13	57	3
M. kansasii	5	2	0	5	1	4	0
M. fortuitum	9	5	3	3	1	5	3
M. chelonae	2	0	0	2	1	1	0
M. xenopi	2	0	0	2	2	0	0
M. simiae	1	1	0	1	0	0	0
M. gordonae	11	4	1	4	1	9	5
M. flavescens	2	1	0	2	1	0	0
All MYCO	244*	180* 15*		203	27	168	17

***NOTE:** Fourteen **MGIT** ONLY isolates are not included in these data. Presumptive identification was performed with no final confirmation of ID.

SEPTI-CHEK SITES

Table 3-Detection of Mycobacteria Positive Isolates in Clinical Evaluations

Isolate	Total Isolates	Total MGIT	MGIT Only	Total SEPTI – CHEK	SEPTI-CHEK Only	Total CONV	CONV Only
MTB	30	25	1	29	2	26	0
MAC	34	26	5	28	2	25	0
M. kansasii	1	1	1	0	0	0	0
M. gordonae	2	2	2	0	0	0	0
All MYCO	67*	54*	9*	57	4	51	0

***NOTE:** Five **MGIT** ONLY isolates are not included in these data. Presumptive identification was performed with no final confirmation of ID.

XI. AVAILABILITY

Cat. No.	Description
245111	**BBL™ MGIT™** Mycobacteria Growth Indicator Tubes, 4 ml, carton of 25 tubes.
245113	**BBL™ MGIT™** Mycobacteria Growth Indicator Tubes, 4 ml, carton of 100 tubes.
245116	**BBL™ MGIT™** OADC, 15 ml, carton of 6 vials. Each vial sufficient for 25 **MGIT** tubes.
245114	**BBL™ MGIT™ PANTA™** Antibiotic Mixture, lyophilized, carton of 6 vials. Each vial sufficient for 25 **MGIT** tubes.
	BBL™ Lowenstein-Jensen Medium Slants, package of 10 (20 x 148 mm tubes with cap).
	BBL™ Lowenstein-Jensen Medium Slants, carton of 100 (20 x 148 mm tubes with cap).
	BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit, ten 75 ml bottles of NALC-NaOH solution and 5 packages of phosphate buffer.
	BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit, ten 150 ml bottles of NALC-NaOH solution and 10 packages of phosphate buffer.
	BBL™ Middlebrook and Cohn 7H10 Agar, carton of 100.
	BBL™ Middlebrook 7H9 Broth, 8mL, package of 10 tubes.
	BBL™ Normal Saline, 5 mL, package of 10.
	BBL™ Normal Saline, 5mL carton of 100.
	BBL™ Taxo ™ X Factor Strips, 1 vial, 30 discs.

XII. REFERENCES

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TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free, (800) 638-8663.

Approved By:__________________________

Date Effective:__________________________

Supervisor______________________________ Date________________

Director ______________________________ Date________________

Reviewed:

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