BACT/ALERT®
BEST PRACTICES

WHICH BOTTLE FOR WHICH APPLICATION?

For best overall recovery when culturing samples that may contain aerobic and anaerobic microorganisms, it is strongly recommended that **more than one atmospheric type of culture bottle be utilized** (e.g.,one anaerobic and one anaerobic).

STANDARD MEDIA		
BACT/ALERT® IAST	Aerobic	Standard culture media for microbial growth.
BACT/ALERT® INST	Anaerobic	
NEUTRALIZING MEDIA		
BACT/ALERT® IFA PLUS	Aerobic	Neutralizing culture media contain resin beads to neutralize the effect of external factors as antibiotics, detergents etc.
BACT/ALERT® IFN PLUS	Anaerobic	
HIGH-ACID PRODUCT MEDIA		
BACT/ALERT® ILYM	Aerobic	The Lactic yeast & molds media is specific for high acid products and enhances the recovery of yeast & molds.

WHY IS THE GROWTH PROMOTION TEST SO IMPORTANT?

- bioMérieux QC testing helps ensure optimal quality of our culture media for microbial testing. However, transportation can still significantly impact the batch of bottles that you receive. Performing a GPT, following Pharmacopeias guidance, will validate the bottle quality after transportation.
- The strains must be cultivated under the best conditions: some species require specific temperature/atmosphere/duration of incubation. Please make sure to perform the test applying these conditions in order to obtain the best shaped strain.
- A range of ready-to-use BIOBALL® strains is available to perform the GPT.

- Discover the list of available strains: ***go.biomerieux.com/BIOBALL-strains***

HOW DO I PROPERLY INOCULATE AND INCUBATE MY BOTTLES?

- Make sure to disinfect at the following steps: septum, needle, or syringe.
- If using both an aerobic and anaerobic bottle, **transfer to the anaerobic bottle first** to avoid any oxygen trapped in the syringe to be transferred to the bottle.
- No more than 10mL of sample should be tested in a bottle. When testing a non-nutritional sample, the sample volume added to the bottle might need to be less than 10mL.
- Multiple inoculations into a bottle should be avoided due to the potential for air to enter the bottle, this may significantly delay detection times.
- To optimize detection times, bottles should be loaded into the BACT/ALERT® Microbial Detection systems as soon as possible following inoculation. Procedures for loading and unloading bottles into the instrument are given in the User Manual.

STANDARD MEDIA		
BACT/ALERT® IAST	Aerobic	*No specific recommendation*
BACT/ALERT® INST	Anaerobic	The bottle must **NOT be vented.** We recommend **the use of a 27-gauge needle** to prevent disruption of the anaerobic environment.
NEUTRALIZING MEDIA		
BACT/ALERT® IFA PLUS	Aerobic	*No specific recommendation*
BACT/ALERT® IFN PLUS	Anaerobic	We recommend the use of the **smallest gauge needle** that will accommodate dispensing the test sample to prevent disruption of the anaerobic environment. **A 23-gauge needle is recommended.**
HIGH-ACID PRODUCT MEDIA		
BACT/ALERT® ILYM	Aerobic	The seal can be removed. The bottle can be vented to introduce oxygen, depending on the types of organisms expected in the sample. Venting is not necessary if the septum has been removed for sample addition. **To vent the iLYM bottle:** • Consider venting the bottle in a protected environment. • For large volume sample you may wish to vent after sample inoculation to take advantage of the vacuum which helps draw the sample into the bottle. • Disinfect the septum with alcohol pad or equivalent. • Place a sterile airway needle/subculture unit into the septum and allow the bottle to vent for 1-5min. Alternatively use a sterile syringe with the plunger removed.

FACTORS IMPACTING TIME-TO-DETECTION (TTD)

1 - Oxygen Introduction During Inoculation of anaerobic bottles (iNST, iFN PLUS)

The way bottles are handled during inoculation can significantly impact the growth of strict anaerobic organisms. Introducing oxygen by using larger gauge needles than the ones mentioned in the IFUs, or not following proper inoculation steps, may delay microbial growth and increase the Time to Detection (TTD).

To illustrate this, we compared the TTD of *C. sporogenes* in the iNST bottle using different needle sizes in both control conditions (1 mL) and in saline* (10 mL).

**Here, the saline is used to simulate the presence of a product in the bottle.*

Needle Size	Control (1 mL)	Control (10 mL)
27G	~1.0 day	~1.0 day
22G	~1.0 day	~1.2 days
18G	~2.3 days	~5.6 days

These results show that TTD can be significantly delayed by up to 4–5 days, depending on the technique and syringe used.

2 - Strain Preparation

TTD can also be affected by the strain preparation protocol. The source of the strain (e.g., ATCC vs. in-house), its rehydration, and the suspension method can all impact growth, particularly during Growth Promotion Testing (GPT). Ensuring consistent preparation helps reduce TTD variability and supports reproducibility.

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