Chameleon™ BTES Multidifferential Agar

Chameleon™ BTES Agar is a chromogenic multidifferential media that can be utilized as an alternative to traditional media specified in the pharmacopeia, as well as agar films.

1 PLATE DETECTION OF:

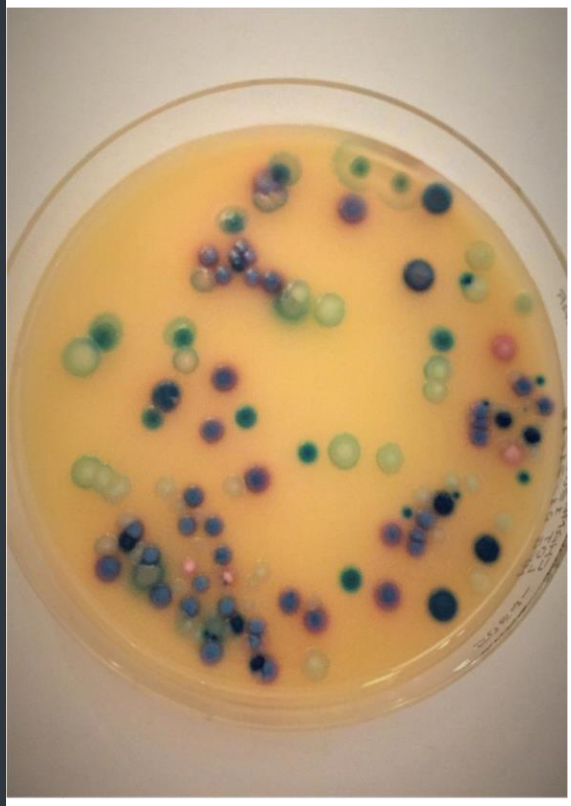
Total Bile-Tolerant Gram-Negative (BTGN) Organisms including differentiation of *Escherichia coli, Salmonella*, and Total Coliforms

REDUCED PREPARATION

Why not save time and money? Multiple enumeration broths, sample transfers, and incubation steps are not required with Chameleon™ BTES.
Simply dilute in a sterile diluent, spread plate 0.1mL, and incubate at 33-37°C for 36-48 hours.

Our media package has been reviewed and approved by:

RJ Lee Group



BTGN Bacteria on Chameleon™ BTES Multidifferential Agar

Our product complies with pharmaceutical standards and we have the data to prove it!

- Validated as per USP <1223> *Validation of Alternative Microbiological Methods,* for Microbial Examination of Non-Sterile Products
- Method verification and suitability testing completed as per USP <61> *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* and USP <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*
- A commercially prepared Microbial Panel Proficiency Test for Cannabis was completed and compared favorably to both agar films and traditional USP-NF specified media

Method Validation & Equivalency:

Compendial test methods captured in USP General Chapter <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* include the Test for Bile-Tolerant Gram-Negative Bacteria, the Test for Escherichia coli, and the Test for Salmonella. These compendial methods were completed as written using traditional broths and agars as well as Chameleon™ BTES agar and S2M test instructions. Validation parameters for quantitative tests were completed and included acceptance criteria for accuracy, precision, specificity, LOD, LOQ, linearity, operational range, robustness, ruggedness, method suitability, and equivalency between the alternate and compendial methods. All validation acceptance criteria were satisfied.

The equivalency study data demonstrated that recoveries and cultural responses obtained for the Chameleon™ BTES alternate method were either equal to or better than those obtained with traditional media. Additionally, chi-square analysis was performed and indicated that the populations (i.e. - traditional media versus Chameleon™ BTES Agar) were not significantly different. An error of estimate calculated over the CFU range tested established that Chameleon™ BTES had lower values than traditional media. The study also confirmed that enumeration broths and steps are not required with Chameleon™ BTES agar.

S² Media

Chameleon™ BTES Multidifferential Agar

EXPECTED RESPONSES:

Bile-Tolerant Gram-Negative (BTGN) Bacteria – Total count of all organisms present regardless of colony color.

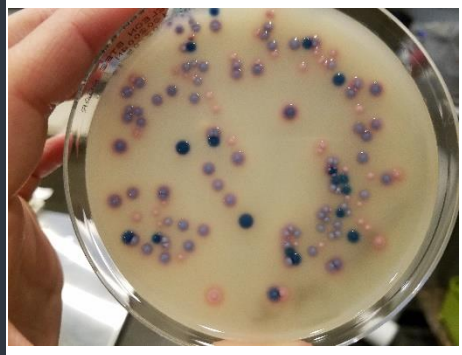
Escherichia coli – Pink colonies **with bile precipitate;**

Salmonella – Teal colonies with a white outer ring, which becomes more pronounced after 36 hours incubation. (Resembles a bullseye pattern, which is unique to *Salmonella* ssp. on this agar);

Total Coliforms – Total count of all colonies that are pink, purple, and purple-blue.

Gram-Positive Bacteria – Partial to complete inhibition;

Fungi (Mold and Yeast) – Partial to complete inhibition. (Antimycotic agents deteriorate after 3 days of incubation at 33-37°C and organisms can break through at this point; however, the colonies have characteristics easily identifiable such as hyphae and spores)



Total Coliforms on Chameleon™ BTES Multidifferential Agar

Method Verification & Suitability:

Verification and suitability of Chameleon™ BTES Agar for the microbial examination of cannabis was completed in accordance with:

- ✓ USP <61> *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests: Growth Promotion Test, Suitability of the Counting Method in the Presence of Product* where the Plate Count Method was utilized.
- ✓ USP <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms, Growth-Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls*.

Accuracy and precision criteria for quantitative tests were established and utilized spiked recovery samples, positive / negative controls, and products controls. All acceptance criteria were fulfilled. The verification established that cannabis neither enhances nor inhibits signals generated on Chameleon™ BTES Agar. In other words, there is no sample matrix interference.

Proficiency Test for Cannabis:

State regulator agencies use proficiency testing programs for laboratory licensure and compliance to promote accurate testing for cannabis quality and safety. The standardized test panel is designed to evaluate performance of accredited testing laboratories independently, as well as in relation to one another. Laboratories completing PT panels must use their internal processes and procedures (i.e. – sample handling, storage, and test methods) to complete the test panels.

The comparison study was designed around WA i502 and Ch. 324-55 WAC, which currently require a total count of bile-tolerant gram-negative (BTGN) bacteria as well as testing for the absence or presence of both *E. coli* and *Salmonella*. Testing for total coliforms and a total count of *E. coli* were also performed. (Note: *Enterobacteriaceae* is the standardized challenge for BTGN organisms. All organisms recovered at BTGN as opposed to only those expressing the *Enterobacteriaceae* response on the correlating medium.) The test preparations were prepared and compared on traditional plates (USP <62>, film agars, and Chameleon™ BTES multidifferential agar.

The study demonstrated that Chameleon™ BTES agar, agar films, and traditional media specified in USP <62> provide comparable recoveries for total counts of BTGN bacteria and coliforms, as well as the absence or presence of E. coli and Salmonella. The study also exhibited that Chameleon™ BTES agar notably simplifies the testing process by using a single sterile diluent, a single sample preparation, a single incubation temperature, and a single plate to complete testing for total BTGN bacteria, total coliforms, and the absence or presence of *E. coli* and *Salmonella*.

DOCUMENTS AVAILABLE FOR REVIEW UPON REQUEST:

- Validation & Equivalency Summary Report
- Verification & Suitability Summary Report
- WP2018-01R0 Proficiency Test Comparison
- Method Comparison Infographic
- Expected Response Guide
- Chameleon™ BTES Agar Test Instructions

S2 Media

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