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Full-Process External Controls for Sample-to-Answer Molecular Sepsis Diagnostic Systems Ensure Test and System Performance

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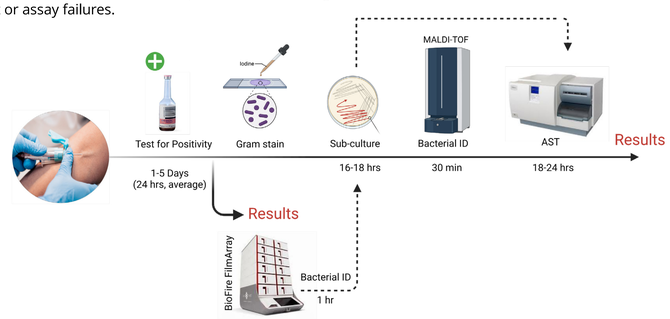
Background

Sepsis is a leading cause of death in the United States. Conventional sepsis testing methods (Scheme 1) can take at least 24 to 72 hours to get actionable results, which is not ideal given the limited time available for addressing deadly bloodstream infections. The Infectious Diseases Society of America (IDSA) recently released guidelines advocating for the use of rapid molecular diagnostic tests in combination with conventional phenotypic methods. This strategy enables timely and accurate diagnosis of sepsis-causing microorganisms, ultimately reducing mortality and hospitalization costs [1,2].

Given the complexity of automated multi-step sample-to-answer workflows for rapid molecular sepsis tests, it is crucial to verify instrument performance and accuracy of the assay. This can be accomplished by using full process external controls that monitor and validate each step of the workflow to ensure the accuracy and reliability of the test results. However, the availability of such controls is limited, and commercially available controls often do not contain patient sample components associated with human blood and blood culture media. Additionally, internal controls, integrated into the test cartridge by the manufacturer, do not include sample matrix components or the targets tested by the panel. This is a critical limitation, especially when the instrument and test are expected to remove inhibitors from the sample. An ideal control should ensure that the extraction and purification processes are working appropriately.

Here, we describe the development and performance evaluation of MDx-Chex™ for BCID2, a commercially available full-process quality control kit (FDA-cleared as a class II assayed control) for use with the BIOFIRE® BCID2 Panel (Scheme 2). We show that MDx-Chex for BCID2 effectively challenges each step of the BIOFIRE BCID2 Test. MDx-Chex for BCID2 can be used for assay verification, lot-to-lot performance tracking and to reduce the occurrence of incorrect results due to instrument or assay failures.



Scheme 1. Sepsis diagnosis workflow. Conventional sepsis testing methods could take several days to produce results, which delays de-escalation of empiric therapy and initiation of antibiotic therapy. Integration of rapid molecular sepsis tests, such as the BIOFIRE BCID2 Test, into the existing conventional workflow allows for faster turnaround time, enabling earlier administration of effective treatments, improving patient care, and reducing healthcare costs.

MDx-Chex for BCID2 is an FDA-Cleared Quality Control Kit for Use with the BIOFIRE BCID2 Panel

Intact and inactive microbes contained in each control vial

Control 1-GN:

Gram-negative bacteria:
Acinetobacter calcoaceticus-baumannii complex
Bacteroides fragilis
Enterobacter cloacae complex
Escherichia coli
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Proteus spp.
Salmonella spp.
Serratia marcescens
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia

Antimicrobial resistance genes:
KPC, CTX-M, IMP, NDM, OXA-48-like, VIM, *mcr-1*

Control 2-GPY:

Gram-positive bacteria:
Enterococcus faecalis
Enterococcus faecium
Listeria monocytogenes
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Yeast:
Candida albicans
Candida auris
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis
Cryptococcus neoformans/gatti

Antimicrobial resistance genes: *mecA/C*, MREJ, *vanA/B*

Scheme 2. MDx-Chex for BCID2 is a full-process quality control kit for use with the BIOFIRE BCID2 Panel. The control kit contains two separate vials that cover targets tested on the BIOFIRE BCID2 Panel: Control 1-GN, which contains Gram (-) bacteria, and Control 2-GPY, which contains Gram (+) bacteria and yeasts. Each vial contains intact, inactivated microorganisms which are suspended in a matrix of blood cells and culture media components.

Methods

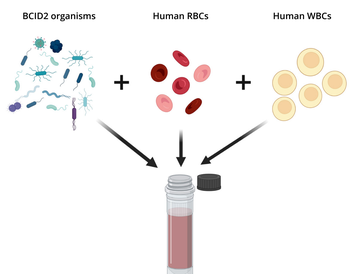
Quality Control Sample: MDx-Chex for BCID2 is comprised of inactivated microorganisms in a matrix of stabilized human blood cells and culture media components (known as PCR inhibitors), which simulates a clinical patient sample. The control kit contains two separate vials, one for Gram (-) bacteria and one for the Gram (+) bacteria and yeasts, and covers all targets tested with the BIOFIRE BCID2 Panel.

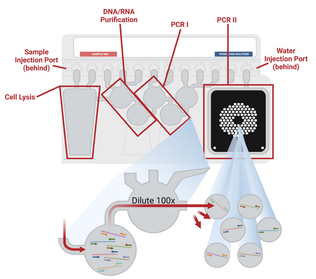
Performance evaluation: Precision of the controls was evaluated by testing three lots of MDx-Chex for BCID2 using BIOFIRE BCID2 Panel. Reproducibility (multi-site precision) of the controls was assessed internally at Streck (La Vista, NE) and externally at three different clinical sites.

Sample testing: 200 µL aliquots of each control sample were tested on the BIOFIRE FilmArray Torch and 2.0 Systems using the BIOFIRE BCID2 Panel (Cat No: # RFIT-ASY-0147) per manufacturer's instructions.

Results

MDx-Chex for BCID2 has a Patient-like Matrix and Tests Every Automated Step in the BIOFIRE BCID2 Assay





Representative BCID2 cartridge created using BioRender

	Sample Processing	Cell Lysis		DNA Purification	Two-stage PCR	
	Barcode tracking	Intact organisms	Contains human blood cells	Removal of inhibitors Hemoglobin Human gDNA Culture media	Test for PCR inhibition	Amplification/ detection
MDx-Chex for BCID2	✓	✓	✓	✓	✓	✓
Competitor A	x	x	x	x	x	✓
Competitor B	x	✓	x	x	x	✓
MDx-Chex for BCID2 is the only control on the market which fulfills the CAP, CLSI, ISO and CFR requirements.						

Scheme 3. Development of MDx-Chex for BCID2 for use with the BIOFIRE BCID2 Panel. Intact BCID2 microorganisms are inactivated and constituted in a matrix of stabilized human red and white blood cells and blood culture media components (i.e., those identified as PCR inhibitors). This unique control resembles patient samples and complies with guidelines provided by regulatory bodies that emphasize the use of positive and negative controls that challenge each step of the assay. When compared to other commercially available kits, MDx-Chex for BCID2 is the only kit that provides controls in a patient-like sample matrix.

Results (continued)

MDx-Chex for BCID2 Positive and Negative Controls Ensure Accuracy and Reliability of BCID2 Test Results

Table 1. MDx-Chex for BCID2 Precision Study.

Category	#Observed/Expected Results	Percent Agreement	95% Confidence Interval
Positive: Control 1-GN and Control 2-GPY, Combined	114/120	95%	94 - 100%
Negative: Control 1-GN and Control 2-GPY, Combined	120/120	100%	97 - 100%
Denominator = total # of results for Control 1-GN and Control 2-GPY controls. GPY = Gram-positive and yeast control, GN = Gram-negative control.			

Precision of the controls was internally evaluated by testing three independent control lots on the FilmArray Torch and 2.0 Systems using the BIOFIRE BCID2 Panel. Overall precision of the controls was ≥ 95% positive agreement (114 of 120 tests produced expected positive results). Overall negative agreement was 100% (120 of 120 tests produced expected negative results).

Table 2. MDx-Chex for BCID2 Reproducibility Study.

Category	Site #1	Site #2	Site #3	Site #4	Percent Agreement (all sites)	95% Confidence Interval
	#Observed/Expected Results	#Observed/Expected Results	#Observed/Expected Results	#Observed/Expected Results		
Positive: Control 1-GN and Control 2-GPY, Combined	59/60	57/60	58/60	56/60	95.8% (230/240)	92 - 98%
Negative: Control 1-GN and Control 2-GPY, Combined	60/60	60/60	60/60	59/60	99.6% (239/240)	98 - 100%
Denominator = total # of results for Control 1-GN and Control 2-GPY controls. GPY = Gram-positive and yeast control, GN = Gram-negative control.						

Three independent lots of MDx-Chex for BCID2 were evaluated at four different testing sites on the FilmArray Torch and 2.0 Systems using the BIOFIRE BCID2 Panel. Overall, reproducibility of the control was ≥ 96 % positive agreement (230 of 240 tests produced expected positive results). Overall negative agreement across all testing sites was ≥ 99 % (239 of 240 tests produced expected negative results).

Conclusions

Overall, performance data presented here demonstrates MDx-Chex for BCID2 is an excellent choice for BIOFIRE BCID2 quality control testing. MDx-Chex for BCID2 has the added benefit of being a full-process quality control that can effectively challenge the limitations of the assay and instrument by controlling for all steps in the automated molecular diagnostic process for the BCID2 Panel. With the recent recommendations by IDSA regarding the use of quality controls, healthcare providers now have an opportunity to increase their compliance with CAP and CLSI regulatory guidelines by adopting MDx-Chex for BCID2 into their quality control programs, thereby ensuring reliability of test results and improving patient care.

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References

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