Sepsis Diagnostics: Patient-Like Full-Process Controls Ensure Accuracy and Reliability for Molecular-Based Testing

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INTRODUCTION

In recent years, the use of automated DNA-based sepsis tests to complement gold standard culture-based methods have increased in frequency. While automation simplifies molecular diagnostic workflows, the integration of steps into a proverbial "black box" creates the potential for variability in instrument and test performance. To ensure expected performance, effective quality control measures are paramount (Figure 1). However, many commercially available controls are either solely comprised of synthetic materials that have been diluted in buffers or may have intact organisms that are not included in a patient-like matrix. Thus, these formulations fail to comprehensively measure the assay, as they are unable to detect all panel targets or cannot verify the system's ability to process and remove sample matrix effects from inhibitors in patient blood and culture media. Furthermore, internal controls do not effectively evaluate matrix inhibition when the quality control matrix doesn't reflect a patient sample. The consequences of these gaps in coverage were highlighted in a recent medical device correction for the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel that identified misdetection of *C. tropicalis* by a commercially available quality control kit that employs synthetic DNA targets. This example underscores the need to shift from "traditional" quality control designs to use of controls mirroring patient samples in composition and test experience. Patient-like, full-process controls are essential to adequately evaluate system and test performance, including manufacturer changes to the assay.

Here, we present MDx-Chex® for BC-GP and MDx-Chex® for BC-GN as a quality control solution for routine molecular diagnostic sepsis testing. These FDA-cleared, class II assayed, patient-like full process controls are intended for use with Luminex VERIGENE® BC-GP and BC-GN sepsis panels. MDx-Chex for BC-GP and BC-GN contain intact, inactivated microorganisms constituted in a matrix representative of a blood culture sample (Figures 2 and 3). Our data demonstrate overall precision and reproducibility of 100% positive and negative agreement for MDx-Chex for BC-GP (n = 150 total tests, 95% CI) and ≥ 98% positive agreement and 100% negative agreement for MDx-Chex for BC-GN (n = 150 total tests, 95% CI) (Tables 1 and 2). Collectively, the controls ensure reliable and accurate VERIGENE panel-based sepsis testing, enabling physicians and health care professionals to maintain assay quality while improving compliance with accrediting standards and guidelines.

Full-Process Quality Controls Play Crucial Role in Validating Analytical Variables of Automated Sepsis Tests

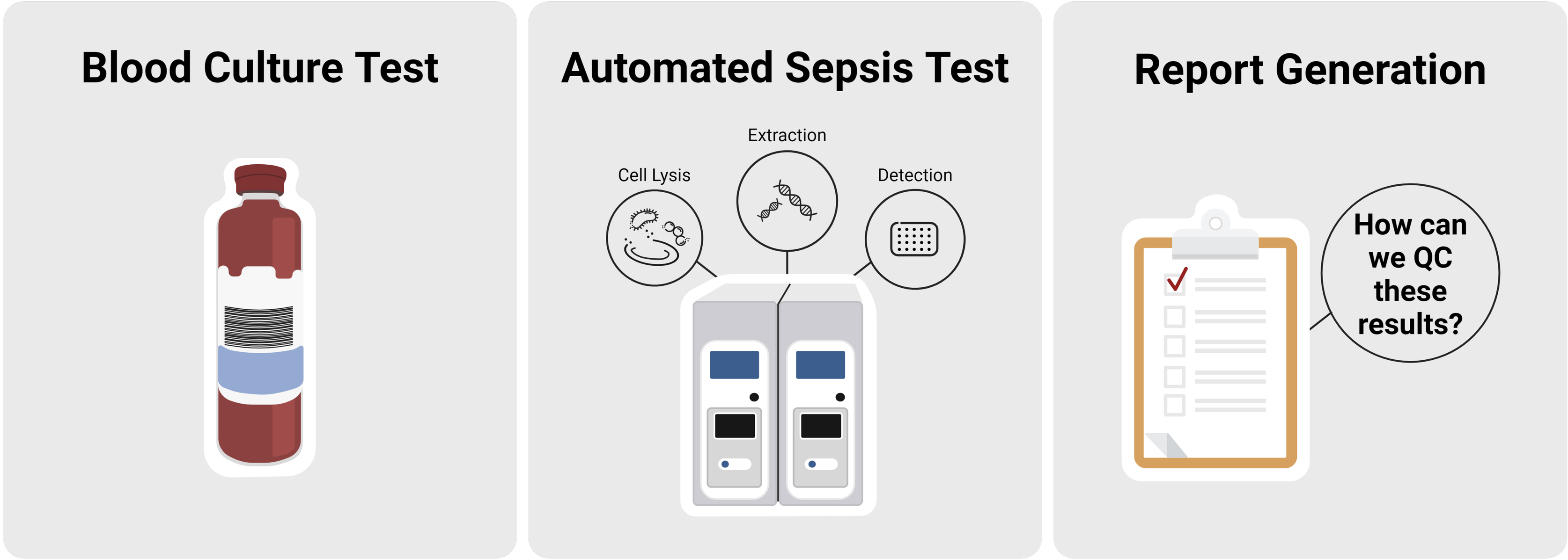

Figure 1. While automated sepsis testing streamlines workflows, finding a control that challenges the entire analysis process to accurately evaluate and identify deviations and false results can be difficult.

INTRODUCTION (cont.)

MDx-Chex for BC-GP and MDx-Chex for BC-GN Contain Intact, Inactivated Microorganisms Specific to the Luminex VERIGENE Sepsis Panels

MDx-Chex for BC-GP

Gram-positive bacteria:
+ *Enterococcus faecalis*
+ *Enterococcus faecium*
+ *Listeria spp.*
+ *Staphylococcus spp.*
+ *Staphylococcus aureus*
+ *Staphylococcus epidermidis*
+ *Staphylococcus lugdunensis*
+ *Streptococcus spp.*
+ *Streptococcus agalactiae*
+ *Streptococcus anginosus group*
+ *Streptococcus pneumoniae*
+ *Streptococcus pyogenes*

Antimicrobial resistance genes:
+ *mecA*
+ *vanA*
+ *vanB*

MDx-Chex for BC-GN

Gram-negative bacteria:
+ *Acinetobacterspp.*
+ *Citrobacterspp.*
+ *Enterobacterspp.*
+ *Escherichia coli*
+ *Klebsiella oxytoca*
+ *Klebsiella pneumoniae*
+ *Proteus spp.*
+ *Pseudomonas aeruginosa*

Antimicrobial resistance genes:
+ CTX-M
+ IMP
+ KPC
+ NDM
+ OXA
+ VIM

Figure 2. MDx-Chex for BC-GP and BC-GN are full-process quality control kits designed for use with the Luminex VERIGENE Sepsis Panels. The controls feature intact, inactivated microorganisms suspended in a matrix of blood cells and culture media, ensuring accurate target detection across manufacturer test revisions. Each MDx-Chex for BC-GP and BC-GN control kit contains a positive control vial with gram-positive or gram-negative bacteria specific to Luminex VERIGENE BC-GP and BC-GN tests, respectively, and a negative control vial containing only blood matrix and culture media (i.e., no bacteria).

METHODS

Control materials: The MDx-Chex for BC-GP control kit contains 10 separate vials: 5 positive control vials containing intact, inactivated gram-positive bacteria specific to the Luminex VERIGENE BC-GP panel and 5 negative control vials. The MDx-Chex for BC-GN kit contains 10 separate vials: 5 positive control vials containing intact, inactivated gram-negative bacteria specific to the Luminex VERIGENE BC-GN panel and 5 negative controls. Negative controls in both kits contain only blood matrix and culture media.

Performance evaluation: Precision was measured by testing three lots of each control kit on the Luminex VERIGENE using the BC-GP and BC-GN Panels according to the manufacturer's instructions. Reproducibility (multi-site precision) of the controls was assessed internally at Streck and externally at 2 clinical sites. For each test, 350 µl of MDx-Chex for BC-GP (positive and negative) and 700 µl of MDx-Chex for BC-GN (positive and negative) was used.

RESULTS

MDx-Chex for BC-GP and MDx-Chex BC-GN Challenge and Verify the Entire Analytical Process of the Luminex VERIGENE BC-GP and BC-GN Assay

	Streck	Competitor A	Competitor B
Preanalytical Variables			
Sample barcoding	Yes	No	No
Controls technician sample handling	Yes	No	No
Preanalytical Variables			
Lysing			
Contains non-infectious intact organisms	Yes	No	Yes
Contains human RBCs and WBCs	Yes	No	No
Lyses all panel organisms	Yes	No	Yes
Nucleic Acid Extraction			
Patient-like sample matrix	Yes	No	No
Inhibitors			
RBCs (i.e., hemoglobin)	Yes	No	No
WBCs (i.e., interleukin, human genomic DNA)	Yes	No	No
Culture media chemicals	Yes	No	No
Specificity			
All targets present	Yes	Yes	Yes
Off target (human DNA) present	Yes	No	No
Detection			
All DNA targets	Yes	No	Yes
FDA Class II Clearance	Yes	No	No

Figure 3. MDx-Chex for BC-GP and BC-GN validate the entire analytical process of the Luminex VERIGENE BC-GP and BC-GN Panels including nucleic acid extraction, DNA purification, target DNA-probe hybridization, and staining.

MDx-Chex for BC-GP and MDx-Chex for BC-GN Display High Precision and Reproducibility

Table 1. Precision Study of MDx-Chex for BC-GP and BC-GN Controls

Category	#Observed/Expected Results*	Percent Agreement	95% Confidence Interval
MDx-Chex for BC-GP; Positive Control	60/60	100%	94% - 100%
MDx-Chex for BC-GP; Negative Control	60/60	100%	94% - 100%
MDx-Chex for BC-GN; Positive Control	59/60	98%	91% - 100%
MDx-Chex for BC-GN; Negative Control	60/60	100%	94% - 100%

** Expected result for the positive control is positive and for the negative control is negative.*

To evaluate precision of the controls, three lots of each control kit were tested internally on the Luminex VERIGENE using BC-GP and BC-GN Panels. Overall precision of the MDx-Chex for BC-GP controls was 100% agreement for both positive and negative controls (60 of 60 tests produced expected results for each control type). MDx-Chex for BC-GN positive control had 98% positive agreement (59 of 60 tests produced expected results), and MDx-Chex for BC-GN negative control had 100% negative agreement (60 of 60 tests produced expected results).

RESULTS (continued)

MDx-Chex for BC-GP and MDx-Chex for BC-GN Display High Precision and Reproducibility

Table 2. Reproducibility Study of MDx-Chex for BC-GP and BC-GN Controls

Category	Site #1	Site #2	Site #3	Percent Agreement (all sites)	95% Confidence Interval
	#Observed/Expected Results*	#Observed/Expected Results*	#Observed/Expected Results*		
MDx-Chex for BC-GP; Positive Control	30/30	30/30	30/30	100% (90/90)	96% - 100%
MDx-Chex for BC-GP; Negative Control	30/30	30/30	30/30	100% (90/90)	96% - 100%
MDx-Chex for BC-GN; Positive Control	29/30	30/30	30/30	99% (89/90)	94% - 100%
MDx-Chex for BC-GN; Negative Control	30/30	30/30	30/30	100% (90/90)	96% - 100%

** Expected result for the positive control is positive and for the negative control is negative.*

The reproducibility of the controls was evaluated by testing three lots of each control kit at three different clinical testing sites. Overall reproducibility of the MDx-Chex for BC-GP controls was 100% agreement for both positive and negative controls, with all 90 tests yielding expected results for each control type. MDx-Chex for BC-GN had 99% positive agreement (89 of 90 tests produced expected results) and 100% negative agreement, (90 of 90 tests produced expected results) for positive and negative controls, respectively.

CONCLUSION

Full-process external quality controls such as MDx-Chex for BC-GP and MDx-Chex for BC-GN are indispensable to molecular sepsis testing and health care, as they are crucial for ensuring test accuracy, reliability, and adherence to CLSI and CAP quality standards. Integration of these controls into a sepsis diagnostics workflow not only minimizes errors, bolsters confidence in results, and improves patient care, but also underscores a continuous commitment to quality and safety in sepsis testing procedures.

Contact BD@streck.com for more information about working with Streck!