

# Example protocol for laboratory performance verification of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel with MDx-Chex® for BCID2

## Overview

CLIA-certified laboratories are required to establish quality control procedures to verify the accuracy of diagnostic tests and instrumentation used to analyze human specimens. Such quality control procedures should monitor the entire testing process and include positive and negative tests to verify the accuracy of the results. This technical note provides an example performance verification procedure using Streck's MDx-Chex for BCID2 quality control with the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel. MDx-Chex for BCID2 is a patient-like, full-process control compatible with the BIOFIRE BCID2 Panel. MDx-Chex for BCID2 includes pre-pooled microorganisms split between two vials suspended in a simulated blood culture matrix. The formulation of this control evaluates the BCID2 Panel just like a patient sample, increasing testing efficiency by reducing the number of sample handling steps and BCID2 tests required to complete a verification protocol.

## Intended Use

MDx-Chex for BCID2 is intended for use as an external positive and negative assayed control to monitor the performance of the qualitative detection of yeast, Gram positive and Gram negative bacteria, as well as associated antimicrobial resistance genes, by the BIOFIRE FilmArray® Blood Culture Identification 2 (BCID2) Panel on FilmArray systems.

## Product Description

MDx-Chex for BCID2 contains 43 bacteria, yeasts and antimicrobial resistance gene targets packaged in two separate vials: Control 1-GN containing gram-negative bacteria and associated antimicrobial resistance mechanisms and Control 2-GPY containing gram-positive bacteria, yeasts, and their resistance mechanisms (organisms included in each vial are listed below). Microorganisms are intact (inactivated) and formulated in a patient-like simulated blood culture matrix containing stabilized red blood cells, white blood cells and blood culture media components. MDx-Chex for BCID2 contains all targets assayed for the BIOFIRE BCID2 Panel and monitors performance of each automated sample processing step, including cell lysis, DNA purification, PCR inhibition/amplification and detection. In addition, each control vial functions as a negative control for the microorganism targets included in the opposing vial included in the MDx-Chex kit.

**Control 1-GN:** Gram-negative bacteria: *Acinetobacter colcoeticus-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* and MREJ, *vanA/B*.

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### Example Performance Verification Protocol

#### Performance Verification: Required Materials

Streack MDx-Chex for BCID2 kit and the BIOFIRE FilmArray BCID2 Panel (30 tests per kit) includes all materials needed to complete the following performance verification protocol. Table 1 compares the recommended verification materials when using MDx-Chex for BCID2 vs. other commercially available quality controls.

**Note:** MDx-Chex for BCID2 contains inactivated organisms and materials of human and animal origin and should be considered potentially infectious. Universal precautions should be utilized during handling.

| Material                                 | Part #                                                      | Competitor A | Competitor B | Streack |
|------------------------------------------|-------------------------------------------------------------|--------------|--------------|---------|
| BIOFIRE® BCID2 Panel (30-test kit)       | BIOFIRE Diagnostics, LLC RFIT-ASY-0147                      | √            | √            | √       |
| BIOFIRE BCID2 Panel Instructions for Use | BIOFIRE Diagnostics, LLC RFIT-PRT-0841                      | √            | √            | √       |
| BIOFIRE BCID2 Panel Quick Guide          | BIOFIRE Diagnostics, LLC RFIT-PRT-0867                      | √            | √            | √       |
| Blood culture media                      | BACT/ALERT® FA PLUS, 410851, 442192a (or equivalent)        | √            | √            |         |
| Human whole blood                        | BioIVT, HUMANWBCACDAUZN (or equivalent, with anticoagulant) | √            |              |         |
| McFarland turbidity standard             | Fisher Scientific, R20411 (or equivalent)                   | √            |              |         |
| Phosphate buffered saline, pH 7.4        | Hardy Diagnostics, R201 (or equivalent)                     | √            |              |         |
| Polystyrene tubes with cap               | VWR, 82050-278 or 82050-350 (or equivalent)                 | √            | √            |         |
| 10-mL syringe and 18 gauge needle        | VWR, 75846-756 and BD-305196 (or equivalent)                | √            | √            |         |
| Disposable transfer pipets, graduated    | VWR, 414004-024 (or equivalent)                             | √            | √            |         |
| Serological pipet, 5 mL                  | VWR, 82050-478 (or equivalent)                              | √            | √            |         |
| Control organism                         | ZeptoMetrix NATBCP2-BIO                                     |              | √            |         |
| 36 individual microbial strains          | Various numbers                                             | √            |              |         |
| 2 mL or 5 mL sample tubes                | Various manufacturers                                       |              | √            |         |

Table 1. Recommended material for verification protocols.

#### Performance Verification: MDx-Chex for BCID2 Protocol Overview

MDx-Chex for BCID2 may be used to verify detection of each microorganism and associated antimicrobial resistance genes included in the BIOFIRE BCID2 Panel. Use of MDx-Chex for BCID2 takes advantage of the multiplex format of the BCID2 test and provides increased efficiency by evaluating multiple targets, between two control vials, producing at least 20 positive and 20 negative results per test. Each test uses 200 µL of sample from each control vial; no pooling or spiking microorganisms into a blood culture matrix is required. The protocol will evaluate day-to-day and user-to-user variability, sample matrix effect, and generate multiple positive and negative detections for each target in the panel. The estimated time for completion of this protocol is 2 days. Figure 1 illustrates an example testing schema for verification of a single BIOFIRE module. However, this workflow may be adapted to verify the performance of additional instruments/modules (Figure 2).

**Note:** The number of samples tested per day must be determined by the individual laboratory and, per CLIA regulation, it is ultimately the responsibility of the Laboratory Director to ensure the verification procedure meets the appropriate standards for applicable accrediting agencies.

## Example protocol for laboratory performance verification of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel with MDx-Chex® for BCID2

| BIOFIRE Modules (n = 1) | Module 1            |                      |                     |                      |
|-------------------------|---------------------|----------------------|---------------------|----------------------|
| Day 1                   | Control-1 GN/User A | Control-2 GPY/User B | Control-1 GN/User B | Control-2 GPY/User A |
| Day 2                   | Control-1 GN/User B | Control-2 GPY/User A | Control-1 GN/User A | Control-2 GPY/User B |

Figure 1. Example of a verification workflow for one BIOFIRE FilmArray Torch module.

### 2 Modules

| BIOFIRE Modules (n = 2) | Module 1            |                      | Module 2            |                      |
|-------------------------|---------------------|----------------------|---------------------|----------------------|
| Day 1                   | Control-1 GN/User A | Control-2 GPY/User B | Control-1 GN/User B | Control-2 GPY/User A |
| Day 2                   | Control-1 GN/User B | Control-2 GPY/User A | Control-1 GN/User A | Control-2 GPY/User B |

### 4 Modules

| BIOFIRE Modules (n = 4) | Module 1            |                      | Module 2            |                      |
|-------------------------|---------------------|----------------------|---------------------|----------------------|
| Day 1                   | Control-1 GN/User A | Control-2 GPY/User B | Control-1 GN/User B | Control-2 GPY/User A |
|                         | Module 3            |                      | Module 4            |                      |
| Day 2                   | Control-1 GN/User B | Control-2 GPY/User A | Control-1 GN/User A | Control-2 GPY/User B |

Figure 2. Example of a verification workflow for use with multiple BIOFIRE FilmArray Torch modules.

### Performance Verification: MDx-Chex for BCID2 Protocol Example

Follow the MDx-Chex for BCID2 Instructions for Use to prepare controls. The user should determine the total number of samples to be run needed for verification based on the needs of the individual laboratory. If the lab chooses not to perform the verification on each instrument, BIOFIRE advises to distribute control replicates evenly among the instruments and modules included in the verification. Each MDx-Chex for BCID2 control kit contains 2 vials and 5 runs per control vial. The following protocol is for one BIOFIRE FilmArray 2.0 instrument or Torch module (Figure 1) but can be adapted for multiple modules or BIOFIRE Systems (Figure 2).

#### Day 1

1. Obtain required materials (see Table 1).
2. Remove Control 1-GN and Control 2-GPY vials from the MDx-Chex for BCID2 box and, if stored in the refrigerator, warm to room temperature (18 °C to 30 °C) for at least 15 minutes prior to use.  
Note: Each vial contains enough material to perform five tests.
3. Following MDx-Chex for BCID2 Instructions for Use, test replicates as indicated in Figure 1 for each of the Control 1-GN and Control 2-GPY replicates evaluated by user A and user B.  
Note: [https://www.streck.com/wp-content/uploads/sync/MDx-Chex\\_for\\_BCID2/01\\_Instructions\\_\(IFU\)/01\\_MDx-Chex\\_for\\_BCID2\\_IFU.pdf](https://www.streck.com/wp-content/uploads/sync/MDx-Chex_for_BCID2/01_Instructions_(IFU)/01_MDx-Chex_for_BCID2_IFU.pdf)
4. After completion of the Day 1 testing, the MDx-Chex for BCID2 kit may be returned to storage until Day 2.

#### Day 2

1. To evaluate day-to-day variation, repeat steps 1-3 above using the same vials for the third and fourth replicates of Control 1-GN and Control 2-GPY.
2. After completion of testing, the remaining volume from each vial may be returned to storage at 2 ° to 25 °C for up to the expiry date or until all control material is used from each vial.

### Performance Verification: Evaluation of Verification Results

1. See MDx-Chex for BCID2 assay for interpretation of results.
2. Positive Results: All indicated microorganisms and resistance genes included in each control vial should be detected in all respective replicates across multiple days and users.
3. Negative Results: Microorganisms and resistance mechanisms not included in each respective control vial should produce negative results for the indicated organisms across each day and user.

See [streck.com/patents](https://www.streck.com/patents) for patents that may be applicable to this product.

## Example protocol for laboratory performance verification of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel with MDx-Chex® for BCID2

### Performance Verification: Increased Testing Efficiency with MDx-Chex for BCID2

MDx-Chex for BCID2 is a pre-pooled, full-process, quality control that contains all BCID2 Panel microorganisms distributed between two control vials and provided in a simulated blood culture matrix. The format of this control utilizes the same workflow as a patient sample and is designed to challenge the multiplex nature of the BIOFIRE BCID2 Panel, resulting in increased testing efficiency for laboratories who need to verify performance of the BCID2 Panel on their FilmArray Systems. Specifically, MDx-Chex for BCID2 does not require the purchase of additional materials (Table 1) to complete BCID2 verification and uses 40% fewer BCID2 pouches than other commercially available controls (Table 2). MDx-Chex for BCID2 is the first pre-pooled control comprised of intact microorganisms and blood cells that closely resembles a patient sample in appearance and composition. MDx-Chex for BCID2 does not introduce extra pre-analytical variables caused by the pooling and spiking of microorganisms into blood culture media prior to testing, as with other controls.

| Verification Protocol | Organisms per Pool      | # of Sample Pools | Replicates per Pool | Pouches Required | Expected (+) Results       | Expected (-) Results | Approximate Days of Testing | Pool/Vial OVS (Days) <sup>g</sup> |
|-----------------------|-------------------------|-------------------|---------------------|------------------|----------------------------|----------------------|-----------------------------|-----------------------------------|
| Streck                | 14 or 16                | 2 <sup>a</sup>    | 4                   | 8                | 4 per target               | 4 per target         | 2                           | 90                                |
| Competitor A          | 6 or 7                  | 5                 | 4                   | 20               | ≥4 per target <sup>b</sup> | 12-16 per target     | 4 <sup>c</sup>              | 3                                 |
| Competitor B          | 1, 8, or 9 <sup>d</sup> | 5                 | 4                   | 20               | ≥4 per target <sup>e</sup> | 4                    | 2 <sup>f</sup>              | 2                                 |

Table 2. Overview of MDx-Chex for BCID2 Verification Protocols vs. other commercially available quality controls.

<sup>a</sup>Streck materials are ready to use and do not require pooling.

<sup>b</sup>The expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification. The proposed verification procedure recommends multiple *K. pneumoniae* strains; therefore, the number of expected *K. pneumoniae* group positives would be 8 and the number of expected negatives would be 12.

<sup>c</sup>The approximate number of days for testing assumes a BIOFIRE® system configured with one instrument/module.

<sup>d</sup>Depending on the material used for verification, pooling of organisms may not be appropriate and the values in the table may need to be modified.

<sup>e</sup>The expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification. The proposed verification procedure recommends two *E. coli* strains; therefore, the number of expected *E. coli* positives would be 8 and the number of expected negatives would be 12.

<sup>f</sup>The approximate number of days for testing assumes a system configured with one instrument/module and does not include time to grow microbial cultures.

<sup>g</sup>Open-Vial Stability (OVS) is the number of days that the material is viable once opened or pooled.

## Example protocol for laboratory performance verification of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel with MDx-Chex® for BCID2

| Benefits of Streck MDx-Chex for BCID2 control over other available controls                                                                                           |        |                 |                 |                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-----------------|-----------------|-----------------|
|                                                                                                                                                                       | Streck | Competitor A    | Competitor B    | Competitor C    |
| <b>Pre-analytical Variables</b>                                                                                                                                       |        |                 |                 |                 |
| Sample tracking (via barcode)                                                                                                                                         | Yes    | No              | No              | No              |
| Controls technician sample handling                                                                                                                                   | Yes    | No <sup>†</sup> | No <sup>†</sup> | No <sup>†</sup> |
| <b>Instrument Variables Tested</b>                                                                                                                                    |        |                 |                 |                 |
| <b>Lysis</b>                                                                                                                                                          |        |                 |                 |                 |
| Contains non-infectious intact organisms                                                                                                                              | Yes    | No              | Yes             | No <sup>‡</sup> |
| Contains intact human RBCs and WBCs                                                                                                                                   | Yes    | No              | No              | No              |
| Lyses all panel organisms                                                                                                                                             | Yes    | No              | Yes*            | Yes*            |
| <b>Nucleic Acid Isolation and Purification</b>                                                                                                                        |        |                 |                 |                 |
| Patient-like sample matrix                                                                                                                                            | Yes    | No              | No              | No              |
| <b>PCR Inhibitors</b>                                                                                                                                                 |        |                 |                 |                 |
| RBCs (i.e., hemoglobin)                                                                                                                                               | Yes    | No              | No              | No              |
| WBCs (i.e., interleukin, human genomic DNA)                                                                                                                           | Yes    | No              | No              | No              |
| Culture media chemicals                                                                                                                                               | Yes    | No              | No              | No              |
| <b>PCR Amplification Specificity</b>                                                                                                                                  |        |                 |                 |                 |
| All DNA targets present                                                                                                                                               | Yes    | Yes             | Yes*            | Yes*            |
| Off target (human gDNA) present                                                                                                                                       | Yes    | No              | No              | No              |
| <b>Detection</b>                                                                                                                                                      |        |                 |                 |                 |
| All DNA targets present                                                                                                                                               | Yes    | Yes             | Yes*            | Yes*            |
| <b>Workflow Efficiency</b>                                                                                                                                            |        |                 |                 |                 |
| Simulates a patient sample                                                                                                                                            | Yes    | No              | No              | No              |
| Pre-pooled cellular components                                                                                                                                        | Yes    | No              | No              | No <sup>†</sup> |
| Excludes live culture                                                                                                                                                 | Yes    | Yes             | Yes             | No              |
| Pre-screened components                                                                                                                                               | Yes    | Yes             | Yes             | No              |
| Requires pooling of multiple components (i.e., microorganisms, blood culture media chemicals, and human blood components) not part of patient sample testing workflow |        |                 |                 |                 |
| *If all panel organism are pooled                                                                                                                                     |        |                 |                 |                 |
| †Barcode allows the control lot number, expiration date, and level to be captured in the software                                                                     |        |                 |                 |                 |
| ‡Uses live intact organisms that have not been inactivated; requires live cultures                                                                                    |        |                 |                 |                 |

Table 3. MDx-Chex for BCID2 Attributes.

### Reference Documents:

1. Streck MDx-Chex® for BCID2 Instructions for Use.
2. Protocols for Laboratory Verification of Performance of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel: Laboratory Protocols for Use with a ZeptoMetrix NATtrol™ Verification Panel.
3. Protocol for Laboratory Verification of Performance of the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel: A Laboratory Protocol for Use with Live Organisms.