MRSA Assay (Panther Fusion®)

For *in vitro* diagnostic use.

For US export only.

CONTENTS

General Information

Intended Use

The Panther Fusion® MRSA assay is an automated *in vitro* diagnostic test that uses Invader Plus® chemistry for the qualitative detection and differentiation of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swab specimens. This assay is intended for use on the Panther Fusion system to aid in the prevention and control of MRSA/SA infections in healthcare settings.

Summary and Explanation of the Test

Staphylococcus aureus (*S. aureus*) is considered part of normal human flora and may colonize the anterior nares, throat, perineum, groin, and skin.[1] The majority of carriers are asymptomatic and the colonizing bacteria do not cause disease. However, in healthcare settings *S. aureus* infections can be serious or fatal. Invasive *S. aureus* infection symptoms range from mild skin infections (boils and abscesses) to bacteremia, sepsis, endocarditis, osteomyelitis, and pneumonia.[1]

The widespread use of the β-lactam antibiotic methicillin, a derivative of penicillin, led to the emergence of certain antibiotic-resistant *S. aureus* strains termed methicillin-resistant *S. aureus*. Resistance to methicillin in MRSA is largely mediated by the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), an enzyme involved in cell wall synthesis that is resistant to inhibition by β-lactam antibiotics.[1] The *mecA* gene is present within the staphylococcal chromosomal cassette *mec* element (SCC*mec*). Genetic excisions within the SCC*mec* element can lead to the lack of a functional *mecA* gene, resulting in a so-called “empty cassette variant” carried by certain methicillin-susceptible *S. aureus* (MSSA) strains. An alternative resistance mechanism gene, *mecC*, was described in *S. aureus* in 2011.[2,3] It is, therefore, necessary to specifically target both *mecA* and *mecC* genes in addition to the *orfX/SCCmec* junction to properly identify MRSA.

MRSA is considered a significant cause of healthcare-associated infections (HAIs) in the EU.[4] As a result of its highly invasive nature and limited susceptibility to treatment, MRSA is an immense clinical burden with high morbidity and mortality.[5] Due to high prevalence among hospitalized patients, accurate and fast identification of MRSA is necessary to initiate effective antimicrobial therapy and slow the spread of MRSA infections.[6] Molecular methods for the detection of MRSA have been introduced as a faster alternative to traditional, time-consuming culture methods.

Principles of the Procedure

The Panther Fusion system fully automates specimen processing (cell lysis, nucleic acid capture, amplification and detection) for the Panther Fusion MRSA assay. An internal control (IC-X) is added automatically to each specimen via working Fusion Capture Reagent-X (wFCR-X) to monitor for interference during specimen processing, amplification and detection caused by reagent failure or inhibitory substances.

Note: *The Panther Fusion system adds IC-X to FCR-X. After IC-X is added to FCR-X, it is referred to as wFCR-X.*

Sample processing and nucleic acid capture: Specimens are first incubated in an alkaline reagent (Panther Fusion Enhancer Reagent-X; FER-X) to lyse the cells. Nucleic acid released during the lysis step hybridizes to magnetic particles in FCR-X. The capture particles are separated from residual specimen matrix in a magnetic field by a series of wash steps with a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a reagent of low ionic strength (Panther Fusion Elution Buffer).

Multiplex PCR amplification and Invader® detection: Lyophilized single unit dose reaction master mix is reconstituted with Panther Fusion Reconstitution Buffer II and combined with the eluted nucleic acid in a reaction tube. Panther Fusion Oil reagent is added to prevent evaporation during the Invader Plus reaction.

An Invader Plus reaction is a combination of polymerase chain reaction (PCR) and Invader chemistries. PCR-based target amplification occurs with target-specific forward and reverse primers. Target detection and signal generation is achieved via Invader chemistry. During the detection phase a primary, unlabeled, probe and an invading oligonucleotide hybridize to the target DNA, forming a ternary DNA complex which is recognized and cleaved by a Cleavase® enzyme. This cleavage reaction releases a target-specific cleavage product from the primary probe. The target-specific cleavage product then hybridizes to a corresponding fluorescence resonance energy transfer (FRET) cassette, leading to another cleavage reaction. Each time a FRET cassette is cleaved, the corresponding fluorophore and quencher are separated, generating an increase in detectable fluorescence signal.[7] The assay uses target-specific primary probes and paired FRET cassettes with spectrally distinct fluorophores for the *orfX*/SCC*mec*, *mecA/C*, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and internal control targets. The assay targets a GAPDH isoform specific for *S. aureus*. The Panther Fusion MRSA assay software computes a cycle threshold (Ct) result from the accumulated fluorescent signal in each fluorescent channel to qualitatively determine the presence of each target.

The targets and corresponding fluorescent channels used in the Panther Fusion MRSA assay are listed in the table below:

Target	Channel
orfX/SCC*mec* junction	FAM
mecA/C gene	HEX
GAPDH gene	ROX
Internal Control	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Carefully read this entire package insert and the *Panther Fusion System Operator's Manual*.

C. Panther Fusion Enhancer Reagent-X (FER-X) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

E. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[8]

F. Use only supplied or specified disposable laboratory ware.

G. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

H. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

I. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

J. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of bacteria or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

K. Do not use the ESwab collection kit if it is damaged and do not use it after the expiration date.

L. Do not use reagents and controls after the expiration date.

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

N. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

O. Avoid microbial and nuclease contamination of reagents.

P. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

Q. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

R. Do not use fluid packs that are damaged or leaking. Contact Hologic Technical Support if this occurs.

S. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

T. Some of the reagents used with the Panther Fusion MRSA assay are labeled with risk and safety symbols.

Note: *Hazard Communication information reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

EU Hazard Information
 **Panther Fusion Oil** ***Polydimethylsiloxane 100%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
    **Panther Fusion Enhancer Reagent-X (FER-X)** ***Lithium Hydroxide Monohydrate 5-10%*** **DANGER** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapours/spray P280 - Wear protective gloves/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/physician P280 - Wear eye protection/ face protection

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	Onboard/ Open Stability[1]	Opened Storage
Panther Fusion MRSA Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C[2]
Panther Fusion Capture Reagent-X (FCR-X)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-X (FER-X)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-X (IC-X)	2°C to 8°C	(In wFCR-X)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer II	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion MRSA Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control II	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] Onboard stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion MRSA assay cartridge, FCR-X, FER-X and IC-X. Onboard stability for Panther Fusion Reconstitution Buffer II, Panther Fusion Elution Buffer, and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[2] If the assay cartridge is removed from the Panther Fusion system, store it in an air-tight container with desiccant at the recommended storage temperature.

B. Working FCR-X and FER-X are stable for 60 days when capped and stored at 15°C to 30°C. Do not refrigerate.

C. Discard any unused reagents that have surpassed their onboard stability.

D. Controls are stable until the date indicated on the vials.

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens - Clinical material collected from a patient and placed in an appropriate transport system. For the Panther Fusion MRSA assay, this is the ESwab Collection and Transport System.

Samples - Represents a more generic term to describe any material for testing on the Panther Fusion System including specimens and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

A. Specimen Collection

Collect one nasal ESwab specimen from both nostrils according to your facility's standard practice or use the following as guidance:

1. Wash hands and put on clean gloves.
2. Open swab packaging and remove the swab from its packaging.
3. Carefully insert the flocked part of the swab into the patient's nostril.
4. Press gently and roll the swab along the inside of the nostril 3 to 5 times.
5. Repeat the process in the other nostril using the same swab.

Note: *To avoid contamination, be careful not to touch the swab shaft below the break point.*

6. Open the tube containing 1 mL of liquid Amies, place the specimen swab into the tube, and break the swab shaft at the break point.
7. Recap the tube and discard the remaining part of the swab shaft.
8. Label the tube if necessary.
9. Remove gloves and wash hands.

Note: *If the liquid Amies spills before the swab is placed in the tube, place the specimen swab in a new tube containing 1 mL of liquid Amies. If the tube spills after placing the swab in the tube, collect a new nasal swab specimen.*

B. Specimen Transport and Storage before Testing

After collection, transport and store the specimen in the tube for up to 48 hours at 15°C to 30°C or for up to 5 days at 2°C to 8°C.

C. Specimen Storage after Testing

1. Place the specimen tubes upright in a tube rack.
2. Place a new cap on specimens that have been tested.
3. If tested specimens need to be shipped, remove the penetrable cap and replace with a non-penetrable cap. Maintain specimen storage conditions during transport as described under *Specimen Transport and Storage before Testing*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Reagents and Materials Provided

Assay Packaging

Components[1]	Cat. No.	Storage
Panther Fusion MRSA Assay Cartridges 96 Tests Panther Fusion MRSA assay cartridge, 12 tests, 8 per box	PRD-04803	2°C to 8°C
Panther Fusion MRSA Assay Controls Panther Fusion MRSA Positive Control tube, 5 per box Panther Fusion Negative Control II tube, 5 per box	PRD-04805	2°C to 8°C
Panther Fusion Internal Control-X 960 Tests Panther Fusion Internal Control-X tube, 4 per box	PRD-04476	2°C to 8°C
Panther Fusion Extraction Reagent-X 960 Tests Panther Fusion Capture Reagent-X bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-X bottle, 240 tests, 4 per box	PRD-04477	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer II 1920 Tests Panther Fusion Reconstitution Buffer II, 960 Tests, 2 per box	PRD-04804	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent, 960 tests, 2 per box	PRD-04335	15°C to 30°C

[1] Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution Buffer.

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion Module Upgrade	PRD-04173
Panther Fusion System	PRD-04172
Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with Panther Fusion assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)

Material	Cat. No.
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Liquid Handling (LiHa) Disposable Tips, 1000 µL	10612513 (Tecan)
Copan Liquid Amies Elution Swab (ESwab™) Collection and Transport System, or the equivalent BD™ Liquid Amies Elution Swab (ESwab) Collection and Transport System	480C or 480CE (Copan) 220245 (Becton Dickinson)
Aptima penetrable caps	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
Vortex mixer	—
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable powderless gloves	—

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

Note: *Refer to the Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Preparation

1. Remove the IC-X, FCR-X and FER-X bottles from storage.
2. Open the IC-X, FCR-X and FER-X bottles, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-X, FCR-X and FER-X bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds IC-X to the FCR-X bottle. After IC-X is added to FCR-X, it is referred to as wFCR-X. If wFCR-X and FER-X are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

1. Vortex each specimen for 5 seconds. Do not invert tube.
2. Remove the tube cap and swab from the tube.
3. Discard the tube cap and swab according to laboratory procedures.
4. Place a penetrable cap onto the tube.
5. Inspect specimen tubes before loading into the rack. If a specimen tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to dislodge bubbles and bring contents to the bottom.

Note: *To avoid a processing error, ensure the specimen volume is greater than 500 µL. There is sufficient volume to perform 2 Panther Fusion reactions from a specimen collected with the ESwab collection kit.*

D. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion MRSA Positive Control and the Panther Fusion Negative Control II can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and processed for the Panther Fusion MRSA assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. The Panther Fusion MRSA Positive Control and the Panther Fusion Negative Control II may appear cloudy or contain precipitate which will not interfere with the test results. Letting the controls reach room temperature before processing will allow the precipitate to dissolve. **Do not vortex the controls.**
4. Each control tube can be tested once.
5. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A set of controls is currently in process on the system.

Quality Control

The Panther Fusion MRSA assay software may invalidate a run or specimen result if problems occurred while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the Panther Fusion MRSA Positive Control and the Panther Fusion Negative Control II must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active assay cartridge lot has expired.

The Panther Fusion system is configured to require that assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and a new set of assay controls is required prior to starting any new specimens.

If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected specimens and requires a new set of assay controls be tested prior to starting any new specimens.

Internal Control

An internal control is added to each sample during automated specimen processing on the Panther Fusion system. During processing, the internal control acceptance criteria are automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for any assay target. Specimens that fail to meet that criteria are reported as Invalid. Each specimen with an Invalid result must be retested.

The Panther Fusion system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion MRSA assay software automatically determines the results for specimens and controls. Results for SA and MRSA are reported separately. A result may be SA negative and MRSA negative, SA positive and MRSA negative, SA positive and MRSA positive, or invalid. Specimens with invalid results must be retested.

Table 1 shows the possible results reported with corresponding result interpretations.

Table 1: Test Interpretation

orfX*/SCC*mec **(FAM)**	***mecA/C*** **(HEX)**	**GAPDH (ROX)**	**Internal Control (RED677)**	**Result**	
				MRSA	**SA**
+	+	+	+ / -	Positive	Positive
+	-	+	+ / -	Negative	Positive
-	+	+	+ / -	Negative	Positive
-	-	+	+ / -	Negative	Positive
+	-	-	+ / -	Negative	Negative
-	+	-	+ / -	Negative	Negative
+	+	-	+ / -	Negative	Negative
-	-	-	+	Negative	Negative
-	-	-	-	Invalid	Invalid

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. The Panther Fusion MRSA assay has only been validated for use with nasal swab specimens collected with the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System or the equivalent BD Liquid Amies Elution Swab (ESwab) Collection and Transport System.

E. Collect nasal swab specimens by following the procedures in the package insert for the ESwab Collection and Transport System.

F. New MRSA or SA strains with mutations or polymorphisms in primer- or probe-binding regions may not be detected with the Panther Fusion MRSA assay.

G. The Panther Fusion MRSA assay may generate a false positive MRSA result when testing a mixed infection nasal specimen containing both methicillin-resistant coagulase-negative staphylococci and empty cassette SA.

H. *S. argenteus*, a newly identified coagulase-positive species of the *Staphylococcus* genus that is closely related to *S. aureus*, is rare but may result in a false positive result in the Panther Fusion MRSA assay.

Panther Fusion System Assay Performance

Assay Reproducibility

Panther Fusion MRSA assay reproducibility was evaluated at three sites using a 5-member reproducibility panel. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed twice per day (one run per operator), for at least five days. Each run had three replicates of each panel member.

The panel members are described in Table 2, along with a summary of the agreement with expected results for each panel member. Table 3 presents the mean and variability analysis between sites, between operators, between days, between runs and within runs, and overall (total) for Ct.

Table 2: Percent Agreement with Expected Result

Panel Member		% Agreement			
Description	**Concentration**	**Site 1**	**Site 2**	**Site 3**	**Total Agreement**
MRSA Moderate Positive	MRSA at 2-3X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MRSA Low Positive	MRSA at 1-2X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
SA Moderate Positive	SA at 2-3X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
SA Low Positive	SA at 1-2X LoD	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
Negative	SNM unspiked	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)

LoD = limit of detection, SNM = simulated nasal matrix.

Table 3: Ct Value Variability

Panel Member		Target	POS n	Mean Ct	Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Description	**Concentration**				**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
MRSA Moderate Positive	MRSA at 2-3X LoD	*orfX*/SCC*mec*	90	34.0	0.3	0.8	0.0	0.0	0.2	0.6	0.0	0.0	0.5	1.4	0.6	1.7
		mec A/C	90	35.1	0.3	0.9	0.0	0.0	0.2	0.6	0.1	0.4	0.4	1.2	0.6	1.7
		GAPDH	90	33.2	0.3	0.9	0.1	0.3	0.1	0.4	0.1	0.4	0.4	1.3	0.6	1.7
MRSA Low Positive	MRSA at 1-2X LoD	*orfX*/SCC*mec*	90	35.2	0.2	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.8	0.7	1.9
		mec A/C	90	36.2	0.3	0.7	0.0	0.0	0.1	0.3	0.1	0.3	0.5	1.4	0.6	1.6
		GAPDH	90	34.2	0.3	0.8	0.0	0.0	0.0	0.0	0.2	0.6	0.4	1.3	0.6	1.6
SA Moderate Positive	SA at 2-3X LoD	GAPDH	90	32.9	0.4	1.2	0.0	0.0	0.2	0.6	0.0	0.0	0.4	1.1	0.6	1.7
SA Low Positive	SA at 1-2X LoD	GAPDH	90	33.9	0.4	1.2	0.0	0.0	0.2	0.5	0.2	0.7	0.4	1.2	0.6	1.9
Negative	SNM only (unspiked)	IC	90	35.2	0.2	0.5	0.0	0.0	0.1	0.2	0.2	0.7	0.4	1.3	0.5	1.5

Ct = threshold cycle, CV = coefficient of variation, LoD = limit of detection, POS= positive, SD = standard deviation, SNM = simulated nasal matrix.

Clinical Performance

This study was performed to demonstrate clinical performance for the Panther Fusion MRSA assay. Clinical performance was evaluated by comparing results with the Panther Fusion MRSA assay to results with an IVD nucleic acid test (NAT) reference assay.

Nasal swab specimens were collected at a US hospital with the Copan ESwab liquid Amies transport system. An aliquot of the specimen was tested with an IVD NAT reference assay. The remnant specimen was then frozen, shipped to Hologic, and tested with the Panther Fusion MRSA assay.

A total of 805 specimens were tested for SA and MRSA with the Panther Fusion MRSA assay and the reference assay.

Compared to the reference method, the Panther Fusion MRSA assay sensitivity and specificity were 95.6% and 96.8%, respectively, for detection of MRSA (Table 4) and 95.9% and 95.7%, respectively, for detection of SA (Table 5).

Table 4: Panther Fusion MRSA Assay Performance Compared to Reference Assay for Detection of MRSA

MRSA		Reference Assay POS	Reference Assay NEG	Total
Panther Fusion MRSA Assay	POS	109	22[1]	131
	NEG	5[1]	669	674
Total		114	691	805
Sensitivity		95.6% (109/114) (95% CI: 90.1% to 98.1%)		
Specificity		96.8% (669/691) (95% CI: 95.2% to 97.9%)		
PPV		83.2% (109/131) (95% CI: 76.9% to 88.6%)		
NPV		99.3% (669/674) (95% CI: 98.3% to 99.7%)		
Percent Agreement		96.6% (778/805) (95% CI: 95.2% to 97.7%)		

NEG = negative, NPV = negative predictive value, POS = positive, PPV = positive predictive value.

[1] Specimens generating discordant MRSA test results between the Panther Fusion MRSA assay and the reference assay were further evaluated using an enrichment culture method.

Of the 22 MRSA false positive Panther Fusion MRSA assay specimens, 12 were found to be MRSA positive after enriched culture discordant resolution.

Of the 5 MRSA false negative Panther Fusion MRSA assay specimens, 4 were found to be MRSA negative after enriched culture discordant resolution.

Table 5: Panther Fusion MRSA Assay Performance Compared to Reference Assay for Detection of SA

SA		Reference Assay POS	Reference Assay NEG	Total
Panther Fusion MRSA Assay	POS	234	24[1]	258
	NEG	10[1]	537	547
Total		244	561	805
Sensitivity		95.9% (234/244) (95% CI: 92.6% to 97.8%)		
Specificity		95.7% (537/561) (95% CI: 93.7% to 97.1%)		
PPV		90.7% (234/258) (95% CI: 86.5% to 93.7%)		
NPV		98.2% (537/547) (95% CI: 96.7% to 99.0%)		
Percent Agreement		95.8% (771/805) (95% CI: 94.2% to 97.0%)		

NEG = negative, NPV = negative predictive value, POS = positive, PPV = positive predictive value.

[1] Specimens generating discordant SA test results between the Panther Fusion MRSA assay and the reference assay were further evaluated using an enrichment culture method.

Of the 24 SA false positive Panther Fusion MRSA assay specimens, 11 were found to be SA positive after enriched culture discordant resolution.

Of the 10 SA false negative Panther Fusion MRSA assay specimens 7 were found to be SA negative after enriched culture discordant resolution.

Analytical Sensitivity

The 95% confidence intervals for the limit of detection (LoD) of MRSA and SA with the Panther Fusion MRSA assay were determined by testing simulated nasal matrix (SNM) spiked at multiple concentrations with two MRSA strains and one SA strain. Twenty-one replicates were tested with three reagent lots at each concentration for a total of 63 replicates. Target specific LoD concentrations were determined by Probit analysis and verified by testing an additional ≥20 replicates with one reagent lot. The obtained CFU/mL representing the LoD value for each strain was confirmed by plate count (Table 6).

Table 6: Analytical Sensitivity

Strain	Source (ID)	SCC*mec* Type	Limit of Detection (CFU/mL)
S. aureus (SA), Seattle 1945	ATCC (25923)	N/A	1,833
Methicillin-resistant *S. aureus* (MRSA), NYBK2464	ATCC (BAA-41)	II	2,383
Methicillin-resistant *S. aureus* (MRSA), HPV107	ATCC (BAA-44)	I	1,183

Analytical Reactivity (Inclusivity)

A total of 106 MRSA strains and 22 SA strains were tested with the Panther Fusion MRSA assay in SNM near the assay LoD. All samples tested were correctly identified with the Panther Fusion MRSA assay.

MRSA strains representing 27 countries; SCC*mec* types I, II, III, IV, IV (a-e), IVg, IVh, V, VI, VII, VIII, IX and XI; 14 clonal complexes (CC); 32 sequence types (ST) including ST772 (Bengal Bay clone); and several strains with low and high oxacillin minimum inhibitory concentrations (MIC) were detected with the Panther Fusion MRSA assay. The following PFGE types were reactive in the Panther Fusion MRSA assay: USA100-1200 (including USA300-0114, and Iberian). The Panther Fusion MRSA assay correctly identified and reported 9 empty cassette variant strains and 8 BORSA strains as MRSA negative/SA positive.

Analytical Specificity

The analytical specificity of the Panther Fusion MRSA assay was evaluated by testing 96 non-target organisms commonly present in the nose (Table 7). Bacteria (77 strains) and yeast (2 strains) were tested at concentrations of 10^6 CFU/mL or IFU/mL or copies/mL. Viruses (16 strains) were tested at concentrations of 10^5 PFU/mL. Each organism was added to SNM and tested in the presence and absence of MRSA or SA at 3X LoD.

No cross-reactivity was observed. No interference was observed in the presence of the organism.

Table 7: Microorganisms Commonly Found in Nasal Specimens and Tested for Cross-Reactivity

Viruses		
Adenovirus Type 1	Measles Virus	Influenza A H1N1
Adenovirus Type 7A	Mumps Virus	Parainfluenza Virus Type 1
Cytomegalovirus	Parainfluenza Virus Type 3	Parainfluenza Virus Type 2
Enterovirus Type 68	Respiratory Syncytial Virus Type B	Rhinovirus Type 1A
Human Metapneumovirus (hMPV) 18 Type B2	Coronavirus Strain 229E	
Influenza B	Epstein Barr Virus	
Bacteria		
Acinetobacter baumannii	*Legionella pneumophila*	*Staphylococcus equorum*
Acinetobacter haemolyticus	*Legionella wadsworthii*	*Staphylococcus felis*
Bacillus cereus	*Listeria monocytogenes*	*Staphylococcus gallinarum*
Bordetella pertussis	*Micrococcus luteus*	*Staphylococcus haemolyticus*
Candida albicans	*Moraxella catarrhalis*	*Staphylococcus hominis*
Candida glabrata	*Mycobacterium tuberculosis avirulent*	*Staphylococcus intermedius*
Chlamydia pneumoniae	*Mycoplasma pneumoniae*	*Staphylococcus kloosii*
Citrobacter freundii	*Neisseria gonorrhoea*	*Staphylococcus lentus*
Citrobacter koseri	*Neisseria meningitidis*	*Staphylococcus pasteuri*
Corynebacterium aquaticus (Leifsonia aquatica)	*Pasteurella aerogenes*	*Staphylococcus pulvereri*
Corynebacterium bovis	*Proteus mirabilis*	*Staphylococcus saprophyticus*
Corynebacterium flavescens	*Proteus vulgaris*	*Staphylococcus sciuri*
Corynebacterium genitalium	*Providencia stuartii*	*Staphylococcus simulans*
Cryptococcus neoformans	*Pseudomonas aeruginosa*	*Staphylococcus warneri*
Enterobacter aerogenes	*Pseudomonas fluorescens*	*Staphylococcus xylosus*
Enterobacter cloacae	*Salmonella typhimurium (Salmonella enterica subsp. enterica)*	*Streptococcus agalactiae*
Enterococcus faecalis	*Serratia marcescens*	*Streptococcus anginosus*
Enterococcus faecium	*Shigella sonnei*	*Streptococcus mitis*
Enterococcus flavescens	*Staphylococcus arlettae*	*Streptococcus mutans*
Enterococcus gallinarum	*Staphylococcus auricularis*	*Streptococcus pneumoniae*
Enterococcus hirae	*Staphylococcus capitis*	*Streptococcus pyogenes*
Escherichia coli	*Staphylococcus caprae*	*Streptococcus salivarius*
Haemophilus influenzae	*Staphylococcus carnosus*	*Streptococcus sanguinis*
Klebsiella oxytoca	*Staphylococcus chromogenes*	*Streptococcus suis*
Klebsiella pneumoniae	*Staphylococcus cohnii subsp. Urealyticum*	*Yersinia enterocolitica*
Lactobacillus casei	*Staphylococcus delphini*	
Lactobacillus crispatus	*Staphylococcus epidermidis (MRSE)*	

Competitive Interference

Mixed infections of MRSA with SA, MRSA with *Staphylococcus epidermidis* (MRSE), and SA with MRSE were evaluated with the Panther Fusion MRSA assay by testing the assay target (MRSA or SA) near the limit of detection in the presence of a competing microbial organism at high concentration. The results shown in Table 8 indicate that the sensitivity of MRSA and SA detection was not affected by mixed infections under the conditions tested.

Table 8: Competitive Interference

Competing Microorganism		Target		Panther Fusion MRSA Assay Result	
Description	**Concentration**	**Description**	**Concentration**	**MRSA**	**SA**
SA	1.8 x 10^7 CFU/mL	MRSA	3X LoD	+	+
MRSE	1.8 x 10^7 CFU/mL	MRSA	3X LoD	+	+
MRSE	2.7 x 10^7 CFU/mL	SA	3X LoD	-	+

CFU = colony forming unit, LoD = limit of detection.

Interference

Potentially interfering substances that may be present in the specimens were evaluated with the Panther Fusion MRSA assay. Clinically relevant concentrations of the multiple endogenous and exogenous substances (Table 9) were tested in the absence and presence of MRSA and SA, respectively, near the LoD. None of the substances at the concentrations tested impacted the performance of the Panther Fusion MRSA assay.

Table 9: Potentially Interfering Substances

Type	Substance	Active Ingredients	Concentration
Endogenous	Blood	100% Human Blood	5% v/v
	Mucin	Bovine Mucin from Submaxillary Gland	0.5% w/v
Over-The-Counter Drugs	Afrin	0.05% Oxymetazoline Hydrochloride	15% v/v
	Dristan Nasal Mist	0.05% Oxymetazoline Hydrochloride	15% v/v
	Otrivin	0.1% Xylometazoline Hydrochloride	15% v/v
	Saline Nasal Spray	0.65% Sodium Chloride (0.65%)	15% v/v
	Neo-Synephrine	1.0% Phenylephrine Hydrochloride	15% v/v
	Chloroseptic Throat Lozenge	0.4% Benzocaine (15 mg in 1 lozenge) and 0.3% Methanol (10 mg in 1 Lozenge)	15% w/v
	Zicam Nasal Gel	0.05% Oxymetazoline Hydrochloride	15% w/v
	Flonase	0.05% Fluticasone Propionate	15% v/v
	NasalCrom Nasal Spray	Cromolyn Sodium	15% v/v

Table 9: Potentially Interfering Substances (continued)

Type	Substance	Active Ingredients	Concentration
Prescription Drugs	Taro-Mupirocin, Mupirocin Ointment USP, 2%	Mupirocin	0.5 mg/mL
	Relenza	5 mg Zanamivir	2.0 mg/mL
	Tobramycin	Tobramycin	4.5 mg/mL
	Flunisolide Nasal Solution USP, 0.025%	Flunisolide	0.12 mg/mL
	Beconase AQ	Beclomethasone	0.4 mg/mL

v/v = volume/volume, w/v = weight/volume.

Carryover/Cross-Contamination

Carryover/cross-contamination was evaluated in nine separate runs on three instruments. Each run included interspersed negative samples (SNM) and high positive samples (SNM containing 1 x 10^7 CFU/mL MRSA). The carryover rate was 0.0%.

Assay Precision

Panther Fusion MRSA assay precision was evaluated with contrived specimens at or near the LoD by three operators on two separate runs per day, using three reagent lots on three Panther Fusion instruments over 35 days.

Table 10 shows the positivity rate (%) and percent agreement (95% CI). Table 11 shows the mean and variability analysis of the Ct values between instruments, between lots, between operators, between days, between runs and within runs, and overall Ct.

Table 10: Percent Agreement to the Expected Result

Target	Panel Member		% Positive for Target Type (Positive n/Valid n)	% Agreement (95% CI)
	Description	Concentration (in SNM)		
MRSA	MRSA Moderate Positive	MRSA at 2-3X LoD	100.0% (160/160)	100.0% (97.7 - 100%)
	MRSA Low Positive	MRSA at 1-2X LoD	99.4% (159/160)	99.4.0% (96.5 - 99.9%)
SA	SA Moderate Positive	SA at 2-3X LoD	100.0% (160/160)	100.0% (97.7 - 100%)
	SA Low Positive	SA at 1-2X LoD	100.0% (162/162)	100.0% (97.7 - 100%)
Negative	Negative	SNM only (unspiked)	0.0% (0/162)	100.0% (97.7 - 100%)

CI = confidence interval, LoD = limit of detection, SNM = simulated nasal matrix.

Table 11: Ct Value Variability

Panel Member	Target	POS n	Mean Ct	Between Instruments		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
MRSA Moderate Positive	*orfX*/SCC*mec*	160	33.8	0.0	0.0	0.0	0.0	0.2	0.6	0.2	0.5	0.2	0.6	0.4	1.1	0.5	1.5
	mec A/C	160	35.2	0.1	0.3	0.1	0.3	0.3	1.0	0.2	0.5	0.2	0.5	0.4	1.0	0.6	1.7
	GAPDH	160	33.4	0.1	0.4	0.1	0.2	0.3	0.8	0.1	0.4	0.2	0.5	0.3	0.9	0.5	1.5
MRSA Low Positive	*orfX*/SCC*mec*	160	35.1	0.0	0.1	0.0	0.1	0.2	0.5	0.1	0.3	0.0	0.0	0.6	1.8	0.7	1.9
	mec A/C	160	36.5	0.1	0.3	0.1	0.4	0.3	0.9	0.2	0.5	0.0	0.0	0.6	1.7	0.7	2.0
	GAPDH	159	34.6	0.1	0.4	0.1	0.2	0.3	0.8	0.1	0.4	0.0	0.0	0.5	1.5	0.6	1.9
SA Moderate Positive	GAPDH	160	33.3	0.2	0.5	0.0	0.0	0.3	0.8	0.0	0.0	0.2	0.5	0.4	1.2	0.5	1.6
SA Low Positive	GAPDH	162	34.3	0.2	0.6	0.2	0.5	0.2	0.4	0.0	0.0	0.2	0.7	0.4	1.2	0.6	1.6
Negative	IC	162	35.4	0.6	1.8	0.0	0.0	0.4	1.1	0.3	0.7	0.3	0.8	0.6	1.6	1.0	2.9

Ct = threshold cycle, CV = coefficient of variation, POS = positive, SD = standard deviation.

Bibliography

1. Murray, P., Rosenthal, K., Kobayashi, G., and Pfaller, M. 2002. Medical Microbiology (4th Ed.), pp. 207-216. Mosby, St. Louis, MO.
2. García-Álvarez, L., Holden, M.TG., Lindsay, H., Webb, C.R., Brown, D.FJ., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J., and Holmes, M.A. 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis. 11(8): 595–603. doi: 10.1016/S1473-3099(11)70126-8.
3. Shore, A.C., Deasy, E.C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., and Coleman, D.C. 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 55(8): 3765–3773. doi: 0.1128/AAC.00187-11.
4. Köck, R., Becker, K., Cookson, B., van Gemert-Pijnen, J.E., Harbarth, S., Kluytmans, J., Mielke, M., Peters, G., Skov, R.L., Struelens, M.J., Tacconelli, E., Navarro Torné, A., Witte, W., and Friedrich, A.W. 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 15(41), pii=19688. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19688.
5. Ventola, C.L. 2015. The antibiotic resistance crisis: Part 1: Causes and threats. Pharm Ther. 40(4):277–283.
6. Bode, L.G.M., Kluytmans, J.A.J.W., Wertheim, H.F.L., et al. 2010. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. N Engl J Med. 362(1):9-17.
7. Allawi, H.T., Li, H., Sander, T., et al. 2006. Invader Plus method detects herpes simplex virus in cerebrospinal fluid and simultaneously differentiates types 1 and 2. J Clin Microbiol. 44(9), 3443-3447.
8. Clinical & Laboratory Standards Institute. Document M29: Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI website, https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

EC REP
Hologic N.V.
Da Vincilaan 5
1930 Zaventem
Belgium

Customer Support: +1 800 442 9892
customersupport@hologic.com

Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

AW-18028-001 Rev. 001
2018-06