510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K200839

B Applicant

Clever Culture Systems

C Proprietary and Established Names

APAS Independence with IC Chromogenic MRSA BD Analysis Module; APAS Independence with IC Chromogenic MRSA TFS/S Analysis Module

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QQY	Class II	21 CFR 866.2190 - Automated Image Assessment System For Microbial Colonies On Solid Culture Media	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the APAS Independence with two independent MRSA Analysis Modules

B Measurand:

Digital images of colonies cultured on chromogenic agar plates to determine presence or absence of methicillin-resistant *Staphylococcus aureus* (MRSA)

C Type of Test:

The APAS Independence when using its MRSA Analysis Modules is an *in vitro* diagnostic test system for automated assessment of microbial colonies on chromogenic culture media. The system is for use with anterior nares specimens inoculated on chromogenic agar to aid in screening for MRSA.

III Intended Use/Indications for Use:

A Intended Use(s):

The APAS Independence is an in vitro diagnostic system comprised of an instrument and software analysis module(s) for specific indications that are used to automate imaging and interpretation of microbial colonies on plates of solid culture media.

B Indication(s) for Use:

The APAS Independence is an in vitro diagnostic system comprised of an instrument for automated imaging of agar culture plates and a software analysis module for the following use:

1. The APAS Independence, when using its IC MRSA Chromogenic BD analysis module, automates culture plate imaging and interpretation to detect the presence or absence of colonies with colors suggestive of methicillin-resistant *Staphylococcus aureus* (MRSA) growth on Beckton Dickson BBL CHROMagar MRSA II agar that has been inoculated with anterior nares swabs and incubated at 36°C ± 1°C for 24 hours.

The APAS Independence, when using its IC MRSA Chromogenic BD analysis module, provides an aid in routine screening for colonization with MRSA. It provides one of two screening results: Presumptive MRSA or Negative. All culture plates that are identified as Presumptive MRSA by the APAS Independence, when using the IC MRSA Chromogenic BD analysis module require review by a trained microbiologist.

2. The APAS Independence, when using its IC MRSA Chromogenic TFS/S analysis module, automates culture plate imaging and interpretation to detect the presence or absence of colonies with colors suggestive of methicillin-resistant *Staphylococcus aureus* (MRSA) growth on Thermo-Fisher Spectra MRSA agar that has been inoculated with anterior nares swabs and incubated at 36°C ± 1°C for 24 hours.

The APAS Independence, when using its IC MRSA Chromogenic TFS/S analysis module, provides an aid in routine screening for colonization with MRSA. It provides one of three screening results: Presumptive MRSA, Presumptive non-MRSA, or Negative. All culture plates that are identified as Presumptive MRSA or Presumptive non-MRSA by the APAS Independence, when using the IC MRSA Chromogenic TFS/S analysis module, require review by a trained microbiologist.

C Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only
- The APAS Independence with MRSA Analysis Module is only for use with BBL CHROMagar MRSA II Spectra MRSA chromogenic agar plates inoculated with anterior nares swab specimens.
- The APAS Independence with IC MRSA Chromogenic BD is qualified for use with BBL CHROMagar MRSA II agar from Becton Dickinson (product numbers 215228 and 215229). It has not been evaluated for use with non-chromogenic plates or other chromogenic plates.
- The APAS Independence with IC MRSA Chromogenic TFS/S is qualified for use with Spectra MRSA agar from Thermo-Fisher Scientific (product numbers R01821(A) and R01822(A). It has not been evaluated for use with non-chromogenic plates or other chromogenic plates.
- Organisms other than MRSA may produce a mauve color on this media and produce a presumptive positive result. Laboratories should employ confirmation procedures for all Presumptive MRSA results.
- Some MRSA strains may not produce characteristic chromogenic reactions on the media. Laboratories should consider alternative procedures when considering such strains, or employ the APAS flag functions to assist.

D Special Instrument Requirements:

APAS Independence

IV Device/System Characteristics:

A Device Description:

The APAS Independence is an automated system for the screening of culture plates to assess the presence of microbial growth. The APAS Independence system consists of the following:

- an automated plate handling mechanism that moves culture plates through the instrument,
- an imaging station to capture images of a culture plate, and
- software for analysis of the image, determination of growth and presentation of reports.

A list of the major sub-components of the APAS Independence is provided in **Table 1**. With the exception of the MRSA module software, these components remain the same as those used in the predicate (APAS Independence with Urine Analysis Module, K183648).

Table 1. Major sub-systems of the APAS Independence

Component		Function
Hardware	Imaging Station	LED illumination of culture plates and image capture using a CCD camera
	Automated Plate Handling System	Movement, positioning and sorting of culture plates within the instrument
Software	APAS Controller PC	Controls image capture, analysis, report generation and result storage
	Plate Controller PC	Controls movement of culture plates between the input carriers, imaging station and output carriers or stacks

Component		Function
	Instrument Controller Software	Provides the user interface for operation of the instrument and coordinates the functions of the APAS and Plate Controller PCs
	Analysis Module Software	Installed on the APAS Controller PC to provide the configuration and instructions for image capture and analysis
	LIMS Interface Software	Installed on the Instrument Controller PC to import diagnostic details and provide context for interpretation of MRSA culture results. Imported information may be applied to the system: "*LIMS Force Flag*": automatically forces an APAS result to "Review" irrespective of the growth characteristics observed "*LIMS Complementary Test Flag*": automatically changes a "Negative" designation to "Review" based on user defined rules applied to additional diagnostic information
Quality Control	Color Calibration Tool	Multicolored disk for calibration/checking of system optics
	System Check	Simulated culture plates used to confirm instrument function

The APAS Independence is intended to be installed with multiple, independent software (analysis) modules, each of which will provide an assessment of growth for a specific clinical indication. More than one analysis module may be developed for the same Indication for Use to allow APAS to assess growth on culture plates from multiple agar manufacturers. In this application, two independent MRSA analysis modules have been designed to interpret microbial growth as follows:

- APAS Independence with IC Chromogenic MRSA BD Analysis Module is designed to interpret microbial growth on BBL CHROMagar MRSA II agar from Becton Dickinson
- APAS Independence with IC Chromogenic MRSA TFS/S Analysis Module is designed to interpret microbial growth on Remel Spectra MRSA agar from Thermo Fisher Scientific.

The APAS Independence with the IC Chromogenic MRSA BD and TFS/S Analysis Modules images and evaluates chromogenic agar plates (BBL CHROMagar MRSA II and Remel Spectra MRSA, respectively) to determine and report plate results. Each module has its own reporting scheme (described in **Table 2**) based on the analytical and clinical performance data and corresponding risk mitigations.

Table 2. Reporting results for the IC Chromogenic MRSA BD and TFS/S analysis modules

Chromogenic Agar Result	Chromogenic Agar Description	IC Chromogenic Analysis Modules	
		BD Result	TFS/S Result
Presumptive MRSA Growth	Colonies or growth suggestive of MRSA	Presumptive MRSA	Presumptive MRSA

Chromogenic Agar Result	Chromogenic Agar Description	IC Chromogenic Analysis Modules	
		BD Result	TFS/S Result
Presumptive non-MRSA Growth	Colonies or growth not suggestive of MRSA	Negative	Presumptive non-MRSA
No Growth	No colonies or growth	Negative	Negative

B Principle of Operation:

APAS Independence when using a MRSA analysis module is designed to detect the presence or absence of presumptive methicillin-resistant *Staphylococcus aureus* (MRSA) growth on chromogenic MRSA agar plates. No quantification of growth is required since it is not used to inform clinical decisions. The system comprises an imaging station to capture images of chromogenic MRSA agar plates and a plate handling system that moves plates between the input carriers, imaging station and output carriers or stacks. The software conducts analysis of the images, assessment of microbial colonies (if present), and result designation.

The APAS Independence with MRSA Analysis Modules is indicated for screening MRSA chromogenic agar plates (BBL CHROMagar MRSA II and Remel Spectra MRSA) inoculated with anterior nares swab specimens and incubated for 24 hours. The system takes digital images of each plate that are analyzed automatically to determine if presumptive MRSA colonies are present. For each plate, the APAS Independence performs the following tasks:

a. digitally combines several images of the plate for analysis,
b. categorizes each pixel of the image according to the visually different areas on the plate (e.g., type of growth or agar background),
c. determines how pixels are arranged in relation to each other (e.g., clumps of related pixels may represent a colony),
d. counts the number of colonies per colony type (although colony counts are not reported in the MRSA Analysis Modules),
e. differentiates between MRSA and non-MRSA colony types and reports presumptive results accordingly,
f. incorporates data from the Laboratory Information System (LIS) to provide a final report; when applicable based on clinical context ("flagged"), a "negative" result may be modified to "presumptive MRSA growth" to alert the microbiologist that further investigation may be warranted.

When using the IC MRSA Chromogenic BD Analysis module (BD module), all plates with presumptive MRSA growth are designated for review by a trained microbiologist for determination of status and follow up testing according to conventional laboratory practice. Plates with presumptive non-MRSA growth or no growth are not designated for review and may be discarded according to the standard operating procedures of the laboratory.

When using the IC MRSA Chromogenic TFS/S Analysis module (TFS/S module), all plates with presumptive MRSA growth or presumptive non-MRSA growth are designated for review by a trained microbiologist for determination of status and follow up testing according to conventional laboratory practice. Plates with no growth are not designated for review and may be discarded according to the standard operating procedures of the laboratory.

1. Instrument Name:
   APAS Independence

2. Specimen Identification:
   Plates are identified using an integrated barcode scanner within the Imaging Module of the APAS Independence. The identity of each plate is displayed on the results screen and transmitted automatically to the LIS.

3. Specimen Sampling and Handling:
   The operator loads carriers containing culture plates into the Input Module of the APAS Independence from which they are moved automatically, one plate at a time, to the Imaging Module where the barcode is read, the lid is removed, and images of the agar surface are captured. Upon completion of image capture and analysis, the plate lid is replaced, and the system automatically sorts each plate based on its designation into the appropriate carrier in the Output Module.

4. Calibration:
   The APAS Independence requires daily color calibration prior to analysis of culture plates. Calibration is performed using the Color Check Tool provided with the instrument, a multicolored disk for checking and correcting color of the system optics. Instruction for the daily Color Check is included in the APAS Independence User Manual. In the event of a Color Check failure, the check should be repeated. If the repeated failure occurs, the instrument should not be used until it has been serviced.

5. Quality Control:
   After daily calibration (Color Check), a System Check must be performed followed by biological Quality Control testing.

   The System Check Tool comprises a pair of disks with small dots replicating colonies for checking overall system functionality and associated software. Instruction for daily System Check is provided in the User Manual for the APAS Independence.

   Daily biological Quality Control testing is performed using a reference culture plate inoculated with a MRSA positive strain (e.g., *Staphylococcus aureus* ATCC 43300). Instructions for testing is provided in the User Manuals for the APAS Independence with IC MRSA Chromogenic BD Analysis Module and APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module.

   In the event of a System Check or Quality Control testing failure, the check/testing should be repeated. If the repeated failure occurs, the instrument should not be used until it has been serviced. In the event of a System Check fail, the instrument should be locked to prevent processing of additional culture plates until service has been performed.

V Substantial Equivalence Information:

A Predicate Device Name(s):

APAS Independence with Urine Analysis Module

B Predicate 510(k) Number(s):

K183648

C Comparison with Predicate(s):

Table 3. Comparison with Predicate

Device & Predicate Device(s):	DEVICE: K200839	PREDICATE: K183648
Device Trade Name	APAS Independence with IC Chromogenic MRSA BD Analysis Module; APAS Independence with IC Chromogenic MRSA TFS/S Analysis Module	APAS Independence with Urine Analysis Module
General Device Characteristic Similarities		
Intended Use	The APAS Independence is an *in vitro* diagnostic system comprised of an instrument and software analysis module(s) for specific indications that are used to automate imaging and interpretation of microbial colonies on plates of solid culture media.	Same
Imaging Station	Light Emitting Diode (LED) illumination of culture plates and image capture using a Charge Coupled Device (CCD) camera	Same
Plate Handling	Automated	Same
APAS Controller PC	Controls image capture, analysis, report generation and result storage	Same
Instrument Controller PC	Provides the user interface for the APAS Independence and controls plate movement	Same
Analysis Module	Installed on the APAS Controller PC to provide the configuration and instructions for image capture and analysis	Same
Laboratory Information System (LIS) Interface	Analysis result for each plate sent to the LIS. Sample ID details retrieved from the LIS.	Same
Calibration	Performed daily using a manufacturer-provided Color Check Tool	Same
System Check	Performed daily using the manufacturer-provided System Check Tool to verify instrument and software functionality	Same
General Device Characteristic Differences		

Device & Predicate Device(s):	DEVICE: K200839	PREDICATE: K183648
Indications For Use	The APAS Independence is an *in vitro* diagnostic system comprised of an instrument for automated imaging of agar culture plates and a software analysis module for the following use: 1. The APAS Independence, when using its IC MRSA Chromogenic BD analysis module, automates culture plate imaging and interpretation to detect the presence or absence of colonies with colors suggestive of methicillin-resistant Staphylococcus aureus (MRSA) growth on Beckton Dickson BBL™ CHROMagar™ MRSA II agar that has been inoculated with anterior nares swabs and incubated at 36°C ± 1°C for 24 hours. The APAS Independence, when using its IC MRSA Chromogenic BD analysis module, provides an aid in routine screening for colonization with MRSA. It provides one of two screening results: Presumptive MRSA or Negative. All culture plates that are identified as Presumptive MRSA by the APAS Independence, when using the IC MRSA Chromogenic BD analysis module require review by a trained microbiologist. 2. The APAS Independence, when using its IC MRSA Chromogenic TFS/S analysis module, automates culture plate imaging and interpretation to detect the presence or absence of colonies with colors suggestive of methicillin-resistant Staphylococcus aureus (MRSA) growth on Thermo-Fisher Spectra™ MRSA agar that has been inoculated with anterior nares swabs and incubated at 36°C ± 1°C for 24 hours. The APAS Independence, when using its IC MRSA Chromogenic TFS/S analysis module, provides an aid in routine screening for colonization with MRSA. It provides one of three screening results: Presumptive MRSA, Presumptive non-MRSA, or Negative. All culture plates that are identified as Presumptive MRSA or	The APAS Independence is an *in vitro* diagnostic system comprised of an instrument for automated imaging of agar culture plates and a software analysis module for the following use: The APAS Independence, when using its urine analysis module, automates urine culture plate imaging and interpretation to detect the presence or absence of microbial growth on sheep blood and MacConkey agar culture plates that are inoculated with a 1µL sample volume. The APAS Independence, when using its urine analysis module, provides a semiquantitative assessment of colony counts that are used as an aid in the diagnosis of urinary tract infection. All urine culture plates that are identified as positive for growth by the APAS Independence, when using its urine analysis module, must be reviewed by a trained microbiologist.

Device & Predicate Device(s):	DEVICE: K200839	PREDICATE: K183648
	Presumptive non-MRSA by the APAS Independence, when using the IC MRSA Chromogenic TFS/S analysis module, require review by a trained microbiologist.	
IFU Target	Presumptive MRSA colonies	Microbial growth
Sample type	Anterior nares specimens	Urine samples
Biological Quality Control	Performed daily using standardized suspensions of *Staphylococcus aureus* ATCC 43300 (MRSA positive strain)	Performed daily using standardized suspensions of *Escherichia coli* and *Enterococcus faecalis*

VI Standards/Guidance Documents Referenced:

- **ISO 14971**: Medical devices - Application of Risk Management to Medical Devices; 2007.
- **IEC 61010-1**: Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements; 2010, 3rd edition.
- **IEC 62304**: Medical device software - Software life cycle processes; 2006.
- **IEC 62366-1**: Medical devices – Part 1: Application of usability engineering to medical devices; 2015.
- **IEC 61326-1**: Electrical equipment for measurement, control and laboratory use - EMC Requirements – Part 1: General requirements; 2012.
- **IEC 61326-2-6**: Electrical equipment for measurement, control and laboratory use - EMC Requirements - Part 2-6: Particular Requirements - *In vitro* diagnostic (IVD) medical Equipment; 2012.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

The organisms listed in **Table 4** were used to evaluate the analytical performance of the APAS Independence with MRSA Analysis Modules on both on BBL CHROMagar MRSA II (referred throughout as "BD") and Remel Spectra MRSA (referred throughout as "TFS/S") chromogenic agar plates. The organism codes were internal and used for the purpose of tracking during the studies.

Table 4. Organisms tested in analytical studies

Organism Name (Strain)	Code	Colony Appearance		Interpretation / Designation	Used in Study
		BD	TFS/S		
Bacillus cereus (Stock)	MISC6	Pink	Blue	Negative	Accuracy, Digital Image Quality
Bacillus licheniformis (NCTC 10341)	MISC7	Purple	Blue	Negative	Accuracy, Digital Image Quality
Bacillus spp. (Stock)	MISC2	Purple	Blue	Negative	Accuracy, Digital Image Quality

Organism Name (Strain)	Code	Colony Appearance		Interpretation / Designation	Used in Study
		BD	TFS/S		
Enterococcus faecalis (ATCC 29212)	EF8	Fine blue smear	Fine blue smear	Negative	Accuracy
Staphylococcus aureus (Stock)	MRSA3	Mauve	Denim blue	Presumptive Positive	Accuracy, Reproducibility, LoD, Interference, Range of Assay
Staphylococcus aureus (Stock)	MRSA5	Mauve	Denim blue	Presumptive Positive	Accuracy, Range of Assay
Staphylococcus aureus (ATCC 43300)	MRSA9	Mauve	Denim blue	Presumptive Positive	Accuracy, Reproducibility, LoD, Biological QC, Digital Image Quality
Staphylococcus aureus (ATCC 1707)	MRSA26	Mauve	Denim blue	Presumptive Positive	Accuracy, Digital Image Quality
Staphylococcus aureus (ATCC 13277)	MRSA29	Mauve	Denim blue	Presumptive Positive	Accuracy, Digital Image Quality
Staphylococcus aureus (ATCC 13435)	MRSA31	Mauve	Denim blue	Presumptive Positive	Accuracy, Digital Image Quality
Staphylococcus aureus (ATCC 13626)	MRSA33	Mauve	Denim blue	Presumptive Positive	Accuracy, Digital Image Quality
Staphylococcus haemolyticus (Stock)	SH3	Small white	Small white	Negative	Accuracy, Digital Image Quality
Staphylococcus haemolyticus (Stock)	SH6	Small white	Small white	Negative	Accuracy, Reproducibility, LoD, Interference, Range of Assay, Digital Image Quality
Staphylococcus haemolyticus (Stock)	STSP2	Small white	Small white	Negative	Reproducibility
Staphylococcus warneri (Stock)	STSP4	Small white	Small white	Negative	Range of Assay

BD: BBL CHROMagar MRSA II chromogenic agar
TFS/S: Remel Spectra MRSA chromogenic agar

1. Precision/Reproducibility:

Reproducibility
The reproducibility of presumptive MRSA and non-presumptive MRSA colony detection by the APAS Independence with MRSA Analysis Modules was evaluated with three instruments. Saline was used to evaluate no growth, three dilutions of two MRSA strains (MRSA3 and MRSA9) were used to evaluate presumptive MRSA growth (i.e., mainly confluent, partial confluent, and single colony growth), and three dilutions of two *S. haemolyticus* strains (SH3 and SH6) were used to evaluate non-presumptive MRSA growth (i.e., mainly confluent, partial confluent, and single colony growth) on BD and TFS/S chromogenic agar plates. Each dilution was plated in triplicate and incubated at 35°C 2 ±°C for 24 hours. Five replicate images of each plate were taken at three different orientations (0°, 120° and 270°) on three instruments for a total of 135 images per dilution (3 replicate plates x 5 replicate images x 3 orientations x 3 instruments = 135 images per dilution).

Results of the reproducibility study with the BD and TFS/S MRSA analysis modules are shown in **Table 5** and **Table 6**, respectively. The results demonstrate that the reproducibility between instruments is acceptable.

Table 5. Reproducibility of the APAS Independence with the BD MRSA Analysis Module

Organism Code[1]	Dilution	Percent Agreement							
		Instrument 1		Instrument 2		Instrument 3		Combined	
		Growth[2]	Color[3]	Growth[2]	Color[3]	Growth[2]	Color[3]	Growth[2] [95% CI]	Color[3] [95% CI]
MRSA3	1	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	2	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
MRSA9	1	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	2	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
SH3	1	100% (45/45)	80% (36/45)	100% (45/45)	93.3% (42/45)	100% (45/45)	75.6% (34/45)	100% (135/135) [97.2-100]	83.0% (112/135) [75.7-88.4]
	2	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	97.8% (44/45)	100% (135/135) [97.2-100]	99.3% (134/135) [95.9-99.9]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	93.3% (42/45)	100% (135/135) [97.2-100]	97.8% (132/135) [93.7-99.2]
SH6	1	100% (45/45)	80% (36/45)	100% (45/45)	75.6% (34/45)	100% (45/45)	55.6% (25/45)	100% (135/135) [97.2-100]	70.4% (95/135) [62.2-77.4]
	2	100% (45/45)	97.8% (44/45)	100% (45/45)	88.9% (40/45)	100% (45/45)	82.2% (37/45)	100% (135/135) [97.2-100]	89.6% (121/135) [83.3-93.7]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	95.6% (43/45)	100% (135/135) [97.2-100]	98.5% (133/135) [94.8-99.6]
Saline	-	100% (45/45)	100% (45/45)	97.8% (44/45)	100% (45/45)	95.6% (43/45)	100% (45/45)	97.8% (132/135) [93.7-99.2]	100% (135/135) [97.2-100]

[1] Organism code, as noted in **Table 4**.
[2] Value calculated by dividing the number of images with the expected growth (i.e., growth or no growth detected) by the total number of images.
[3] Value calculated by dividing the number of images with the expected color (i.e., at least one colored colony or no color detected) by the total number of images.

Table 6. Reproducibility of the APAS Independence with the TFS/S MRSA Analysis Module

Organism Code[1]	Dilution	Percent Agreement							
		Instrument 1		Instrument 2		Instrument 3		Combined	
		Growth[2]	Color[3]	Growth[2]	Color[3]	Growth[2]	Color[3]	Growth[2] [95% CI]	Color[3] [95% CI]
MRSA3	1	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	2	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
MRSA9	1	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	2	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
SH3	1	100% (45/45)	75.6% (34/45)	100% (45/45)	93.3% (42/45)	100% (45/45)	95.6% (43/45)	100% (135/135) [97.2-100]	88.1% (119/135) [81.6-92.6]
	2	100% (45/45)	97.8% (44/45)	100% (45/45)	100% (45/45)	100% (45/45)	84.4% (38/45)	100% (135/135) [97.2-100]	94.1% (127/135) [88.7-97.0]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
SH6	1	100% (45/45)	82.2% (37/45)	100% (45/45)	80.0% (36/45)	100% (45/45)	82.2% (37/45)	100% (135/135) [97.2-100]	81.5% (110/135) [74.1-87.1]
	2	100% (45/45)	95.6% (43/45)	100% (45/45)	100% (45/45)	100% (45/45)	91.1% (41/45)	100% (135/135) [97.2-100]	95.6% (129/135) [90.6-97.9]
	3	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (45/45)	100% (135/135) [97.2-100]	100% (135/135) [97.2-100]
No Organism (Saline)	-	66.7% (30/45)	100% (45/45)	66.7% (30/45)	100% (45/45)	68.9% (31/45)	100% (45/45)	67.4% (91/135) [59.1—74.7]	100% (135/135) [97.2-100]

[1] Organism code, as noted in **Table 4**.
[2] Value calculated by dividing the number of images with the expected growth (i.e., growth or no growth detected) by the total number of images.
[3] Value calculated by dividing the number of images with the expected color (i.e., at least one colored colony or no color detected) by the total number of images.

Accuracy

The accuracy in which the APAS Independence with MRSA Analysis Modules is able to correctly assign "presumptive MRSA" and "presumptive non- MRSA" colonies was evaluated with a range of organisms noted in **Table 4**. Organisms were serially diluted in saline and streaked onto BBL CHROMagar MRSA II (BD) and Remel Spectra MRSA (TFS/S) chromogenic agar plates in 2 replicates or 7 replicates to produce either light or confluent growth, respectively, after 24-hour incubation at 36°C ±1°C. At least 100 isolated colonies per plate were digitally labelled by a microbiologist and compared to the corresponding APAS result.

Results of the accuracy study with the BD and TFS/S analysis modules are shown in **Table 7** and **Table 8**, respectively. The results demonstrate that the APAS Independence can accurately detect presumptive MRSA colonies when grown on BD or TFS/S chromogenic agar plates. However, it cannot accurately detect presumptive non-MRSA colonies. This reduced specificity is acceptable since plates with "no growth" or "presumptive non-MRSA growth" do not pose a risk for patient management and are therefore discarded.

Table 7. Accuracy of the APAS Independence with the BD MRSA Analysis Module

		Microbiologist Assigned		
		Presumptive MRSA Colony	**Presumptive Non-MRSA Colony**	**Total**
APAS Assigned	**Presumptive MRSA Colony**	793	23	816
	Presumptive Non-MRSA Colony	3	208	211
	Colony Not Detected	2	77	79
	Total	798	308	1106
Percent Agreement		99.4% (793/798)	67.5% (208/308)	90.5% (1101/1106)

Table 8. Accuracy of the APAS Independence with the TFS/S MRSA Analysis Module

All Colonies Combined		Microbiologist Assigned		
		Presumptive MRSA Colony	**Presumptive Non-MRSA Colony**	**Total**
APAS Assigned	**Presumptive MRSA Colony**	772	262	1034
	Presumptive Non-MRSA Colony	2	226	228
	Colony Not Detected	2	56	58
	Total	776	544	1320
Percent Agreement		99.5% (772/776)	41.5% (226/544)	75.5% (997/1320)

Digital Image Quality Study

A Digital Image Quality study was performed to evaluate the accuracy of microbiologist's interpretation of a digital image compared to reading the agar plate manually (visual read). The data generated in this study were analyzed in two different ways: microbiologist interpretation of a digital image compared to a manual plate read (Table 9-Table 15) and reproducibility of microbiologists' interpretation of a digital image read (Table 16).

a) *Microbiologist interpretation of APAS-generated digital image compared to manual plate read*

The purpose of this study was to assess whether an APAS-generated digital image of a chromogenic agar plate read by a microbiologist can be interpreted equivalent to a manual reading of the chromogenic agar plate (i.e., manual plate read). Three hundred clinical samples (received as swabs and cultured on appropriate agar plates for routine MRSA surveillance) and 100 contrived samples were evaluated. Contrived samples were diluted such that two dilutions were evaluated: medium growth (100-250 CFU/plate) and low growth (1-50 CFU/plate). A panel of three microbiologists blindly interpreted all 400 agar plates and the corresponding 400 digital images that were randomized after a two week "wash out" period to avoid any potential for bias based on prior readings.

A summary of the results with the BD and TFS/S analysis modules is provided in **Table 9**. Data shown by each microbiologist are provided in **Table 10**, **Table 11** and **Table 12** for the BD analysis module as well as in **Table 13**, **Table 14** and **Table 15** for the TFS/S analysis module.

Table 9. Digital Image Quality Summary

		No. of Results	Percent Agreement[1]		
			No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth
BD	Microbiologist 1	400	95.8% (252/263)	89.6% (69/77)	91.7% (55/60)
	Microbiologist 2	400	98.1% (264/269)	98.7% (74/75)	98.2% (55/56)
	Microbiologist 3	400	95.4% (250/262)	97.6% (82/84)	98.1% (53/54)
	Combined	**1200**	**96.4% (766/794)**	**95.3% (225/236)**	**95.9% (163/170)**
TFS/S	Microbiologist 1	400	93.8% (182/194)	87.1% (88/101)	92.4% (97/105)
	Microbiologist 2	400	96.0% (193/201)	90.8% (89/98)	92.1% (93/101)
	Microbiologist 3	400	94.2% (180/191)	86.8% (92/106)	96.1% (99/103)
	Combined	**1200**	**94.7% (555/586)**	**88.2% (269/305)**	**93.5% (289/309)**

[1]Percent agreement determined by dividing number of digital image read results by the number of manual plate read results from the same microbiologist for each designation (i.e., "no growth", "presumptive non-MRSA growth", "presumptive MRSA growth").

The digital image quality study determined that an APAS-generated digital image using the BD module can be equivalently interpreted by a microbiologist compared to reading the plate manually (visual read). This was achieved >95% of the time for each of the three result designations. Importantly, MRSA growth agreement was >95%, which is acceptable to support use of a two-rule designation set (i.e., presumptive MRSA growth and negative) in that only plates with presumptive MRSA growth are required for microbiologist review.

The digital image quality study determined that an APAS-generated digital image using the TFS/S module cannot be equivalently interpreted by a microbiologist compared to reading the plate manually. Agreement was <95% of the time for each result designation. Importantly, presumptive MRSA growth agreement was 93.5%, which is not acceptable to support use of a two-rule designation set. As such, the TFS/S module will use a three-rule designation set (i.e., presumptive MRSA growth, presumptive non-MRSA growth, and negative) in that plates with any growth (presumptive MRSA or presumptive non-MRSA) are required for microbiologist review.

Table 10. APAS Independence with BD MRSA analysis module – Microbiologist 1 Digital Image vs Manual Plate Interpretation

BD analysis module Microbiologist 1		Manual Plate			
		No Growth	**Presumptive Non-MRSA Growth**	**Presumptive MRSA Growth**	**Total**
Digital Image	**No Growth**	252	8	2	262
	Presumptive Non-MRSA Growth	11	69	3	83
	Presumptive MRSA Growth	-	-	55	55
	Total	263	77	60	400
	No Growth Agreement: 95.8% (252/263) Presumptive Non-MRSA Agreement: 89.6% (69/77) Presumptive MRSA Agreement: 91.7% (55/60)				

Table 11. APAS Independence with BD MRSA analysis module – Microbiologist 2 Digital Image vs Manual Plate Interpretation

BD analysis module Microbiologist 2		Manual Plate			
		No Growth	**Presumptive Non-MRSA Growth**	**Presumptive MRSA Growth**	**Total**
Digital Image	**No Growth**	264	1	-	265
	Presumptive Non-MRSA Growth	5	74	1	80
	Presumptive MRSA Growth	-	-	55	55
	Total	269	75	56	400
	No Growth Agreement: 98.1% (264/269) Presumptive Non-MRSA Agreement: 98.7% (74/75) Presumptive MRSA Agreement: 98.2% (55/56)				

Table 12. APAS Independence with BD MRSA analysis module – Microbiologist 3 Digital Image vs Manual Plate Interpretation

BD analysis module Microbiologist 3		Manual Plate			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
Digital Image	No Growth	250	1	-	251
	Presumptive Non-MRSA Growth	10	82	1	93
	Presumptive MRSA Growth	2	1	53	56
	Total	262	84	54	400
	No Growth Agreement: 95.4% (250/262) Presumptive Non-MRSA Agreement: 97.6% (82/84) Presumptive MRSA Agreement: 98.1% (53/54)				

Table 13. APAS Independence with TFS/S MRSA analysis module – Microbiologist 1 Digital Image vs Manual Plate Interpretation

TFS/S analysis module Microbiologist 1		Manual Plate			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
Digital Image	No Growth	182	7	3	192
	Presumptive Non-MRSA Growth	10	88	5	103
	Presumptive MRSA Growth	2	6	97	105
	Total	194	101	105	400
	No Growth Agreement: 93.8% (182/194) Presumptive Non-MRSA Agreement: 87.1% (88/101) Presumptive MRSA Agreement: 92.4% (97/105)				

Table 14. APAS Independence with TFS/S MRSA analysis module – Microbiologist 2 Digital Image vs Manual Plate Interpretation

TFS/S analysis module Microbiologist 2		Manual Plate			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
Digital Image	No Growth	193	8	4	205
	Presumptive Non-MRSA Growth	4	89	4	97
	Presumptive MRSA Growth	4	1	93	98
	Total	201	98	101	400
	No Growth Agreement: 96.0% (193/201) Presumptive Non-MRSA Agreement: 90.8% (89/98) Presumptive MRSA Agreement: 92.1% (93/101)				

Table 15. APAS Independence with TFS/S MRSA analysis module – Microbiologist 3 Digital Image vs Manual Plate Interpretation

TFS/S analysis module Microbiologist 3		Manual Plate			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
Digital Image	No Growth	180	12	3	195
	Presumptive Non-MRSA Growth	10	92	1	103
	Presumptive MRSA Growth	1	2	99	102
	Total	191	106	103	400
	No Growth Agreement: 94.2% (180/191) Presumptive Non-MRSA Agreement: 86.8% (92/106) Presumptive MRSA Agreement: 96.1% (99/103)				

b) Reproducibility of the APAS-generated digital image interpretation

The data obtained from the digital image quality study was further analyzed to evaluate the ability of an APAS-generated digital image to be reproducibly interpreted by a panel of microbiologists. Here, each microbiologist's interpretation of a digital image was compared to the final panel interpretation of the digital image (i.e., majority result). The study determined that a digital image generated with both the BD and TFS/S analysis modules can be reproducibly interpreted by a microbiologist. This was achieved >95% of the time for each of the three result designations (summarized in **Table 16**), which is acceptable.

Table 16. Digital Image Reproducibility Summary

		No. of Results	Percent Agreement[1]		
			No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth
BD analysis module	Microbiologist 1	400	98.9% (260/263)	97.6% (80/82)	100% (55/55)
	Microbiologist 2	400	100% (263/263)	97.6% (80/82)	100% (55/55)
	Microbiologist 3	400	95.4% (251/263)	100% (82/82)	100% (55/55)
	Combined	**1200**	**98.1% (774/789)**	**98.4% (242/246)**	**100% (165/165)**
TFS/S analysis module	Microbiologist 1	400	98.0% (192/196)	95.1% (98/103)	98.0% (99/101)
	Microbiologist 2	400	100% (196/196)	93.2% (96/103)	97.0% (98/101)
	Microbiologist 3	400	99.0% (194/196)	97.1% (100/103)	98.0% (99/101)
	Combined	**1200**	**99.0% (582/588)**	**95.1% (294/309)**	**97.7% (296/303)**

[1]Percent agreement determined by dividing number of individual microbiologist digital read results by the number of panel digital image microbiologist results for each designation (i.e., "no growth", "presumptive non-MRSA growth", "presumptive MRSA growth").

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Microbial Interference

A study was conducted to assess the ability of APAS Independence with MRSA Analysis Modules to accurately detect MRSA in the presence of non-MRSA growth within the recommended incubation time for each respective plate (i.e., 20 – 26 hours for BD and 24 hours for TFS/S chromogenic agar plates).

Two MRSA (MRSA3 and MRSA5) isolates and two non-MRSA (*Staphylococcus haemolyticus*, SH6 and *Staphylococcus warneri*, STSP4) organisms were prepared as pure and mixed cultures and diluted accordingly to produce culture plates with mainly confluent growth (>100 colonies per plate) or light growth (1 – 100 colonies per plate) in five replicates. After 24 hours of incubation, a panel of three microbiologists evaluated the presence or absence of growth and color indicative of MRSA. The majority panel result (i.e., final panel interpretation) was compared to the APAS result to determine agreement for growth and color.

The data provided in **Table 17** and **Table 18** supports interpretation of the BD and TFS/S chromogenic agar plates after 24 hours of incubation.

Table 17. Microbial Interference - APAS Independence with the BD MRSA Analysis Module

Organism(s) Plated (Organism Code[2])	Expected Growth		Percent Agreement[1]	
	MRSA	Other	Growth	Color
MRSA (MRSA3)	light	-	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA3)	confluent	-	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA5)	light	-	100.0% (3/3)	100.0% (3/3)
MRSA (MRSA5)	confluent	-	100.0% (7/7)	100.0% (7/7)
S. haemolyticus (SH6)	-	light	100.0% (5/5)	100.0% (5/5)
S. haemolyticus (SH6)	-	confluent	100.0% (5/5)	60.0% (3/5)
S. warneri (STSP4)	-	light	100.0% (5/5)	100.0% (5/5)
S. warneri (STSP4)	-	confluent	100.0% (5/5)	80.0% (4/5)
MRSA (MRSA3) and *S. haemolyticus* (1:1)	confluent	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA5) and *S. warneri* (1:1)	confluent	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA3) and *S. haemolyticus* (1:100)	light	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA5) and *S. warneri* (1:100)	light	confluent	100.0% (5/5)	100.0% (5/5)

[1] Agreement between majority panel microbiologist result (i.e., final panel interpretation) and APAS result.
[2] Organism code, as noted in **Table 4**.

Table 18. Microbial Interference - APAS Independence with the TFS/S MRSA Analysis Module

Organism(s) Plated (Organism Code[2])	Expected CFU		Percent Agreement[1]	
	MRSA	Other	Growth	Color
MRSA (MRSA3)	light	-	100.0% (4/4)	100.0% (4/4)
MRSA (MRSA3)	confluent	-	100.0% (6/6)	100.0% (6/6)
MRSA (MRSA5)	light	-	100.0% (2/2)	100.0% (2/2)

Organism(s) Plated (Organism Code[2])	Expected CFU		Percent Agreement[1]	
	MRSA	Other	Growth	Color
MRSA (MRSA5)	confluent	-	100.0% (8/8)	100.0% (8/8)
S. haemolyticus (SH6)	-	light	100.0% (5/5)	100.0% (5/5)
S. haemolyticus (SH6)	-	confluent	100.0% (5/5)	80.0% (4/5)
S. warneri (STSP4)	-	light	100.0% (5/5)	100.0% (5/5)
S. warneri (STSP4)	-	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA3) and *S. haemolyticus* (1:1)	confluent	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA5) and *S. warneri* (1:1)	confluent	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA3) and *S. haemolyticus* (1:100)	light	confluent	100.0% (5/5)	100.0% (5/5)
MRSA (MRSA5) and *S. warneri* (1:100)	light	confluent	100.0% (5/5)	100.0% (5/5)

[1] Agreement between majority panel microbiologist result (i.e., final panel interpretation) and APAS result.
[2] Organism code, as noted in **Table 4**.

Analytical Specificity / Limit of Blank

A study was conducted to evaluate the potential influence of various external factors on image quality and the ability of the APAS Independence with MRSA Analysis Modules to provide correct results in the presence of known factors. Chromogenic agar plates (BD or TFS/S) were prepared to concurrently simulate a combination of four interference mechanisms (label, applicator, transport media and pen marking). Three blank applicators (flocked swab, cotton swab and 10 µL loop) were used to inoculate chromogenic agar plates using three different transport media (Stuart Transport, Amies Transport and saline) and no transport media (dry). One of four different labels were applied to plates that were either marked with felt-tip pen or not (3 applicators x 4 transport media x 4 labels x 2 marks = 96 plates per chromogenic agar). Although each interference mechanism was evaluated concurrently (i.e., four variables evaluated on each plate), performance is stratified by each interference variable in **Table 19**.

Table 19. Analytical Specificity (Limit of Blank) Summary

Interference Mechanism	Interference Variable	Total number of images evaluated	BD Agreement		TFS/S Agreement	
			Expected Growth (%)[1]	Expected Color (%)[2]	Expected Growth (%)[1]	Expected Color (%)[2]
Label Type	Paper 1D barcode on base	24	20/24 (83.3)	24/24 (100)	58.3 (14/24)	100.0 (24/24)
	Paper 2D barcode on base	24	19/24 (79.2)	23/24 (95.8)	66.7 (16/24)	100.0 (24/24)
	Plastic 2D barcode on base	24	18/24 (75.0)	24/24 (100)	54.2 (13/24)	100.0 (24/24)
	Paper 1D barcode on side	24	17/24 (70.8)	24/24 (100)	58.3 (14/24)	91.7 (22/24)
	Cotton Swab	32	21/32 (65.6)	31/32 (96.9)	62.5 (20/32)	100.0 (32/32)

Interference Mechanism	Interference Variable	Total number of images evaluated	BD Agreement		TFS/S Agreement	
			Expected Growth (%)[1]	Expected Color (%)[2]	Expected Growth (%)[1]	Expected Color (%)[2]
Applicator Type	Flocked Swab	32	27/32 (84.3)	32/32 (100)	50.0 (16/32)	93.8 (30/32)
	Loop	32	26/32 (81.2)	32/32 (100)	65.6 (21/32)	100.0 (32/32)
Transport Media Type	Amies Transport	24	22/24 (91.7)	24/24 (100)	62.5 (15/24)	95.8 (23/24)
	Stuart Transport	24	13/24 (54.2)	23/24 (95.8)	12.5 (3/24)	95.8 (23/24)
	Saline	24	21/24 (87.5)	24/24 (100)	91.7 (22/24)	100.0 (24/24)
	Dry	24	18/24 (75.0)	24/24 (100)	70.8 (17/24)	100.0 (24/24)
Pen Markings	No Pen	48	36/48 (75.0)	47/48 (97.9)	62.5 (30/48)	97.9 (47/48)
	With Pen	48	38/48 (79.2)	48/48 (100)	56.2 (27/48)	97.9 (47/48)

[1] Value calculated by dividing the number of images with the expected growth (i.e., no growth detected) by the total number of images.
[2] Value calculated by dividing the number of images with the expected color (i.e., no color detected) by the total number of images.

In general, the interference variables did interfere with the ability of the APAS Independence with MRSA Analysis Modules to provide accurate "no growth" result, which may produce false positives growth. However, since BD plates with "presumptive MRSA growth" and TFS/S plates with "presumptive MRSA growth" and "presumptive non-MRSA growth" are reviewed by microbiologists before confirmatory testing, the risk for an erroneous result being reported for patient management is mitigated by the review. In addition, plates with "no growth" or "presumptive non-MRSA growth" do not pose a risk for patient management and are therefore discarded.

Analytical Specificity / Interference

The ability in which the APAS Independence with MRSA Analysis Modules can accurately detect presumptive MRSA and presumptive non-MRSA colonies in the presence of known interferences was evaluated. Three applicators (flocked swab, cotton swab and 10 µL loop) were used to inoculate chromogenic agar plates using two different media (Stuart Transport and Amies Transport). Plates were labelled with one of four different labels applied to the base or side of the plate that were either marked with felt-tip pen or not (3 applicators x 2 media x 4 labels x 2 marks = 48 plates per organism, per chromogenic agar). One MRSA (MRSA3) and one non-MRSA (*S. haemolyticus*, SH6*)* isolate were prepared to a 0.5 McFarland suspension and diluted accordingly to produce predominantly isolated colonies or heavy growth, respectively, when cultured. Plates were incubated at 36°C ±1°C for 24 hours then imaged. Performance is summarized in **Table 20**.

Table 20. Analytical Specificity (Interference) Summary

Organism (Code[1])	Interference Mechanism	Interference Variable	Number of images evaluated	BD Agreement		TFS/S Agreement	
				Growth detected (%)[2]	Color detected (%)[3]	Growth detected (%)[2]	Color detected (%)[3]
MRSA (MRSA3)	Label Type	Paper 1D barcode on base	12	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)
		Paper 2D barcode on base	12	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)

Organism (Code[1])	Interference Mechanism	Interference Variable	Number of images evaluated	BD Agreement		TFS/S Agreement	
				Growth detected (%)[2]	Color detected (%)[3]	Growth detected (%)[2]	Color detected (%)[3]
		Plastic 2D barcode on base	12	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)
		Paper 1D barcode on side	12	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)
	Applicator Type	Cotton Swab	16	16/16 (100)	16/16 (100)	16/16 (100)	16/16 (100)
		Flocked Swab	16	16/16 (100)	16/16 (100)	16/16 (100)	16/16 (100)
		Loop	16	16/16 (100)	16/16 (100)	16/16 (100)	16/16 (100)
	Media Type	Amies Transport	24	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
		Stuart Transport	24	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
	Pen Markings	No Pen	24	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
		With Pen	24	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
S. haemolyticus (SH6*)*	Label Type	Paper 1D barcode on base	12	12/12 (100)	5/12 (41.7)	12/12 (100)	12/12 (100)
		Paper 2D barcode on base	12	12/12 (100)	2/12 (16.7)	12/12 (100)	12/12 (100)
		Plastic 2D barcode on base	12	12/12 (100)	6/12 (50.0)	12/12 (100)	12/12 (100)
		Paper 1D barcode on side	12	12/12 (100)	12/12 (100)	12/12 (100)	11/12 (91.7)
	Applicator Type	Cotton Swab	16	16/16 (100)	7/16 (43.8)	16/16 (100)	15/16 (93.8)
		Flocked Swab	16	16/16 (100)	8/16 (50.0)	16/16 (100)	16/16 (100)
		Loop	16	16/16 (100)	10/16 (62.5)	16/16 (100)	16/16 (100)
	Media Type	Amies Transport	24	24/24 (100)	12/24 (50.0)	24/24 (100)	24/24 (100)
		Stuart Transport	24	24/24 (100)	13/24 (54.2%)	24/24 (100)	23/24 (95.8)
	Pen Markings	No Pen	24	24/24 (100)	13/24 (54.2%)	24/24 (100)	23/24 (95.8)
		With Pen	24	24/24 (100)	12/24 (50.0)	24/24 (100)	24/24 (100)

[1] Organism code, as noted in **Table 4**.
[2] Value calculated by dividing the number of images with the expected growth (i.e., growth detected or no growth detected) by the total number of images.
[3] Value calculated by dividing the number of images with the expected color (i.e., no color detected) by the total number of images.

In general, the interference variables tested had no impact on the ability of the BD and TFS/S MRSA Analysis Modules to detect presumptive MRSA growth (100% detection). False positive results were obtained with the *S. haemolyticus* samples; however, the risk of false positive results is mitigated since all plates with "presumptive MRSA growth" are reviewed by a microbiologist prior to confirmatory testing.

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The device labeling indicates that the APAS Independence with MRSA Analysis Module requires daily color calibration and a System Check using dedicated tools provided with the instrument. In addition, daily biological Quality Control testing must be performed using reference culture plates inoculated with a MRSA positive strain (e.g., *Staphylococcus aureus* ATCC 43300). In order to pass, results from the culture media must meet the expected designation (i.e., "presumptive MRSA growth"). There were 22 QC plates tested during the clinical study and 8 QC plates tested during the analytical studies. All QC results passed each day of testing with both the BD and TFS/S MRSA analysis modules.

6. Detection Limit:

   Limit of Detection
   A study was conducted to determine the smallest colony size that can be detected by the APAS Independence with MRSA Analysis Modules. The effect of colony size on the ability of APAS Independence with MRSA Analysis Modules to detect presumptive MRSA colonies was evaluated. Two strains of MRSA (MRSA3 and MRSA9) used to evaluate presumptive MRSA growth were prepared as 0.5 McFarland suspensions in saline, diluted and plated in six replicates to grow < 100 colonies per plate. Plates were incubated at 36°C ±1°C until pinpoint colonies were observed. Then, plates were imaged and returned to the incubator at 1-hour intervals until the colonies on the plates were approximately 2 mm in diameter (up to 24 hours).

   Table 21 summarizes the percent of colonies detected by APAS by colony size (pixels and extrapolated mm). The smallest colony size (SCZ) that was detected as presumptive MRSA >95% was determined to be the LoD. The SCZ detected by the BD analysis module was 0.815 mm for MRSA3 and 0.906 mm for MRSA9. The SCZ detected by the TFS/S analysis module was 0.634 mm for MRSA3 and 0.543 mm for MRSA 9.

Table 21. Limit of Detection (Smallest Colony Size Detected)

		APAS-Detected MRSA Colonies / Total Number of Colonies (% of total)			
Colony Size (diameter)		**BD**		**TFS/S**	
Pixels	**Extrapolated mm**	**MRSA3[1]**	**MRSA9[1]**	**MRSA3[1]**	**MRSA9[1]**
1 – 3	0.091 – 0.272	0/399 (0%)	1/264 (0.4%)	0/285 (0%)	3/163 (1.8%)
4 – 5	0.362 – 0.453	4/258 (1.6%)	3/175 (1.7%)	12/159 (7.5%)	98/228 (43.0%)
6	0.543	7/135 (5.2%)	9/93 (9.7%)	63/89 (70.8%)	129/132 (97.7%)
7	0.634	21/113 (18.6%)	18/79 (22.8%)	145/148 (98.0%)	100/100 (100%)
8	0.725	66/91 (72.5%)	36/84 (42.9%)	197/198 (99.5%)	71/71 (100%)
9	0.815	117/123 (95.1%)	50/74 (67.6%)	167/167 (100%)	43/43 (100%)
10	0.906	147/150 (98.0%)	84/86 (97.7%)	-	-
11	0.996	130/130 (100%)	95/96 (99.0%)	-	-
12	1.087	156/156 (100%	90/90 (100%)	-	-

[1] Organism code, as noted in **Table 4**.

7. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

   The clinical performance of the APAS Independence with MRSA Analysis Modules was evaluated in a two-part study. The overall data from both parts were used collectively in the evaluation of performance.

   For part 1 of the clinical study, the goal was to evaluate performance of the APAS Independence with MRSA Analysis Modules by inoculating prospective and contrived clinical samples onto chromogenic agar media and comparing APAS-generated results to a panel (i.e., majority) result. This part of the study was performed at a single clinical site (outside United States, oUS) using remnant swab specimens that were leftover from routine, standard of care testing from various healthcare facilities. Each swab specimen was subjected to PCR testing for the detection of MRSA. Positive PCR samples were sent to a confirmatory lab for culture. Both positive and negative PCR samples were inoculated onto chromogenic agar and shipped to a central laboratory for incubation and imaging. A portion of PCR-negative samples were also used to generate contrived-negative and contrived-positive samples to supplement the study. The on-screen (digital) images were interpreted by a panel of three oUS microbiologists. The majority result was considered the truth state (i.e., final panel interpretation) and used as a comparison to the APAS-generated result.

   For part 2 of the clinical study, the goal was to assess the ability of a panel of three US microbiologists to interpret the same set of randomized digital images already acquired and reported in part 1. The microbiologists were blinded to the other interpretations and plate source (clinical or contrived). The majority result was compared to the historical APAS result obtained in the first part of the study.

   The following 1590 samples were enrolled in the clinical study:
   - 1100 PCR-negative clinical samples
   - 25 PCR-positive clinical samples
   - 60 PCR-negative, contrived-negative samples
   - 405 PCR-negative, contrived-positive samples

Thirteen samples were excluded from the BD analysis due to defects in the agar resulting in a total of 1573 BD plates analyzed. Ten samples were excluded from the TFS/S analysis due to defects in the agar, resulting in a total of 1580 TFS/S plates analyzed.

Contrived samples were used due to the low incidence of MRSA in the population. This was acceptable since the APAS Independence with MRSA Analysis Modules only images and interprets microbial colonies (i.e., it is not a selective and differential medium). Leftover PCR-negative swab specimens were spiked (1 – 10,000 CFU/plate) with MRSA organisms known to produce a positive reaction on the agar ("presumptive MRSA growth") or organisms known to grow on the agar but not produce a positive reaction ("presumptive non-MRSA growth"). Some organisms included in the contrived samples are representative of the most common strains from the US, Europe, United King and Australia (**Table 22**). The other contrived samples were spiked with well-characterized stock clinical isolates obtained by LBT Innovations and identification confirmed using MALDI-TOF with methicillin susceptibility/resistance confirmed using well-accepted methods (i.e., oxacillin MIC assay using an FDA-cleared device or cefoxitin disk diffusion assay).

Table 22. Global MRSA strains used in simulated samples

MRSA Strain (Organism Number)	Type	Other Name & Information
NCTC 13395 (MRSA30)	HA	IRISH 2/ IBERIAN ST 247. It is CC8 but different from USA 300.
NCTC 13277 (MRSA29)	HA	MRSA-252
NCTC 13656 (MRSA34)	CA	PVL-negative CA-MRSA strain belonging to clonal complex 59, a clone that originated in East Asia.
NCTC 13435 (MRSA31)	CA	PVL-positive CA-MRSA strain belonging to clonal complex 80, commonly known as European clone of CA MRSA
NCTC 13616 (MRSA32)	HA	EMRSA 15, widely distributed in Europe, this HA strain has now been found in the USA
ATCC 1707 (MRSA26)	CA	USA 400, ST1-IV
ATCC 1717 (MRSA27)	CA/HA	USA 300, ST8-IV, causes most community-associated MRSA infections and is an increasingly common cause of health care–associated MRSA infections
ATCC 1761 (MRSA28)	HA	USA100 MRSA

HA: Hospital-associated MRSA strain
CA: Community-associated MRSA strain

Part 1 Clinical Study

Performance of the APAS Independence with MRSA Analysis Modules for the first part of the clinical study (i.e., oUS site) is shown in **Table 23** (clinical samples), **Table 24** (contrived samples), and **Table 25** (clinical and contrived samples combined). The overall agreement of the BD MRSA analysis module (when evaluating combined samples) is 82.6% for "no growth" detection, 84.6% for "presumptive non-MRSA growth", and 100% for "presumptive MRSA growth". The overall agreement of the TFS/S MRSA analysis module (when evaluating clinical and contrived samples, combined) is 81.8% for "no growth" detection, 83.8% for "presumptive non-MRSA growth", and 99.2% for "presumptive MRSA growth".

Table 23. Clinical Study Part 1 - *Clinical Samples*

Clinical Samples		Panel of oUS Microbiologists							
		BD (clinical)				TFS/S (clinical)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	730	11	-	741	508	4	1	513
	Presumptive Non-MRSA Growth	148	179	-	327	114	316	3	433
	Presumptive MRSA Growth	3	13	34	50	1	62	113	176
	Total	881	203	34	1118	623	382	117	1122
		No Growth Agreement: 82.9% (730/881) Presumptive Non-MRSA Agreement: 88.2% (179/203) Presumptive MRSA Agreement: 100% (34/34)				No Growth Agreement: 81.5% (508/623) Presumptive Non-MRSA Agreement: 82.7% (316/382) Presumptive MRSA Agreement: 96.6% (113/117)			

Table 24. Clinical Study Part 1 - *Contrived Samples*

Clinical Samples		Panel of oUS Microbiologists							
		BD (contrived)				TFS/S (contrived)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	24	-	-	24	18	1	-	19
	Presumptive Non-MRSA Growth	8	47	-	55	2	67	-	69
	Presumptive MRSA Growth	-	17	359	376	-	7	363	370
	Total	32	64	359	455	20	75	363	458
		No Growth Agreement: 75.0% (24/32) Presumptive Non-MRSA Agreement: 73.4% (47/64) Presumptive MRSA Agreement: 100% (359/359)				No Growth Agreement: 90.0% (18/20) Presumptive Non-MRSA Agreement: 89.3% (67/75) Presumptive MRSA Agreement: 100% (363/363)			

Table 25. Clinical Study Part 1 - *Combined Samples*

Combined Samples		Panel of oUS Microbiologists							
		BD (combined)				TFS/S (combined)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	754	11	-	765	526	5	1	532
	Presumptive Non-MRSA Growth	156	226	-	382	116	383	3	502
	Presumptive MRSA Growth	3	30	393	426	1	69	476	546
	Total	913	267	393	1573	643	457	480	1580
		No Growth Agreement: 82.6% (754/913) Presumptive Non-MRSA Agreement: 84.6% (226/267) Presumptive MRSA Agreement: 100% (393/393)				No Growth Agreement: 81.8% (526/643) Presumptive Non-MRSA Agreement: 83.8% (383/457) Presumptive MRSA Agreement: 99.2% (476/480)			

Part 2 Clinical Study

Performance of the APAS Independence with MRSA Analysis Modules for the second part of the clinical study (i.e., US site) is shown in **Table 26** (clinical samples), **Table 27** (contrived samples), and **Table 28** (clinical and contrived samples combined). The overall agreement of the BD MRSA analysis module (when evaluating combined samples) is 82.7% for "no growth" detection, 84.0% for "presumptive non-MRSA growth", and 99.7% for "presumptive MRSA growth". The overall agreement of the TFS/S MRSA analysis module (when evaluating clinical and contrived samples, combined) is 79.7% for "no growth" detection, 76.4% for "presumptive non-MRSA growth", and 99.5% for "presumptive MRSA growth".

Table 26. Clinical Study Part 2 - *Clinical Samples*

Clinical Samples		Panel of US Microbiologists							
		BD (clinical)				TFS/S (clinical)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	731	10	-	741	509	3	1	513
	Presumptive Non-MRSA Growth	149	177	1	327	130	302	1	433
	Presumptive MRSA Growth	3	15	32	50	2	102	72	176
	Total	883	202	33	1118	641	407	74	1122
		No Growth Agreement: 82.8% (731/883) Presumptive Non-MRSA Agreement: 87.6% (177/202) Presumptive MRSA Agreement: 97.0% (32/33)				No Growth Agreement: 79.4% (509/641) Presumptive Non-MRSA Agreement: 74.2% (302/407) Presumptive MRSA Agreement: 97.3% (72/74)			

Table 27. Clinical Study Part 2 - *Contrived Samples*

Contrived Samples		Panel of US Microbiologists							
		BD (contrived)				TFS/S (contrived)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	24	-	-	24	18	1	-	19
	Presumptive Non-MRSA Growth	6	49	-	55	2	67	-	69
	Presumptive MRSA Growth	-	18	358	376	-	8	362	370
	Total	30	67	358	455	20	76	362	458
		No Growth Agreement: 80.0% (24/30) Presumptive Non-MRSA Agreement: 73.1% (49/67) Presumptive MRSA Agreement: 100% (358/358)				No Growth Agreement: 90.0% (18/20) Presumptive Non-MRSA Agreement: 88.2% (67/76) Presumptive MRSA Agreement: 100% (362/362)			

Table 28. Clinical Study Part 2 - *Combined Samples*

Combined Samples		Panel of US Microbiologists							
		BD (combined)				TFS/S (combined)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
APAS	No Growth	755	10	-	765	527	4	1	532
	Presumptive Non-MRSA Growth	155	226	1	382	132	369	1	502
	Presumptive MRSA Growth	3	33	390	426	2	110	434	546
	Total	913	269	391	1573	661	483	436	1580
		No Growth Agreement: 82.7% (755/913) Presumptive Non-MRSA Agreement: 84.0% (226/269) Presumptive MRSA Agreement: 99.7% (390/391)				No Growth Agreement: 79.7% (527/661) Presumptive Non-MRSA Agreement: 76.4% (369/483) Presumptive MRSA Agreement: 99.5% (434/436)			

An analysis was conducted to assess concordance of results between part 1 and part 2 of the clinical study. As shown in **Table 29**, the combined data from part 1 (oUS) was used as the comparator for the combined data from part 2 (US). The overall agreement of the BD MRSA analysis module is 98.6% for "no growth" detection, 94.8% for "presumptive non-MRSA growth", and 99.2% for "presumptive MRSA growth". The overall agreement of the TFS/S MRSA analysis module is 99.1% for "no growth" detection, 94.5% for "presumptive non-MRSA growth", and 90.6% for "presumptive MRSA growth".

Table 29. Clinical Study Part 1 (oUS) vs Part 2 (US) - *Combined Samples*

Combined Samples		oUS Results							
		BD (combined)				TFS/S (combined)			
		No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total	No Growth	Presumptive Non-MRSA Growth	Presumptive MRSA Growth	Total
US Results	No Growth	900	13	-	913	637	24	-	661
	Presumptive Non-MRSA Growth	13	253	3	269	6	432	45	483
	Presumptive MRSA Growth	-	1	390	391	-	1	435	436
	Total	913	267	393	1573	643	457	480	1580
		No Growth Agreement: 98.6% (900/913) Presumptive Non-MRSA Agreement: 94.8% (253/267) Presumptive MRSA Agreement: 99.2% (390/393)				No Growth Agreement: 99.1% (637/643) Presumptive Non-MRSA Agreement: 94.5% (432/457) Presumptive MRSA Agreement: 90.6% (435/480)			

Overall Performance of the APAS Independence with the MRSA Analysis Modules

The overall performances for the BD MRSA and TFS/S MRSA analysis modules were evaluated to determine the possible claims that could be supported and how performance issues could be mitigated. As a result, considering data and supportive metrics from other studies, it was concluded that either a two-designation rule set (i.e., presumptive MRSA growth and negative) or a three-designation rule set (i.e., presumptive MRSA growth, presumptive non-MRSA growth, and negative) would be appropriate.

a) *Overall Performance of the APAS Independence with the BD MRSA Analysis Modules*

The overall performance of the BD MRSA analysis modules for detection of presumptive MRSA growth (both in the analytical studies and clinical study) is acceptable to support a two-designation rule set (i.e., presumptive MRSA growth and negative). As such, only plates with "presumptive MRSA growth" require review by a microbiologist. The acceptability of the two-designation rule set is based on the following performance metrics:

- >95% agreement for "presumptive MRSA growth" in the digital image quality study **(Table 9**)
- >98% agreement for "presumptive MRSA growth" in part 1 (**Table 25**) and part 2 clinical data (**Table 28**)
- >95% agreement for "presumptive MRSA growth" when comparing part 1 and part 2 clinical data (**Table 29**)

b) Overall Performance of the APAS Independence with the TFS/S MRSA Analysis Modules
The overall performance of the TFS/S MRSA analysis modules for detection of presumptive MRSA growth (both in the analytical studies and clinical study) is acceptable to support a three-designation rule set (i.e., presumptive MRSA growth, presumptive non-MRSA growth, and negative). As such, all plates with growth ("presumptive MRSA growth" or "presumptive non-MRSA growth) require review by a microbiologist. The acceptability of the three-designation rule set is based on the following performance metrics:

- <95% agreement for "presumptive MRSA growth" in the digital image quality study **(Table 9**)
- >98% agreement for "presumptive MRSA growth" in part 1 (**Table 25**) and part 2 clinical data (**Table 28**)
- <95% agreement for "presumptive MRSA growth" when comparing part 1 and part 2 clinical data (**Table 29**)

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

A summary of the number and percentage of samples with the different result interpretations observed during parts 1 (oUS) and 2 (US) of the clinical study, as determined by the panel of oUS and US microbiologists (reference), and by the APAS Independence with MRSA Analysis Modules, is described in **Table 30**.

Table 30. Microbiologists (Reference) and APAS Results from Clinical Study

Interpretation	BD[1]			TFS/S		
	Microbiologists		APAS	Microbiologists		APAS
	oUS	US		oUS	US	
No Growth	913 (58.0%)	913 (58.0%)	765 (48.6%)	643 (40.7%)	661 (41.8%)	532 (33.7%)
Presumptive Non-MRSA Growth	267 (17.0%)	269 (17.1%)	382 (24.3%)	457 (28.9%)	483 (30.6%)	502 (31.8%)
Presumptive MRSA Growth	393 (25.0%)	391 (24.9%)	426 (27.1%)	480 (30.4%)	436 (27.6%)	546 (34.6%)
TOTAL	1573	1573	1573	1580	1580	1580

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.