October 28, 2021

Clever Culture Systems
Julie Winson
Regulatory Affairs Manager
Seestrasse 204a
Bach, CH-8806
Switzerland

Re: K200839
Trade/Device Name: APAS Independence with IC Chromogenic MRSA BD Analysis Module; APAS Independence with IC Chromogenic MRSA TFS/S Analysis Module
Regulation Number: 21 CFR 866.2190
Regulation Name: Automated Image Assessment System For Microbial Colonies On Solid Culture Media
Regulatory Class: Class II
Product Code: QQY
Dated: March 27, 2020
Received: March 31, 2020

Dear Julie Winson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar, Ph.D. (ABMM)
Chief,
General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K200839

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

Indications for Use *(Describe)*

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

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

5. 510(k) Summary

5.1 Submitter

Clever Culture Systems AG
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Date prepared: 19 October 2021

5.2 Device

The information presented in this 510(k) premarket notification submission is for **two devices**, as follows:

Name of Devices	**APAS Independence with IC MRSA Chromogenic BD Analysis Module** **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module**
Model Numbers	AI-16001 - APAS Independence instrument AI-16053 - IC MRSA Chromogenic BD Analysis Module AI-16057 - IC MRSA Chromogenic TFS/S Analysis Module
Common or Usual Name	(Both Devices) APAS Independence with MRSA Analysis Module
Classification Name:	Automated image assessment system for microbial colonies on solid culture media (21 CFR 866.2190).
Regulatory Class:	II (special controls).
Product Code	QQY

5.3 Predicate Device

The predicate device is APAS Independence with Urine Analysis Module, K183648.

This predicate has not been subject to a design related recall.

5.4 Device Description

APAS Independence is a device designed to be used in a microbiology laboratory to automate the initial screening of specimens for the presence of growth on culture plates. It is an *in vitro* diagnostic device and has no direct contact with patients.

The APAS Independence consists of an automated plate handling mechanism to move culture plates through the instrument, an imaging station to capture images of culture plates, and software for image analysis (e.g., determination of growth) and presentation of reports.

The APAS Independence is intended to be installed with multiple 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 to allow APAS to assess growth on culture plates from multiple agar manufacturers sold for the same indications.

This submission includes two MRSA analysis modules that have been developed for the same indication, which is to be used in a microbiology laboratory to automate the initial screening for the presence of presumptive methicillin-resistant *Staphylococcus aureus* (MRSA) growth on culture plates. It is indicated for the screening of MRSA colonization from swabs, where a specimen is collected from the anterior nares by non-invasive sampling techniques and plated onto specified chromogenic MRSA agars. No quantification of growth is required.

APAS Independence with IC MRSA Chromogenic BD Analysis Module is designed to interpret growth on BBL™ CHROMagar™ MRSA II agar from Becton Dickinson, and **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module** is designed to interpret growth on Spectra™ MRSA agar from Thermo Fisher Scientific (Remel).

Table 5-1. shows reported final plate designation and relationship to plate interpretation result for each module, respectively.

Table 5-1: APAS final designation

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

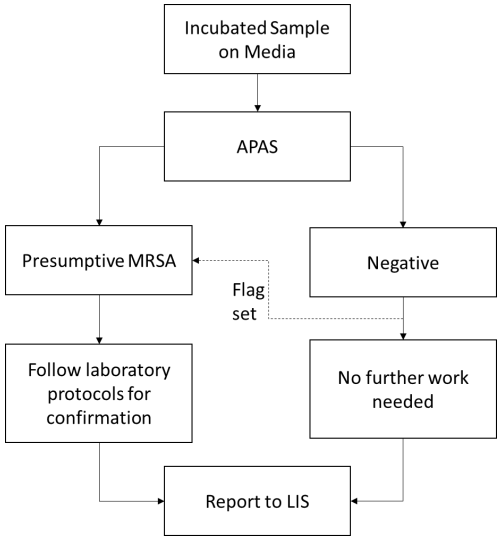
Both modules provide a triaging step within the workflow of a microbiology laboratory. Each module takes two key inputs (agar plate images and flags) and computes a final plate designation so that the plate can be efficiently and appropriately routed to the next step in the laboratory workflow.

For **APAS Independence with IC MRSA Chromogenic BD Analysis Module**, the final designation will be either **Presumptive MRSA** or **Negative**.

- **Presumptive MRSA** designation indicates that the culture plate was determined to contain colored colonies which are suspected to be MRSA. Investigation and confirmation by a microbiologist are required.
- **Negative** designation indicates that the culture plate was determined to not contain colored colonies indicative of MRSA. No further investigations will be required.

Figure 5-1 provides an illustration of the expected workflow after the **IC MRSA Chromogenic BD Analysis Module** has produced a screening result.

Figure 5-1: Typical workflow of APAS Independence with IC MRSA Chromogenic BD analysis module



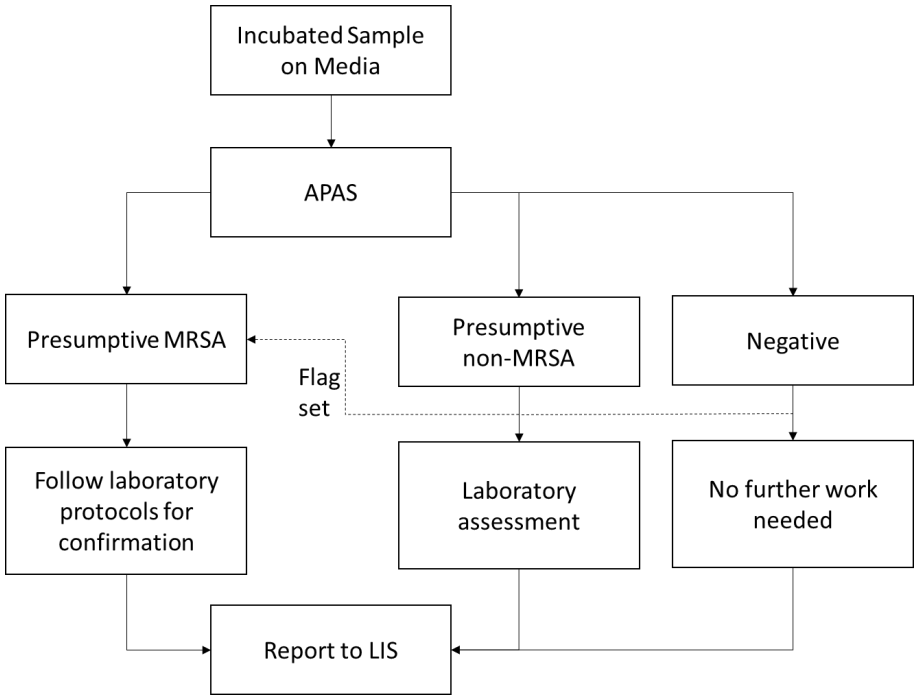
When APAS Independence with IC MRSA Chromogenic BD analysis module completes the analysis of each plate, the APAS-generated result is sent to the LIS. In the case of a Negative designation which does not require any further work, the final laboratory report can be issued according to standard reporting protocols without any investigation. Any Presumptive MRSA designations, however, will require investigation according to laboratory protocols for confirmation.

For **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module** the final designation will be either Presumptive MRSA, Presumptive non-MRSA, or Negative.

- **Presumptive MRSA** designation indicates that the culture is likely to contain colored colonies which are suspected to be MRSA. Investigation and confirmation by a microbiologist are required.
- **Presumptive non-MRSA** designation indicates that the culture is likely to contain growth of organisms not typically suspected to be MRSA. Review is required by a microbiologist.
- **Negative** designation indicates that no growth is present on the plate. No further investigations will be required.

Figure 5-2 provides an illustration of the expected workflow after the **IC MRSA Chromogenic TFS/S Analysis Module** has produced a screening result.

Figure 5-2: Typical workflow of APAS Independence with IC MRSA Chromogenic TFS/S analysis module



When APAS Independence with IC MRSA Chromogenic TFS/S analysis module completes the analysis of each plate, the APAS-generated result is sent to the LIS. In the case of a Negative designation, which does not require any further work, the final laboratory report can be issued according to standard reporting protocols without any investigation. Any Presumptive MRSA or Presumptive non-MRSA designation, however, will require investigation according to laboratory protocols for confirmation prior to a laboratory report being issued.

Figure 5-3 shows a photograph of the instrument from the front. It shows the input area on the left, the imaging area in the middle and the output area on the right. The user controls the instrument via the screen at the top middle of the instrument.

Figure 5-3: APAS Independence

5.5 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.

5.6 Indications for Use

The indication for use for APAS Independence, when using one of the MRSA analysis modules included in this application, is one of the following:

5.6.1 APAS Independence with IC MRSA Chromogenic BD Analysis Module

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

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.

5.6.2 APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module

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

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.

5.6.3 Predicate device APAS Independence with Urine Analysis Module

The indication for use of the predicate device, which is the APAS Independence with Urine Analysis Module, is as follows:

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 semi-quantitative 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.

The difference in indications for use reflects the different therapeutic screening purposes supported by APAS. Neither the predicate urine analysis module nor either of the MRSA analysis modules provide a final diagnostic result; for each module, a positive screening result is confirmed by a microbiologist.

The same questions of safety and effectiveness for all analysis modules are addressed by fulfilling the requirements of the Special Controls for automated image assessment systems for microbial colonies on solid culture media.

5.7 Comparison of Technological Characteristics with the Predicate Device

The nominated predicate device is the APAS Independence with Urine Analysis Module which was cleared via application K183648.

APAS Independence when using both MRSA analysis modules included in this submission uses the same technology and methods to provide an interpretation of growth from anterior nares swabs taken by non-invasive techniques and cultured for screening for specific indications.

Both devices have been developed by Clever Culture Systems (CCS). Table 5-2 provides a side-by-side comparison of the main components of APAS Independence as used in the predicate and the two new devices, showing that no changes were necessary to accommodate the two new modules.

Table 5-2: Comparison of Main Components Between Predicate and Subject Device

Component		**Predicate Device**	**Subject Devices**
		APAS Independence with Urine Analysis Module (K183648)	**APAS Independence with IC MRSA Chromogenic BD Analysis Module** **And** **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module**
Hardware	**Imaging Station**	Light Emitting Diode (LED) illumination of culture plates and image capture using a Charge Coupled Device (CCD) camera	Same
	Plate Handling Mechanism	An automated system that accepts input carriers of multiple plates, moves plates through the instrument and sorts them to output carriers and stacks based on the analysis result. Accurately positions the plate for imaging	Same
Software	**APAS Controller PC**	Controls image capture, analysis, report generation and result storage	Same

Component		Predicate Device	Subject Devices
		APAS Independence with Urine Analysis Module (K183648)	**APAS Independence with IC MRSA Chromogenic BD Analysis Module** **And** **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module**
	Instrument Controller PC	User interface for operation of the APAS Independence. Oversight of plate movements	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
	Laboratory Network Interactions	Interfaces with an external LIS. Optionally interfaces with NTP, LDAP, DNS and DHCP servers.	Same
QC	**Qualification Tools**	Color Check Tool: - Multicolored disk for checking and correcting color of the system optics. System Check Tool: - A pair of disks with small dots replicating colonies for checking overall system functionality and associated software	Same

Table 5-3 and Table 5-4 provide a side-by-side comparison of the main characteristics of the *Analysis Modules* used in the predicate and subject devices. The differences do not raise different questions about safety and effectiveness, which are addressed by determining analytical performance and clinical performance.

Table 5-3: Comparison of Characteristics Between Predicate and Subject Analysis Modules

Characteristic	Predicate Device	Subject Device
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
Target population	All patients	Same
Anatomical site	Biological samples taken by non-invasive techniques	Same
Sample Type	Urine samples	Anterior nares swab samples
Where used	Microbiology laboratory	Same

Characteristic	Predicate Device	Subject Device
Media used	Urine analysis module uses media manufactured by Remel, USA: • Trypticase Soy Agar with 5% sheep blood, Product Code R01202A • MacConkey Agar with crystal violet, Product Code R01552A	IC MRSA Chromogenic BD analysis module uses media manufactured by Beckton Dickinson • BBL™ CHROMagar™ MRSA II, Product Codes 215228 (20pk) and 215229 (120pk) IC MRSA Chromogenic TFS/S analysis module uses media manufactured by Thermo Fisher Scientific • Spectra™ MRSA, Product Codes R01821(A) (10pk) and R01822(A) (100pk)

Table 5-4: Comparison of Indications for Use Between Predicate and Subject Analysis Modules

Predicate Urine Analysis Module	IC MRSA Chromogenic BD analysis module	IC MRSA Chromogenic TF/S analysis module
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 semi-quantitative 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.	The APAS Independence is an *in vitro* diagnostic system comprised of an instrument for automated imaging of agar plates and a software analysis module for the following use: 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.	The APAS Independence is an in vitro diagnostic system comprised of an instrument for automated imaging of agar plates and a software analysis module for the following use: 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.

5.8 Performance Data

This section addresses the software verification and validation conducted for each MRSA analysis module, and the performance requirements specified as Special Controls for a device of this type, which require that pre-market notification submissions include:

1) detailed documentation of the analytical studies performed to characterize device performance to support the intended use, as appropriate.
2) detailed documentation from clinical studies performed on a population that is consistent with the intended use population.
   a) The clinical studies must establish the device performance based on comparison to results obtained by an acceptable reference method, as appropriate.
   b) The clinical study documentation must include the study protocol with a predefined statistical analysis plan and the final report documenting support for the Indications for Use and the results of the statistical analysis, as appropriate.

5.8.1 Analysis Module Software Verification and Validation

Software verification and validation testing were conducted, and documentation was provided as recommended by FDA's Guidance for Industry and Staff, *Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*. The software for this device was considered as a "moderate" level of concern as a malfunction of, or latent design flaw in the software could lead to an erroneous diagnosis or a delay in delivery of appropriate medical care that would likely lead to a Minor injury to the patient. See Section 16 for additional details.

5.8.2 Digital Image Quality, Digital Image Interpretation

When conducting clinical studies and some analytical performance studies, the reference result against which APAS performance was compared was generated by microbiologists interpreting growth on digital images of plates (plate image) rather than from the physical plate (plate-in-hand). Studies were conducted to determine:

a) If the interpretation of the digital image of the same plate is reproducible across microbiologists, as this is the method used to generate performance data in support of the current 510(k) application.
b) Whether the plate-in-hand interpretation and plate image interpretation of the same plate are equivalent across microbiologists to determine if a microbiologist can equivalently interpret presumptive MRSA on the plate and the digital image, as this provide confidence that the digital image interpretation is a true representation of the agar plate interpretation.

5.8.2.1 Point (a) Reproducibility of plate image interpretations

The reproducibility of plate image interpretations was assessed by comparing the individual assessments of plate images by three microbiologists with a single panel result (i.e., majority vote) for each image. Table 5-5 shows the percent agreement of the three microbiologists with the panel result for detection of presumptive MRSA, presumptive non-MRSA, and no growth on each of the BD BBL and TFS Spectra media.

Table 5-5: Microbiologist agreement with panel result

		Percent Agreement		
		No growth	Presumptive non-MRSA	Presumptive MRSA
BD	Microbiologist 1	98.9% (260/263)	97.6% (80/82)	100.0% (55/55)
	Microbiologist 2	100.0% (263/263)	97.6% (80/82)	100.0% (55/55)
	Microbiologist 3	95.4% (251/263)	100.0% (82/82)	100.0% (55/55)
	Combined	98.1% (774/789)	98.4% (242/246)	100.0% (165/165)
TFS/S	Microbiologist 1	98.0% (192/196)	95.1% (98/103)	98.0% (99/101)
	Microbiologist 2	100.0% (196/196)	93.2% (96/103)	97.0% (98/101)
	Microbiologist 3	99.0% (194/196)	97.1% (100/103)	98.0% (99/101)
	Combined	99.0% (582/588)	95.1% (294/309)	97.7% (296/303)

Table 5-5 shows that for BD media, when interpreting growth from digital images, microbiologists agree with the panel 100% of the time for detection of presumptive MRSA, over 97% of the time for presumptive non-MRSA, and over 95% of the time for no growth. Combining (averaging) these results yields 100%, 98.4% and 98.1%, respectively. These results support the use of digital images by a panel of microbiologists to establish a reference result against which the APAS result can be compared.

Table 5-5 shows that for TFS/S media, when interpreting growth from digital images, microbiologists agree with the panel more than 97% of the time for detection of presumptive MRSA, 93% of the time for presumptive non-MRSA, and 98% of the time for no growth. Combining (averaging) these results yields 97.7%, 95.1% and 99.0%, respectively. These results support the use of digital images by a panel of microbiologists to establish a reference result against which the APAS result can be compared.

5.8.2.2 Point (b) Equivalency of Plate-in-Hand vs. Plate Image

The equivalency of plate-in-hand vs. plate image measurements were assessed by measuring the percent of time a microbiologist's interpretations of the plate and image were in agreement for presumptive MRSA and applying an acceptance criterion of 95% for the combined result. In this comparison, the plate-in-hand interpretation is considered to be the truth state.

Table 5-6: Microbiologist Image vs. Plate-in-hand agreement

		Percent Agreement Microbiologist Image vs Plate-in-Hand		
		No growth	Presumptive Non-MRSA	Presumptive MRSA
BD	Microbiologist 1	95.8% (252/263)	89.6% (69/77)	91.7% (55/60)
	Microbiologist 2	98.1% (264/269)	98.7% (74/75)	98.2% (55/56)
	Microbiologist 3	95.4% (250/262)	97.6% (82/84)	98.1% (53/54)
	Combined	96.5% (766/794)	95.3% (225/236)	95.9% (163/170)
TFS/S	Microbiologist 1	93.8% (182/194)	87.1% (88/101)	92.4% (97/105)
	Microbiologist 2	96.0% (193/201)	90.8% (89/98)	92.1% (93/101)
	Microbiologist 3	94.2% (180/191)	86.8% (92/106)	96.1% (99/103)
	Combined	94.7% (555/586)	88.2% (269/305)	93.5% (289/309)

Table 5-6 shows that on average, microbiologists interpret the plate image equivalent to the plate-in-hand using the BD media >95% of the time for each result designation. Presumptive MRSA

agreement is >95%, supporting the use of a 2-designation approach (Presumptive MRSA and Negative) with this media.

Table 5-6 shows that on average, microbiologists interpret the plate image equivalent to the plate-in-hand using the TFS media <95% of the time for each result designation. Presumptive MRSA agreement is <95%, supporting the use of a 3-designation approach (Presumptive MRSA, Presumptive non-MRSA and Negative) with this media.

5.8.3 Quality Control Test Results

During the IVD and analytical performance studies, quality control tests were conducted using the methods described in the APAS Independence User Manual. Daily instrument checks were conducted for Colour Check and System Check using the tools provided with the instrument and both tests passed on all testing days.

In addition, biological quality control testing was performed using specially prepared positive QC plates as described in the user manuals for each of the analysis modules. These QC plates were prepared from a 0.5 McFarland suspension in saline of *S. aureus* MRSA ATCC 43300 which was serially diluted with sterile saline to make a suspension containing approximately 10^5 cells per mL and then inoculated onto BBL™ CHROMagar™ MRSA II plates and Spectra™ MRSA plates using a 10 µL loop for quadrant streaking. The plates were incubated at 36°C ±1°C for 24 hours.

A positive QC was performed for each instrument used per experiment for each day of the IVD study and analytical studies. APAS produced the expected presumptive positive MRSA result in all cases.

5.8.4 Analytical Performance Studies

5.8.4.1 Accuracy - Trueness

This study tested the ability of APAS to correctly detect colonies of MRSA and non-MRSA.

A range of methicillin-resistant *Staphylococcus aureus* and other non-MRSA organisms that may grow on the media, were used to produce 0.5 McFarland suspensions of pure cultures. These were serially diluted in sterile saline to create suspensions which, when streaked, produced either light or confluent growth. The plates were incubated at 36°C ± 1°C for 24 hours then imaged using APAS. Across the set of images produced for each of the organisms plated, at least 100 isolated colonies were digitally labelled by a microbiologist as displaying the distinctive morphology associated with colonies of MRSA or non-MRSA on the specific agar, where non-MRSA means colonies which will grow on the media but do not react with the chromogen. The APAS result for each colony was compared with the result by the microbiologist.

Table 5-7 shows that APAS with **IC MRSA Chromogenic BD analysis module** was able to correctly detect the typical morphology associated with MRSA 99.3 % of the time.

Table 5-7: Trueness - IC MRSA Chromogenic BD

All colonies combined		**Microbiologist Assigned**		
		MRSA Colony	**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

All colonies combined		Microbiologist Assigned		
		MRSA Colony	Non-MRSA Colony	Total
Agreement	By colony morphology	99.4%	67.5%	
	All colony morphologies	90.5%		

Table 5-8 shows that APAS with **IC MRSA Chromogenic TFS/S analysis module** was able to correctly detect the typical morphology associated with MRSA 99.5% of the time.

Table 5-8: Trueness - IC MRSA Chromogenic TFS/S

All colonies combined		Microbiologist Assigned		
		MRSA Colony	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
Agreement	By colony morphology	99.5%	41.5%	
	All colony morphologies	75.6%		

The measured performance of both modules met the performance target of 95%.

5.8.4.2 Accuracy - Precision

The reproducibility of plate interpretation performed by the APAS when installed with each module was evaluated on different instruments over multiple runs. The test determined:

- Repeatability of interpretation within an instrument when the same plate is presented to the imaging system at the same angle and at different angles, and
- Reproducibility of interpretations across instruments.

Testing was performed using 3 dilutions of two strains of MRSA, (one isolated from the wild and one ATCC strain), and 3 dilutions of two strains of *Staphylococcus haemolyticus*, both of which were isolated from the wild and which will grow on BD and Spectra media but do not react with the chromogen. The dilutions were chosen to produce plates with mainly confluent growth (dilution 1), partially confluent growth (dilution 2) and only isolated colonies (dilution 3), respectively, when 10µL of each dilution is plated using the quadrant streak method. Each dilution was inoculated in triplicate and the plates incubated at 35°C ± 2°C for 24 hours. Five replicate images of each plate were taken at 3 different orientations (0°, 120° and 270°) on 3 different APAS Independence instruments.

The results tables report the agreement of the APAS-generated interpretation with the expected outcome (presence or absence of presumptive MRSA (Color) and Growth), along with the agreement across instruments, expressed as a percent. Confidence intervals are calculated for the measure of reproducibility across instruments.

Table 5-9 provides the results for IC MRSA Chromogenic BD Analysis Module and Table 5-10 provides the results for IC MRSA Chromogenic TFS/S Analysis Module. The results demonstrate reproducibility of APAS-generated results with multiple plate orientations and across multiple instruments.

Table 5-9: Precision (Repeatability and Reproducibility) IC MRSA Chromogenic BD

Organism	Dilution	Percent Agreement							
		Instrument 1		Instrument 2		Instrument 3		Combined	
		Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1] (95% CI)	Color[2] (95% CI)
MRSA – Wild Strain 1	1	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	2	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
MRSA – ATCC 43300	1	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	2	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
Saline (negative control)	-	100.0% (45/45)	100.0% (45/45)	97.8% (44/45)	100.0% (45/45)	95.6% (43/45)	100.0% (45/45)	97.8% (132/135) 93.7%-99.2%	100.0% (135/135) 97.2%-100.0%
S. haemolyticus – Wild Strain 1	1	100.0% (45/45)	80.0% (36/45)	100.0% (45/45)	93.3% (42/45)	100.0% (45/45)	75.6% (34/45)	100.0% (135/135) 97.2%-100.0%	83.0% (112/135) 75.7%-88.4%
	2	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	97.8% (44/45)	100.0% (135/135) 97.2%-100.0%	99.3% (134/135) 95.9%-99.9%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	93.3% (42/45)	100.0% (135/135) 97.2%-100.0%	97.8% (132/135) 93.7%-99.2%
S. haemolyticus – Wild Strain 2	1	100.0% (45/45)	80.0% (36/45)	100.0% (45/45)	75.6% (34/45)	100.0% (45/45)	55.6% (25/45)	100.0% (135/135) 97.2%-100.0%	70.4% (95/135) 62.2%-77.4%
	2	100.0% (45/45)	97.8% (44/45)	100.0% (45/45)	88.9% (40/45)	100.0% (45/45)	82.2% (37/45)	100.0% (135/135) 97.2%-100.0%	89.6% (121/135) 83.3%-93.7%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	95.6% (43/45)	100.0% (135/135) 97.2%-100.0%	98.5% (133/135) 94.8%-99.6%

[1] 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

[2] 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

CI: confidence interval; LB: lower bound of the 95% CI; UB: upper bound of the 95% CI

Table 5-10: Precision (Repeatability and Reproducibility) IC MRSA Chromogenic TFS/S

Organism	Dilution	Percent Agreement							
		Instrument 1		Instrument 2		Instrument 3		Combined	
		Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1] (95% CI)	Color[2] (95% CI)
MRSA – Wild Strain 2	1	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	2	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
MRSA – ATCC 43300	1	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	2	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
Saline (negative control)	0	66.7% (30/45)	100.0% (45/45)	66.7% (30/45)	100.0% (45/45)	68.9% (31/45)	100.0% (45/45)	67.4% (91/135) 59.1%-74.7%	100.0% (135/135) 97.2%-100.0%
S. haemolyticus – Wild Strain 1	1	100.0% (45/45)	75.6% (34/45)	100.0% (45/45)	93.3% (42/45)	100.0% (45/45)	95.6% (43/45)	100.0% (135/135) 97.2%-100.0%	88.1% (119/135) 81.6%-92.6%
	2	100.0% (45/45)	97.8% (44/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	84.4% (38/45)	100.0% (135/135) 97.2%-100.0%	94.1% (127/135) 88.7%-97.0%
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%
S. haemolyticus – Wild Strain 2	1	100.0% (45/45)	82.2% (37/45)	100.0% (45/45)	80.0% (36/45)	100.0% (45/45)	82.2% (37/45)	100.0% (135/135) 97.2%-100.0%	81.5% (110/135) 74.1%-87.1%
	2	100.0% (45/45)	95.6% (43/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	91.1% (41/45)	100.0% (135/135) 97.2%-100.0%	95.6% (129/135) 90.6%-97.9%

Organism	Dilution	Percent Agreement							
		Instrument 1		Instrument 2		Instrument 3		Combined	
		Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1]	Color[2]	Growth[1] (95% CI)	Color[2] (95% CI)
	3	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (45/45)	100.0% (135/135) 97.2%-100.0%	100.0% (135/135) 97.2%-100.0%

[1] 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

[2] 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

CI: confidence interval; LB: lower bound of the 95% CI; UB: upper bound of the 95% CI

5.8.4.3 Analytical Sensitivity – Limit of Detection of Colony Size

A study was conducted to determine the smallest colony size that can be reproducibly detected as presumptive MRSA 95% of the time. The study was conducted using 2 strains of MRSA, one ATCC strain and one wild strain.

Five 10-fold dilutions of a 0.5 McFarland suspension were prepared of each organism and the fifth dilution (approximately 10^3 CFU/mL) was further diluted 1:2. 100µL of the final dilution was spread onto 6 replicate plates and incubated at 36°C ± 1°C until pinpoint colonies were detected. The plates were then imaged and returned to the incubator at 1-hour intervals until the colonies on the plate were approximately 2mm in diameter.

Selecting from the images at each timepoint, the operator digitally labelled multiple isolated colonies to measure the colony diameter. The image of each plate was then analysed to generate the corresponding APAS result. The smallest colony diameter that was successfully detected for growth and color by APAS >95% of the time was identified as the smallest colony size for each organism (bolded result).

Results of the study demonstrated that the smallest MRSA colony size that can be detected 95% of the time by APAS is 0.82 mm for MRSA wild strain and 0.91 mm for MRSA ATCC 43300 using the BD module (Table 5-11) and 0.63 mm for MRSA wild strain and 0.54 mm for MRSA ATCC 43300 using the TFS/S module (Table 5-12).

Table 5-11: LoD of Colony Size – IC MRSA Chromogenic BD

Colony Size (Diameter)		Detection Rate[1]	
Pixels	**Extrapolated mm**	**MRSA Wild Strain**	**MRSA ATCC 43300**
1-3	0.09-0.27	0.0% (0/399)	0.4% (1/264)
4-5	0.36-0.45	1.5% (4/258)	1.7% (3/175)
6	0.54	5.2% (7/135)	9.7% (9/93)
7	0.63	18.6% (21/113)	22.8% (18/79)
8	0.72	72.5% (66/91)	42.9% (36/84)
9	0.82	**95.1% (117/123)**	67.6% (50/74)
10	0.91	98.0% (147/150)	**97.7% (84/86)**
11	1.00	100.0% (130/130)	99.0% (95/96)
12	1.09	100.0% (156/156)	100.0% (90/90)

[1] Value calculated by dividing the number of presumptive MRSA colonies detected by APAS by the total number of colonies located by a microbiologist.

Table 5-12: LoD of Colony Size – IC MRSA Chromogenic TFS/S

Colony Size (Diameter)		Detection Rate[1]	
Pixels	**Extrapolated mm**	**MRSA Wild Strain 1**	**MRSA ATCC 43300**
1-3	0.09-0.27	0.0% (0/285)	1.8% (3/163)
4-5	0.36-0.45	7.6% (12/159)	43.0% (98/228)
6	0.54	70.8% (63/89)	**97.7% (129/132)**
7	0.63	**98.0% (145/148)**	100.0% (100/100)
8	0.72	99.5% (197/198)	100.0% (71/71)
9	0.82	100.0% (167/167)	100.0% (43/43)
10	0.91	100.0% (103/103)	100.0% (12/12)
11	1.00	100.0% (21/21)	100.0% (2/2)

[1] Value calculated by dividing the number of presumptive MRSA colonies detected by APAS by the total number of colonies located by a microbiologist.

5.8.4.4 Analytical Sensitivity – Detection of MRSA in Mixtures

This test evaluated the ability of APAS to detect small amounts (1-100 colonies) and large amounts (>100 colonies) of MRSA when in the presence of other growth (>100 white colonies) after incubation for 24 hours.

Five, 10-fold dilutions of a 0.5 McFarland suspension were prepared with two strains of MRSA, one strain of *S. haemolyticus,* and one strain of *S. warneri*, all of which had been isolated from the wild. A 1:1 mixture and a 1:10 mixture of MRSA with a non-MRSA organism were prepared for two different combinations of MRSA and non-MRSA. The plates were incubated at 36°C ± 1°C for 24 hours. The images were read and interpreted by APAS and by 3 microbiologists, each of whom recorded the presence or absence of MRSA colonies on the plate. The majority vote was taken as the truth state against which the APAS result was compared.

The results for APAS Independence with IC Chromogenic MRSA BD Analysis Module are shown in Table 5-13 and show that APAS correctly identifies the presence of MRSA colonies ≥95% of the time at 24 hours.

The results for APAS Independence with IC Chromogenic MRSA TFS/S Analysis Module are shown in Table 5-14**.** and show that APAS correctly identifies the presence of MRSA colonies ≥95% of the time at 24 hours.

Table 5-13: Detection of MRSA in Mixtures - IC MRSA Chromogenic BD

Organisms Plated	Mixture Ratios	CFU		Agreement[1]	
		MRSA	Other	Presence or absence of MRSA	Presence or absence of Growth
MRSA (Strain 1) *S. haemolyticus*	1:1	>100	>100	100.0% (5/5)	100.0% (5/5)
	1:100	1-100	>100	100.0% (5/5)	100.0% (5/5)
MRSA (Strain 2) *S. warneri*	1:1	>100	>100	100.0% (5/5)	100.0% (5/5)
	1:100	1-100	>100	100.0% (5/5)	100.0% (5/5)

[1] Agreement between majority panel microbiologist result (i.e., truth state) and APAS result.

Table 5-14: Detection of MRSA in Mixtures - IC MRSA Chromogenic TFS/S

Organisms Plated	Mixture Ratios	CFU		Agreement[1]	
		MRSA	Other	Presence or absence of MRSA	Presence or absence of Growth
MRSA (Strain 1) *S. haemolyticus (Strain 2)*	1:1	>100	>100	100.0% (5/5)	100.0% (5/5)
	1:100	1-100	>100	100.0% (5/5)	100.0% (5/5)
MRSA (Strain 2) *S. warneri*	1:1	>100	>100	100.0% (5/5)	100.0% (5/5)
	1:100	1-100	>100	100.0% (5/5)	100.0% (5/5)

[1] Agreement between majority panel microbiologist result (i.e., truth state) and APAS result.

5.8.4.5 Analytical Specificity – Limit of Blank

This test examined the ability of APAS to correctly identify when there is no growth present on the agar, and therefore determine the likelihood of false positives caused by known interferences.

A test matrix of plates with no growth were prepared using 3 different applicators (flocked swab, cotton swab and 10µL loop) to inoculate plates using 3 different media (Stuart Transport, Amies Transport and saline) and no media (dry). The plates were labelled with one of 4 different labels applied to the base or side and were either marked with felt-tip pen or not. The test therefore consisted of a matrix of 96 plates (3 applicators x 4 media x 4 labels x 2 marks).

Table 5-15 shows that for APAS with IC MRSA Chromogenic BD, there was good agreement that colored colonies indicative of MRSA were not present. The table also shows that growth may be detected, but that it was unlikely to be reported as presumptive MRSA.

Table 5-15: Limit of Blank – IC MRSA Chromogenic BD

Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement that no growth is present (%)[1]	Agreement that no colored colonies are present (%)[2]
Label Type	Paper, 1D, Base	24	83.3 (20/24)	100.0 (24/24)
	Paper, 2D, Base	24	79.2 (19/24)	95.8 (23/24)
	Plastic, 2D, Base	24	75.0 (18/24)	100.0 (24/24)
	Paper, 1D, Side	24	70.8 (17/24)	100.0 (24/24)
Applicator	Cotton swab	32	65.6 (21/32)	96.9 (31/32)
	Flocked swab	32	84.4 (27/32)	100.0 (32/32)
	10uL Loop	32	81.2 (26/32)	100.0 (32/32)
Transport Media	Amies Transport	24	91.7 (22/24)	100.0 (24/24)
	Dry	24	75.0 (18/24)	100.0 (24/24)
	Saline	24	87.5 (21/24)	100.0 (24/24)
	Stuart Transport	24	54.2 (13/24)	95.8 (23/24)
Pen	Present	48	79.2 (38/48)	100.0 (48/48)
	Absent	48	75.0 (36/48)	97.9 (47/48)

[1] Value calculated by dividing the number of images with no detected growth by the total number of images
[2] Value calculated by dividing the number of images with zero detected colored colonies by the total number of images

Table 5-16 shows that for APAS with IC MRSA Chromogenic TFS/S, there was good agreement that colored colonies indicative of MRSA were not present. The table also shows that growth may be detected, but that it was unlikely to be reported as presumptive MRSA.

Table 5-16: Limit of Blank – IC MRSA Chromogenic TFS/S

Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement that no growth is present (%)[1]	Agreement that no colored colonies are present (%)[2]
Label Type	Paper, 1D, Base	24	58.3 (14/24)	100.0 (24/24)
	Paper, 2D, Base	24	66.7 (16/24)	100.0 (24/24)
	Plastic, 2D, Base	24	54.2 (13/24)	100.0 (24/24)
	Paper, 1D, Side	24	58.3 (14/24)	91.7 (22/24)
Applicator	Cotton swab	32	62.5 (20/32)	100.0 (32/32)
	Flocked swab	32	50.0 (16/32)	93.8 (30/32)
	10uL Loop	32	65.6 (21/32)	100.0 (32/32)
Transport Media	Amies Transport	24	62.5 (15/24)	95.8 (23/24)
	Dry	24	70.8 (17/24)	100.0 (24/24)
	Saline	24	91.7 (22/24)	100.0 (24/24)
	Stuart Transport	24	12.5 (3/24)	95.8 (23/24)
Pen	Present	48	56.2 (27/48)	97.9 (47/48)
	Absent	48	62.5 (30/48)	97.9 (47/48)

[1] Value calculated by dividing the number of images with no detected growth by the total number of images
[2] Value calculated by dividing the number of images with zero detected colored colonies by the total number of images

5.8.4.6 Analytical Specificity – Interference with detection of MRSA

This test evaluated the ability of APAS to correctly detect MRSA in the presence of known interferences and to determine if a heavy growth of non-MRSA is detected by APAS as MRSA, as heavy growth of some non-MRSA organisms may display a chromogen reaction.

Pure 0.5 McFarland suspensions of MRSA and *S. haemolyticus* (representing non-MRSA) were serially diluted to produce an inoculum for each organism which would produce predominantly isolated colonies of MRSA and heavy growth of *S. haemolyticus* when cultured.

A test matrix of plates per organism was quadrant streaked using 3 different applicators (flocked swab, cotton swab and 10µL loop) to inoculate plates using 2 different media (Stuart Transport and Amies Transport). The plates were labelled with one of 4 different labels applied to the base or side and were either marked with felt-tip pen or not. The test therefore consisted of 48 plates (3 applicators x 2 media x 4 labels x 2 marks) per organism. The inoculated plates were incubated at 36°C ± 1°C for 24 hours and then imaged.

The images were processed to generate the APAS report for each plate and then the reported presence or absence of MRSA and non-MRSA were compared with the expected result for growth and color, which was the presence of colored growth and no colored growth, respectively.

The results for APAS Independence with IC MRSA Chromogenic BD Analysis Module are shown in Table 5-17. These results show that the interference modes tested had minimal impact on the ability of APAS to detect the presence of MRSA. The results also show that when a heavy inoculum of *S. haemolyticus* is present, false positive detections as presumptive MRSA occurred in this interference study.

Table 5-17: Analytical Specificity - Interference – IC MRSA Chromogenic BD

Organism	Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement with Expected Growth[1] (%)	Agreement with Expected Color[2] (%)
MRSA	Label Type	Paper, 1D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Plastic, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 1D, Side	12	100.0 (12/12)	100.0 (12/12)
	Applicator	Cotton swab	16	100.0 (16/16)	100.0 (16/16)
		Flocked swab	16	100.0 (16/16)	100.0 (16/16)
		10uL Loop	16	100.0 (16/16)	100.0 (16/16)
	Transport Media	Amies Transport	24	100.0 (24/24)	100.0 (24/24)
		Stuart Transport	24	100.0 (24/24)	100.0 (24/24)
	Pen	Present	24	100.0 (24/24)	100.0 (24/24)
		Absent	24	100.0 (24/24)	100.0 (24/24)
S. haemolyticus (heavy growth)	Label Type	Paper, 1D, Base	12	100.0 (12/12)	41.7 (5/12)
		Paper, 2D, Base	12	100.0 (12/12)	16.7 (2/12)
		Plastic, 2D, Base	12	100.0 (12/12)	50.0 (6/12)
		Paper, 1D, Side	12	100.0 (12/12)	100.0 (12/12)

Organism	Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement with Expected Growth[1] (%)	Agreement with Expected Color[2] (%)
	Applicator	Cotton swab	16	100.0 (16/16)	43.8 (7/16)
		Flocked swab	16	100.0 (16/16)	50.0 (8/16)
		10uL Loop	16	100.0 (16/16)	62.5 (10/16)
	Transport Media	Amies Transport	24	100.0 (24/24)	50.0 (12/24)
		Stuart Transport	24	100.0 (24/24)	54.2 (13/24)
	Pen	Present	24	100.0 (24/24)	50.0 (12/24)
		Absent	24	100.0 (24/24)	54.2 (13/24)

[1] Value calculated by dividing the number of images with detected growth by the total number of images
[2] Value calculated by dividing the number of images where the detection or non-detection of colored colonies matches expectation, by the total number of images

The results for APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module are shown in Table 5-18. These results show that the interference modes tested had minimal impact on the ability of APAS to detect the presence of MRSA. The results also show that the heavy growth of *S. haemolyticus* did not cause false detections of presumptive MRSA on this media.

Table 5-18: Analytical Specificity - Interference – IC MRSA Chromogenic TFS/S

Organism	Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement with Expected Growth[1] (%)	Agreement with Expected Color[2] (%)
MRSA	Label Type	Paper, 1D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Plastic, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 1D, Side	12	100.0 (12/12)	100.0 (12/12)
	Applicator	Cotton swab	16	100.0 (16/16)	100.0 (16/16)
		Flocked swab	16	100.0 (16/16)	100.0 (16/16)
		10uL Loop	16	100.0 (16/16)	100.0 (16/16)
	Transport Media	Amies Transport	24	100.0 (24/24)	100.0 (24/24)
		Stuart Transport	24	100.0 (24/24)	100.0 (24/24)
	Pen	Present	24	100.0 (24/24)	100.0 (24/24)
		Absent	24	100.0 (24/24)	100.0 (24/24)
S. haemolyticus (heavy growth)	Label Type	Paper, 1D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Plastic, 2D, Base	12	100.0 (12/12)	100.0 (12/12)
		Paper, 1D, Side	12	100.0 (12/12)	91.7 (11/12)
	Applicator	Cotton swab	16	100.0 (16/16)	93.8 (15/16)
		Flocked swab	16	100.0 (16/16)	100.0 (16/16)
		10uL Loop	16	100.0 (16/16)	100.0 (16/16)

Organism	Interference Mechanism	Interference Variable	Total number of Images evaluated	Agreement with Expected Growth[1] (%)	Agreement with Expected Color[2] (%)
	Transport Media	Amies Transport	24	100.0 (24/24)	100.0 (24/24)
		Stuart Transport	24	100.0 (24/24)	95.8 (23/24)
	Pen	Present	24	100.0 (24/24)	95.8 (23/24)
		Absent	24	100.0 (24/24)	100.0 (24/24)

[1] Value calculated by dividing the number of images with detected growth by the total number of images
[2] Value calculated by dividing the number of images where the detection or non-detection of colored colonies matches expectation, by the total number of images

5.8.5 IVD Studies

The clinical performance of each analysis module was assessed in a prospective study, where the performance of APAS with each analysis module was evaluated separately against two reference panels of 3 clinical microbiologists interpreting growth from the same APAS-generated digital image.

Device	Agar	Growth interpreted by;
APAS Independence with IC MRSA Chromogenic BD Analysis Module	BBL CHROMagar MRSA II From Becton Dickinson	APAS and Reference Panel
APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module	Spectra™ MRSA From Thermo Fisher Scientific (Remel)	APAS and Reference Panel

Left-over specimens from standard of care screening culture for colonization with MRSA were collected and inoculated onto chromogenic agar plates at a single site in Australia. The plates were then incubated and imaged with the APAS.

The APAS result for each image was compared against each of two reference results; one panel was located in the USA and the other in Australia. Each reference result was achieved using the majority result from the respective panel of 3 independent microbiologists.

Due to the low positivity rate for MRSA detection in the study population, simulated samples were prepared to supplement the study. The simulated swab samples were spiked with known MRSA and non-MRSA organisms. The use of simulated samples allowed the inclusion of unique strains of MRSA that provided global representation of the most common MRSA clades, including those found in the United States.

The total number of both clinical and simulated samples included in the final analysis for the IC MRSA Chromogenic BD Analysis Module (BD) and IC MRSA Chromogenic TFS/S Analysis Module (TFS/S) from the 1590 enrolled samples is shown in Table 5-19, stratified by the proportions of clinical and simulated samples.

Table 5-19: Number of samples per specimen category

Type	Samples recruited / included	Plates Imaged (BD)	Plates imaged (TFS/S)
PCR- MRSA Negative	1100	1093	1097
PCR- MRSA Positive	25	25	25
Simulated Negative	60	60	60
Simulated Positive	405	395	398
Total	1590	1573	1580

Each specimen and simulated sample were inoculated onto a culture plate and incubated for 24 hours at 36±1°C prior to analysis by APAS Independence and by the reference panels.

Performance was assessed by comparing the final interpretation of digital plate images established by the microbiologists (reference method) with the corresponding interpretation by APAS. The primary performance objective was positive percent agreement of presumptive MRSA growth.

5.8.5.1 Results – IC MRSA Chromogenic BD

Of the 1590 samples enrolled in the study, 13 were excluded due to defects in the agar and thus 1573 were analyzed, being 1118 non-simulated samples, 395 simulated MRSA-positive samples and 60 simulated negative MRSA samples. The study results are shown in Table 5-20, Table 5-21 and Table 5-22.

Table 5-20: APAS Performance against Australian Reference Panel - IC MRSA Chromogenic BD

		AU Panel Result			
		Presumptive MRSA	Presumptive non-MRSA	Negative	Total
APAS Result	Presumptive MRSA	393	30	3	426
	Presumptive non-MRSA	0	226	156	382
	Negative	0	11	754	765
	Total	393	267	913	1573
Presumptive MRSA Agreement	100.0% (393/393: 95% CI 99.0% - 100.0%)				
Presumptive Non-MRSA Agreement	84.6% (226/267: 95% CI 79.8% - 88.5%)				
Negative Agreement	82.6% (754/913: 95% CI 80.0% - 84.9%)				

Table 5-21: APAS Performance against US Reference Panel – IC MRSA Chromogenic BD

		US Panel Result			
		Presumptive MRSA	Presumptive non-MRSA	Negative	Total
APAS Result	Presumptive MRSA	390	33	3	426
	Presumptive non-MRSA	1	226	155	382
	Negative	0	10	755	765
	Total	391	269	913	1573
Presumptive MRSA Agreement	99.7% (390/391: 95% CI 98.6% - 100.0%)				
Presumptive Non-MRSA Agreement	84.0% (226/269: 95% CI 79.2% - 87.9%)				
Negative Agreement	82.7% (755/913: 95% CI 80.1% - 85.0%)				

Table 5-22. Presumptive MRSA agreement results stratified by sample type – IC MRSA Chromogenic BD

Panel	Population	Statistic	Cases	Correct	Estimate	Lower 95% CI	Upper 95% CI
US	All	PPA[1]	391	390	**99.7%**	98.6%	100.0%
		NPA[2]	1182	1146	97.0%	95.8%	97.8%
	Simulated	PPA	358	358	100.0%	98.9%	100.0%
		NPA	97	79	81.4%	72.6%	87.9%
	Non-simulated	PPA	33	32	97.0%	84.7%	99.5%
		NPA	1085	1067	98.3%	97.4%	98.9%
AU	All	PPA	393	393	**100.0%**	99.0%	100.0%
		NPA	1180	1147	97.2%	96.1%	98.0%
	Simulated	PPA	359	359	100.0%	98.9%	100.0%
		NPA	96	79	82.3%	73.5%	88.6%
	Non-simulated	PPA	34	34	100.0%	89.8%	100.0%
		NPA	1084	1068	98.5%	97.6%	99.1%

[1]Positive percent agreement. APAS agreement with the panel that presumptive MRSA is present
[2]Negative percent agreement. APAS agreement with the panel that presumptive MRSA is absent

5.8.5.2 Results – IC MRSA Chromogenic TFS/S

Of the 1590 samples enrolled in the study, 10 were excluded due to defects in the agar so that a total of 1580 was analyzed, being 1122 non-simulated samples, 398 simulated MRSA-positive samples and 60 simulated negative MRSA samples.

The study results are shown in Table 5-23 Table 5-24 and Table 5-25

Table 5-23: Performance against Australian Reference Panel – IC MRSA Chromogenic TFS/S

		AU Panel Result			
		Presumptive MRSA	Presumptive non-MRSA	Negative	Total
APAS Result	Presumptive MRSA	476	69	1	546
	Presumptive non-MRSA	3	383	116	502
	Negative	1	5	526	532
	Total	480	457	643	1580
Presumptive MRSA Agreement	99.2% (476/480: 95% CI 97.9% - 99.7%)				
Presumptive Non-MRSA Agreement	83.8% (383/457: 95% CI 80.2% - 86.9%)				
Negative Agreement	81.8% (526/643: 95% CI 78.6% - 84.6%)				

Table 5-24: Performance against US Reference Panel – IC MRSA Chromogenic TFS/S

		US Panel Result			
		Presumptive MRSA	Presumptive non-MRSA	Negative	Total
APAS Result	Presumptive MRSA	434	110	2	546
	Presumptive non-MRSA	1	369	132	502
	Negative	1	4	527	532
	Total	436	483	661	1580
Presumptive MRSA Agreement	99.5% (434/436: 95% CI 98.3% - 99.9%)				
Presumptive Non-MRSA Agreement	76.4% (369/483: 95% CI 72.4% - 80.0%)				
Negative Agreement	79.7% (527/661: 95% CI 76.5% - 82.6%)				

Table 5-25: Presumptive MRSA agreement stratified by sample type for MRSA performance – IC MRSA Chromogenic TFS/S

Panel	Population	Statistic	Cases	Correct	Estimate	Lower 95% CI	Upper 95% CI
US	All	PPA[1]	436	434	**99.5%**	98.3%	99.9%
		NPA[2]	1144	1032	90.2%	88.4%	91.8%
	Simulated	PPA	362	362	100.0%	98.9%	100.0%
		NPA	96	88	91.7%	84.4%	95.7%
	Non-simulated	PPA	74	72	97.3%	90.7%	99.3%
		NPA	1048	944	90.1%	88.1%	91.7%
AU	All	PPA	479	475	**99.2%**	97.9%	99.7%
		NPA	1101	1030	93.6%	91.9%	94.9%
	Simulated	PPA	362	362	100.0%	98.9%	100.0%
		NPA	96	88	91.7%	84.4%	95.7%
	Non-simulated	PPA	117	113	96.6%	91.5%	98.7%
		NPA	1005	942	93.7%	92.1%	95.1%

[1]Positive percent agreement. APAS agreement with the panel that presumptive MRSA is present

[2]Negative percent agreement. APAS agreement with the panel that presumptive MRSA is absent

5.8.5.3 Agreement between the Australian and US reference panels

An analysis of the results from the Australian and US panels of microbiologists was performed to determine the extent of agreement in interpretation of the same digital images. This data is presented in Table 5-26 and Table 5-27 where the AU panel result is treated as being the truth.

Table 5-26: Agreement of US panel with Australian panel – images of BD plates

		AU Microbiologist panel			
		MRSA Growth	Non-MRSA Growth	No growth	Total
US Microbiologist panel	MRSA Growth	390	1	0	391
	Non-MRSA Growth	3	253	13	269
	No growth	0	13	900	913
	Total	393	267	913	1573
Agreement – Presumptive MRSA	99.2% (390/393: 95% CI 97.8% - 99.7%)				
Agreement – Presumptive Non-MRSA	94.8% (253/267: 95% CI 91.4% - 96.9%)				
Agreement - No growth	98.6% (900/913: 95% CI 97.6% - 99.2%)				

Table 5-27: Agreement of US panel with Australian panel – images of TFS plates

		AU Microbiologist			
		MRSA Growth	Non-MRSA Growth	No growth	Total
US Microbiologist	MRSA Growth	435	1	0	436
	Non-MRSA Growth	45	432	6	483
	No growth	0	24	637	661
	Total	480	457	643	1580
Agreement – Presumptive MRSA	90.6% (435/480: 95% CI 87.7% - 92.9%)				
Agreement – Presumptive Non-MRSA	94.5% (432/457: 95% CI 92.0% - 96.3%)				
Agreement - No growth	99.1% (637/643: 95% CI 98.0% - 99.6%)				

The tables show that the US panel had 99.8% agreement with the Australian panel for presumptive MRSA when interpreting images of BD plates and 90.6% agreement for images of TFS plates, indicating that the Australian panel reported more MRSA than the US panel.

5.9 Conclusions

5.9.1 2 vs 3 Designation Rule Set

The overall performances for the BD MRSA and TFS/S MRSA analysis modules have been 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.

The overall performance of the BD MRSA analysis module 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" (95.9%, Table 5-6) in the digital image quality study
- >98% agreement for "presumptive MRSA growth" in the Australian (100.0%, Table 5-20) and US clinical studies (99.7%, Table 5-21)
- >95% agreement for "presumptive MRSA growth" when comparing the Australian and US microbiologist panel results (99.2%, Table 5-26)

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" (93.5%, Table 5-6) in the digital image quality study
- >98% agreement for "presumptive MRSA growth" in the Australian reference panel (99.2%, Table 5-23) and US clinical studies (99.5%, Table 5-24)
- <95% agreement for "presumptive MRSA growth" when comparing the Australian and US microbiologist panel results (90.6%, Table 5-27)

5.9.2 Substantial Equivalence

Both **APAS Independence with IC MRSA Chromogenic BD Analysis Module** and **APAS Independence with IC MRSA Chromogenic TFS/S Analysis Module** have equivalent safety and effectiveness to the predicate device, APAS Independence with Urine Analysis Module (K183648) because:

- They have the same intended use.
- The technological characteristics are very similar.
- The differences in technological characteristics do not raise different questions about safety and effectiveness.
- The same questions of safety and effectiveness are addressed by fulfilling the requirements of the Special Controls for automated image assessment systems for microbial colonies on solid culture media.
- The safety and effectiveness of the MRSA modules compared with the urine module has been assured by applying the same 98% Positive Percent Agreement target for the decision point where a microbiologist review is required. For urine analysis and IC MRSA Chromogenic

TFS/S, the decision is whether growth is present on the agar. For IC MRSA Chromogenic BD, the decision is whether chromogen activation is detected, indicating a presumptive MRSA result.