510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K102922

B. Purpose for Submission:

To obtain a substantial equivalent determination for a Premarket notification for the HardyCHROM™ MRSA product.

C. Measurand:

Methicillin Resistant *Staphylococcus aureus (MRSA)*

D. Type of Test:

Detection of MRSA using a selective and differential chromogenic media

E. Applicant:

Hardy Diagnostics

F. Proprietary and Established Names:

HardyCHROM™ MRSA

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1700

2. Classification:

   Class II

3. Product code:

   JSO: Culture media, Antimicrobial susceptibility test, excluding Mueller Hinton Agar

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   HardyCHROM™ MRSA is a selective and differential chromogenic medium recommended for the qualitative detection of nasal colonization by methicillin-resistant Staphylococcus aureus (MRSA) to aid in the prevention and control of MRSA infections in health care settings. The test is performed on anterior nares swabs from patients and healthcare workers to screen for MRSA colonization. HardyCHROM™ MRSA is not intended to diagnose MRSA infection nor to guide or monitor therapy for MRSA infections. Concomitant cultures are necessary for susceptibility testing or epidemiological typing. A negative result does not preclude MRSA nasal colonization.

2. Indication(s) for use:

   HardyCHROM™ MRSA is a selective and differential chromogenic medium recommended for the qualitative detection of nasal colonization by methicillin-resistant Staphylococcus aureus (MRSA) to aid in the prevention and control of MRSA infections in health care settings. The test is performed on anterior nares swabs from patients and healthcare workers to screen for MRSA colonization. HardyCHROM™ MRSA is not intended to diagnose MRSA infection nor to guide or monitor therapy for MRSA infections. Concomitant cultures are necessary for susceptibility testing or epidemiological typing. A negative result does not preclude MRSA nasal colonization.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

HardyCHROM™ MRSA is translucent and light amber in color and contains chromogens that release chromophores when cleaved by enzymes that are produced by MRSA strains. Based on colony color, HardyCHROM™ MRSA allows for the reliable detection of most methicillin-resistant *S. aureus* from clinical specimens in less than 24 hours based on the appearance of a pink/magenta colored colony. Non-MRSA strains are either inhibited by the

addition of selective agents or utilize different chromogenic substrates in the media to produce different colored colonies. If none of the substrates are utilized, natural or white colored colonies will be present. If plates are negative for growth after 24 hours, it is recommended to re-incubate for an additional 24 hours.

J. Substantial Equivalence Information:

1. Predicate:

   BBL™ CHROMagar™ MRSA

2. Predicate K number(s):

   K042812

3. Comparison with Predicate

Product Attribute	HardyCHROM™ MRSA	BBL™ CHROMagar™ MRSA
Intended Use	HardyCHROM™ MRSA is a selective and differential chromogenic medium recommended for the qualitative detection of nasal colonization by methicillin-resistant Staphylococcus aureus (MRSA) to aid in the prevention and control of MRSA infections in health care settings. The test is performed on anterior nares swabs from patients and healthcare workers to screen for MRSA colonization. HardyCHROM™ MRSA is not intended to diagnose MRSA infection nor to guide or monitor therapy for MRSA infections. Concomitant cultures are necessary to recover organisms for susceptibility testing or epidemiological typing. A negative result does not preclude MRSA nasal colonization.	BBL™ CHROMagar™ MRSA is a selective and differential medium for the qualitative direct detection of nasal colonization by methicillin resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test is performed on anterior nares swab specimens from patients and healthcare workers to screen for MRSA colonization. BBL™ CHROMagar™ MRSA is not intended to diagnose MRSA infection nor to guide or monitor treatment for infections.
Specimen Type	Anterior nasal swabs	Anterior nasal swabs
Test Methodology	Selective and differential chromogenic prepared culture	Selective and differential chromogenic prepared culture

	medium	medium
Inoculation	Direct from specimen collection device	Direct from specimen collection device
Incubation Temperature	Incubation at 35 -37°C	Incubation at 35 -37°C
Incubation Length	24 hours. Negative plates should be re-incubated for a total of 48 hours to confirm initial findings.	24 hours, if negative re-incubate an additional 24 hours
Selective Agent	Selective agents included to allow for growth of mecA mediated MRSA strains.	Selective agents included to allow for growth of mecA mediated MRSA strains.
Testing Method	Manual	Manual
Growth Detection	Pink to magenta colonies within 24 hours. Negative plates should be re-incubated to 48 hours to confirm initial findings.	Pink to magenta colonies within 24 hours. Negative plates should be re-incubated to 48 hours to confirm initial findings.
Organism Differentiation	Chromogenic substrates facilitate differentiation of MRSA from other organisms.	Chromogenic substrates facilitate differentiation of MRSA from other organisms.
Shelf Life	10 weeks	10 weeks

K. Standard/Guidance Document Referenced (if applicable):

Clinical and Laboratory Standards Institute. 2010. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*, M100-S20. CLSI, Wayne PA.

L. Test Principle:

Methicillin-resistant *Staphylococcus aureus* strains produce pink to magenta colonies as the result of the chromogenic substrates incorporated into the HardyCHROM™ MRSA medium. The addition of specific inhibitory agents allows for the growth of *mec*A mediated MRSA strains while preventing growth of methicillin sensitive *Staphylococcus aureus* (MSSA) strains. Additional selective agents have been added to increase the sensitivity and specificity of the medium by inhibiting gram-negative organisms, yeast, and some gram-positive cocci. Bacteria other than MRSA may utilize additional chromogenic substrates present in the medium and produce blue or green colonies. HardyCHROM™ MRSA can detect most MRSA strains within 24 hours. Negative plates should be re-incubated up to 48 hours.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study with Characterized MRSA & MSSA strains

Reproducibility testing of HardyCHROM™ MRSA was conducted with twenty well-characterized test strains (fifteen MRSA strains that included Pulse-Field Gel Electrophoresis (PFGE) types USA100, USA200, USA300-0114, USA400, USA500, USA600, USA700, USA800, USA1000, USA1100, additional MRSA strains that are representative of prevalent HA(Hospital acquired)-MRSA and CA(community acquired)-MRSA (NRS100, NRS70, NRS71, BAA-43, BAA-44) and five MSSA (NRS72, SF_SNIF58,SF_SNIF104, SF_SNIF129, SF_SNIF142) that were selected from medically important *S. aureus* lineages. SF8300 is a wound isolate of pulse-field type USA300-0114, a subtype implicated in severe disease and in numerous outbreaks. All of the characterized test strains were obtained from the University of San Francisco and (DNA restriction patterns) were determined by pulsed-field gel electrophoresis. (J Clin Microbiol 33:2233-9)

Three different lots of HardyCHROM™ MRSA plates were tested to determine that the HardyCHROM™ MRSA plates reliably detected MRSA strains across different lots at different time intervals. A commercial chromogenic MRSA medium was also evaluated with the same strains and served as a performance standard. An acceptance reproducibility rate of 100% for both inter-lot and overall testing intervals was achieved with the HardyCHROM™ MRSA plates media. All of the characterized MRSA strains showed growth and magenta colony coloration within 24 hours following aerobic incubation at 35°C. All of the MSSA strains were inhibited and showed no growth even after 48 hours of aerobic incubation at 35°C. No discernible differences were observed between the performance testing of HardyCHROM™ MRSA in comparison with the commercial chromogenic MRSA medium.

The fifteen strains of MRSA were tested using a suspension containing approximately 10^5 to 10^6 CFU/ml and the five strains of MSSA were tested at a concentration of approximately 10^6 to 10^7. Ten µl of these suspensions were inoculated onto HardyCHROM™ MRSA. The sensitivity was 100% for the fifteen characterized MRSA strains and 100% specificity for the five characterized MSSA strains.

Staphylococcus aureus strains with other mechanisms of oxacillin resistance such as modified *S. aureus* (MOD-SA) strains) which have altered affinity of penicillin binding proteins for oxacillin and borderline methicillin-resistant

Staphylococcus aureus (BORSA) were not evaluated on HardyCHROM™ MRSA.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Quality Control

Quality control was performed by each of the three testing sites on receipt of each shipment of the medium and on each day of use following parameters in table below. Fresh suspensions of *Staphylococcus aureus* (MRSA) ATCC® 43300 and *Staphylococcus aureus* (MSSA) ATCC® 25923 were prepared in Tryptic Soy Broth at concentrations of approximately 10^5 to10^6 for the MRSA strain and 10^6 to10^7 for MSSA. 10µl of these suspensions were used to inoculate HardyCHROM™ plates. Plates were incubated and examined for 24 and 48 hours for growth of the MRSA strain and inhibition of the MSSA strain. All QC testing results provided expected reactions at each of the three testing sites on each day tested.

Test Organisms	**Incubation**			**Results**
	Time	**Temperature**	**Atmosphere ***	
Staphylococcus aureus ATCC® 43300	24hr	35 to 37ºC	Aerobic	Growth; pink to magenta colonies
Staphylococcus aureus ATCC® 29213	24-48hr	35 to 37ºC	Aerobic	Inhibited

* Do not incubate in CO_2.

d. Detection limit:

Not applicable

e. Analytical specificity:

Cross-Reactivity Study

Common types of nasal flora (*Staphylococcus* and non-*Staphylococcus* organisms) were evaluated for growth and performance on nonselective blood agar plates, HardyCHROM™ MRSA, and BBL™ CHROMagar™ MRSA plates. Testing of other *Staphylococcus* and non-*Staphylococcus* organisms

was conducted to determine if there was any cross reactivity when tested on HardyCHROM™ MRSA medium. A total of 121 strains were tested and included the following genera: *Acinetobacter, Burkholderia, Candida, Corynebacterium, Enterococcus, Enterobacter, Escherichia, Haemophilus, Klebsiella, Leuconostoc, Micrococcus, Moraxella, Neisseria, Proteus, Pseudomonas, Streptococcus* and *Staphylococcus*. *Staphylococcus* strains included MRSA, MSSA, and 20 different species of coagulase negative *Staphylococci* (including methicillin resistant *Staphylococcus epidermidis*). In the cross reactivity study, only *Corynebacterium jeikeium* produced a purple film in the first quadrant at 24 hours. At 48 hours, very small dark purple colonies were present but were only present in the first quadrant. *Staphylococcus intermedius* produced gray-blue colonies. All other organisms were inhibited.

Mixed Infection Study

Cell suspensions at a concentration of 10^8 and 10^9 were prepared of *Staphylococcus aureus* MSSA ATCC® 25923 and *E. coli* ATCC® 25922. Individual 10^3 dilutions (LoD) of *Staphylococcus aureus* MRSA strains (ATCC® 33591 and 43300) were mixed with the non MRSA strains and plated in duplicate on Blood Agar and paired nasal swabs were inoculated with the same suspension. The nasal swabs were used to inoculate the HardyCHROM™ MRSA plates. On HardyCHROM™ MRSA medium, the MRSA strains tested were detected beginning at 16 hours with final counts being reported at 24 hours following aerobic incubation at 35°C. Pink to magenta colonies were detected on all dilutions at 16 hours and colony counts were stable at 21 to 24 hours with no increase in the number of colonies counted. All dilutions plated showed typical pink to magenta colony coloration and colony morphology was consistent with the MRSA strains. No breakthrough was noted of any of the non-MRSA strains at 24 hours and all plates were incubated for a total of 48 hours. There was no change in MRSA strains colony forming units nor was there any breakthrough of non-MRSA at the 48 hour reading.

Interference Study Results – Nasal Sprays

The strains used in this study included MRSA strains ATCC® 33591 and ATCC 43300, as well as ten clinical MRSA isolates. Commonly used nasal sprays that contain a concentration of 1% Phenylephrine Hydrochloride did show an inhibitory affect for microbial growth on the nonselective Blood Agar plates, the HardyCHROM™ MRSA plates, and BBL™ CHROMagar™ MRSA plates. Nasal sprays that did not contain this ingredient showed no interference for growth or chromogenic reactions. The sensitivity and specificity criteria of ≥98% were met for the HardyCHROM™ MRSA plates as compared to BBL™ CHROMagar™ and Blood Agar plates.

Interference Study Results – Human Blood

Human blood could potentially be associated with MRSA strains in a healthcare setting and therefore was evaluated for potential interference of the chromogenic reaction and growth. Isolates used in this study included ATCC® control strains of MRSA (ATCC® 33591, ATCC® 43300) and ten clinical well characterized clinical MRSA isolates. All strains were evaluated for growth and performance on HardyCHROM™ MRSA, a commercial chromogenic MRSA medium, and Blood Agar plates. Results demonstrated that none of the MRSA strains were inhibited on the three types of media evaluated, and there was a 100% recovery rate for all strains after exposure to human blood. No discernible difference was observed between the growth and performance testing of HardyCHROM™ MRSA in comparison with nonselective blood agar plates and the commercial chromogenic MRSA medium.

Interference Study Results – Transport Media

The strains used in this study included MRSA strains ATCC® 33591 and ATCC 43300, as well as ten well-characterized clinical MRSA isolates. All of the strains were evaluated for growth and performance on HardyCHROM™ MRSA, BBL™ CHROMagar™ MRSA, and Blood Agar plates. Testing included the following commonly used types transport media: Stuart's Gel, Stuart's Liquid, Amies Liquid, Amies Gel, and Amies Charcoal. Each transport device tested contained rayon-tipped, plastic shaft swabs Results demonstrated that none of the MRSA strains were inhibited on the three types of media evaluated, and there was a 100% recovery rate for all transport media devices tested. No discernible difference was observed between the growth and performance testing of HardyCHROM™ MRSA in comparison with nonselective blood agar plates and BBL™ CHROMagar™ MRSA plates.

Interference Study – Mucin

Mucin from bovine submaxillary glands (type 1-S) was tested for interference using ATCC® 33591, ATCC® 43300, ten well-characterized clinical MRSA strains, and ten well-characterized clinical MSSA strains. All of the strains were exposed to suspensions of 50% and 75% mucin and after 10 minutes, were plated on blood agar and HardyCHROM™ MRSA. Results were recorded after 24 hours of incubation, with negative plates being held for an additional 24 hours (total incubation 48 hours). Suspensions prepared in mucin were held at room temperature for 24 hours and re-plated. The secondary plates were incubated at 35°C for 24 hours and plates with no growth were re-incubated for a total of 48 hours. Colonies of the MRSA strains grown on HardyCHROM MRSA™ were typical for coloration and size. Counts were stable at 18 hours with no additional growth detected at 24 or 48 hours. All MSSA strains that were tested showed growth only on the

blood agar plate and were completely inhibited at 24 and 48 hours on HardyCHROM MRSA™. Nasal swabs containing mucous or mucin were evaluated determine if there was an effect on the chromogenic reaction and the growth of MRSA strains. Isolates used in this study included ATCC® control strains of MRSA (ATCC® 33591, ATCC® 43300), ten clinical well characterized clinical MRSA isolates and ten well characterized clinical MSSA isolates.

Incubation Study

When compared to traditional culture, 127 of 132 positive MRSA cultures (96.2%) were detected on HardyCHROM™ MRSA at 24 hours. The remaining five MRSA isolates were detected at approximately 48 hours. All testing (clinical and in-house testing) was incubated at 35 to 37 degrees C.

f. Analytical sensitivity

Recovery Study (Limit of Detection (LoD))

ATCC® methicillin-resistant *Staphylococcus aureus* strains (*Staphylococcus aureus* subsp. *aureus* ATCC® 43300 and *Staphylococcus aureus* subsp. *aureus* ATCC® 33591) were tested at seven serial dilutions in duplicate. The dilutions were plated in duplicate to HardyCHROM™ MRSA and Tryptic Soy Agar with 5% Sheep Blood (BAP).

On HardyCHROM™ MRSA medium, MRSA strains tested were detected beginning at 16 hours with final counts being reported at 24 hours following aerobic incubation at 35°C. Pink to magenta colonies were detected on all dilutions at 16 hours and colony counts were stable from 21 to 24 hours with no increase in the number of colonies counted. All dilutions plated showed typical pink to magenta colony coloration. There was no significant difference in counts noted between the two observers. *Staphylococcus aureus* subsp. *aureus* ATCC® 43300 resulted in a 67.6% recovery when present at 10^2 concentration and *Staphylococcus aureus* subsp. *aureus* ATCC® 33591 resulted in a 34.6% recovery at the same dilution. At 10^1, there was 80% recovery and 52.3% recovery respectively. At 10^3 there was no discernable difference in recovery.

g. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Performance of HardyCHROM™ MRSA was evaluated at three geographically diverse hospitals with fresh surveillance specimens collected from the anterior nares. The recovery of methicillin-resistant *S. aureus* on HardyCHROM™ MRSA was compared to routine culture, defined as isolation of staphylococci on Trypticase Soy Agar with 5% blood (TSAB), with *S. aureus* identification confirmed by latex agglutination. All *S. aureus* recovered were tested for *mecA* mediated resistance by PBP2' latex testing and cefoxitin and oxacillin disk diffusion. Antibiotic disk susceptibility testing followed CLSI methods and interpretive criteria (*Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*, M100-S20. 2010). Performance of HardyCHROM™ MRSA was also compared to a commercially available chromogenic medium.

A total of 443 samples were tested against routine culture. A total of 131 specimens were positive on HardyCHROM™ MRSA with concordant results obtained on TSAB and confirmed by PBP2' latex testing and cefoxitin (30µg) and oxacillin disk (1µg) diffusion testing. An additional specimen was positive on HardyCHROM™ MRSA but did not grow on TSAB. Growth was confirmed as MRSA by PBP2' latex testing and cefoxitin (30µg) and oxacillin disk (1µg) diffusion testing. Agreement with PBP2', cefoxitin (30µg) and oxacillin (1µg) disk testing was 93.4% at 24 hours and 97.0% at 48 hours for *mecA* mediated MRSA. Percent agreement was 99.7% for non-MRSA at both 24 and 48 hours.

The combined data from three clinical trial sites, demonstrated the positive and negative percent agreement at 24 and 48 hours of HardyCHROM™ MRSA with traditional culture and the commercial chromogenic MRSA medium as summarized in Table 1 and Table 2 below:

Table 1: Agreement between Traditional Culture, a Commercial Chromogenic Medium and HardyCHROM™ MRSA

	MRSA	**Non-MRSA***
HardyCHROM™ MRSA vs. Traditional Culture 24 hours	93.3% (126/135) (95% CI 89.6 – 98.4%)	99.7% (307/308) (95% CI 98.9 – 100%)
HardyCHROM™ MRSA vs. Traditional Culture 48 hours	97.0% (131/135) 95% CI 89.6 – 98.5%	99.7% (307/308) 95% CI 98.9 – 100%
HardyCHROM™ MRSA vs. Commercial Chromogenic Medium 24 hours	98.3% (118/120) 95% CI 97.5 – 99.3%	97.5% (315/323) 95% CI 89.6 – 98.4%
HardyCHROM™ MRSA vs. a Commercial Chromogenic Medium 48 hours	98.3% (122/124) 95% CI 97.5 – 99.3%	96.9% (309/319) 95% CI 89.6 – 98.4%

*****Organisms producing other types of non-*mec*A resistance were not evaluated on this medium (e.g. MOD-SA and BORSA)

Table 2: Comparison between HardyCHROM™ MRSA and Cefoxitin 30µg Disk 24 hours

HardyCHROM™ MRSA 24 hours	Cefoxitin 30µg 24 hours			Percent Agreement
	MRSA	**Non-MRSA[a]**	**Total**	
MRSA	126	1[b]	127	Positive Percent Agreement – 93.3% (95% CI 89.6 – 98.4%)
Non-MRSA [a,c]	9	307	316	Negative Percent Agreement – 99.7% (95% CI 98.9 – 100%)
Total	135	308	443	

a. Organisms producing other types of non-*mec*A resistance were not evaluated on this medium (e.g. MOD-SA and BORSA).
b. 1/1 Blood Agar Plate negative specimen was confirmed as MRSA positive by cefoxitin disk diffusion.
c. Nine MRSA isolates were detected by traditional culture methods, but did not grow on HardyCHROM™ MRSA. Discrepant results were confirmed as MRSA positive by cefoxitin disk diffusion.

The challenge clinical isolates were obtained from a private culture collection and included 10 MRSA and 10 MSSA well-characterized strains. All isolates were coagulase positive and were characterized by PBP2' and cefoxitin testing. The challenge set was tested at three testing sites for three days using three different lots of media and by three different operators performing tested in triplicate on each day of testing. The ten strains of MRSA were tested using a suspension containing approximately 10^5 to 10^6 CFU/ml and the ten strains of MSSA were tested at a concentration of approximately 10^6 to 10^7. Ten µl of these suspensions were inoculated onto HardyCHROM™ MRSA. At each clinical study site, all isolates showed expected results on the HardyCHROM™ MRSA for each operator at each individual site and across all three sites on each testing day.

b. Matrix comparison:

Not applicable

3. Clinical studies:

   a. Clinical Sensitivity:

   Not Applicable

   b. Clinical specificity:

   Not Applicable

   c. *Other clinical supportive data (when a. and b. are not applicable):*

   Not Applicable

4. Clinical cut-off:

   Not Applicable

5. Expected values/Reference range:

   Not Applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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