Nanosphere

JAN - 8 2014

510(k) Summary

The Summary for this 510(k) submission is submitted in accordance with the requirements of SMDA 1900 and CFR 807.92

510(k) Number:

K132843: Verigene® Gram Negative Blood Culture Nucleic Acid Test (BC-GN)

Summary Preparation Date:

January 6, 2014

Submitted by:

Nanosphere, Inc.
4088 Commercial Avenue
Northbrook, IL 60062
Phone: 847-400-9000
Fax: 847-400-9176

Contact:

Mark A. Del Vecchio
Vice President, Regulatory Affairs

Proprietary Names:

For the instrument:

Verigene® System

For the assay:

Verigene® Gram Negative Blood Culture Nucleic Acid Test (BC-GN)

Common Names:

For the instrument:

Bench-top molecular diagnostics workstation

For the assay:

Gram-negative blood culture assay
Acinetobacter spp. blood culture assay
Citrobacter spp. blood culture assay
Enterobacter spp. blood culture assay
Proteus spp. blood culture assay
Klebsiella pneumoniae blood culture assay
Klebsiella oxytoca blood culture assay
Pseudomonas aeruginosa blood culture assay
Escherichia coli blood culture assay
CTX-M resistance marker blood culture assay
OXA resistance marker blood culture assay
KPC resistance marker blood culture assay
VIM resistance marker blood culture assay
NDM resistance marker blood culture assay
IMP resistance marker blook culture assay

Regulatory Information:

Regulation section:

866.3365 Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures

Classification:

Class II

Panel:

Microbiology (83)

Product Code(s):

PEN Gram-Negative Bacteria and Associated Resistance Markers

Other codes used by predicate devices:

PAM Gram-Positive Bacteria and their Resistance Markers
PEO Fungal Organisms, Nucleic Acid-Based Assay
OOI Real Time Nucleic Acid Amplification System

Predicate Devices:

FilmArray Blood Culture Identification (BCID) Panel (K130914, Biofire Diagnostics)
Verigene Gram-Positive Blood Culture (BC-GP) Nucleic Acid Test (K122514 / K113450)

Intended Use:

The Verigene® Gram Negative Blood Culture Nucleic Acid Test (**BC-GN**), performed using the sample-to-result Verigene System, is a qualitative multiplexed *in vitro* diagnostic test for the simultaneous detection and identification of selected gram-negative bacteria and resistance markers. **BC-GN** is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-negative bacteria as determined by gram stain.

BC-GN detects and identifies the following:

Bacterial Genera and Species	***Resistance Markers***
Acinetobacter spp.	CTX-M (bla_{CTX-M})
Citrobacter spp.	KPC (bla_{KPC})
Enterobacter spp.	NDM (bla_{NDM})
Proteus spp.	VIM (bla_{VIM})
Escherichia coli[1]	IMP (bla_{IMP})
Klebsiella pneumoniae	OXA (bla_{OXA})
Klebsiella oxytoca	
Pseudomonas aeruginosa	

[1] BC-GN will not distinguish *Escherichia coli* from *Shigella* spp. (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei)*

BC-GN is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, is not used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for antimicrobial susceptibility testing (AST), for identification of organisms not detected by **BC-GN**, to detect mixed infections that may not be detected by BC-GN, for association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

Technological Characteristics:

The Verigene Gram Negative Blood Culture Nucleic Acid Test (BC-GN) is a molecular assay which relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial nucleic acid sequences detected by the BC-GN test, unique Capture and Mediator oligonucleotides are utilized, with gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have regions which bind to a different portion of the same nucleic acid targets and also have sequences which allow binding of gold nanoparticle probes. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

The BC-GN test is performed on the Verigene System, a sample-to-result, fully automated, bench-top molecular diagnostics workstation consisting of two components: the Verigene Reader and the Verigene Processor *SP*. For the BC-GN test, the Verigene System allows automated nucleic acid extraction from positive bacteria-containing blood culture specimens and target detection of bacteria-specific DNA. The BC-GN test utilizes single-use disposable test consumables and a self-contained Verigene Test Cartridge for each sample tested.

The Reader is the Verigene System's central control unit and user interface, and, with a touch-screen control panel and barcode scanner, guides the user through test processing, imaging, and test result generation. The Verigene Processor *SP* executes the test procedure, automating the steps of (1) Sample Preparation– cell lysis and magnetic bead-based bacterial DNA isolation from blood culture samples, and (2) Hybridization– detection and identification of bacterial-specific DNA in a microarray format by using gold nanoparticle probe-based technology. Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are utilized for each sample tested with the BC-GN test.

To obtain the test results after processing is complete, the user removes the Test Cartridge from the Processor *SP*, and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a bacterial nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Reader.

Performance Data - Analytical Testing

Analytical Sensitivity / Limit of Detection (LOD)

The following analytical sensitivity (LOD) has been established for the detection of each of the eight (8) BC-GN test bacterial targets and six (6) resistance markers, established by testing a total of 12 bacterial strains, most of which contained the listed resistance markers.

Target	***Resistance Marker(s)***	***No. of Strains Tested***	***LOD (CFU/mL)***
Acinetobacter spp.	OXA	2	$4.0x10^5$ to $4.6x10^6$
Citrobacter spp.	VIM	2	$6.9x10^6$ to $1.3x10^7$
Enterobacter spp.	KPC	2	$4.1x10^6$ to $1.1x10^7$
Proteus spp.	-	2	$1.9x10^5$ to $7.7x10^5$
Klebsiella pneumoniae	OXA; CTX-M	1	$1.2x10^7$
Klebsiella oxytoca	CTX-M	1	$2.0x10^7$
Escherichia coli	NDM	1	$3.7x10^6$
Pseudomonas aeruginosa	IMP	1	$2.3x10^7$

Analytical Reactivity (Inclusivity)

Analytical reactivity was evaluated by triplicate testing of a large number of gram-negative bacterial species, both with and without antibiotic resistance markers, as appropriate. The panel organisms were selected to cover the genetic diversity of each BC-GN target and resistance marker. A total of 195 strains of 44 different organisms were tested including 36 strains of 12 *Acinetobacter* species, 41 strains of 11 *Citrobacter* species, 29 strains of 8 *Enterobacter* species, 16 strains of 5 *Proteus* species, 8 strains of 4 *Shigella* species, 10 strains of *Klebsiella oxytoca*, 25 strains of *Klebsiella pneumoniae*, 13 strains of *Pseudomonas aeruginosa*, and 17 strains of *Escherichia coli*. Of these 195 strains, 79 contained one or more resistance markers associated with 11 different bacterial species including a total of 38 strains containing CTX-M, 17 containing OXA, 12 containing IMP, 10 containing VIM, 10 containing KPC, and 9 containing NDM. The bacterial and resistance targets for all of the 195 strains were correctly detected (including the expected cross-reactivity with the 4 *Shigella* species) with three exceptions. Two (2) of the 13 *Acinetobacter radioresistens* strains observed false negative results for the OXA marker for 9 of 24 replicates tested. In both strains however, the bacterial target was correctly identified as "Acinetobacter spp." in all replicates tested. One of the *Citrobacter amalonaticus* strains observed false negative results Citrobacter spp. for two of nine replicates tested.

Analytical Specificity (Exclusivity)

Analytical specificity was assessed using organisms phylogenetically related to panel organisms detected by the BC-GN test, organisms without gene sequence information, common blood-borne pathogens, as well as organisms potentially present as contaminants in blood culture specimens. The organisms tested were divided into two distinct panels. The first panel consisted of the 195 "BC-GN panel" organisms, which in total comprised the analytical inclusivity study samples. There were no false positive results observed for any of the samples, indicating no cross-reativity of any of the panel members.

The second panel consisted of 172 "non-BC-GN panel" organisms which were not expected to be detected by the BC-GN test, including 88 gram-negative bacteria (including *Acinetobacter baumanii* containing OXA-51), 71 gram-positive bacteria, six (6) gram-negative cocci bacteria, and seven (7) yeast strains. Of these 172 strains tested, 159 demonstrated no cross-reactivity with the BC-GN test, while thirteen (13) organisms were determined to cross-react with the tests, as listed below. In addition, BC-GN will not distinguish *E. coli* from *Shigella* spp. including *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*.

BC-GN Test Target for Which Cross Reactivity Observed	***Cross Reactive Organism***
Citrobacter spp.	*Buttiauxella gaviniae*
	Enteric group 137
Enterobacter spp.	*Klebsiella variicola*
	Leclercia adecarboxylata
Escherichia coli	*Escherichia albertii*
	S. dysenteriae
	S. flexneri
	S. boydii
	S. sonnei
Klebsiella oxytoca	*Kluyvera ascorbata*
	Raoultella ornithinolytica
	Raoultella planticola
	Cedecea davisae
CTX-M	*Kluyvera georgiana**
	Leminorella grimontii
	Enterococcus raffinosus
	Candida parapsilosis
	blaKLUA
	blaKLUG
	blaKLUY

*Organism confirmed by bi-directional sequencing to contain CTX-M

Interfering Substances

The potential inhibitory effects of substances that may be present in patient blood specimens and a blood culture bottle additive present in different blood culture media were tested with the BC-GN test at biologically or experimentally relevant concentrations. The design of the study took into consideration that the BC-GN test sample preparation process inherently acts to minimize the potential for an interferent present in the blood culture samples to impact the test. The effects of potential interfering substances were evaluated with one representative strain of each of the eight (8) BC-GN test bacterial targets and six (6) resistance markers in the presence of several endogenous substances. Hemoglobin, triglycerides, conjugated and unconjugated bilirubin, γ-globulin and Sodium Polyanethol Sulfonate (SPS) were selected for testing. Control samples containing no interferents were also tested. No interfering effects were observed.

Fresh vs. Frozen Samples

The stability of specimens subjected to multiple freeze/thaw cycles was evaluated by conducting a fresh versus frozen specimen study. This study was performed to demonstrate that frozen storage of samples does not affect the accuracy of test results such that frozen clinical blood culture samples could be utilized in the evaluation of the BC-GN test. This study was conducted using positive blood culture specimens which were tested before and after freezing (at or below -70 °C) to allow a comparison. Representative strains of each of the eight (8) BC-GN test bacterial targets and six (6) resistance markers, as well as two additional organisms, *Hafnia alvei* and *Staphylococcus epidermidis* which served as negative organism controls, were grown to bottle positivity. Cultured samples were tested at baseline (fresh/unfrozen) and then frozen at or below -70 °C, thawed and tested, and the freeze/thaw and testing of the specimen repeated to evaluate two freeze-thaw cycles. A comparison of matched sets of specimens demonstrated 100% agreement between the fresh and frozen samples for all the analytes and for all the conditions tested. The results from this study demonstrate that the BC-GN test results are not compromised when samples are exposed to up to two freeze/thaw cycles.

Competitive Inhibition / Mixed Growth

A competitive inhibition study was conducted to evaluate the impact of combinations of gram-negative organisms present in the same blood culture bottle at similar concentrations prior to culturing. Combinations of bacterial organisms representative of all BC-GN test target analytes were co-inoculated into individual blood culture bottles at clinically-relevant starting concentrations and incubated to positivity. The BC-GN test correctly detected the bacteria and resistance marker(s) for four (4) of the eight target organisms, irrespective of combination(*A. baumannii*/OXA, *C. freundii*/VIM, *K. pneumoniae*/OXA and CTX-M, and *P. mirabilis*) present in co-inoculated blood culture bottles, demonstrating that these organisms are not subject to competitive inhibition from BC-GN test panel members when both are present at concentrations expected in routine clinical practice. For the remaining four (4) organisms, at least one of the expected bacterial targets or resistance markers was not detected. This was due most likely to the slower growth rates of several organisms relative to other co-infected organisms, as faster growing organisms may reach a higher concentration at bottle positivity compared with a slower growing organism. This may lead to slower-growing organism concentrations at bottle positivity that are below the limit of detection of the test.

A second competitive inhibition study was conducted to further evaluate representative combinations of the four (4) organisms for which at least one false negative result was observed during the first study. This involved retesting nine specific organism combinations. This study was designed to confirm whether false negative results observed with these combinations were due to the slower growth rates of the undetected organisms relative to the detected organisms. Therefore, growth rate was eliminated as an experimental variable by testing mixed culture organisms at "bottle positivity" concentrations and above the LoD for each organism. Of the nine (9) combinations, eight (8) combinations yielded expected calls. For one combination, *K. oxytoca*/CTX-M and *E. coli*/NDM, the *K. oxytoca* target was detected at a rate of 78% (7/9); however, 100% detection was observed for the other three targets in this sample (*E. coli*, NDM, and CTX-M). This demonstrated that except in one instance, growth rate, not competitive inhibition was a contributing factor to the initial observed false negative results.*Carry-over / Cross-contamination*

Twelve Verigene *SP* instruments were used to assess the potential for carryover/cross-contamination by alternately running "high positive" samples followed by negative samples. Representative strains of the eight (8) bacterial target organisms and six (6) resistance markers were used to prepare the high positive samples. All of the high positive samples yielded the expected "Detected" results for the intended bacteria/marker and "Not Detected" results for the other analytes. The negative samples gave a "Not Detected" call for all analytes. There was no evidence of carryover/cross-contamination observed.

Cutoff Verification

In order to verify the assay cut-off, the target mean intensity values observed with the BC-GN test were examined for a panel of eighteen (18) contrived specimens, representing all of the BC-GN test analytes and two (2) additional organisms not detected by the BC-GN test, which served as negative organism controls. Taking into consideration replicate testing, a complete set of 1:10 dilutions of all the organisms, and the number of probes on the BC-GN test microarray, 2460 data points were compiled for the threshold evaluation, using logistic fit and ROC statistics.

Validation of Additional Blood Culture Bottle Types

In order to achieve a"universal bottle-type" claim, the performance of the BC-GN test was evaluated for twelve (12) types of blood culture media (in addition to the BACTEC PLUS-Aerobic/F bottle used for the other analytical validation studies) using three different automated blood culture monitoring systems, providing a significant representation of commercially-available blood culture media bottles and monitoring systems (see listing of culture bottles tested below). This study was conducted to demonstrate that BC-GN test target organisms grow to a sufficient concentration in the various bottle types and additionally to demonstrate the stability of bacterial DNA is sufficiently stable over an extended time period of 36 hours once bottle positivity has been reached.

Blood Culture Bottle *(Manufacturer)*		***Blood Culture System***
BD BACTEC (Becton Dickenson)	Standard/10 Aerobic/F	BACTEC 9050
	PLUS - Anaerobic/F	
	Peds Plus/F	
	Standard/Anaerobic/F	
	Lytic/10 Anaerobic/F	
BacT/ALERT (BioMerieux)	SA Standard Aerobic	BacT/ALERT 3D
	FA FAN® Aerobic	
	PF Pediatric FAN	
	SN Standard Anaerobic	
	FN FAN Anaerobic	
VersaTREK (Thermo Scientific)	REDOX 1 EZ Draw® / Aerobic	VersaTREK
	REDOX 2 EZ Draw ® / Anaerobic	

In the study, representative BC-GN test bacterial organisms (all eight bacteria and six resistence markers) were inoculated into each of the twelve different bottle types which were spiked with anti-coagulated human whole blood. The bottles were placed on the appropriate culture system and upon reaching bottle positivity, an aliquot of each sample was tested with the BC-GN test in triplicate. To evaluate specimen stability in the various bottles, one bottle of each organism was then stored for 36 hours at each of three temperature conditions: refrigerated, ambient, and on the blood culture system, after which BC-GN testing was performed. The test results demonstrated that these twelve (12) blood culture bottles are appropriate for use with the BC-GN test and that specimens are stable in those bottles at various temperature storage conditions for up to 36 hours after reaching bottle positivity.

Precision / Repeatability

Precision was evaluated by testing an 18-member panel, containing eight (8) unique specimens representing each target analyte (both bacterial and resistance markers) detected by the BC-GN test, as well as two negative controls, one consisting of negative blood culture media and the second containing an organism not detected by the BC-GN test (*Hafnia alvei*). The 18-member panel was tested in-house by Nanosphere twice daily by two operators over twelve (12) non-consecutive days for a total of forty-eight replicates per sample. Except for the negative controls, organisms were tested at Bottle Positivity and Bottle Positivity + 8 hours incubation. A total of 864 tests were conducted and the final call rate for the study was 99.9% (863/864). There was one inaccurate call involving a *Klebsiella pneumoniae*/OXA/CTX-M specimen, whereby BC-GN detected "K. oxytoca", in addition to the correct expected calls, resulting in a call accuracy of 99.9% (862/863) for the study. The results from the study are contained in the table below.

Sample		***Bottle Positivity***		***Bottle Positivity + 8 hours***	
Organism/Specimen	***Resistance Marker(s)***	*Final Call Rate*	*Accuracy*	*Final Call Rate*	*Accuracy*
Negative Control – Blood Culture Media	N/A	100% (48/48) 92.6-100	100% (48/48) 92.6-100	-	-
Hafnia alvei	N/A	97.9% (47/48) 88.9-100	100% (47/47) 92.5-100	-	-
Acinetobacter baumanii	OXA	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Citrobacter freundii	VIM	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Enterobacter cloacae	KPC	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Escherichia coli	NDM	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Klebsiella pneumoniae	OXA, CTX-M	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	97.9% (47/48) 88.9-100
Klebsiella oxytoca	CTX-M	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Proteus mirabilis	N/A	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100
Pseudomonas aeruginosa	IMP	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100	100% (48/48) 92.6-100

Reproducibility

Reproducibility was evaluated by testing an 18-member panel (the same sample panel that was tested for the evaluation of precision with one exception), containing eight (8) unique specimens representing each target analyte detected by BC-GN, as well as two negative controls, one consisting of negative blood culture media only and the second containing an organism not detected by BC-GN (*Morganella morganii*). The 18-member panel was tested at three (3) external sites twice daily in triplicate on five (5) non-consecutive days for a total of 90 replicates per sample. Except for the negative controls, organisms were tested at Bottle Positivity and Bottle Positivity + 8 hours incubation. The final call rate and accuracy for the study was 100% (1620/1620). The results from the study are contained in the table below.

Sample		***Bottle Positivity***		***Bottle Positivity + 8 hours***	
Organism/Specimen	***Resistance Marker(s)***	*Final Call Rate*	*Accuracy*	*Final Call Rate*	*Accuracy*
Negative Control – Blood Culture Media	N/A	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	-	-
Morganella morganii	N/A	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	-	-
Acinetobacter baumanii	OXA	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Citrobacter freundii	VIM	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Enterobacter cloacae	KPC	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Escherichia coli	NDM	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Klebsiella pneumoniae	OXA, CTX-M	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Klebsiella oxytoca	CTX-M	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Proteus mirabilis	N/A	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)
Pseudomonas aeruginosa	IMP	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)	100% 90/90 (96.0-100)

Method Comparison

The combined results for the method comparison study conducted at thirteen (13) investigational sites are presented below. A total of 1412 specimens were analyzed, 604 of which were prospectively-collected fresh specimens, 272 of which were prospectively-collected frozen specimens, 239 of which were selected frozen specimens, and 297 of which were simulated frozen specimens.. Performance of the BC-GN test related to the detection of the target organisms was determined by comparing the BC-GN test result to results obtained using standard culture-based automated phenotypic bacterial identification reference methods. For the resistance markers, reference method testing included the combination of PCR amplification and bidirectional sequencing confirmation.

Summary of Clinical Test Performance—Bacterial Results (n=1412)

Organism	***Percent Agreement***	
	Positive *(95% CI)*	***Negative*** *(95% CI)*
Acinetobacter spp.	98.2% 55/56 (90.5-100)	99.9% 1355/1356 (99.6-100)
Citrobacter spp.	100% 49/49 (92.8-100)	99.9% 1362/1363 (99.6-100)
Enterobacter spp.	97.6% 120/123 (93.0-99.5)	99.4% 1281/1289 (98.8-99.7)
Proteus spp.	100% 58/58 (93.8-100)	99.9% 1353/1354 (99.6-100
Escherichia coli	99.8% 517/518 (98.9-100)	99.4% 889/894 (98.7-99.8)
Klebsiella pneumoniae	93.1% 285/306 (89.7-95.7)	100% 1106/1106 (99.7-100)
Klebsiella oxytoca	92.2% 59/64 (82.7-97.4)	99.6% 1342/1348 (99.0-99.8)
Pseudomonas aeruginosa	97.6% 124/127 (93.3-99.5)	100% 1285/1285 (99.7-100)

Summary of Clinical Test Performance—Linked Resistance Marker Results (n=1266)

Resistance Marker Target	*Percent Agreement*	
	Positive (95% CI)	*Negative (95% CI)*
CTX-M	98.7% 151/153 (95.4-99.8)	99.9% 1112/1113 (99.5-100)
OXA	95.3% 61/64 (86.9-99.0)	99.9% 1201/1202 (99.5-100)
KPC	100% 51/51 (93.1-100)	100% 1215/1215 (99.7-100)
VIM	100% 41/41 (91.4-100)	100% 1225/1225 (99.7-100)
NDM	100% 41/41 (91.4-100)	100% 1225/1225 (99.7-100)
IMP	100% 48/48 (92.6-100)	100% 1218/1218 (99.7-100)

Substantial Equivalence

As demonstrated by the information stated below, the Verigene Gram Negative Blood Culture Nucleic Acid Test (BC-GN test) has been shown to be as safe and effective as the BioFire FilmArray Blood Culture Identification (BCID) Panel Kit (K130914) and the Nanosphere Verigene Gram-Positive Blood Culture (BC-GP) Nucleic Acid Test (K122514). The BC-GN test has similar intended use and indications, technological characteristics, and performance characteristics. The minor differences between the BC-GN test and its predicate device raises no new issues of safety or effectiveness. Clinical and analytical performance data demonstrate that the BC-GN test is as safe and effective as the predicate device. Thus, the BC-GN test is substantially equivalent.

Similarities between the Verigene BC-GN Test and Predicate Device #1

Items	*Device*	*Predicate #1*
	Verigene BC-GN Test	***BioFire FilmArray Blood Culture Identification Panel***
510(k)#	K132843	K130914
Regulation	866.3365	Same
Product Code	PEN	PEN
Device Class	II	Same
Intended Use	Qualitative *in vitro* diagnostic test for detection and identification of microorganism nucleic acids	Same
Indications for Use	To aid in the diagnosis of bacterial bloodstream infections	Same
Warnings and Precautions	Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identification of organisms not detected by the test, differentiation of mixed growth, association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.	Same
Contraindication(s)	Not to be used to monitor bloodstream infections/treatment for bacterial infection.	Same
Organisms and Resistance Markers Detected	*Proteus* spp. *Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aeruginosa* KPC	Same (see below for differences)
Sample Processing and Purification	On-board, automated DNA extraction	Same
Technology Principles	Multiplex nucleic acid-based	Same (see below for differences)
Interpretation of Results	Diagnostic Software/Decision Algorithm	Same
Sensitivity* (Positive % Agreement)	*Acinetobacter* spp.: 98.2% (90.5-100%) *Enterobacter* spp.: 97.5% (93.0-99.5%) *Escherichia coli*: 99.6% (98.9-100%) *Klebsiella oxytoca*: 92.2% (82.7-97.4%) *Klebsiella pneumoniae*: 93.1% (89.7-95.7%) *Proteus* spp.: 100% (93.8-100%) KPC: 100% (93.1-100%)	*A. baumannii*: 100% (93.0-100%) *Enterobacteriaceae*: 98.4% (96.9-99.3%) *Escherichia coli*: 98.0% (94.4-99.6%) *K. oxytoca*: 92.2% (82.7-97.4%) *K. pneumoniae*: 97.1% (91.9-99.4%) *Proteus* spp.: 100% (91.0-100%) KPC: 100% (91.0-100%)
Specificity* (Negative % Agreement)	*Acinetobacter* spp.: 100% (99.6-100%) *Enterobacter* spp.: 99.6% (98.8-99.7%) *Escherichia coli*: 99.4% (98.7-99.8%) *Klebsiella oxytoca*: 99.5% (99.0-99.8%). *Klebsiella pneumoniae:* 100% (99.7-100%) *Proteus* spp.: 100% (99.6-100%) KPC: 100% (99.7-100%)	*A. baumannii*: 99.8% (99.5-99.9%) *Enterobacteriaceae*: 99.8% (99.4-99.9%) *Escherichia coli*: 99.8% (99.4-99.9%) *K. oxytoca*: 99.9% (99.7-100%) *K. pneumoniae*: 99.6% (99.2-99.8%) *Proteus* spp.: 100% (99.8-100%) KPC: 100% (99.2-100%)
End Users	Operators with no clinical lab experience to experienced clinical laboratory technologists.	Same

* Data for predicate obtained from BioFire FilmArray Blood Culture Identification Panel (K130914) Decision Summary.

Differences between the Verigene BC-GN Test and Predicate Device #1

Items	*Device*	*Predicate #1*
	Verigene BC-GN Test	***BioFire FilmArray Blood Culture Identification Panel***
510(k)#	K132843	K130914
Organisms and Resistance Markers Detected	*Acinetobacter* spp. *Citrobacter* spp. *Enterobacter* spp. CTX-M NDM VIM IMP OXA	*Acinetobacter baumannii* *Enterobacteriaceae* *Haemophilus influenza* *Neisseria meningitidis* (encapsulated) *Serratia marcescens*
Test Format	Disposable single-use, multi-chambered fluidic test cartridge	Disposable single-use, pouch containing freeze-dried reagents
Controls	Two Internal Processing Controls (whole organism complete assay control and single-stranded DNA Hybridization control)	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.
DNA Amplification method	None	Nested, multiplex PCR with DNA melt analysis.
Time to Result	2 hours	Less than one hour
Detection Method	Gold/Ag nanoparticle probe detection of bacterial-specific DNA on complementary oligo- microarray	Amplicon detection by melt curve analysis of fluorescence signal from DNA binding dye.
Instrumentation	Verigene Reader and Processor SP	FilmArray Instrument

Similarities between the Verigene BC-GN Test and Predicate Device #2

Items	*Device*	*Predicate #2*
	Verigene BC-GN Test	*Verigene BC-GP Test*
510(k)	K132843	K122514
Regulation	866.3365	Same
Product Code	PEN	PAM
Device Class	Class II	Same
Intended Use	Qualitative multiplex *in vitro* diagnostic test for the detection and identification of microorganism nucleic acids	Same
Indications for Use	To aid in the diagnosis of infectious diseases	Same
Time to Result	~2.5 hrs	~2.0 hrs
Test Cartridge	Disposable single-use, multi-chambered fluidic cartridge.	Same
Sample Prep	Automated onboard extraction of nucleic acids performed on the Processor *SP* using silica coated magnetic beads and chaotropic salts.	Same
Quality control	Internal procedural/instrument quality controls; internal negative control, sample processing control; external positive and negative assay controls	Same
Detection Method	Gold/Ag nanoparticle probe detection of bacterial-specific DNA on complementary oligo- microarray	Same
Method Steps/ Complexity	Single pipetting step specimen, prior to NA extraction acid isolation, hybridization, and direct detection	Same
Interpretation of Results	Diagnostic Software/ Decision Algorithm	Same
Software	Custom embedded software application running under embedded operating system	Same
Reader	Optical intensities are analyzed after target-specific hybridization of probes	Same
End Users	Operators with no clinical lab experience to experienced clinical laboratory technologists	Same

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

NANOSPHERE, INC
MARK DEL VECCHIO
4088 COMMERCIAL AVENUE
NORTHBROOK IL 60062

January 8, 2014

Re: K132843
Trade/Device Name: Verigene® Gram Negative Blood Culture Nucleic Acid Test (BC-GN)
Regulation Number: 21 CFR 866.3365
Regulation Name: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures
Regulatory Class: II
Product Code: PEN, NSU
Dated: December 20, 2013
Received: December 27, 2013

Dear Mr. Del Vecchio:

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. 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 Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K132843

Device Name: Verigene® Gram Negative Blood Culture Nucleic Acid Test (BC-GN)

Indications For Use:

The Verigene® Gram Negative Blood Culture Nucleic Acid Test (BC-GN), performed using the sample-to-result Verigene System, is a qualitative multiplexed *in vitro* diagnostic test for the simultaneous detection and identification of selected gram-negative bacteria and resistance markers. BC-GN is performed directly on blood culture media using blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain gram-negative bacteria as determined by gram stain.

BC-GN detects and identifies the following:

Bacterial Genera and Species	***Resistance Markers***
Acinetobacter spp.	CTX-M (bla_{CTX-M})
Citrobacter spp.	KPC (bla_{KPC})
Enterobacter spp.	NDM (bla_{NDM})
Proteus spp.	VIM (bla_{VIM})
Escherichia coli[1]	IMP (bla_{IMP})
Klebsiella pneumoniae	OXA (bla_{OXA})
Klebsiella oxytoca	
Pseudomonas aeruginosa	

[1]BC-GN will not distinguish *Escherichia coli* from *Shigella* spp. (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei)*

BC-GN is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections; however, is not used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for antimicrobial susceptibility testing (AST), for identification of organisms not detected by BC-GN, to detect mixed infections that may not be detected by BC-GN, for association of antimicrobial resistance marker genes to a specific organism, or for epidemiological typing.

Prescription Use __X_____ AND/OR Over-The-Counter Use _____

(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of Center for Devices and Radiological Health (CDRH)

John Hobson -S
2014.01.07 10:37:53
-05'00'