November 7, 2019

Centers for Disease Control and Prevention
Julie Villanueva
Chief, Laboratory Preparedness and Response Branch
1600 Clifton Road NE, MS: H24-11
Atlanta, Georgia 30329

Re: K192871
Trade/Device Name: B. anthracis Real-time PCR Assay
Regulation Number: 21 CFR 866.3045
Regulation Name: In vitro diagnostic device for Bacillus spp. detection
Regulatory Class: Class II
Product Code: NHT
Dated: October 7, 2019
Received: October 8, 2019

Dear Julie Villanueva:

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,

Kristian Roth, Ph.D.
Branch Chief
Bacterial Multiplex and Medical Countermeasures 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: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K192871

Device Name
B. anthracis Real-time PCR Assay

Indications for Use *(Describe)*
The B. anthracis Real-Time PCR Assay is an in vitro diagnostic test for the qualitative detection of plasmid and chromosomal DNA sequences from B. anthracis. The assay can be used to test human respiratory samples, whole blood, serum, plasma, swabs from lesions, cerebrospinal fluid, pleural fluid, and bacterial culture isolates from individuals suspected of having anthrax.

Results generated from direct specimen testing are presumptive for the identification of B. anthracis. Results generated from culture isolate testing should be used in conjunction with other conventional methods for identification of Bacillus anthracis isolates as part of the LRN Bacillus anthracis Testing Algorithm. The diagnosis of anthrax infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidences, in addition to the identification of B. anthracis from cultures or detection directly in clinical specimens.

Use is limited to Laboratory Response Network (LRN) designated laboratories.

The B. anthracis Real-time PCR Assay is also intended for environmental specimen testing for biothreat detection and response. FDA has not evaluated claims related to the use of this assay on environmental specimens.

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.

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5. 510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Assigned 510(k) number:	K192871
Submitted by:	Centers for Disease Control and Prevention 1600 Clifton Road, MS: H24-11 Atlanta, GA 30329-4027
Contact Person:	Julie Villanueva, Ph.D. Laboratory Preparedness and Response Branch Chief Division of Preparedness and Emerging Infections National Center for Emerging and Zoonotic Infectious Diseases Centers for Disease Control and Prevention (Registration number: 1050190) 1600 Clifton Road, NE, MS: H24-11 Atlanta, GA 30329-4027 (404) 639-3851 (office) fkb8@cdc.gov
Date prepared:	October 31, 2019

Device trade name:	*B. anthracis* Real-time PCR Assay
Classification name and regulation: (if applicable)	*In vitro* diagnostic device for *Bacillus spp.* detection; 21 CFR 866.3045
Predicate device(s):	*B. anthracis* Real-time PCR Assay (K140426)

Background

Anthrax is a zoonotic disease caused by *B. anthracis* that is transmissible to humans through handling or consumption of contaminated animal products. Infection can also occur through inhalation of *B. anthracis* spores from contaminated animal products such as wool or hides. Infection caused by human-to-human contact has been reported only rarely, and only via the cutaneous route (Versalovic, 2011). There have been 3 major presentations of anthrax in humans: cutaneous, ingestion, and inhalation. In cases of cutaneous anthrax, patients typically present with a painless blister or skin ulcer with a black area in the center. Inhalation anthrax is typically associated with cold or flu-like symptoms, cough, chest discomfort, shortness of breath, fatigue, and muscle aches. Symptoms of gastrointestinal anthrax typically include nausea, loss of appetite, bloody diarrhea, fever and severe stomach pain.

Prior to the development of the LRN *B. anthracis* Real-time PCR Assay, identification of *B. anthracis* was determined by using phenotypic differences between *B. anthracis* and the rest of the *B. cereus* group. (i.e. lack of motility and hemolysis, susceptibility to penicillin, colony morphology, susceptibility to

lysis by gamma phage) (Hoffmaster, 2002). However, these methods require growth of the microorganism and can take at least 24 hours incubation to obtain a result. Due to the prevalence of *B. anthracis* in the environment, and its past use as a biological weapon, it has long been an organism of concern. The use of *B. anthracis* in the bioterrorism attacks of 2001 resulting in cases of inhalation and cutaneous anthrax increased public health concern and reinforced the worry that it would be used in the same way again. For these reasons, there was a need for rapid testing to aid in the identification of *B. anthracis*. The Laboratory Response Network (LRN) is part of a national bioterrorism preparedness initiative and one of the major goals of this initiative is the development and validation of rapid and specific assays for agents likely to be used in a bioterrorism event. Accordingly, scientists at the Centers for Disease Control and Prevention have developed several real-time PCR based assays to detect *B. anthracis* and other potential agents of bioterrorism in an effort to meet the need for rapid detection.

Device Description

The *B. anthracis* Real-time PCR Assay uses a fluorogenic probe, consisting of an oligonucleotide with a reporter dye (FAM) attached to the 5' end and a quencher dye (BHQ1) attached at or near the 3' end. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe causing the reporter dye to separate from the quencher dye and a fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes and the fluorescence intensity is monitored during the PCR. The Taq polymerase used in this assay is inactive at room temperature. It must be activated by incubation at 95°C, which also minimizes the production of nonspecific amplification products.

Each extracted DNA sample is tested with three *B. anthracis* primer and probe sets run as individual reactions. The primer and probe sets target genes encoding virulence factors as well as conserved regions of DNA from the *B. anthracis* chromosome. All primer and probe sets must be positive for the overall result of the *B. anthracis* Real-time PCR Assay to be interpreted as positive. Any result that is positive for some, but not all target regions, is still considered equivocal and follow-up laboratory investigation should be performed per the LRN *Bacillus anthracis* testing algorithm.

Intended Use

The *B. anthracis* Real-Time PCR Assay is an *in vitro* diagnostic test for the qualitative detection of plasmid and chromosomal DNA sequences from *B. anthracis*. The assay can be used to test human respiratory samples, whole blood, serum, plasma, swabs from lesions, CSF, pleural fluid, and bacterial culture isolates from individuals suspected of having anthrax.

Results generated from direct specimen testing are presumptive for the identification of *B. anthracis*. Results generated from culture isolate testing should be used in conjunction with other conventional methods for identification of *Bacillus anthracis* isolates as part of the LRN *Bacillus anthracis* Testing Algorithm. The diagnosis of anthrax infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidences, in addition to the identification of B. anthracis from cultures or detection directly in clinical specimens.

Use is limited to Laboratory Response Network (LRN) designated laboratories.

The *B. anthracis* Real-time PCR Assay is also intended for environmental specimen testing for biothreat detection and response. FDA has not evaluated claims related to the use of this assay on environmental specimens.

Device Comparison

The following table summarizes the similarities and differences between the cleared assay and the new submission for this device.

	Predicate ***B. anthracis* Real-time PCR Assay (K140426)**	Device ***B. anthracis* Real-time PCR Assay (new)**
Intended Use	The *B. anthracis* Real-Time PCR Assay is an *in vitro* diagnostic test for the qualitative detection of plasmid and chromosomal DNA sequences from *B. anthracis*. The assay can be used to test human respiratory samples, whole blood, serum, plasma, swabs from lesions, CSF, pleural fluid, and bacterial culture isolates from individuals suspected of having anthrax. Results generated from direct specimen testing are presumptive for the identification of *B. anthracis*. Results generated from culture isolate testing should be used in conjunction with other conventional methods for identification of *Bacillus anthracis* isolates as part of the	Unchanged

	LRN *Bacillus anthracis* Testing Algorithm. The diagnosis of anthrax infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidences, in addition to the identification of B. anthracis from cultures or detection directly in clinical specimens. **Use is limited to Laboratory Response Network (LRN) designated laboratories.** The *B. anthracis* Real-time PCR Assay is also intended for environmental specimen testing for biothreat detection and response. FDA has not evaluated claims related to the use of this assay on environmental specimens.	
Principle of Operation	Nucleic acid amplification and fluorescent probe detection	Unchanged
Targets	BA1 – pXO2 DNA BA2 – pXO1 DNA BA3 – *B. anthracis* chromosomal region DNA	Unchanged
Sample Types	• Swabs from lesions and vesicular material • Whole Blood (EDTA or sodium citrate) • Serum/Plasma • Respiratory specimens (transtracheal aspirates, bronchial lavage, and sputum) • Cerebrospinal fluid • Pleural Fluid • Bacterial culture isolates • Environmental samples collected for investigational or surveillance use	Unchanged

Instrumentation	• Applied Biosystems 7500 Fast Dx Real-Time PCR • Cepheid SmartCycler I • Cepheid SmartCycler II	• Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument • Cepheid SmartCycler I • Cepheid SmartCycler II • QuantStudio Dx Real-Time PCR Instrument
Software/ Hardware	Software/Hardware not included as part of device; may be run on the manufacturer installed and validated software from the AB 7500 Fast Dx, SmartCycler I, or SmartCycler II	Software/Hardware not included as part of device; may be run on the manufacturer installed and validated software from the AB 7500 Fast Dx, SmartCycler I, SmartCycler II, or QuantStudio Dx real-time PCR instruments
Master mix	• Quanta PerfeCTa MultiPlex qPCR SuperMix, Low ROX • Roche LightCycler FastStart DNA Master HybProbe	Unchanged

Establishment of Performance Characteristics

Inquiries regarding performance characteristics for the *B. anthracis* Real-time PCR Assay should be directed to the Centers for Disease Control and Prevention.

Analytical Limit of Detection (LoD)

The limit of detection for the *B. anthracis* Real-time PCR Assay was determined through an analytical sensitivity study.

Analytical Sensitivity and Specificity

Inquiries regarding performance characteristics for the *B. anthracis* Real-time PCR Assay should be directed to the Centers for Disease Control and Prevention.

Clinical Performance

Inquiries regarding clinical performance characteristics for the *B. anthracis* Real-time PCR Assay should be directed to the Centers for Disease Control and Prevention.