

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number: k030370

B. Analyte: *Bacillus anthracis* cell surface protein

C. Type of Product: Assay kit, Immunochromatographic, *Bacillus Anthracis* Differential Antibody

D. Applicant: Tetracore, Inc.

E. Proprietary and Established Names of the Product: Redline Alert™ Test

F. Regulatory Information:

1. Regulation section: Unclassified
2. Classification: Unclassified
3. Product code: NPO
4. Panel: 83

G. Intended Use:

Intended use(s): The Tetracore *RedLine Alert*™ Test is intended for the rapid, *in vitro* qualitative presumptive identification of *Bacillus anthracis* from non-hemolytic *Bacillus* colonies cultured on sheep blood agar plates. The test is intended for use in clinical, public health, and hospital laboratories in conjunction with other markers and testing for the presumptive identification of *Bacillus anthracis*.

Warning: The *RedLine Alert*™ Test has not been evaluated for use with spore preparations, suspicious powders, or samples other than colonies from culture growth.

1. Indication(s) for use: non-hemolytic *Bacillus* colonies cultured on sheep blood agar plates
2. Special conditions for use statement(s): used in conjunction with other markers and testing for the presumptive identification of *B. anthracis*
3. Special instrument requirements: none

H. Device Description:

RedLine Alert™ Test is an immunochromatographic lateral flow cassette-type device. It has a nitrocellulose membrane (backed with a vinyl adhesive support) that is striped with a polyclonal rabbit IgG (*B. anthracis* Sterne vegetative and spore mix used as immunogen) for the T line and a 2nd polyclonal antibody for the C (control) line. The T-line polyclonal is obtained from rabbits immunized with *B. anthracis* Sterne, purified by Protein-G chromatography. The membrane is encased in a plastic cassette-type casing.

A mouse monoclonal antibody reagent is purified by Protein A chromatography, sterile filtered, preserved in 0.1% NaAzide and conjugated to colloidal gold, and then added to a glass fiber pad that is assembled in the cassette under the cellulose sample pad. An additional cellulose pad is affixed in the cassette assembly at the distal end of the membrane strip to facilitate migration. To use the device, colony isolation buffer is also needed. The kit contains tubes with 7.5 mL of TRIS, EDTA, KCl, Triton-X100 and NaAzide formulated buffer (pH 8.0). Aliquots of 200 µL are used for performing a test.

I. Substantial Equivalence Information (if known):

1. Predicate device name(s): preamendments
2. Predicate 510(k) number(s): preamendments
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Identification and differentiation of <i>B. anthracis</i> from other non-hemolytic <i>Bacillus</i> -like species	Identification and differentiation of <i>B. anthracis</i> from other <i>Bacillus</i> -like species
Indications	Adjunct to microbiological culture procedures	Same
Differences		
Item	Device	Predicate
Antibody type	Monoclonal	Polyclonal
Conjugate tag	Colloidal gold	FITC
Format	Lateral flow single unit	Fluorescent microscopy

J. Standard/Guidance Document Referenced (if applicable): NA

K. Test Principle: Alert™ is an immunochromatographic test format with manual preparation of test suspension and visual interpretation of endpoint (presence/absence of a colored line in the T and C stripe regions). A visible colored line appears in the cassette window if antigen (a cell surface protein) homologous with the monoclonal conjugate (murine MAB-colloidal gold) is present in the colony tested. This cell surface protein is also found in some *B. cereus* and *B. thuringiensis* strains. However, those strains are invariably hemolytic when cultured on sheep blood agar.

L. Performance Characteristics (provide the information described below as appropriate to support the substantial equivalence determination):

1. Analytical performance:

- a. *Precision/Reproducibility*: During inclusivity, exclusivity, and other analytical testing, test replicates of the same organism were done. These were done from different preparations of the same culture and were read by 2 different observers. Intensity of colored lines was also documented by using a densitometric reader (a reflected light optical reader) giving sample values (SV), a semiquantitative scoring. Three of 52 colonies tested were scored as either indeterminate or a faint band by one or more observers.
 - b. *Linearity/Assay reportable range*: Variations in cultural growth conditions were assessed. These included 8-48 h incubation times, temperature incubation at both 30° and 37° C, ambient air and CO₂ incubation, and density of test inoculum. Conclusions from these studies:
12-24 h cultures, incubated either ambient or CO₂, and with incubation temperature as low as 30°C provide reliable results.
 - c. *Traceability (controls, calibrators, or method)*: A positive control material is included in the kit: individual polypropylene tubes of lyophilized supernatant from liquid broth cultures of *B. anthracis* Sterne. Each cassette contains an internal procedural control, antibody (anti-murine) striped onto the test membrane that captures excess and unbound conjugate. The monoclonal reactive with the cell surface protein is proprietary and there is no standard reference material available.
 - d. *Detection limit*: NA – the assay is intended for use with colonies, using a prescribed amount of inoculum. Inclusivity studies were performed with 145 different *B. anthracis* strains (organisms from 28 different countries on 6 continents and from 12 different states within the US), representing each of the 5 VNTR (variable-number tandem repeats) groups and 53 of the 89 described genotypes. One strain had unusually small atypical colonies. The Alert™ showed positive results for 143/145 (98.6%).
 - e. *Analytical specificity*: Exclusivity studies were done with 49 nonhemolytic *Bacillus* and *Bacillus*-like spp., 52 hemolytic *Bacillus* and *Bacillus*-like spp. and 26 non-*Bacillus* common pathogens. All non-hemolytic colony types were negative when tested with Alert™; 12 of 52 hemolytic *Bacillus* spp. were positive with Alert™.
 - f. *Assay cut-off*: NA
2. Comparison studies:
- a. *Method comparison with predicate device*: There is no predicate device to use for comparison testing. There are established methods for identification of *B. anthracis* and for differentiating *Bacillus* spp. by evaluating different phenotypic characteristics of culture growth, and microscopic and colony morphology. A field evaluation of the Alert™ was done in a laboratory performing testing for *B. anthracis* surveillance of a contaminated worksite. At this laboratory, 633 nonhemolytic isolates were tested with gamma phage and Alert™. Of these, 410 were identified as *B. anthracis* and 223 as non-*B. anthracis* isolates. Results for Alert™ and gamma phage susceptibility agreed for all of these culture isolates. [Note: all *B. anthracis* in this

evaluation were Ames strain so sensitivity across a spectrum of different strains cannot be inferred].

- b. *Matrix description and comparison*: NA – the Alert™ is only indicated for use with colonies recovered on sheep blood agar culture plates.
- 3. Clinical Studies:
 - a. *Clinical sensitivity*: NA
 - b. *Clinical specificity*: NA
 - b. *Other clinical supportive data (when a and b are not applicable)*: NA
- 4. Clinical cut-off (where applicable): NA
- 5. Expected values/Reference range: NA

M. Conclusion: Substantially equivalent