510(k) SUBMISSION TEMPLATE

A. 510(k) Number: K040407

B. Analyte: Antibodies to Protective Antigen (PA) protein, *Bacillus anthracis*

C. Type of Test: ELISA

D. Applicant: Immunetics, Inc.

E. Proprietary and Established Names of the Product: QuickELISA™ Anthrax-PA Kit

F. Regulatory Information:

1. Regulation section: Unclassified
2. Classification: Unclassified
3. Product code: **NRL** - ENZYME LINKED IMMUNOABSORBENT ASSAY, ANTIBODY, B. ANTHRACIS
4. Panel: 83

G. Intended Use and Indication for Use:

1. Intended use(s): The Immunetics® QuickELISA™ Anthrax-PA Kit is intended for use in the qualitative detection of antibodies to the Protective Antigen (PA) protein of *B. anthracis* in human serum. The assay should be used only on serum samples from individuals with a clinical history, signs or symptoms consistent with anthrax infection as an aid in the diagnosis of anthrax, or from recipients of anthrax vaccine.

2. Indication(s) for use: The assay should be used only on serum samples from individuals with a clinical history, signs or symptoms consistent with anthrax infection as an aid in the diagnosis of anthrax, or from recipients of anthrax vaccine.

3. Special conditions for use statement(s):
   - The diagnosis of anthrax infection must be made based on history, signs, symptoms, exposure likelihood, and when possible other laboratory data, in addition to the presence of antibodies to *B. anthracis* PA. Negative results in ELISA should not be used in isolation from other evidence to exclude anthrax.
   - The assay has not been evaluated in individuals without clinical signs or symptoms who were presumed exposed and who subsequently developed cutaneous or inhalation anthrax. There is no information available to interpret the meaning of a positive or negative test result for such individuals.
   - The assay has not been evaluated with specimens from patients infected by the gastrointestinal route; expected results with such infections are unknown.
   - The minimum level of anti-PA antibodies that confers protection following vaccination is not known. The QuickELISA measures total antibody and the

relationship between this value and protective immunity has not been established.
- The affinity and/or avidity of anti-PA IgG and IgM for the rPA antigen have not been determined with this assay.
- This assay is for Rx use.

4. Special instrument requirements: ELISA reader, dual wavelength; ELISA plate washer; microplate shaker

H. Device Description:

The QuickELISA assay kit includes necessary reagents to perform laboratory testing, using immunochemical reactions in an ELISA plate format. Conjugates are manufactured from a recombinant protein product produced by an avirulent plasmidless *B. anthracis* strain. Kit reagents include positive controls (sera from rabbits immunized with recombinant PA), Conjugate A (streptavidin-rPA), Conjugate B (rPA-horseradish peroxidase), a negative control (normal human serum), microwell strips (precoated with biotinylated bovine serum albumin, sample diluent, TMB substrate, wash buffer, and stop reagent. An ELISA reader (dual wavelength), ELISA plate washer and microplate shaker are needed to perform the testing. The cutoff was determined based on preclinical testing to be above the highest negative sample and validated to detect approximately 300 ng/mL of PA-specific antibody (pool of vaccinee sera).

I. Substantial Equivalence Information (if known):

1. Predicate device name(s): preamendments device, US Army Biological Laboratories reagents for modified agar diffusion assay
2. Predicate 510(k) number(s): NA
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Detection of anti-PA antibodies	Same
Specimen Type	Serum	Same
Negative Control	Normal human serum	Same
Differences		
Item	**Device**	**Predicate**
Antigen	Recombinant PA (Protective Antigen)	Purified PA from Sterne strain culture
Assay Endpoint	Spectrophotometric measure (Net OD-450)	Visualized band of identity
Positive Control	Pooled sera from rabbits immunized with rPA	Serum dilution from human vaccinee
Testing Time	Overnight (18-24 h)	35 min (with agitation); 80 min (without agitation)

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

K. Test Principle: Immunochemical using ELISA microwell plate format.

L. Performance Characteristics:

1. Analytical performance:
   a. *Precision(Repeatability/Reproducibility):*

Reproducibility Panel Sample	*Agitated Incubations: Intra-Assay*		*Agitated Inter-Assay CV %*	*Agitated Inter-Lot CV %*	*Agitated Inter-Site CV %*	*Stationary Intra-Assay CV %*	*Stationary vs. Agitated CV %*
	Net OD-450	*CV %*					
PC	**2.233**	**4.4**	**2.0**	**11.2**	**35.4**	**10.8**	**15.1**
Low PC	**0.860**	**4.1**	**1.5**	**10.2**	**13.8**	**13.2**	**11.6**
NC	**0.018**	**7.2**	**6.2**	**5.4**	**18.1**	**10.8**	**27.9**
HiP1	**3.809**	**6.2**	**0.4**	**2.2**	**3.9**	**6.6**	**1.3**
HiP2	**3.830**	**5.6**	**0.5**	**0.9**	**3.5**	**7.6**	**1.2**
HiP3	**3.806**	**5.2**	**2.0**	**1.7**	**1.9**	**6.9**	**0.8**
LoP1	**2.225**	**6.1**	**2.5**	**22.6**	**15.3**	**3.2**	**19.0**
LoP2	**0.609**	**7.9**	**3.0**	**26.3**	**4.0**	**4.5**	**27.1**
LoP3	**0.442**	**8.3**	**6.4**	**20.5**	**10.0**	**7.9**	**22.9**
N1	**0.019**	**9.5**	**21.5**	**19.2**	**7.9**	**10.9**	**12.8**
N2	**0.021**	**8.0**	**23.1**	**14.9**	**23.9**	**9.9**	**12.8**
N3	**0.021**	**5.9**	**9.5**	**18.5**	**19.8**	**3.0**	**37.3**
N4	**0.021**	**12.2**	**6.4**	**13.6**	**20.9**	**2.3**	**33.5**
N5	**0.023**	**14.3**	**8.6**	**24.2**	**20.2**	**6.1**	**32.4**
REF-1	**0.357**	**8.6**	**8.4**	**15.7**	**13.7**	**7.6**	**15.4**
MeP1	**2.575**	**6.7**	**7.8**	**16.0**	**15.9**	**5.0**	**5.2**

   b. *Linearity/Assay measuring (reportable) range*: NA
   c. *Calibrators, and Controls:* Pooled sera from rabbits immunized with rPA
   d. *Traceability of the assay:* NA
   e. *Analytical limits at low levels*: approx. 300 ng of PA-specific antibody
   f. *Analytical specificity*:
      Sera from 225 individuals with other antibody responses associated with various disease conditions, and 583 sera from healthy blood donors were tested with QuickELISA. Positive results were obtained for 5/583 (0.9%) blood donors and 1/225 (0.4%) of patients with other disease conditions.
   g. *Analytical characterization of cut-off:*
      Titrations of pooled human sera from AVA vaccinees (standardized using radial immunodiffusion and nephelometry with US National Reference Preparation for Specific Human Serum Proteins, and mass value using USFDA reference serum, 1983 *H. influenzae* type b)
2. Comparison studies using clinical specimens:
   a. *Method comparison*: NA
   b. *Matrix description and comparison*: NA
3. Clinical Studies :
   a. *Clinical sensitivity and clinical specificity:* NA – anthrax infection would not be expected in a US population.

b. *Other clinical supportive data, statistical analysis and results if applicable:* Archived sera from patients diagnosed with inhalational or cutaneous anthrax (n=13 US patients and 6 non-US patients), along with 49 AVA vaccine recipients were tested with QuickELISA. The anthrax sera were collected 17 days to 15 months post-infection and after development of symptoms. All sera from patients with anthrax were positive when tested with the QuickELISA.

4. Clinical cut-off :
   The cutoff is determined by adding 0.1 to the average of duplicate Negative Control Net OD-450. This level was determined to be higher than readings obtained from sera from unvaccinated/uninfected individuals in preclinical testing.
5. Reference interval (Expected values):
   Anthrax is virtually non-existent in the US. All AVA-vaccinated individuals that have been evaluated are reported to develop an anti-PA immune response. The magnitude of the Net OD-450 measurement is not correlated with antibody level or an endpoint titer.

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