Toxin *BioThreat Alert*®
Multiplex Strip

READ ALL INSTRUCTIONS BEFORE BEGINNING THE ASSAY

INTENDED USE

The Toxin *BioThreat Alert*® Multiplex Strip is a hand-held biological agent detection and identification device based on the Lateral Flow Assay principle. Each strip can detect up to five toxins, *Ricinus communis* Agglutinin II causative agent for Ricin poisoning, Abrin toxin produced by *Abrus precatorious*, Staphylococcal Enterotoxin B (SEB) the causative agent for Staphylococcal food poisoning, and Botulinum Toxin A and/or B the causative agents for Botulism. The Toxin *BioThreat Alert*® Multiplex Strip is intended for use in the field for performing rapid analysis of potential biological threats contained in samples collected from the environment. It is NOT a clinical diagnostic device.

THIS DEVICE IS NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC USE. The user should validate any off label application.

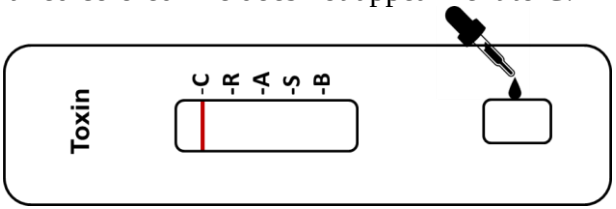
SUMMARY AND EXPLANATION

The *Toxin BioThreat Alert*® Multiplex Strip employs unique antibodies for detection and identification of specific biological agents. These same methods have been employed for over thirty years for the detection of biological threats. After a prepared sample (see Sample Collection and Preparation section) is applied to the *Toxin BioThreat Alert*® Multiplex Strip, self-contained dried components react with specific biological agents present to form a red colored line or band in the results window within minutes.

PRINCIPLE OF THE TEST

The *Toxin BioThreat Alert*® Multiplex Strip is a rapid qualitative test that utilizes a combination of monoclonal and polyclonal antibodies to selectively detect the presence of biological threat agents in aqueous samples. The assay is conducted by the addition of a prepared sample into the test device Sample Port and observation of the formation of red colored lines. The liquid sample rehydrates the labeled antibody, and then migrates via capillary action along the membrane. A positive sample reacts with the agent-specific antibody conjugate and forms a line next to R for Ricin positive, A for Abrin Positive, S for SEB positive, and B for Botulinum A/B positive sample. In the absence of the specific agent, no line will develop, indicating a negative result. To serve as a control for the procedure, a red colored line next to C (control line) should always appear regardless of the presence or absence of specific

biological agents in the sample. The test is invalid if a red colored line does not appear next to C.



PRECAUTIONS

1. THIS DEVICE IS NOT INTENDED FOR MEDICAL OR CLINICAL DIAGNOSTIC USE.
2. The test device should be discarded in a proper biohazard container after testing.
3. The test device should not be used beyond the expiration date.
4. The test device should remain in the sealed pouch until ready for use. Do not use if the pouch is torn or punctured.

STORAGE AND STABILITY

Toxin BioThreat Alert® Multiplex kits should be stored at room temperature (15 - 30°C) for the duration of the shelf life. Each test device must remain sealed in the pouch until ready for use.

SAMPLE COLLECTION AND PREPARATION
(Tetracore *BioThreat Alert*® Sample Collection Kit Recommended)

NOTE: Samples to be tested must be provided in *BioThreat Alert*® Multiplex Sample Buffer,C2T swab (sold separately) sample buffer, or another validated buffer, for use in the *Toxin BioThreat Alert*® Multiplex Strip.

Swab Samples (dry or wet)

1. Fill a sample vial with 1.0 milliliter (mL) of *BioThreat Alert*® Multiplex Sample Buffer. Use a black marker to highlight the 1.0 mL mark on the sample vial.
2. Dry or wet samples to be tested should be collected with an applicator swab; it may be necessary to slightly dampen the swab with *BioThreat Alert*® Multiplex Sample Buffer for dry samples, by placing it in the sample vial and rotating. Swipe the area to be tested, rotating the swab tip to ensure exposure to entire surface containing the sample.
3. For surfaces with a visible powder very lightly brush the swab against the powder to pick up a few granules. A very small amount is more than sufficient for detection.

4. Place the applicator tip of the swab into the sample vial with *BioThreat Alert*® Multiplex Sample Buffer, and mix for 10 seconds by rotating the swab tip. Rotate the tip along the

inside of the vial to remove excess liquid before removing.

5. Close the cap on the vial and agitate vigorously. Allow any large particles to settle to the bottom (15 – 30 seconds).
6. Using the disposable bulb dropper, remove liquid from top liquid layer of the sample vial being careful not to pull up particles that have settled to the bottom.
7. Place 5 drops (0.120 mL) into the sample port of the *BioThreat Alert*® Multiplex Strip.

Liquid Samples

1. Fill the sample vial with 0.5 mL of the *BioThreat Alert*® Multiplex Sample Buffer (0.5 mL mark on the vial).
2. Add liquid sample to the *BioThreat Alert*® Multiplex Sample Buffer in the sample vial up to the 1.0 mL mark on the vial. Close the cap on the vial and agitate vigorously.
3. Using the disposable bulb dropper, remove liquid from top liquid layer of the sample vial.
4. Place 5 drops (0.120 mL) into the sample port of the *BioThreat Alert*® Multiplex Test Strip.

*Solid Samples***

1. Add *BioThreat Alert*® Multiplex Sample Buffer to the 1.0 mL mark on the sample vial.
2. Solid samples must be reduced to a size that will fit in the sample vial without over sampling. Use the scissors, as necessary, to reduce sample size. Sample volume sufficient to fit in the small end of the scoop (provided in

the Tetracore *BioThreat Alert*® Sample Collection Kit) will suffice. In the case of cloth or paper samples, cut a small rectangle of material and add it to the vial. Handle the sample with tweezers to reduce contamination.

3. Agitate the sample vigorously for 10 seconds. Allow any particles to settle to the bottom of the vial.
4. Using the disposable bulb dropper, remove liquid from the top liquid layer of the sample vial being careful not to pull up particles that have settled to the bottom.
5. Place 3-5 drops (0.120 mL) into the sample port of the *BioThreat Alert*® Multiplex Strip.

**These samples may need to be further diluted from the original sample preparation if the solution is too viscous and clogs the filter on the *BioThreat Alert*® Multiplex Strip. Visibly excessive red streaking or incomplete Control Line is evidence of too high a sample concentration. Follow dilution procedure below if necessary.

Dilution Procedure:

1. Add *BioThreat Alert*® Multiplex Sample Buffer up to the 1.0 mL mark on the sample vial.
2. Add the sample to the sample vial, up to the 1.25 mL mark on vial and close.
3. Mix solution by inverting 5 times. Allow any particles to settle to the bottom of the vial (30-60 seconds).

4. Using the disposable bulb dropper, remove liquid from top liquid layer of the sample vial being careful not to pull up particles that have settled to the bottom.
5. Place 3-5 drops (0.120 mL) into the sample port of the *BioThreat Alert*® Multiplex Strip.
6. Repeat dilution procedure using second prep if sample is still too viscous to run properly on a *BioThreat Alert*® Multiplex Strip.

Consideration of the "Hook Effect":
When sampling is performed according to the instructions in this product insert the "high-dose hook effect" does not show a completely **negative result**, but may produce a less intense line. This generally applies to toxins only. If the hook effect is suspected, repeat the dilution procedure. If the strip comes up brighter than before the effect can be confirmed.

Materials Provided

- 10 x Strips
- 10 x Cotton-Tipped Swabs
- 10mL Multiplex Sample Buffer
- 10 x Collection Vials
- 10 x Disposable Bulb droppers
- 1 x Product Insert

Directions for Use

Check for any damage to protective pouch. Allow specimen and/or controls to reach room temperature prior to testing.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with sample or control identifications.
2. Dispense 5 drops (0.120 mL) of specimen into the oval Sample Port (see illustration). Write down the time the strip is loaded.
3. Read results after 20 minutes. Positive results *may be observed* in as short as 30 seconds, depending on the concentration of the biological agent.
4. Strips can be read up to 5 minutes after the incubation period.
5. Confirm positive results on 2 additional strips for a total of 3 positive assays.

NOTE: Colored lines that appear after 30 minutes are not valid and should be ignored.

INTERPRETATION OF RESULTS

Negative Results

The test is negative only if a single colored line appears next to letter C on the strip and the strip has a clean white background.

Positive Results

The test is positive, if in addition to the control line, one or more colored lines appear. These line(s) may appear in the results window next to the respective target. Colored target lines may be lighter or darker than the control line. When more than one colored line appears for the specific targets (R, A, S, or B), retest the assay for

confirmation of result. It is an unlikely result and may indicate an interfering substance.

Invalid Results
The test is invalid if no colored line appears next to C, even if a colored line appears in the Results Window.

Recommendation – If migration of the specimen is slow or no colored line appears next to the C, refer to the Dilution Procedure found under the SAMPLE COLLECTION AND PREPERATION section of this pamphlet and REPEAT THE TEST ON A NEW Toxin *BioThreat Alert*® Multiplex Strip.

LIMITATIONS OF THE PROCEDURE

1. False negative results may occur when levels of specific biological threat agents are below the detectable concentration level (sensitivity) of the test.
2. This test has produced negative results for a number of possible cross reactants that are listed under Specificity Testing. However, unknown cross-reacting substances may cause a positive result.
3. This test provides a presumptive result for presence or absence of a specific biological threat agent. A certified laboratory must confirm all results before a definitive determination is made.
4. Due to chemical interference, false positive results may occur when testing samples containing certain compounds. However, it is

important to note that interference of this kind is very rare and should not be expected. Multiple positives in different assays or multiple target lines within the multiplex assay will potentially indicate such a chemical interference.

EXPECTED VALUES

Negative results are expected in aqueous samples free of specific biological threat agents or samples containing specific biological threat agents at concentrations below the detectable concentration of the test.

Specificity Testing

The substances, listed below, were tested at 100µg/mL and 1µg/mL. None of the substances tested at these concentrations cross react in the assay.

B.globigii Spore
B.globigii Veg.
Bt HD571 Spore
Bt HD571 Veg
Bt AL Hokum Spore
Anthrax Sterne Spore
Bovine Serum Albumin
Ovalbumin
F.tularensis
Naproxen
Tylenol

Dipel Dust
SEB
Y.Pestis
Talc Powder
Bt AL Hokum Veg
BT Worm Killer*
BT Thuricide*
Red Clay**
Gravel**
Mulch**
Ricin Toxin
Bot A Toxin
Bot B Toxin
B.cereus
B.subtillis QST-713 Spore

** These commercial preparations of B. thuringiensis were tested at a 1:10 dilution.*
*** The content of this sample is regionally specific. The possibility of false positive results cannot be excluded for regionally specific samples. Testing on clay, gravel, and mulch samples is not recommended.*

Toxin *BioThreat Alert*® Multiplex Strip

FOR THE RAPID QUALITATIVE DETERMINATION OF UP TO FIVE TOXINS

- **One step test with built-in quality control check**
- **Just add 5 drops of sample and read results in 20 minutes.**
- **Room temperature storage**

NOT FOR IN-VITRO CLINICAL DIAGNOSTIC USE.

Store at 15ºC to 30ºC

AS WITH ALL SCREENING DEVICES, RESULTS MUST BE CONFIRMED BY A CERTIFIED LABORATORY BEOFRE ANY OFFICAL DETERMINATION IS MADE.

Tetracore Inc.
77 Upper Rock Circle, Ste 600
Rockville, MD 20850 USA
TEL: 240-268-5400
FAX: 240-268-1107