positiveID

PositiveID Immunodetection Assay for the Identification of RICIN

SUMMARY

Consistent and sensitive methods are indispensable to protect the American population and its allies from the intentional use of weapons of mass destruction. Ricin, a chemical warfare agent, has been studied since World War II and continued to become a tool of terrorist groups nationally and abroad because of its effortless production [1]. Ricin, also known as RCA-II or RCA_{60}, is derived from the seeds of the castor oil plant *Ricinus communis* [2]. The levels of toxicity and lethality can vary depending on the route of exposure: 0.35-0.7mg by inhalation in humans, 1-20mg of toxin/kg body weight in humans, and injection 0.7-2ug/kg in mice and 1-1.75ug/kg in dogs [2,3].

PositiveID Corporation has developed an immunodetection assay for the rapid identification of Ricin Toxin. This assay was validated in the Microfluidic Bioagent Autonomous Networked Detector system (M-BAND), an instrument developed for the Department of Homeland Security Science & Technology Directorate (DHS S&T), as well as in a commercial ELISA plate reader.

The PositiveID Assay for the Identification of Ricin Toxin

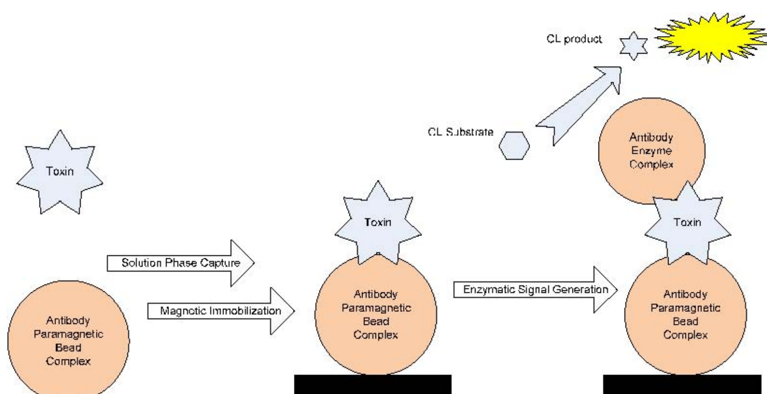

Figure 1. cELISA technology used in the PositiveID assay for the identification of ricin toxin. In this configuration, toxins are captured with specific antibodies linked to paramagnetic beads. Toxin/bead bound complexes are magnetically immobilized and rinsed. A secondary antibody labeled with a chemi-luminescence detection system signals the presence of the captured toxin.

The PositiveID Assay for the identification of Ricin Toxin relies on the capture ELISA (cELISA) configuration, a modified version of the sandwich ELISA (Figure 1). In this configuration, a primary antibody (also referred as the capture antibody) is immobilized onto a surface or a paramagnetic bead. Next, an analyte containing denatured or native proteins is applied through

the capture antibody. As the capture antibodies come in contact with their complementary antigenic targets, the captured proteins are immobilized. Last, a secondary chemi-luminescent (CL) or fluorescently labeled antibody is added through the complex of capture antibody and bound protein. Similar to the primary antibody, the secondary antibody has affinity to the captured protein. The final complex, also referred as the immunostack, is read with the correspondent excitation and emission wavelengths.

Figure 2 depicts the continuous detection of ricin toxin in the M-BAND Instrument. In this example, the system was initially challenged with a Phosphate Buffer Saline (PBS) (Blank). This is followed by a Negative Control (NC) composed of a nonspecific protein. Last, a series of two challenges with 1.5 and 3.0 nanograms of Ricin followed by PBS washes in between are demonstrated.

Continuous Detection of Ricin Toxin in M-BAND System

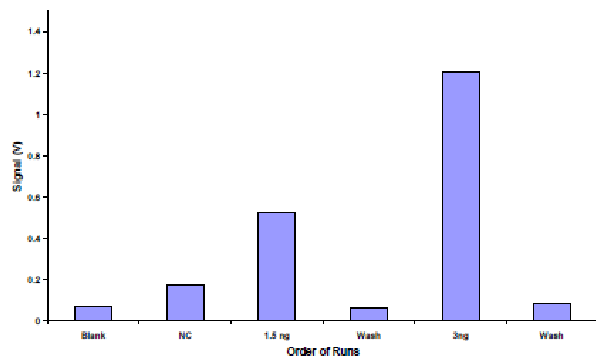

Figure 2. Continuous detection of Ricin Toxin in the M-BAND Instrument developed by PositiveID. Here two challenges composed of 1.5ng and 3.0ng of total ricin toxin are depicted.

In addition to the individual detection of Ricin Toxin, PositiveID also offers a triplex assay for the simultaneous detection of three toxins: Ricin, Botulinum, and Staphylococcal Enterotoxin B (SEB). Figure 3 depicts the performance of the PositiveID Triplex Assay for the detection of these three potential warfare agents.

PositiveID Immunodetection Assay

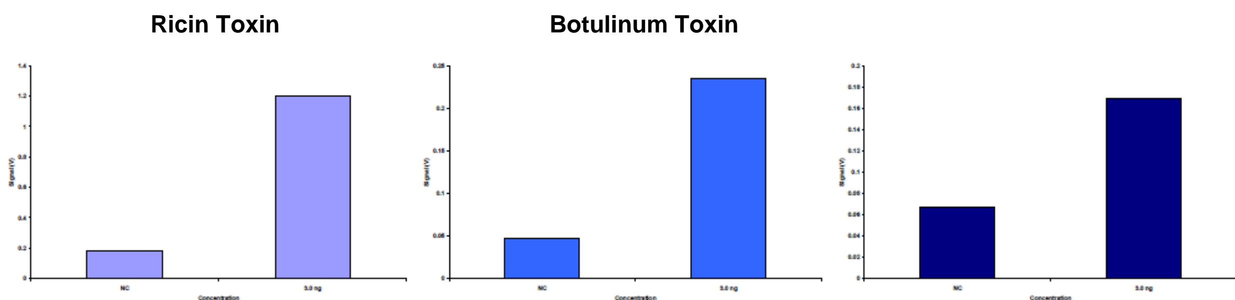

Figure 3. A triplex Assay for the simultaneous detection of Ricin, Botulinum, SEB toxins. In most cases, the Limit of Detection for the triplex assay is between 1-2ng of toxin.

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Discussion

The PSID Assay for the identification of Ricin Toxin relies on proven and well understood technologies. For over four decades, ELISA based technologies have been the gold standard against new emergent immunodetection ones [4]. Compared to a recent evaluation in a Handheld Assay format, the PSID Assay for the identification of Ricin Toxin is equivalent or better [5]. Also, with minimal effort, this assay can be reconfigured to fit other commercial platforms.

References

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