

DETECTION OF PROTEINOUS TOXINS USING THE BIO THREAT ALERT SYSTEM

Kazumitsu IURA^{a*}, Kouichiro TSUGE^a, Yasuo SETO^{a**} and Akiyoshi SATO^b

^a National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

^b Teikoku Sen-i Co. Ltd., 2-5-13 Nihonbashi, Chuo-ku, Tokyo 103-0027, Japan

Received July 15, 2003

Accepted December 9, 2003

Bio Threat Alert システムを用いた蛋白性毒素類の検出

井浦一光^{a*}, 柘 浩一郎^a, 瀬戸康雄^{a**}, 佐藤晃祥^b

^a 科学警察研究所 〒277-0882 千葉県柏市柏の葉6-3-1

^b 帝國纖維株式会社 〒103-0027 東京都中央区日本橋二丁目5-13

Summary

The Guardian Bio Threat Alert (BTA) system for biological warfare agents was investigated for its reliability and capability for detecting proteinous toxins. Staphylococcal enterotoxin B (SEB), botulinum toxins A and B, and ricin gave positive responses of each BTA test strip, and the minimum toxin concentrations indicating positive results were estimated to be about 0.05, 0.1 (0.03) and 0.1 µg/ml, respectively. The calibration curves using the sample values (the intensities of the color developed by the immuno-complex) were linear up to 1.5 µg/ml for SEB and botulinum toxin B; that for ricin was linear up to 0.3 µg/ml, but saturated over the concentration. Various compounds were examined for false BTA responses and for the interference with the response to SEB; only strongly acidic and alkaline solutions showed false negative results.

Key words: Guardian Bio Threat system; Biological warfare agents; Bioterrorism; Proteinous toxins; Staphylococcal enterotoxin B; Botulinum toxin; Ricin; Color test

Introduction

In autumn 2001, terrorism with anthrax occurred in the United States; post letters containing *Bacillus anthracis* spores were sent to several locations via the US postal service, and five people died of anthrax inhalation [1]. Now the threat of bioterrorism has become evident worldwide. Especially, anthrax, brucellosis, plague, Q fever, tularemia, smallpox, viral encephalitides, viral hemorrhagic fevers, botulinum and staphylococcal enterotoxin B are typical biological warfare agents [2]. Ricin is also a candidate to be used for bioterrorism [3]. The biological warfare agents do not manifest the toxicity on public people just after their dispersion. The biological terrorism attacks are rather recognized after a time lag. Usually, the authority takes countermeasures against the bioterrorism, under the system of infection disease surveillance. However, if the biological warfare agents can be detected before the outbreak of the infection, it is very advantageous for their early diagnosis, medical treatments and preventing them from wide-spreading. An on-site detection system is useful for possible bioterrorism at an early stage. Several on-site biological warfare agent detection equipments have been developed [4], and some are now commercially available for military use [5].

After the anthrax attack in USA 2001, many incidents and troubles relating to "white powder like material" occurred [6], but real biological warfare agents have never been detected. In the bioterrorism and also the "white powder" incidents, the first responders (police mobile teams or fire department) rush to the

**Correspondence should be addressed to Yasuo Seto.

*Present address: Tokyo Metropolitan Police Department, 2-2-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8929, Japan

©2004 Japanese Association of Forensic Toxicology. All rights reserved. 0915-9606/04/\$14.00

spots, and suspicious materials such as white powder are sampled to send to local public health research institutes for laboratory tests of *Bacillus anthracis* and other biological warfare agents. However, it is impossible and improper to send all the suspicious materials found on-site to the laboratories, and it is necessary to discriminate the bioterrorism-likely samples from the unrelated ones by on-site screening. Actually, the first responders have managed to adopt the on-site detection methods using commercially available detection kits and also their handmade tools. The Guardian Bio Threat Alert System developed by Alexeter Technologies is now being used by Japanese responders as a rapid and simple detection kit for biological warfare agents. Its detection principle is immuno-chromatography. In this paper, we have investigated the reliability and capability of the system for detecting proteinous toxins, by checking the responses to the real toxins and the interference by various compounds.

Experimental

Chemicals

Bio Threat Alert™ (BTA) Test Strips for ricin, botulinum toxin (BTX), staphylococcal enterotoxin B (SEB), plague and anthrax were obtained from Tetracore LLC (Rockville, MD, USA). Ricin (*Ricinus communis* agglutinin II, RCA60, 2.5 mg/ml solution) was provided from Honen Corporation (Chiyodaku, Tokyo), and used under the permission by the Minister of Economy, Trade and Industry. Botulinum toxin A (BTXA) solution (1 mg/ml in acetate buffer), botulinum toxin B (BTXB) solution (1 mg/ml in acetate buffer) and soluble starch were purchased from Wako Pure Chemicals (Osaka), and used after dilution with phosphate buffered saline (PBS). SEB, albumin from bovine serum (fraction V powder, BSA) and albumin from human serum (fraction V powder, HSA) were obtained from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA), and used after dilution with PBS. Commercially available Nisshin flour Hakuriki (Nisshin Food Co., Tokyo) was used as wheat flour. Other chemicals used were of analytical reagent grade.

The out-dated transfusion blood was obtained as control blood from a local police hospital, and plasma was prepared by centrifugation.

Measurements of samples by the Guardian Bio Threat Alert System

According to the manufacturer's manual, liquid samples can be examined for toxin detection by the BTA system. The PBS solution, containing the target toxins (ricin, SEB, BTXA, BTXB), other nontoxic compounds or a mixture of a toxin and a nontoxic compound, was mixed with an equi-volume of the BTA buffer. The BTA test strip was taken out of the foil pouch, and 150 µl of the mixture solution was poured into the round sample port on the strip (Fig. 1), left for 15 min at room temperature, and inserted into the Guardian BTA Test Strip Reader (Alexeter Technologies, LLC, Wheeling, IL, USA) to obtain a sample value. The reader indicates "POSITIVE" (Fig. 1), when a pink

color appears in both control and sample windows, and the sample value exceeds the threshold value (0.01); and "NEGATIVE", when the value does not exceed the threshold value in the presence of the pink color in the control window. The reader also indicates "FALSE", when the pink color does not appear in the control window.

Safety consideration

SEB, BTXA, BTXB and ricin are highly toxic upon their inhalation and digestion. These compounds should be handled with special care, and they should be destroyed with sodium hypochlorite after examination.

Results

Responses of BTA test strips to toxins

Serially diluted solutions of SEB were examined by the BTA system. The BTA test strip for SEB showed negative response to the PBS solution, and positive response to the SEB solution (higher than 0.1 µg/ml). The typical positive result of the strip (SEB concentration: 1.0 µg/ml) is shown in Fig. 1, where the pink lines were observed in both sample and control windows. The calibration curve using the sample values was linear in the range from 0.1 to 1.5 µg/ml (Fig. 2A). The minimum SEB concentration indicating the positive result (exceeding the threshold value) was about 0.05 µg/ml.

The BTA test strip for BTX showed negative response to the PBS solution, and positive response to the BTXB solution (over 0.1 µg/ml). The calibration curve for BTXB was also linear in the range from 0.1 to 1.5 µg/ml (Fig. 2B). The minimum BTXB concentration indicating the positive result was about 0.1 µg/ml. The BTA test strip for BTX also showed positive response to the BTXA solution at 0.1 µg/ml (sample value: 0.033) and 1.0 µg/ml (0.339). BTXA gave 2–3 fold higher values than those of BTXB, and the minimum BTXA concentration indicating the positive result was about 0.03 µg/ml.

The BTA test strip for ricin indicated negative response to the

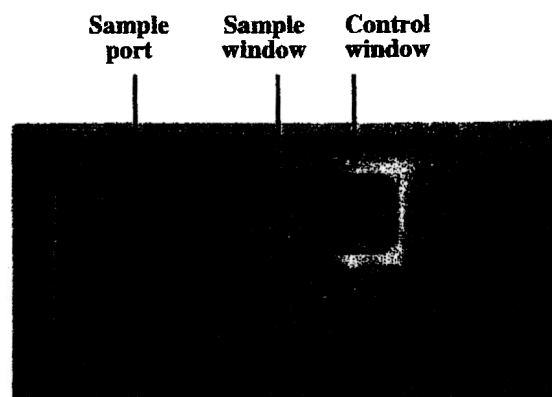


Fig. 1. BTA test strip for SEB indicating a positive result. SEB solution at 1 µg/ml was examined.

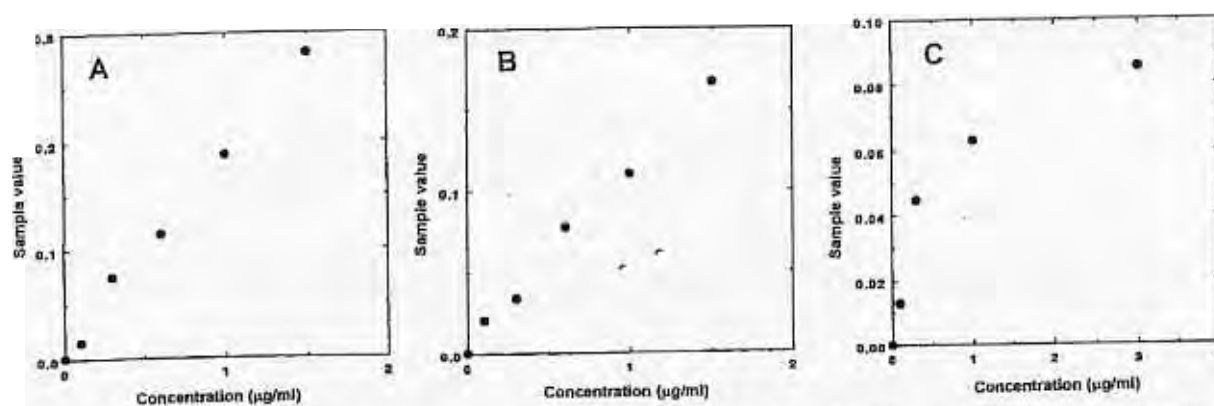


Fig. 2. Calibration curves with the sample values against the toxin concentrations. Each of serially diluted solutions of toxins (A: staphylococcal enterotoxin B; B: botulinum toxin B; C: ricin) was mixed with an equi-volume of the BTA buffer, and 0.15 ml of the mixture was applied to the BTA test strip, and the resulting color intensity was quantitated by the Guardian BTA Test Strip Reader.

PBS solution, and positive response to the ricin solution (higher than 0.1 µg/ml). The calibration curve for ricin was linear in the range of 0 to 0.3 µg/ml; over 0.3 µg/ml, the response was saturated (Fig. 2C). The minimum ricin concentration indicating the positive result was about 0.1 µg/ml.

Interference by various compounds with the responses of the BTA test strips

Various kinds of compounds were added to sample solutions with or without SEB (1 µg/ml), and each mixture solution was subjected to the BTA system for sample value measurement. As shown in Table 1, 1.0% w/v solution of wheat flour, starch, BSA, HSA and human plasma (10-fold diluted) showed negative response of the BTA test strip to SEB, and the sample values were below 0.01. The addition of wheat flour, up to 3.0% w/v, did not change the positive response by the BTA test strip to the SEB solution (1.0 µg/ml). Also, the addition of starch did not change the positive response, but the sample values were lowered with the increase in starch concentration.

The addition of 0.1 M HCl to the sample solution of SEB did not change the result seriously, but more concentrated HCl (final 1.0 M) caused the FALSE response with no color line in the control window. The addition of 0.1 M NaOH to the sample solution of SEB did not change the positive response, though the sample value was lowered. However, 1.0 M NaOH caused the FALSE response.

The addition of sodium dodecyl sulfate (SDS) (final 1 and 5%), sodium cholate (final 5%), sodium deoxycholate (final 5%) and sodium chloride (final 3%) did not change the positive response by the BTA test strip to the SEB solution, although the sample values were lowered in the cases of 1% SDS and 5% sodium deoxycholate.

BSA (1.0%) showed the negative response using the BTA test strips for BTX, ricin, plague and anthrax.

Discussion

The Guardian Bio Threat Alert system is an on-site immunoassay kit for detecting biological warfare agents. Tetracore LLC is providing the BTA test strips for anthrax (*Bacillus anthracis*), ricin (*Ricinus communis*), botulinum toxin (*Clostridium botulinum*), SEB (*Staphylococcus aureus*), plague (*Yersinia pestis*), tularemia (*Francisella tularencis*) and brucella (*Brucella genus*). As detection principle, a toxin, acting as an antigen, is immuno-complexed with a gold colloid-labeled anti-toxin antibody. The mixture of the antigen-antibody complex and unbound antibody flows over the adsorbent pad. During the course of the flow, only the gold colloid-labeled antigen-antibody complex is trapped by another anti-toxin antibody fixed on the bottom of the sample window by the sandwich mechanism. The gold colloid-labeled unbound antibody is trapped by the anti-immunoglobulin antibody fixed on the bottom of the control window. The color line observed in the control window gives the confirmation of normal conditions for the immuno-complex formation, and the color line in the sample window indicates the presence of an antigen (a proteinous toxin) in a sample solution.

In this paper, we have examined the detection capability of the BTA test strips for bacterial toxins, SEB and BTXs A and B, and also plant toxin ricin (Fig. 2). These toxins are very toxic to humans [7]. Human inhalational and oral LD₅₀ values of BTX were reported to be as low as 0.003 and 0.006 µg/kg, respectively. A 100% lethal dose of ricin for humans was reported to be 50-100 µg. Incapacitating and lethal doses of SEB were reported to be 30 and 1.7 µg for humans, respectively. Because of the high costs of the BTA test strips, sufficient experiments, especially for validation data, could not be undertaken. Since the toxins examined were also expensive, the experiments for the BTA responses using large quantities of the toxins were not performed. Even under such limited experiments, we have been able to confirm the high capability of the BTA system for the toxins. Except for ricin, the responses of the BTA strips to BTX B and

Table 1. Effects of various materials and compounds on the response of BTA test strips to SEB

SEB (1 µg/ml)	Compound	Sample value
	None	0.188 ± 0.013 ^a
+	Wheat flour 10 mg/ml	0.002
-	Wheat flour 3.3 mg/ml	0.222
+	Wheat flour 10 mg/ml	0.221
+	Wheat flour 30 mg/ml	0.171
+	Starch 10 mg/ml	0.008
-	Starch 1 mg/ml	0.170
+	Starch 3 mg/ml	0.139
+	Starch 10 mg/ml	0.072
-	Bovine serum albumin 10 mg/ml	0.002
-	Human serum albumin 10 mg/ml	0.008
-	Human plasma 1%(v/v)	0.007
-	Human plasma 10%(v/v)	0.010
+	0.1 M HCl	0.154 ^{b,c}
+	1.0 M HCl	FALSE ^{b,d,e}
+	0.1 M NaOH	0.071 ^{b,f}
+	1.0 M NaOH	FALSE ^{b,e,g}
+	1% Sodium dodecyl sulfate	0.180
+	5% Sodium dodecyl sulfate	0.125 ^b
+	5% Sodium cholate	0.183
+	5% Sodium deoxycholate	0.067
+	3% NaCl	0.211 ^b

^aAverage ± standard deviation of four trials; ^baverage of two trials; ^cpH of the final solution: 2; ^dpH of the final solution: 1; ^eno coloration on the control window; ^fpH of the final solution: 11.5; ^gpH of the final solution: 13.

SEB were linear up to their concentration of 1.5 µg/ml (Fig. 2). The BTA test strips for SEB did not respond to the materials examined (wheat flour, starch, human plasma, BSA and HSA). Various compounds examined (wheat flour, starch, SDS, sodium deoxycholate, sodium cholate and NaCl) did not interfere with the response of the BTA test strips to SEB (Table 1). The detection limits for the toxins were estimated to be 0.03–0.1 µg/ml, under the manufacturer's criteria (comparing with the threshold value). In conclusion, using the BTA system, it is possible to detect low levels of toxins even in the crude samples such as white powder.

References

- 1) Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, Gerberding J, Hauer J, Hughes J, McDade J, Osterholm MT, Parker G, Perl TM, Russell PK, Tonat K: Anthrax as a biological weapon, 2002. *JAMA*, 287, 2236-2252 (2002).
- 2) Franz DR, Jahrling PB, Friedlander AM, McClain DJ, Hoover DL, Bryne WR, Pavlin JA, Christopher GW, Eitzen EM: Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*, 278, 399-411 (1997).
- 3) Ready for the worst. *Japan Times*, January 18, 2003.
- 4) Watt DR, Franz DR: Biological warfare detection. *Anal Chem*, 72, 738A-746A (2000).
- 5) Enserink M: Biodefense hampered by inadequate tests. *Science*, 294, 1266-1267 (2001).
- 6) Powder sent to Koizumi's residence. *Japan Times*, October 18, 2001.
- 7) Burrows WD, Renner SE: Biological warfare agents as threats to potable water. *Environ Health Perspect*, 107, 975-984 (1999).