Rapid detection of ricin in cosmetics and elimination of artifacts associated with wheat lectin

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**A R T I C L E  I N F O**

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**A B S T R A C T**

Ricin can be detected in cosmetics at 0.005 µg/mL in the analytical sample using lateral flow devices (LFDs). Wheat germ, an ingredient used in skin care products is also a potential source of wheat lectin. False positives were observed when wheat lectin was added to LFDs from two manufacturers, irrespective of whether the LFD was specific for ricin, Staphylococcus enterotoxin B (SEB), or botulinum toxin. In contrast, pea and peanut lectins did not cause false positives. Substitution of the buffer supplied with the LFDs with a buffer containing 2.5% non-fat milk powder eliminated the occurrence of false positives. This substitution increased the LOD to 0.01 µg/mL ricin, which is an acceptable level for screening cosmetics for contamination by ricin.

**1. Introduction**

Castor beans of *Ricinus communis* are poisonous to people (Franz and Jaax, 1997; Knight, 1979), animals (Okoye et al., 1987; Purushotham et al., 1986) and insects (Czapla and Johnson, 1990). The most prominent toxin in castor beans is ricin. The inhalation, intravenous, and intraperitoneal LD50 values of ricin in mice are approximately 3–5, 5, and 22 µg/kg body weight, respectively. Inefficient uptake from the intestinal tract reduces the oral toxicity to approximately 20 mg/kg body weight (Franz and Jaax, 1997). Topological application of 50 µg ricin displayed no toxicity with mice (Franz and Jaax, 1997).

Ricin is a class II ribosome inhibiting protein (RIP-II) consisting of two subunits linked by a disulfide bond (Robertus, 1988). A-chain is a 267 amino acid, 32 kDa N-glycosidase that catalyzes the depurination of adenine A4324 of 28S rRNA and thereby blocks protein synthesis (Franz and Jaax, 1997; Gluck et al., 1992; Lord et al., 1994; Robertus, 1988; Robertus, 1991). The second subunit, B-chain is a 262 amino acid, 32 kDa lectin that targets the toxin to cells and facilitates uptake (Lord et al., 1992; Robertus, 1991). The X-ray structure of ricin has been elucidated (Katzin et al., 1991; Montfort et al., 1987; Rutenber et al., 1991) and the enzymatic properties of the toxin characterized (Chen et al., 1998; Day et al., 1996; Endo et al., 1987). Extensive research has also been conducted on the use of the toxin moiety in immuno-targeted therapy (Kreitman, 1999 and 2003; Kreitman and Pastan, 2006; Franz and Jaax, 1997; Schindler et al., 2001; Thorpe, 2004) and on the development of vaccines to counteract ricin toxicosis (Carra et al., 2007; Peek et al., 2007; Vitetta et al., 2006).

Various technologies have been developed for the rapid and sensitive detection of ricin (Huelseweh et al., 2006; Poli et al., 1994; Shyu et al., 2002). One technology, lateral flow devices (LFDs), requires minimal sample preparation and instrumentation, making it ideal for field deployment as an initial screen (Garber et al., 2005). Wheat germ and other sources of plant lectins are routinely used in cosmetics and may affect the reliability of assays employing glycosylated proteins such as antibodies. The compatibility of three common lectins with LFDs was examined along with the
inclusion of 2.5% non-fat milk in the analytical buffer to eliminate false positives. This study demonstrates the effectiveness of LFDs for the screening of cosmetics.

2. Materials and methods

Ricin used by Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) was the generous gift of B. Beaumelle, Ph.D. of Montpellier University. The ricin displayed an intravenous LD50 of 5.88 µg/kg body weight with six-week old female BALB/c mice (personal communication, A. El-Zaouk and D. Sauvaire, AFSSAPS). Lactose and 10 mM PBS were purchased from Sigma Chemical Co. Purified ricin, ricin A-chain, ricin B-chain, and agglutinin (RCA-120) used by the U.S. Food and Drug Administration (FDA) were purchased from Vector Laboratories (Burlingame, CA). Lectins derived from pea (Pisum sativum), peanut (Arachis hypogea), and wheat (Triticum vulgaris) were purchased from Sigma Chemical Co. (catalog numbers L0881, L5380, and L9640, respectively).

LFDs for the detection of ricin, SEB, and botulinum toxin were obtained from Tetracore, Inc. (Rockville, MD, USA) and a U.S. Government supplier and used according to the manufacturer’s instructions. The BioThreat Alert® LFDs manufactured by Tetracore, Inc. can be read either by eye or by using the Guardian Reader™ (Alexeter Technologies, IL, USA). In contrast, the results obtained with the LFDs from the U.S. Government supplier could only be read by eye.

Cosmetics were purchased from local commercial suppliers. Samples were spiked with the ricin provided by B. Beaumelle, Ph.D. of Montpellier University. Eye make up samples were diluted 1:1 with 10 mM PBS / 0.1% Tween-20 / 5% non-fat milk (PBSTM) and analyzed using the LFDs according to manufacturer’s instructions. The more viscous shampoo and body lotion samples were first diluted 1:9 and 1:19, respectively, with PBS and then diluted 1:1 with PBSTM to generate the 50% PBSTM solution for analysis with the LFDs. LOD values for ricin and lectin in buffer were determined based on the concentration which generated a response that exceeded the background response by four standard deviations. Average background responses were typically 0.002. The concentration of ricin in a cosmetic necessary to generate an average response greater than the manufacturer’s recommended cut-off of 0.01 on the Guardian Reader™ defined the concentration of toxin necessary to generate a positive.

3. Results

3.1. Cross-reactivity with wheat lectin

The cross-reactivities of Ricin BioThreat Alert® LFDs towards lectins derived from pea, peanut, and wheat, were examined. Neither the pea nor peanut lectin was detected by the LFDs at concentrations as high as 1 mg/mL and 250 µg/mL, respectively. However, the wheat lectin at ≥15 µg/mL purified protein, or as N1000 ppm stone ground wheat, generated false positive responses (see Figs. 1 and 2). False positives were also generated by wheat lectin with BioThreat Alert® LFDs for the detection of SEB and botulinum toxin (data not shown) and with LFDs manufactured by a U.S. Government source for the detection of ricin and SEB. The addition of lactose at concentrations up to 100 mM in the buffer supplied with the LFDs failed to eliminate the false positives observed with wheat lectin. However, no false positives were observed upon preparing the samples in 50% PBSTM. Substitution of the LFD buffer with 50% PBSTM
increased the LOD from 2.5 ng/mL to 10 ng/mL; the ricin from Vector laboratories displayed a slightly greater increase in the LOD with 50% PBSTM and slightly lower sensitivity than the ricin used to spike the cosmetics.

### 3.2. Cosmetic samples

Table 1 lists the cosmetics tested and the responses generated by samples containing 5 ng/mL ricin in the analytical sample following dilution with PBS and PBSTM. Samples derived from shampoo and body lotion required dilution 1:9 and 1:19, respectively, with PBS prior to mixing 1:1 with PBSTM. As such, the concentration of ricin in the eye make up, shampoo, and body lotion samples prior to dilution were 10 ng/mL, 100 ng/mL, and 200 ng/mL respectively. The additional dilution with PBS prevented the wetting and flow problems typically observed when analyzing viscous samples with LFDs. Further dilution of the shampoo samples, 1:19 with PBS, improved the performance of the LFDs but was not required for analysis.

Interestingly, three different commercial eye makeup removers generated false positive responses with the LFDs in the absence of ricin with an average response of 0.09+0.004. Inclusion of 50% PBSTM in the analytical sample eliminated the problem and enabled the detection of 5 ng/mL ricin. All of the cosmetics listed in Table 1 displayed background responses of ≤0.003 in the presence of 50% PBSTM except eye make up remover C (EMR C) and shampoo D. EMR C and shampoo D displayed backgrounds of 0.01 and 0.004, respectively. It is therefore recommended that a threshold greater than 0.01 be used with the Guardian Reader™. The average responses for EMR C and shampoo D, each containing 5 ng/mL ricin in the analytical sample, were 0.030±0.005 and 0.05±0.01, respectively; both considerably above the background responses.

### 4. Discussion

The Ricin BioThreat Alert® LFDs in combination with the Guardian Reader™ enabled the detection of ricin in cosmetics at levels below those associated with toxic responses. The primary problem associated with using LFDs for the detection of ricin in cosmetics was flow problems with viscous products. However, the high sensitivity of the LFDs made it possible to dilute the samples sufficiently to overcome the flow problems, while still being able to detect ricin at levels less than those associated with toxic events.

The availability of a buffer that prevents false positives due to the presence of lectins has implications affecting all LFDs and immunoassays. The mechanism by which 50% PBSTM prevented false positives or 'nonspecific' binding between the capture and detector antibodies that comprise LFDs is not understood. The explanation that the lectin coupled the two antibodies via their glycosyl groups does not explain why 100 mM lactose failed to suppress false positives. The modest increases in the LOD values for ricin with LFDs were acceptable considering the sensitivity of the assays and elimination of a potential source of false positives.

The mechanism by which eye makeup remover generated false positives was also not understood. Fortunately, 50% PBSTM eliminated these false positives. It is recommended that samples of unadulterated eye makeup remover be analyzed alongside suspicious samples and that a threshold of greater than 0.01 (possibly 0.02) be employed when using the Guardian Reader™. The ingredients listed on the eye makeup remover did not indicate the presence of components that might contain lectins. Thus, it seems unlikely that the mechanism causing the false positives with eye makeup remover and with wheat lectin would be the same, though the mechanism by which 50% PBSTM prevented these nonspecific interactions may be related.


5. Conclusion

Cosmetics suspected of containing ricin can be analyzed using the BioThreat Alert® LFDs provided the samples are sufficiently diluted to prevent flow problems. It is recommended that 50% PBSTM be used with immunoassays whenever samples are suspected of containing wheat lectin. In the event that a cosmetic product generates a positive response with an LFD, the sample should be reanalyzed using 50% PBSTM as the analytical buffer and whenever possible also confirmed using a second, independent method.

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