

## Immunological Detection of Ricin and Castor Seeds in Beverages, Food and Consumer Products

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**Key words:** ricin, abrin, immunological detection, lateral flow assay, LFA, Rizin

### Abstract

The use of ricin or castor seeds in the context of criminal or terrorist attacks poses a significant threat. Two to eight of the readily available seeds can be lethal to adults. In food sensory ricin is unremarkable and has a delayed toxic effect. After metabolism ricin is no longer detectable in the body. When it is suspected, an investigation should be made retrospectively to the environment of the injured person. Food, objects or vessels has to be analyzed for the presence of the toxin. The unmodified toxin can be detected with sufficient sensitivity by a new lateral flow assay, an immunological test. The detection limit in water is 0,05 mg/L, in food between 1 and 2,5 mg/kg. A forensic validation procedure can be performed with peptide analysis.

### 1. Introduction

Abrin is a peptide from Rosary Pea (*Abrus precatorius*), a legume, native to Indonesia, which has spread in tropical and subtropical areas of the world. The content of abrin is about 0,08%. Ricin is a peptide from the castor oil plant (*Ricinus communis*). The castor bean is the source of castor oil (40 – 60 %). The content of Ricin is about 1%. Castor oil world-production is about 600 000 t /year. Ricin and Abrin are nearly identical and have two chains connected by a single disulfide bond. Chain A is an enzyme (N-glycoside hydrolase) and chain B is a lectin, which serves to mediate entry of the A-B protein complex into the cytosol. The effect of ricin is the enzymatic depurination of Adenosine at position 4324 in the Sarcin-Ricin-Loop of the eukaryotic rRNA 28S. This loop is important in binding elongation factor eEF-2 during protein synthesis. Ricin is a schedule 1 part A substance of the Chemical Weapons Convention because it was tested as respirable powder. In Germany the laboratory use of ricin requires a permit from the Foreign Office.

Tab. 1. Toxicity of ricin and abrin.

	LD 50 (oral) man	LD 50 (s.c.) man	LD 50 (pulm.) man
Castor Seeds	2 – 8 seeds, chewed	-	-
Ricin	0,03 bis 20 mg/kg	3 -10 µg/kg	3 -10 µg/kg
Abrin	1 mg/kg	0,04 µg/kg	0,04 µg/kg

The symptoms at oral ingestion are: nausea, emesis, diarrhoea, tachycardia, hypotension and seizures, destruction of red blood cells. Additional symptoms with injection are: hepatic necrosis and acute renal failure, paralysis of the respiratory center. Additional pulmonary symptoms are: pulmonary edema and respiratory failure or severe paralysis.

The possibilities of ricin detection are 1. lateral flow assay (LFA), 2. detection of Ricinin (accompanying alkaloid in castor bean seeds), 3. PCR of DNA (only castor bean seeds), and 4. LC/MS analytic after protein digesting. The only method to detect the unmodified peptide

in its tertiary structure is the immunological LFA test. Detection of ricin and abrin in food and water needs sample preparation (e. g. degreasing, neutralization, extraction with PBS buffer).

## 2. Material and Methods

### 2.1. Sample Preparation

For sample preparation we used organic solvents, buffers and laboratory equipment such as filters, centrifuges and homogenizers. It is important to ensure that the toxins are not denatured by excessive heat, mechanical stress or high mineral content.

### 2.2. Lateral Flow Assay (LFA)

The LFA was from miprolab, Göttingen. The test detects ricin and abrin by an immunological method, indicating the unchanged tertiary structure of both peptide chains by the test line (T). To check the functionality of the test there is an additional control line (C) visible. For the assay 100  $\mu$ L of the PBS buffer extract are pipetted into the sample pad (S) of the test. The sample is sucked through the test and after 20 minutes, the test can be evaluated.

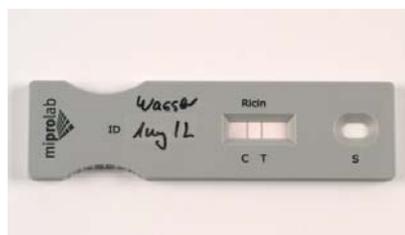


Fig. 1. LFA-test carried out with water (1 mg/L Ricin).

## 3. Results and Discussion

The test was sufficiently sensitive and robust to detect toxic amounts of ricin and abrin in food and water. It could be evaluated visually semi-quantitatively using external calibration.

Tab. 2. Detection limits of ricin for water and food.

Sample material	Detection limit
water	0,05 mg/L
beverages	0,1 mg/L
milk	2,5 mg/L
cereals	1 mg/kg

## 4. Conclusion

In Europe the first time a LFA test for detection of ricin is available. It detects ricin and abrin immunologically in sufficient sensitivity by its unaltered tertiary structure. For the analysis of food, drinking water and objects a short sample preparation is required.

## 5. References

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