

## Original article

**RIDASCREEN® FAST PEANUT, a rapid and safe tool to determine peanut contamination in food**Ulrike Immer,<sup>1\*</sup> Bernhard Reck,<sup>1</sup> Stella Lindeke<sup>1</sup> & Stef Koppelman<sup>2</sup><sup>1</sup> R-Biopharm AG, Landwehrstr. 54, 64293 Darmstadt, Germany<sup>2</sup> TNO Nutrition and Food Research Institute, Zeist, the Netherlands*(Received 20 March 2004; Accepted in revised form 13 April 2004)*

**Summary** During the last years allergenic substances in food have gained more and more attention. Stricter rules for a peanut free food labelling demand a peanut contamination less than 5 ppm. The maintenance of a high quality of food products with regards to allergenic exposure is preferable warranted with rapid tests that can be utilized as an aid for suppliers to control raw materials, to avoid cross contamination during production and to check the final products. A rapid sandwich peanut ELISA was developed, which covers a measuring range of 2.5–20 ppm peanut (detection limit of 1.5 ppm). This test was successfully evaluated in an independent study under the supervision of the AOAC Research Institute, getting the AOAC certificate No. 030404. In-house validation and independent validation data demonstrate the reliability, accuracy and precision of the test.

**Keywords** food allergens, peanut ELISA, peanut in food.

**Introduction**

Even small amounts of allergenic nut proteins in the range of mg kg<sup>-1</sup> (ppm) can induce allergenic immune reactions in highly allergic individuals. Symptoms range from allergic skin reactions up to death due to an anaphylactic shock. Unfortunately peanut as one of the allergenic proteins, which is a very nutritious fruit among the legume family, is also one of the most allergenic foods, capable of causing very dangerous, potentially lethal reactions. It is therefore important to detect hidden allergenic substances as a result of cross-contamination in food producing companies. To protect allergic persons the European Commission has published a new regulation in relation to labelling rules for food ingredients. All ingredients of more than 2% content have to be labelled.

Traces of allergen contamination in food are difficult to detect. To guarantee correct labelling the use of simple and fast methods like ELISAs can be a very effective tool, to help the producers and the consumers to detect peanut contamination in foods.

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R-Biopharm AG has developed a short and simple RIDASCREEN® FAST PEANUT ELISA. R-Biopharm AG, Darmstadt, Germany, which successfully passed the evaluation programme of the AOAC Research Institute as a Performance Tested Method Status with the certificate number 030404 in the first quarter of the year 2003.

**Material and methods**

Polyclonal antibodies (Koppelman *et al.*, 1996) were used to develop an assay in a sandwich format. The antibodies are affinity chromatographically purified against raw and roasted peanut. These antibodies were used to coat a microtitre plate and for conjugation with POD (horseradish peroxidase). Peanut protein in the sample or in the standard is bound to the immobilized antibodies during the 10-min incubation time. After washing the plate an antibody POD conjugate reacts for a further 10 min with the peanut antibody complex on the plate. The plate is washed again and a chromogen (tetramethylbenzidine) substrate reacts with the POD-conjugated complex. A blue colour has been developed and turns to yellow after adding

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a stop solution. The colour can be measured photometrically at 450 nm.

The more colour is developed the more allergenic protein is found in the food sample. The results were expressed as milligram peanut per kilogram (ppm). An extract of a mixture of twenty-four different kinds of peanut, raw and roasted, is used as a standard.

A special extraction buffer was developed which is used in most of the products in R-Biopharm's Allergen ELISA test line. One gram of a 5 g

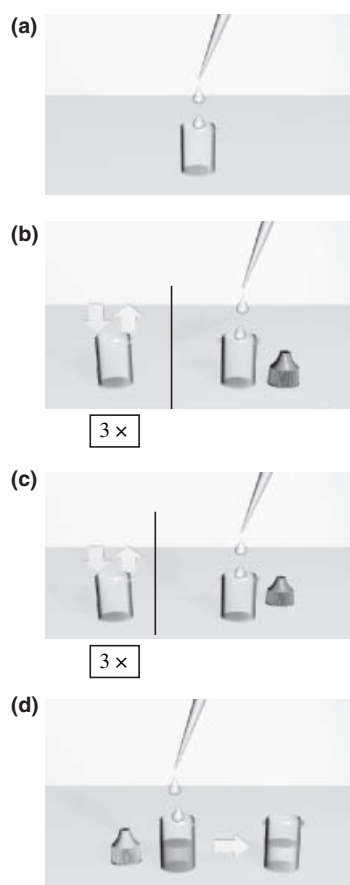


**Figure 1** Test procedure for RIDASCREEN® FAST PEANUT. Prepare a food sample according to the test instructions and place the required number of microwell strips into the frame. (a) Add 100  $\mu$ L of standard or sample and incubated for 10 min at room temperature. (b) Wash three times with washing buffer, add 100  $\mu$ L of conjugate and incubated for 10 min at room temperature. (c) Wash three times with washing buffer, add 100  $\mu$ L of the reddish substrate/chromogen solution mix and incubated for 10 min at room temperature. (d) Add 100  $\mu$ L of stop reagent mix and measured photometrically at 450 nm.

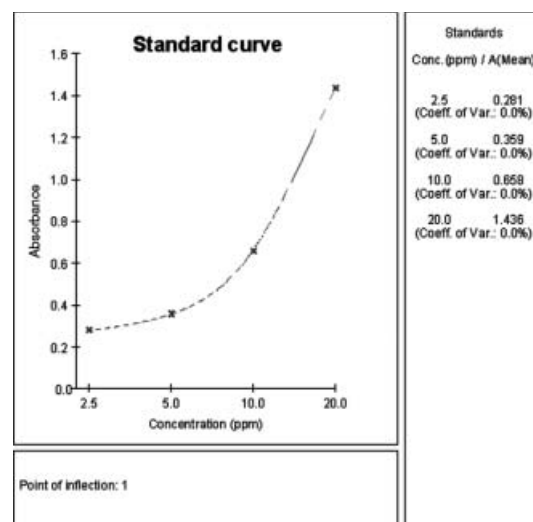


**Figure 2** Typical standard curve of the RIDASCREEN® FAST PEANUT Assay, obtained with the RIDAWIN® software.

ground sample is extracted with 20 mL of pre-heated extraction buffer for 10 min at 60 °C. After this a centrifugation step and or a filtration step has to be carried out to clarify the extract, which can be used directly in the assay.

All components, needed, are included in a test kit, the most components are ready to use and coloured for easier pipetting.

## Results

The layout of the procedure is shown in Fig. 1. After a total of 30 min the result can be obtained. Analysing the data a typical standard curve is obtained, shown in Fig. 2, which was created with the RIDAWIN® software. Using the recommended dilution factor, the result is obtained directly from the standard curve.

The ELISA was validated in an independent study under the leadership of the AOAC Research Institute as a performance tested method and is certified. The measuring range lies between 2.5 and 20 ppm. The limit of detection (LOD) was found at 1.5 ppm and the limit of quantitation at 2.5 ppm peanut. A contaminated sample with a level of 5 ppm peanut can be measured safely. Thirty-four substances were tested for cross-reactivity. No relevant cross reactivity was found.

In the independent study four different matrices (cereals, cookies, ice cream and chocolate) were tested as blank samples and as spiked samples on a level of 5 ppm peanut. In total 480 samples were evaluated in three independent labs with a specificity of 99.2% and a sensitivity of 100%.

In an additionally in-house study further samples, spiked at a level of 5 to 15.5 ppm were tested. All samples were spiked with a peanut butter carboxymethylcellulose suspension. The results are shown in Table 1. All tested blanks were found negative. The peanut butter spiked samples were measured with a recovery of 84–120%, reaching a mean value of 97%. Additionally spiked milk and bitter chocolates were evaluated in house. The samples were spiked at levels between 10 and 250 ppm of peanut (Table 2). Recoveries were between 92 and 137% over the whole range of spiking. To ensure adequate extraction of peanut from dark chocolate, skimmed milk powder has to be added at a concentration of about 35% to the extraction mixture.

**Table 1** In-house study results, obtained after spiking experiments in a range between 5 and 15 ppm peanut (*n* = number of runs)

| Sample               | Spike level (ppm) | Conc. measured (ppm) | SD (ppm) | CV (%) | Recovery (%) | <i>n</i> |
|----------------------|-------------------|----------------------|----------|--------|--------------|----------|
| Cereals              | 0                 | <LOD                 |          |        |              |          |
| Cereals              | 4.5               | 5.4                  | 1.7      | 31.4   | 120.7        | 3        |
| Cereals              | 5.0               | 5.3                  | 1.9      | 35.3   | 105.3        | 3        |
| Cereals              | 6.0               | 6.2                  | 1.7      | 26.8   | 102.9        | 4        |
| Cereals              | 11.1              | 10.9                 | 2.8      | 25.4   | 97.7         | 2        |
| Chocolate            | 0                 | <LOD                 |          |        |              |          |
| Chocolate            | 5.0               | 4.4                  | 1.1      | 25.7   | 88.0         | 2        |
| Chocolate            | 5.4               | 4.6                  | 1.4      | 30.8   | 85.2         | 3        |
| Chocolate            | 5.5               | 5.4                  | 1.4      | 26.2   | 98.2         | 2        |
| Chocolate            | 6.5               | 6.2                  | 1.4      | 21.7   | 95.8         | 4        |
| Chocolate            | 10.0              | 9.5                  | 0.6      | 6.7    | 94.5         | 2        |
| Cookies              | 0                 | <LOD                 |          |        |              |          |
| Cookies              | 4.9               | 4.8                  | 0.8      | 17.3   | 98.0         | 4        |
| Cookies              | 5.0               | 4.6                  | 1.5      | 32.0   | 92.0         | 3        |
| Cookies              | 6.2               | 5.6                  | 0.7      | 12.2   | 89.8         | 3        |
| Ice cream            | 0                 | <LOD                 |          |        |              |          |
| Ice cream            | 5.8               | 6.2                  | 0.5      | 7.9    | 107.3        | 4        |
| Ice cream            | 6.0               | 6.1                  | 0.9      | 15.6   | 99.2         | 4        |
| Ice cream            | 9.1               | 9.0                  | 0.8      | 9.4    | 98.9         | 2        |
| Ice cream            | 10.3              | 9.8                  | 1.3      | 13.0   | 95.1         | 2        |
| Ice cream            | 15.5              | 13.0                 | 0.2      | 1.6    | 83.5         | 2        |
| Mean of recovery (%) |                   |                      |          |        | 97.2         |          |

**Table 2** Spiking experiments with milk and dark chocolate (dark chocolate extracted using an extraction buffer with additional skimmed milk powder)

| Spike (ppm)    | Conc. measured in ppm peanut | Recovery (%) |
|----------------|------------------------------|--------------|
| Milk chocolate |                              |              |
| 0              | <Std 2                       | –            |
| 10             | 13.2                         | 132          |
| 40             | 54.7                         | 136.7        |
| 250            | 297.5                        | 119.0        |
| Dark chocolate |                              |              |
| 0              | <Std 2                       | –            |
| 10             | 9.2                          | 92.0         |
| 40             | 45.3                         | 113.2        |
| 250            | 271.3                        | 108.5        |

## Conclusion

Unlabelled food allergens have caused a lot of food recalls in the last years. R-Biopharm has developed a line of Allergen ELISAs able to detect hidden contamination in food, tests for allergens such as  $\beta$ -lactoglobulin, egg white proteins, peanut, hazelnut, almond and the gliadin proteins, which causes the coeliac disease, are available. Except for  $\beta$ -lactoglobulin, all of these assays are sandwich assays, some of the assays are available in a FAST format involving  $3 \times 10$  min incubation step. The AOAC RI approved RIDASCREEN® FAST PEANUT is an excellent tool for determining peanut contamination at a level of  $\geq 5$  ppm. The assay is fast and simple to perform, the assay is safe and highly specific, with a mean recovery of 97%. The ELISA is also applicable to difficult matrix such as dark chocolate by addition of skimmed milk powder to the extraction buffer, if the content of cocoa is higher than 10%.

The test can be used by food industry to check the food producing process, the incoming raw materials and the final products or can be used by administrative institutes to proof the final products on the market for hidden contamination by allergens for better consumer protection.

## Reference

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