# Validation of sensitive and rapid immunoassay method to detect coconut allergen in foods and beverages

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## Introduction

Coconut (*Cocos nucifera*) is a fruit belonging to *Arecacee* family. widely consumed all over the world in different food preparations, such as sweets, bakery products, sauses, and seasoning.

It contains proteins (7S and 11S globulin) that can trigger allergic reactions in sensitized individuals. Clinical symptoms include hives, skin rushing, and, in severe case, anaphylactic reactions (1). According to the Food Allergen Labelling and Consumer Protection Act (FALCPA) enforced in USA and Canada, coconut is considered a tree nut, included among the big-9 major allergenic foods, and it must be reported as an ingredient on labels.
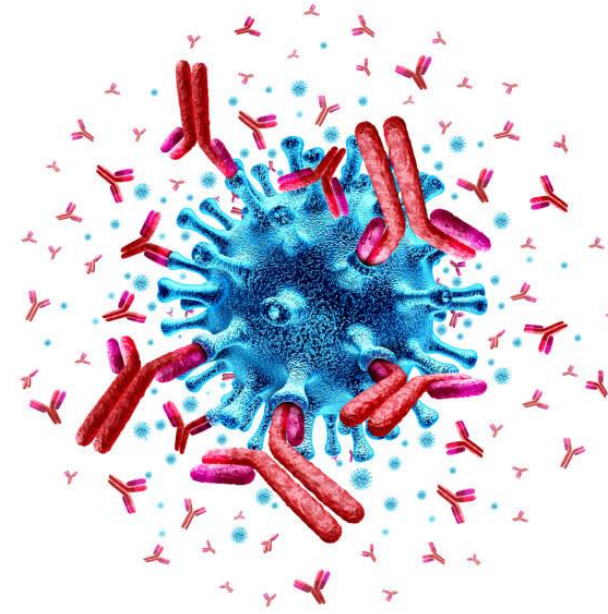
FOOD ALLERGENS

Due the increased consumption of coconut-containing foods imported by extra-EU countries the potential presence of "hidden" coconut allergen could represent a rising health concern to allergic consumers.

The aim of this study is to validate an ELISA qualitative method to detect coconut allergen in food and beverage.

## Material and methods

The SENSISpec Coconut ELISA kit (Gold Standard Diagnostics) for qualitative determination of coconut in food (bakery and dairy products) and beverage. The validation performances evaluated were specificity, sensitivity, ruggedness.

❑ **Food samples and specificity**. The method was tested on three classes of food matrices (blank samples): i) bakery products (cookies), ii) , dairy products (yogurt, drink yogurt, kefir, etc..) iii) beverages (fruit juices and drinks). Specificity analyses were performed on 24 negative samples (bakery products n=8; dairy products n=8; fruit juices n=8).

❑ **Sensitivity.** Sensitivity was tested on the 24 negative samples fortified to a final coconut concentration of 2.5 ppm.

❑ **Ruggedness**. Ruggedness was evaluated according to the Youden approach, modifying incubation temperature, rpm of centrifugation, and conjugate volume.

## Results

Validation performances (specificity, sensitivity, and ruggedness) were verified. Specificity analyses carried out on 24 samples showed β error below 5%. Sensitivity analyses revealed that the test is able to detect analyte at 2.5 ppm, set as limit of detection (LOD). Slight variations (±5%) applied on nominal value of incubation temperature (T=60°C), time of incubation (10 min) and conjugate volume (100 µL), do not significantly affect assay efficiency, indicating that the test is robust.

| Validation of ELISA performance | |
|---|---|
| **Specificity** | tested on 24 food matrices (bakery products, dairy products, beverages) |
| **β error** | < 5% |
| **CC β** | < 2.5 mg/kg |
| **Ruggedness** | evaluated according to the Youden approach |

## Discussion and Conclusion

The ELISA method for the detection of coconut in foods and beverage presented in this study was successfully validated in terms of specificity and sensitivity, LOD and ruggedness. The validated method guarantee reliable and effective results, and it is able to detect coconut occurrence in food matrices in concentration up to 2.5 ppm.

The presented method, proving high analytical specificity and sensitivity, could be a reliable tool to guarantee allergic consumers' protection.

### References

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