Two tetraplex real-time PCR for the detection and quantification of DNA from eight allergens in food

René Köppel · Veronika Dvorak · Franziska Zimmerli · Alda Breitenmoser · Albert Eugster · Hans-Ulrich Waiblinger

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Abstract According to the EU and Swiss legislation, food has to be labelled for allergens to enable allergic consumers to avoid such food and its products. To provide efficient and reliable methods, two novel quantitative multiplex real-time polymerase chain reaction systems were developed and validated. They simultaneously determine DNA of peanut, hazelnut, celery, soy, egg, milk, almond and sesame, respectively. The tests exhibit good specificity and sensitivity in the range of 0.01%. Due to low DNA amounts, lower sensitivities for egg and milk were obtained. First comparisons of ELISA results with PCR results suggest a qualitative accordance, but a low correlation of quantitative results.

Keywords Allergens · Multiplex real-time quantitative PCR · Peanut · Hazelnut · Celery · Soy · Egg · Milk · Almond · Sesame · Validation

Introduction

As much as 2–6% of the population are estimated to exhibit allergic reactions due to the consumption of food containing allergens. To increase the well-being and safety of these people, ingredients that cause allergic reactions must be labelled on foods [1, 2]. To control that these regulations are implemented by the producers, food samples are regularly examined for the presence of allergens and the compliance with the declaration. Currently, there are 20 allergens listed by the food law of European Union and Switzerland. It comprises cereals with gluten, crustacean, egg, fish, milk, molluscs, soy, nuts (almonds, peanuts, cashew, hazelnut, macadamia, walnut, Brazil nut, pecan, pistachio and Queensland nuts), sesame, celery, mustard, lupine and sulphites. Sulphites are analysed by other methods than protein or DNA-based methods. For most of the analytes, polymerase chain reaction (PCR) together with ELISA are well-recognized methods [3, 4], although PCR does not detect proteins that have the allergenic potential. Many qualitative and quantitative PCR systems were described in the past, most of them detecting single analytes [3, 5–11], multiplex qualitatively [12, 13] or duplex quantitatively [14]. In principle, food samples need to be analysed for all allergens. However, these analyses are very laborious and time-consuming. In addition, many allergen combinations are unlikely to be present in certain food products. If analysed separately, the amount of isolated DNA may be insufficient to analyse all possible allergens in a food sample. Therefore, multiplex PCR is a necessary and promising approach to produce results in a more economic and faster way. Here, we present two tetraplex real-time PCR systems called AIAlA A and AIAlA B. AIAlA A simultaneously determines the contents of DNA from peanut, hazelnut, celery and soy, while AIAlA B determines that from almond, sesame, egg (DNA of chicken) and milk (DNA of cow) in food.