

Results of an International Interlaboratory Trial to Determine Twelve Allergens Using Real-time PCR- and ELISA-based Assays

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Abstract: To elucidate the capability of laboratories to determine allergen contents, an international interlaboratory trial was conducted using meat products spiked with 12 allergens. The measurement uncertainty was calculated independent of the applied method simulating realistic situations when comparing analysis certificates from different laboratories. The measurement uncertainty was revealed to be in the best cases +/-100%, in the worst cases quantification exhibited a measurement uncertainty of higher than 200% making quantitative analysis impossible. The measurement uncertainty seemed to depend on the analyte and assays used.

Keywords: Allergen · Determination · ELISA · Real-time PCR · Sausages

1. Introduction

For the wellbeing and safety of persons with allergic reactions, ingredients causing potential allergic reactions must be labeled on each food product.^[1,2] Therefore, food control laboratories examine food products regularly for consequent implementation of these regulations by the producers. Currently, the following allergens are listed by the food law of European Union and Switzerland: cereals with gluten, crustacean, egg, fish, milk, mollusks, soy, nuts (almonds, peanuts, cashew, hazelnut, macadamia, walnut, Brazil nut, pecan and pistachio, Queensland nuts), sesame, celery, mustard, lupin and sulphites.

To determine allergens from animal or plant sources, real-time polymerase chain reaction (PCR) and enzyme linked immunoassays (ELISA) are well-recognized methods.^[3–13] Both methods are prone to matrix effects like loss of analyte during production and inhibition. ELISA does not need expensive equipment whereas

PCR needs a real-time thermocycler. Run in multiplex format, PCR may have an advantage when analyzing mainly unknown samples.

For most allergens no certified reference material is available. In addition, only little information about stability and range of allergen contents which are quantifiable is available. Many studies, such as interlaboratory trials or proficiency studies, address these questions for only a few allergens. This interlaboratory trial tried to gain an overview for meat products like raw and boiled sausages for 12 allergens at once, independent of the applied method.

To overcome the lack of certified reference material, both reference and sample material was produced and provided for the calibration of measurement of all participants.

We decided to make our own meat products because commercial products often contain undeclared allergens. Usually boiled meat products are of finer texture than raw matured products and therefore would be more homogenous and suitable as reference materials. To assess the contribution of homogeneity to the measurement uncertainty both rough cut matured and fine textured boiled meat-products were produced. A set of boiled and a set of raw matured reference sausages were produced from the same starting material (see Table 1) as the unknown samples. These reference sausages were used for the calibration of the assays. Three of the unknown samples belonged to the matured product group (Cevapcici, Landjäger and Salami) and one represented a boiled product (Sucuk). In addition and prior to this in-

Table 1. Reference sausages and samples. Recipe for 100 kg of reference sausage (type Landjäger, raw sausages and type boiled sausages) and sample sausage used in this study. Values are given in kg for the production of 100 kg, taking reduction of the weight during the production process in account.

Fraction %	Kal A LJ	Kal B LJ	Kal C LJ	Kal D LJ	Kal E LJ	Cevap-cicci	Land-jäger	Salami	Sucuk
Beef	1	8	22	31	48	47.6	23.2	5	42
Pork	31	48	22	8	1	14.3		48	3
Horse	48	31	22	1	9	9.5	27.8	45	3
Sheep	8	1	22	48	31	23.8	1.85	2	28
Lard (pork)							39.8		
Water / ice	2.4	2.4	2.4	2.4	2.4	2.4			
Curing salt	2.44	2.44	2.44	2.44	2.44	0.1	2.32		20
Aller-gens and additives	10	10	10	10	10	2.4	5.05		4

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