

特定原材料由来タンパク質定量における ELISA 法の 反応温度に対する影響

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Effects of reaction temperature on the quantification of proteins derived from specific raw materials using ELISA

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Abstract

An enzyme-linked immunosorbent assay (ELISA) is widely used to test for allergens in foods. In this study, we investigated how temperature affects the performance of the “Morinaga FASPEK ELISA II Egg” from the kit under different conditions. ELISA was performed under low-temperature conditions (approximately 5°C), room temperature conditions (approximately 20–30°C), high-temperature conditions (approximately 45°C), and ultra-high-temperature conditions (approximately 65°C). The absorbance values were measured and compared for all conditions. Our results showed that compared to the absorbance at room temperature, the absorbance showed an average decrease of 44.5% and 15.7% under low-temperature and high-temperature conditions, respectively. A calibration curve could not be established under ultra-high-temperature conditions. To verify the main factor contributing to the decrease in absorbance, especially under high-temperature conditions, ELISA was carried out by independently heating the antigen in the standard egg product, the antibody on the immobilised plate, and the enzyme-labelled antibody solution from the kit to high-temperature conditions. No decrease in absorbance was observed upon heating the standard egg product alone. However, an average decrease of 6.2% and 1.7% was observed upon heating the antibody-immobilised plate alone, and heating the enzyme-labelled antibody solution alone, respectively. Therefore, while performing ELISA, it is important to control the temperature in the laboratory, especially that of the antibody-immobilised plate.

Keywords : ELISA 法、抗原、抗体、酵素、温度

ELISA, antigen, antibody, enzyme, temperature

I 緒言

食品アレルギーとは食物に対する免疫学的機序を介する副反応のことである。この多くは I 型アレルギーと呼ばれ免疫グロブリン E (Immunoglobulin E、以下 IgE) が関与する。このような IgE 依存性の食物アレルギーは原因食品 (以下、アレルゲン) を摂取してから 2 時間以内に症状が始まる即時型反応であることが多い。IgE 依存性の食物アレルギーはアナフィラキシーに至ることや患者数の多さから医学的に重要視されている¹⁾。

食品アレルギーのアレルゲンとしては、鶏卵、牛乳、小麦の割合が高い。これらのアレルゲンによる誘発症状としては、皮膚症だけでなく、重症の場合はショック症状を呈し、アドレナリン投与や入院が必要となる場合もある²⁾。

我が国におけるアレルゲンの検査法は平成 27 年 3 月 30 日付け消費者庁次長通知およびその改正通知³⁾における「別添 アレルゲンを含む食品の検査方法 2.1. 定量検査法」に規定されている酵素結合免疫吸着測定法 (Enzyme-linked immunosorbent assay、以下 ELISA 法)