Food IgG antibodies: study of long-term stability of dried blood samples

Gabriele Piuri\(^1\), Katia Basello\(^2\), Attilio Francesco Speciani\(^3\), Andrea Costanzi\(^2\), Francesco Zinno\(^4\)

\(^1\)Inflammation Society, Orpington, United Kingdom; \(^2\)GEK srl c/o Cryolab, Rome, Italy; \(^3\)GEK srl, Milan, Italy; \(^4\)Department of Biomedicine and Prevention, Faculty of Medicine, “Tor Vergata” University, Rome, Italy

**Aims:** A dried blood spot is a common, moderately-invasive method for collecting patient blood from finger pricks. The aim of this study is to evaluate the long-term stability in the collection and detection of food-specific IgG antibodies comparing two different kinds of sample collectors: Whatman Neonatal card 903 (CARD) and Copan Nylon Swab (SWAB). The blood samples are tested by IgG Enzyme-Linked Immuno-Sorbent-Assay (ELISA) for precision and accuracy.

**Methods:** The blood samples are collected from 18 individuals (10 females and 8 males). The collection of samples is carried out in triplicate for both devices. Serum samples are assayed for food-specific IgG. The specific IgG antibody responses are analyzed on a customized ELISA plate (Immunolab GmbH) which detects total IgG for 33 food antigens in serum. Samples were stored at room temperature in the dark. Serum extractions and ELISA assays were carried out after 2 days (T2), 7 days (T7), 14 days (T14), 21 days (T21) and 28 days (T28). Since the Kolmogorov–Smirnov normality test revealed non-normal distribution of the parameters, results were presented as median and interquartile range (IQR).

**Results:** Over time, analyses show progressively smaller results both as regards the CARD and the SWAB (p <0.001). While between T2 and T7 there were no statistically significant changes, in T14 there was a significant reduction of -17% (-0.45 to +0.03) to the initial value that is maintained up to T21. Between T21 and T28, there were no statistically significant differences regarding CARD, while for SWAB there was a further reduction of values of up to -29% (-0.46 to -0.08).

**Discussion:** As discussed by Ligaarden, the concentration of specific IgG for food reflects the use of that food in the diet. In addition, Finkelman showed a IgG-dependent mechanism of anaphylaxis which involves IgG, FcγRs on macrophages, basophils and neutrophils, complement-derived peptides C3a and C5a, B cell Activating Factor (BAFF) and Platelet Activating Factor (PAF). For a more accurate study of the possible interaction between these mechanisms associated with IgG, it is important to know precisely the advantages and disadvantages of the most used methods in this area of investigation.

**Conclusion:** Considering the deterioration of biological samples over time it is important that the dosage of food IgG antibodies in dried blood is carried out as quickly as possible and in any event within 21 days of collection.