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Short Communication

Stability of specific IgE antibodies to common food and inhalant allergens

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ABSTRACT

Objective: To determine the optimum storage temperature for serum allergen specific IgE antibodies (sIgE) to common food and inhalant allergens.**Methods:** Patient sera with sIgE concentrations ≥ 0.7 kIU_A/l were pooled accordingly: pool 1—peanut and hazelnut, pool 2—egg white, cow's milk and cod fish, pool 3—soy, wheat and shrimp and pool 4—dust mite *Dermatophagoides farinae*, dog dander, cat dander, Timothy grass pollen, and silver birch pollen. Aliquots stored frozen, refrigerated and at room temperature were tested in duplicate (Phadia ImmunoCAP® 250) over two weeks. The relative difference was calculated for each sIgE as a percentage of the initial value and compared to the analytical reference change value.**Results:** Minimal effects on specimen stability were noted for all sIgE analyzed under the three storage conditions tested in this study. All changes observed in sIgE concentrations were related to the assay variability and not to sample deterioration.**Conclusion:** Serum allergen specific IgE concentrations are stable at all temperatures studied for up to 17 days.

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1. Introduction

Serum allergen specific IgE (sIgE) levels correlate with an increased likelihood of clinical allergy [1,2]. The use of in vitro allergen specific IgE testing is increasing in primary care where the skin prick/puncture test is impractical and waiting times from patient referral to specialist consultation are often lengthy [3,4]. Furthermore, serum sIgE is particularly relevant in patients with medical conditions contraindicating in vivo testing [5]. Our institution is the sole laboratory that is performing allergy testing for a catchment area of 2 million people in Northern Alberta, Canada.

Currently, sera for allergen sIgE are transported at room temperature. If time between collection and testing is expected to exceed seven days, samples are aliquoted and transported frozen. After testing, all specimens are stored refrigerated under multiple layers of Parafilm® M for up to seven days at our laboratory as per manufacturer recommendations [6]. Specimen stability is a crucial factor for samples that require extended transportation time or prolonged storage to allow physicians time to add on tests.

The aim of this study was to determine the specimen stability for serum sIgE to common food and inhalant allergens over 15 to 17 days as well as the optimum storage temperature.

2. Materials and methods

This study complied with DynaLIFE_{DX} institutional board ethics requirements.

Testing was performed on the Phadia ImmunoCAP® 250. The results are reported quantitatively and can be categorized into standardized classes, in accordance with the manufacturer's instructions [6].

IgE class	IgE (kIU _A /l)	Interpretation
0	<0.35	Negative
I	0.35–0.7	Equivocal
II	0.7–3.5	Positive (+)
III	3.5–17.5	Positive (++)
IV	17.5–50	Strongly positive (+++)
V	50–100	Strongly positive (++++)
VI	>100	Strongly positive (++++)

Sera from several patients with sIgE concentrations ≥ 0.7 kIU_A/l ($\geq$ Class II) were pooled, aliquoted and stored frozen (-23.5 ± 3.3 °C), refrigerated (4.8 ± 2.5 °C) and at room temperature (RT, 21.3 ± 2.5 °C). Samples with gross hemolysis, lipemia or ictericia were excluded from the study to limit potential interferences. Aliquots were tested in duplicate on days 1, 3, 6, 7, 8, 11, 13, 15 and 17. The sera pools were selected to minimize cross reactivity amongst sIgE antibodies accordingly: Pool #1—peanut and hazelnut, Pool #2—egg white, cow's milk and cod fish, Pool #3—soy, wheat and shrimp and Pool #4—dust mite *Dermatophagoides farinae*, dog dander, cat dander, Timothy grass pollen,

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and silver birch pollen. Reliability of the results was assured through determination of standard curve controls and three levels of quality control (QC) evaluated according to specified limits of acceptance. The analytical variation (CVa) for each level of positive QC (2.84 kIU_A/l and 11.80 kIU_A/l) was 8% and 6% respectively.

Data was imported to Excel (Microsoft Office 2010). Paired comparisons and relative percent difference between the baseline and repeated measurements were performed. Percent difference was calculated as follows:

$$\%Diff = \frac{C_f - C_i}{C_i} \times 100$$

where % Diff is the percent difference, C_i is the average of the initial sIgE concentrations on day 1 and C_f is the average of sIgE concentrations on day 15 or 17. Percent difference was considered significant if it exceeded the analytical reference change value (ARCV) and literature values reported for the ImmunoCAP system [7]. ARCV was calculated as follows:

$$ARCV = \pm \left(Z \times \sqrt{2(CVa)^2} \right) = \pm (1.96 \times 1.41 \times CVa) = \pm (2.76 \times CVa).$$

ARCV represents the standard percent difference between replicates belonging to the same statistical normal distribution, $\pm$ represents the bi-directionality of the change, CVa is the assay imprecision and Z is a statistical parameter which is equal to 1.96 at the 95% confidence level [8].

3. Results and discussion

The specific IgE antibodies studied were cod fish (1.19 kIU_A/l, Class II), shrimp (1.09 kIU_A/l, Class II), hazelnut (7.19 kIU_A/l, Class III), dust mite *D. farinae* (7.40 kIU_A/l, Class III), dog (4.42 kIU_A/l, Class III), Timothy grass (2.40 kIU_A/l, Class II), silver birch (4.61 kIU_A/l, Class III), egg (1.35 kIU_A/l, Class II and 7.96 kIU_A/l, Class III), milk (2.14 kIU_A/l, Class II and 16.90 kIU_A/l, Class III), peanut (13.0 kIU_A/l, Class III and 44.9 kIU_A/l, Class IV), wheat (2.42 kIU_A/l, Class II and 5.02 kIU_A/l, Class III), and soy (1.01 kIU_A/l and 1.35 kIU_A/l, Class II). The calculated ARCV was 22% at 2.84 kIU_A/l and 17% at 11.80 kIU_A/l.

Although the percent difference between the sIgE initial and final concentrations was less than the calculated ARCV for all analytes, it was noted that most sIgE concentrations increased by day 15 (vertical

bars, Fig. 1). Thus, evaporation of sera was considered plausible for low volume aliquots.

A second experiment was designed to minimize evaporation. Aliquots contained a larger volume of sera and were sealed with four layers of Parafilm® M as opposed to three layers used on the first trial. Cow's milk, peanut, wheat, egg white and soy sIgE were chosen because they are included in the food mix profile offered by our laboratory. As the name indicates, this is a mixture of allergens contained in a single test, providing a rapid and convenient assessment of common childhood food allergies (Multiallergens, fx5 (6)). This food mix profile is widely ordered by our community physicians as a first tier test for allergy investigation, therefore knowing the specimen stability is essential for follow up testing of positive results.

For logistical reasons the second experiment was extended to day 17. The calculated % Diff between sIgE C_i and C_f was found to be lower than the calculated 17% and 22% ARCV for all analytes. In contrast to the first experiment, it was noted that most sIgE concentrations decreased by day 17 (vertical bars, Fig. 2). To further investigate the shift of sIgE concentrations in the same direction for both experiments, the % Diff was calculated for the two positive QC on the corresponding days (represented by horizontal lines in Figs. 1 and 2). Not surprisingly, the % Diff observed for both levels of QC mimicked the pattern noticed previously for sIgE during the two experiments.

Minimal effects on specimen stability were noted for all the sIgE analyzed under the three storage conditions tested in this study. All changes observed in sIgE concentrations were related to assay variability and not to sample deterioration. Of clinical relevance is the observation that the shift in sIgE results did not impact clinical interpretation. In conclusion, the study demonstrates that serum allergen specific IgE concentrations are stable at all temperatures studied for up to 17 days.

The practical implication of this sIgE stability study is that our laboratory can extend the storage of patient specimens by 10 days in the refrigerator without the need for aliquoting and freezing. This will improve patient care by 1) accepting and testing specimens from remote areas that underwent delayed transportation and 2) allowing physicians to add-on sIgE tests with no re-collection needed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiochem.2016.03.003>.

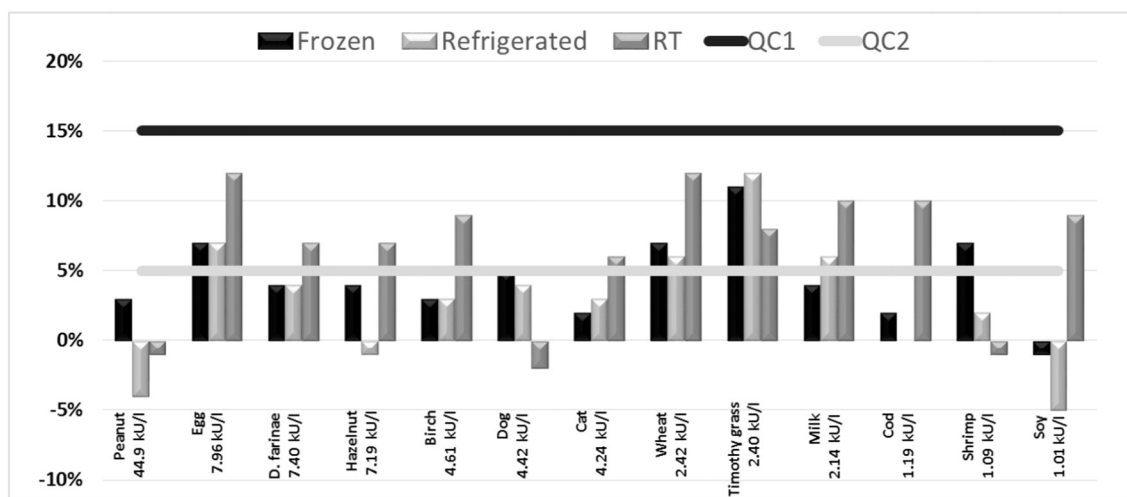


Fig. 1. Influence of storage temperature on allergen specific IgE antibody concentration measured on day 15. Pooled patient sera were stored frozen, refrigerated and at room temperature for 15 days. The allergen specific IgE antibody concentration (sIgE, kIU_A/l) for an aliquot of each pool was measured in duplicate. The percent differences (y-axis) between the initial concentrations (C_i) and the final concentrations (C_f) for each allergen are plotted against the sIgE antibody concentration (x-axis). The horizontal bars represent the percent difference between the QC concentrations obtained on day 1 and day 15. Calculated analytical reference change value was 22% at 2.84 kIU_A/l and 17% at 11.80 kIU_A/l.

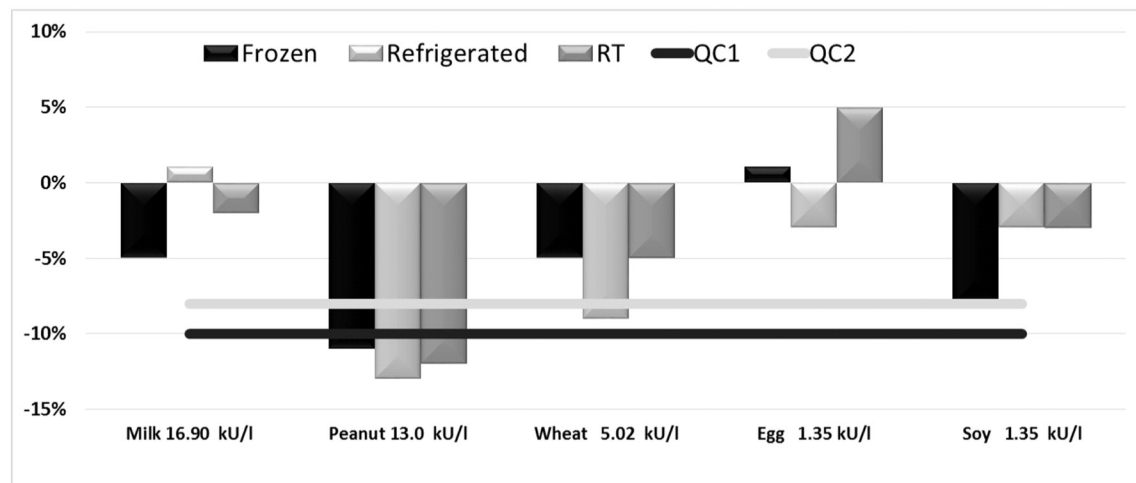


Fig. 2. Influence of storage temperature on allergen specific IgE antibody concentration measured on day 17. Pooled patient sera were stored frozen, refrigerated and at room temperature for 17 days. The allergen specific IgE antibody concentration (sIgE, kIU_A/l) for a food mixture was measured in duplicate. The percent difference (y-axis) between the initial concentration (Ci) and the final concentration (Cf) for each food are plotted against the sIgE antibody concentration (x-axis). The horizontal bars represent the percent difference between the QC concentrations obtained on day 1 and day 15. Calculated analytical reference change value was 22% at 2.84 kIU_A/l and 17% at 11.80 kIU_A/l.

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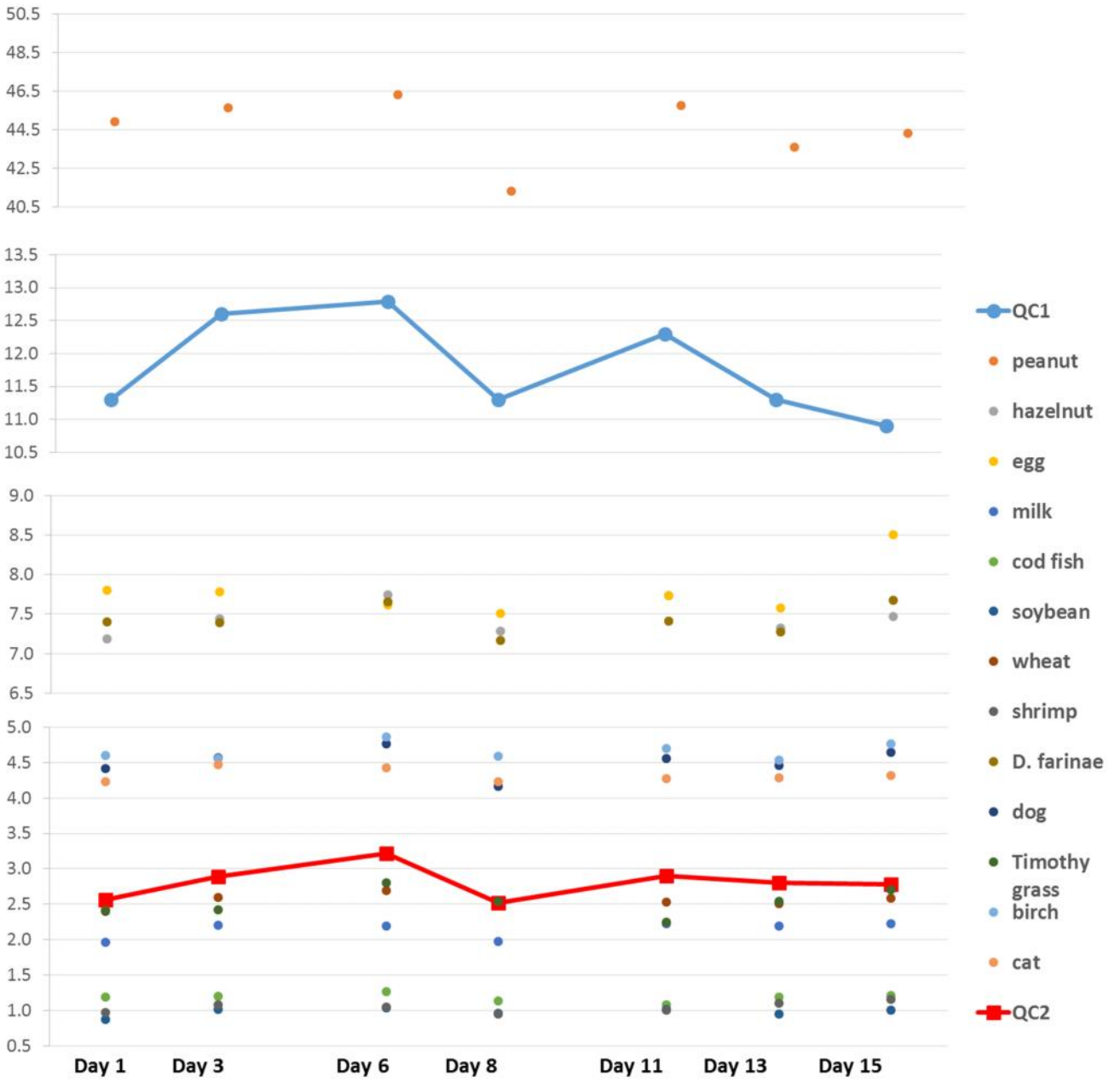
Supplementary Data

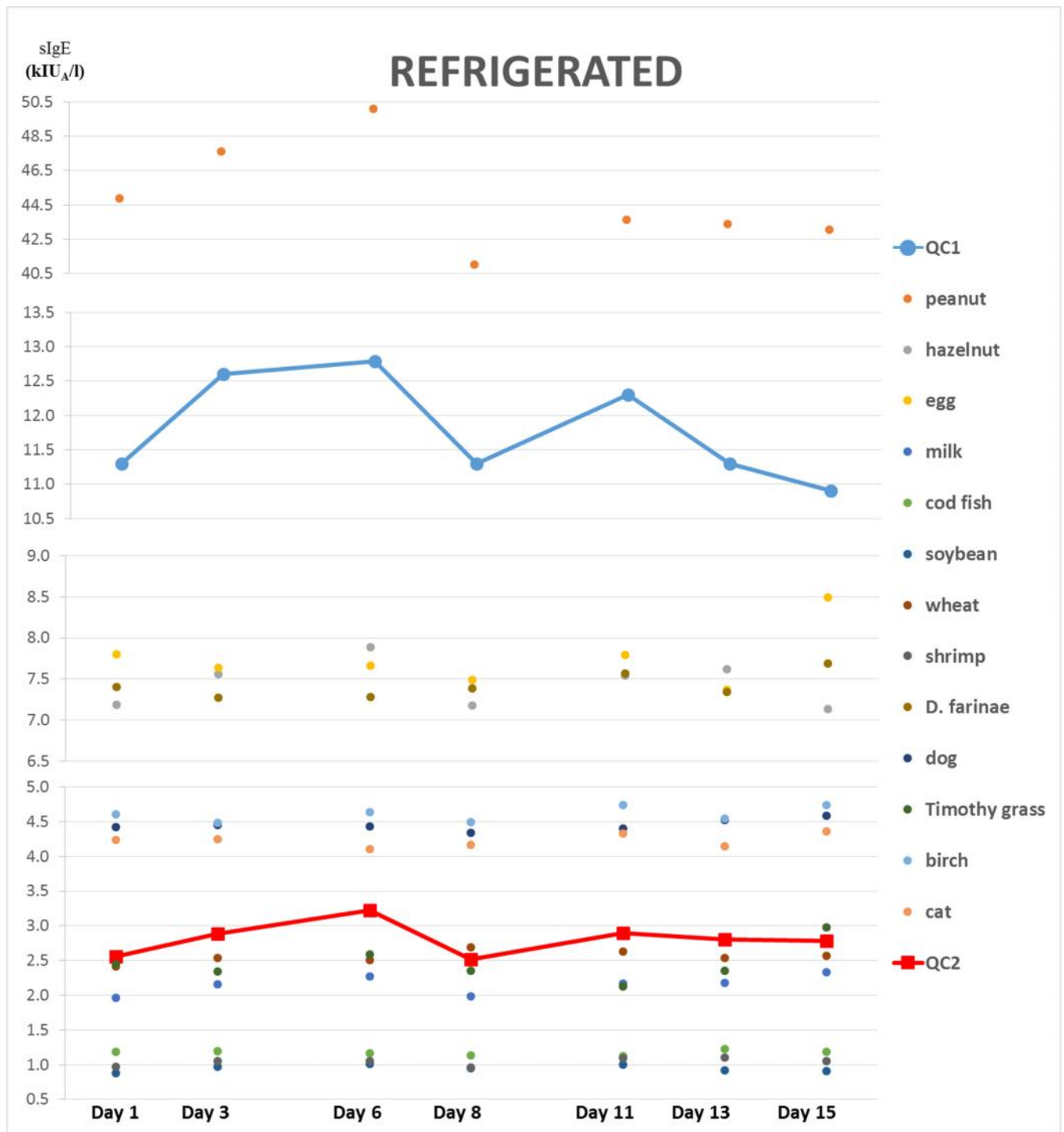
Influence of storage temperature on allergen specific IgE antibody concentration.

Pooled patient sera were stored frozen ($-23.5 \pm 3.3^{\circ}\text{C}$), refrigerated ($4.8 \pm 2.5^{\circ}\text{C}$) and at room temperature (RT, $21.3 \pm 2.5^{\circ}\text{C}$) for 15/17 days. The allergen specific IgE antibody concentration (sIgE, kIU_A/l) for an aliquot of each pool was measured in duplicate on days 1, 3, 6, 7, 8, 11, 13, 15 and 17. Dots represent the average of the duplicates, the sIgE antibody concentration (y-axis) is plotted for each day of analysis (x-axis).

FROZEN

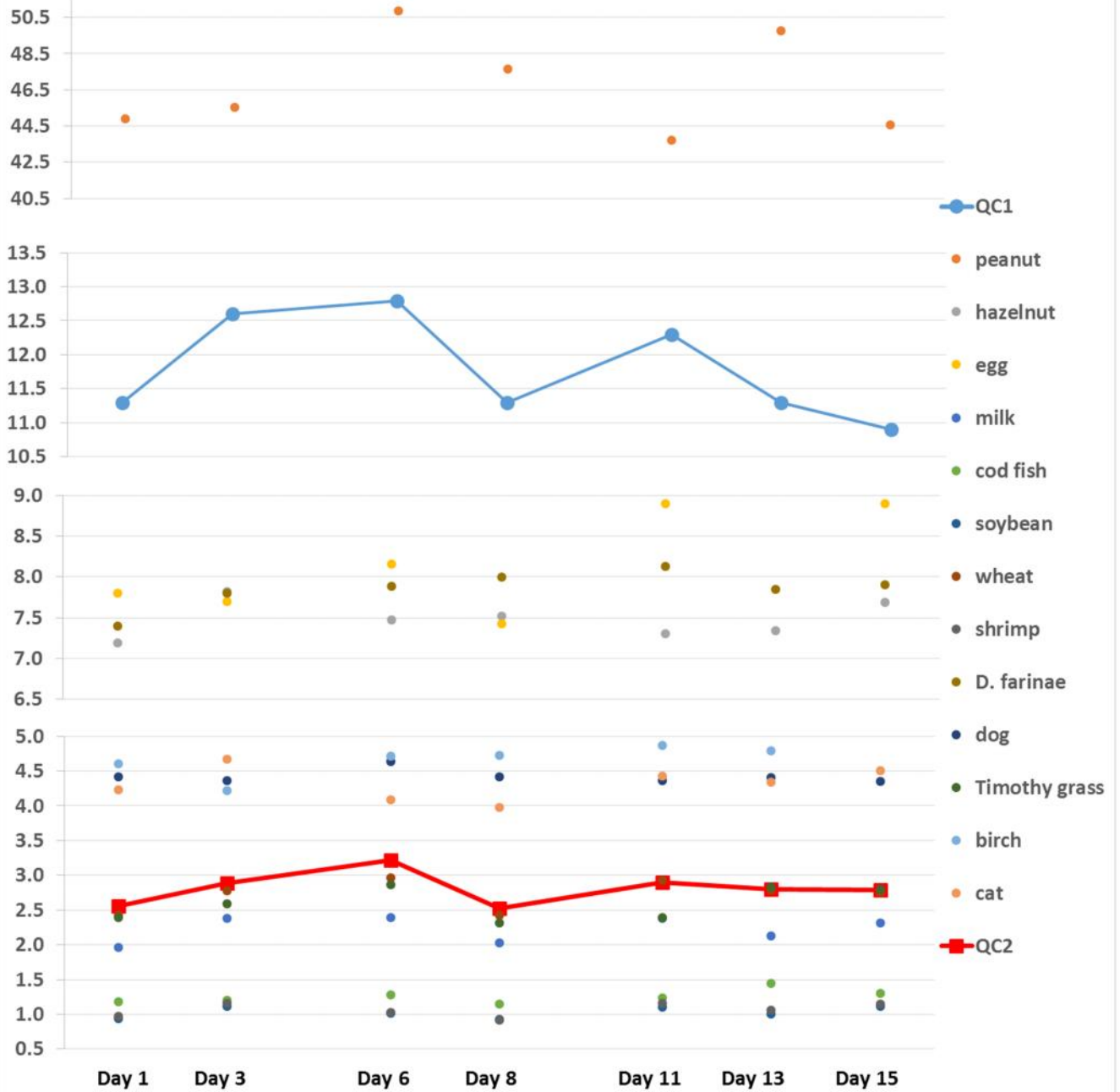
sIgE
(kIU_A/l)

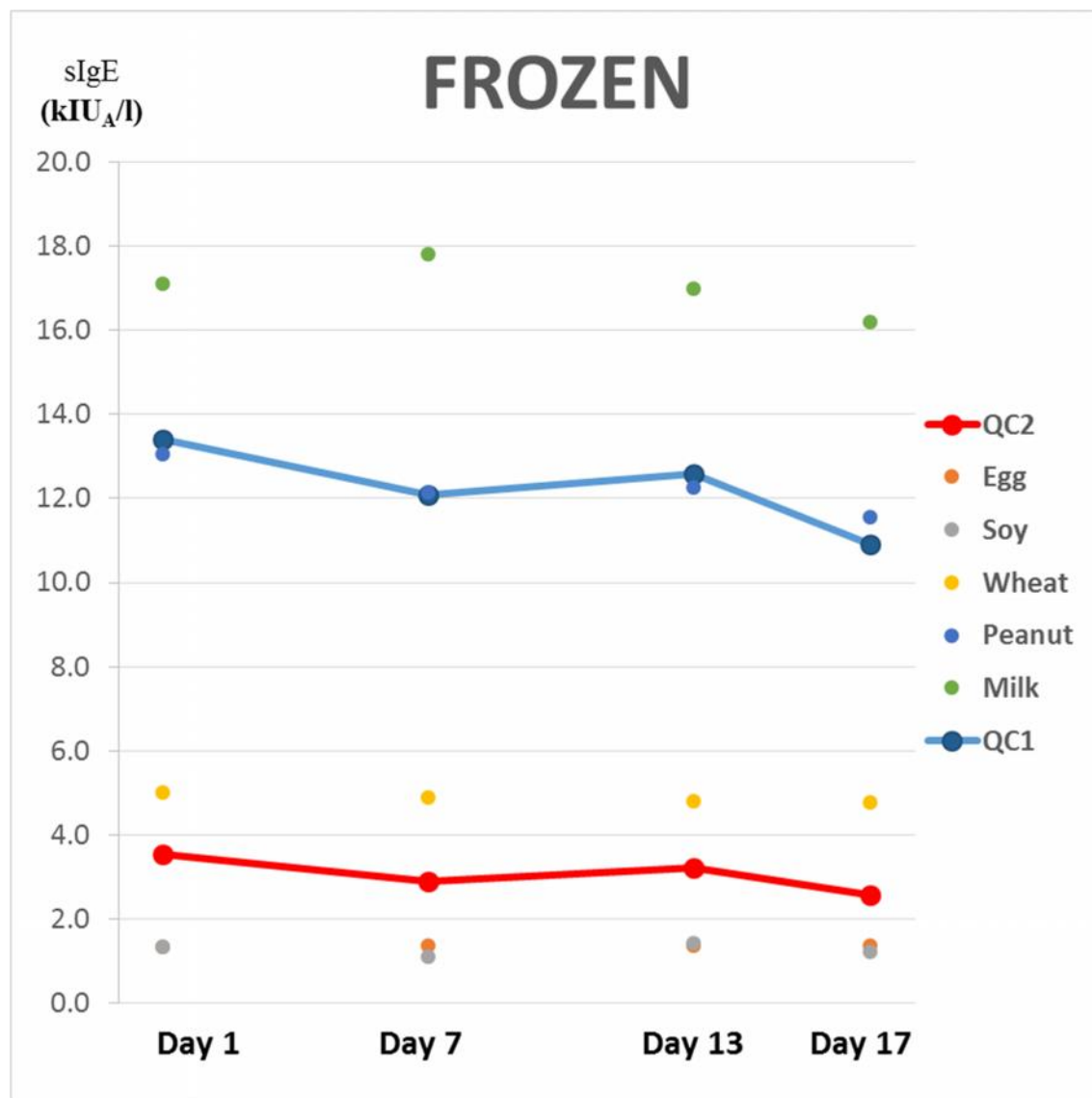


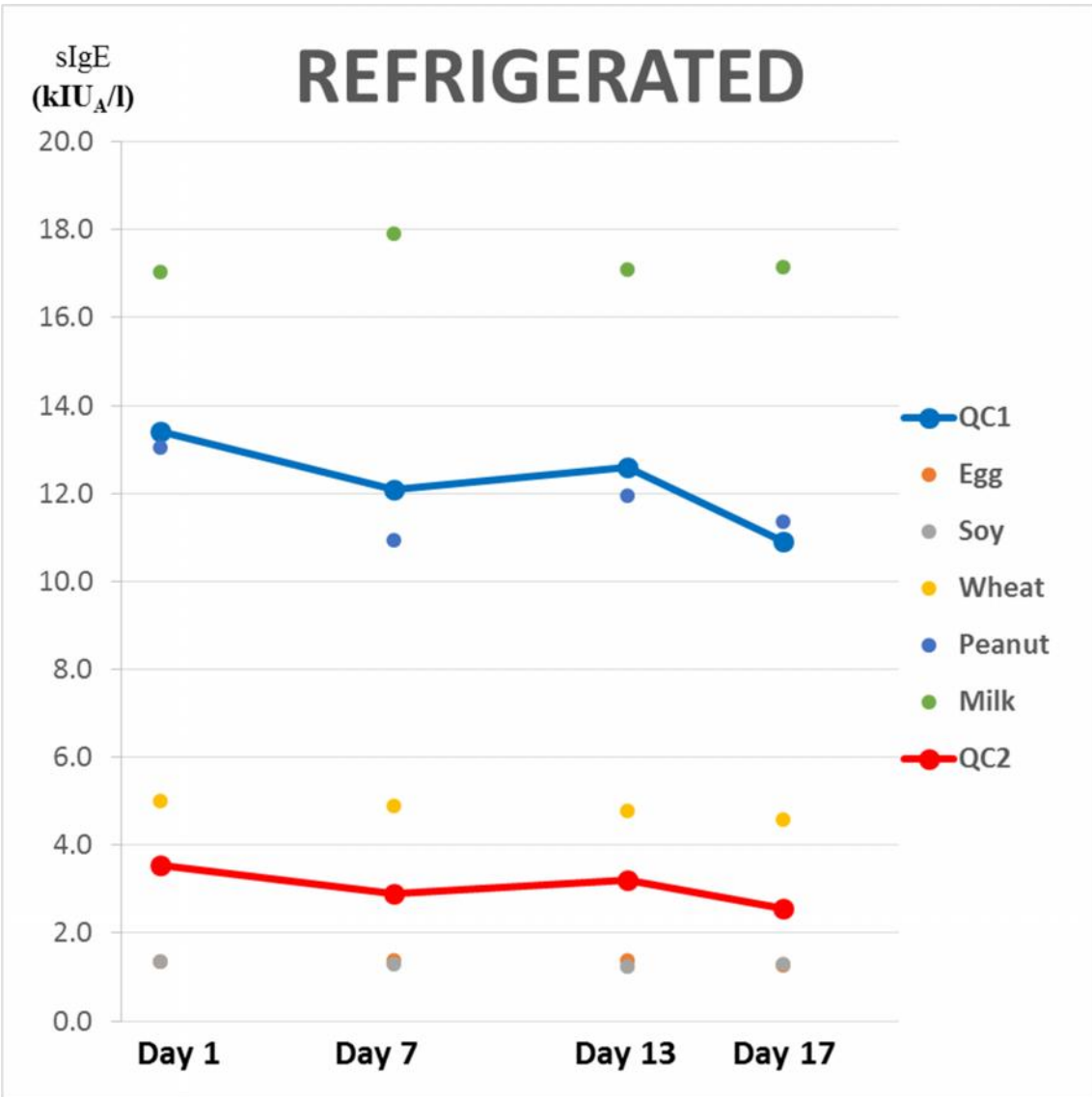


ROOM TEMPERATURE

sIgE
(kIU_A/l)







ROOM TEMPERATURE

sIgE
(kIU_A/l)

