



Stability of milk and gliadin on swabs during 7 days under different storage conditions



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ABSTRACT

Food industries rely on swabbing to control allergen and gluten occurrences in their manufacturing plant. However, collecting allergens from a surface with a swab and further analysis can be affected by numerous aspects, like the operator, the allergen, the swab fabric, the ELISA kit, and the procedure. In addition, food companies often send swabs to external laboratories, but there are no available data to describe the effect of time and temperature on the allergen or gluten stability on the swabs. This study presents the stability of milk and gliadin collected from a stainless-steel surface using different swab types (cotton, Dacron, rayon, polyester, and polyurethane). The protein concentrations were tested on days 0, 1, 2, 3, and 7 by an ELISA technique to follow the protein concentration over time. The swabs with gliadin were stored at 37 °C for 7 days to mimic poor storage conditions. The swabs used to collect milk were stored at 4 and 37 °C. The experiments indicated that, except for two cases, there was no difference in gliadin concentrations between day 1 and the other days; the same observation was made for milk. However, the swab types were not equivalent and some led to false-negative results. Validation of those observations was undertaken at a commercial laboratory. To reduce the risk of false-negatives and the effect of factors influencing the result (operator, swab, procedure, and allergen), it is recommended to swab an area in triplicate.

1. Introduction

Allergies represent a potentially high risk for public health. Allergenic food and ingredients have received increasing attention from industries, regulators, and consumers in the past decades. Moreover, studies have shown that the prevalence of food-induced allergies in children is estimated to be 8% in the USA and 7% in Canada (Gupta et al., 2011; Soller et al., 2012).

Since 2012, the Canadian legislation requires that priority allergens and gluten must be declared on the food label products for the protection of allergic consumers and coeliac patients (Canadian Food Inspection Agency, 2018). Gluten is managed as an allergen based on this regulatory standpoint. In addition, as part of a Hazard Analysis Critical Control Point (HACCP) plan or a Global Food Safety Initiative (GFSI) program, preventive measures have to be taken to control allergens by minimising the risks of their presence in food products due to cross-contamination of ingredients.

One of the key steps in controlling cross-contamination is the

cleaning procedure, which requires document of presence or absence of food allergens on surfaces. Manufacturers can use several analytical methods for this purpose but they mostly rely on rapid detection tests, like lateral flow devices or enzyme-linked immunosorbent assays (ELISA), to assess allergen presence and concentration in ingredients, final products, and on surfaces (Jackson et al., 2008). However, the efficiency of capturing these substances from a surface is likely to be multi-factor-dependent (e.g., operator, swab fabric, surface, pre-wet solvent, and allergen types). Besides, the detection of allergens from swabs is likely to depend on their interaction with the selected pieces of soft material, the capacity of the material to release the proteins in the extraction buffer, the food matrices collected during sampling, the solution, the procedure used to extract, and the detection system. To better understand this aspect of preventive control, several studies have been performed to measure the allergen concentrations on surfaces (Ortiz et al., 2018; Röder et al., 2008; Schelgel, Yong, & Foo, 2007; Wang, Young, & Karl, 2010). These studies relied on swabs and ELISA or SDS-PAGE to measure the allergen presence and highlighted some

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issues regarding protocols, recovery and sensitivity.

A research team recommended strategies to prevent the allergen cross-contamination and suggested swabbing with ELISA testing as part of the cleaning process validation and verification (Jackson et al., 2008). Another study presented data on the egg and milk allergen recovery using different swab fabrics, solvents, and surfaces; a testing procedure was proposed based on their results (Galan-Malo et al., 2017). Meanwhile, it is worth noting that control of cleaning procedures remains a “go”/“no-go” step in a preventive plan, but collecting allergen samples with a swab has not been standardised yet.

In this regard, data on the efficiency of swabbing are greatly needed to support decision-making in the industry to prevent and control allergen contamination. Besides, guidelines from Food and Drink Europe recommend proceeding to swab analysis within 24 h following the swab sampling (FoodDrinkEurope, 2013). However, as a result of a transportation delay or delay at the external laboratory, it may be challenging to respect the 24 h timeline. To our knowledge, no prior study presents allergenic protein stability on a swab over time, which would illustrate a situation when the swab is shipped to sub-contractors for analysis and tested later.

The work assessed the performance of different kinds of swab fabrics to capture, preserve, and release milk proteins or gluten in an extraction buffer for analysis. The authors also monitored the effect of time and storage temperature on the allergen stability on swabs under different storage conditions. In addition, efforts were made to develop a single procedure for swabbing both gliadin and milk with the same kind of swabs.

2. Material and methods

2.1. Gliadin and milk reference materials

Pure gliadin was obtained by the Osborne fractionation. The sample was used as previously described (Schalk, Lexhaller, Koehler, & Scherf, 2017) and resuspended in ethanol at 70%. The gliadin concentrations were assessed using an ELISA detection kit (RIDASCREEN®FAST Gliadin; R7002 lot #: 13436; R-Biopharm, Darmstadt, Germany). The MoniQA Association milk allergen reference material (MQA092014) was used for the milk assay. Concentrations were measured using the ELISA detection kit RIDASCREEN®FAST Milk (R6254 lot #: 12517; R-Biopharm). All ELISA kits were run on the ThunderBolt® platform (Gold Standard Diagnostics, Davis, CA, USA).

2.2. Swab materials

Five swab types were assessed. These included a cotton-based (Greiner Bio-One™, Monroe, NC, USA), a rayon-based (BD, Franklin Lakes, NJ, USA), a polyester-based (Copan, Murrieta, CA USA), a Dacron-based (World Bioproduct, Bothell, WA, USA), and a polyurethane-based swab prototype (r-Biopharm Inc., Washington, MO, USA).

2.3. Surface contamination

Experiments with gliadin were performed on a stainless-steel surface. Milk, butter, gliadin-free oat flour, ham slice, turkey slice, corn oil, and potato peels were incorporated into a bag, applied onto the stainless-steel surface, and left to dry. All food items were separately tested for the absence of gliadin prior to these experiments. There was no evidence that the food items would contain microorganisms capable of degrading gluten. The surface was only cleaned with cold water and Kimwipes™. A 1-ml aliquot of the food mixture was put onto a total flora medium Petri dish and stored at 37 °C for 24 h to assess the level of microbial contamination. Square surfaces of 10 × 10 cm in dimension were delimited, and two gliadin spiking solutions of 40.4 and 80.4 ppm were deposited on the surface as spots before letting them dry. For milk,

assays were performed like those designed for gliadin with 10.2 and 19.8 ppm of milk. The composition of the food material to contaminate the surface was almond milk, chocolate powder, strawberries, wheat flour, corn oil, and sugar.

2.4. Sampling procedure

The protein solution spots were left to dry. After 1 h, gliadin was collected with five swabs types on the 10 × 10 cm surfaces. The polyurethane, cotton, and polyester swabs were tested in triplicate at the two gliadin concentrations for days 0, 1, 2, 3, and 7. The Dacron and rayon swabs were tested on all 7 days. All swabs were pre-wet in PBS, and two operators performed the following procedure: swab firmly in a horizontal direction from the left to the right of the square, in a vertical direction up and down the surface, and diagonally (up left towards right down and down left towards up right). Three swabs per concentration were tested immediately (day 0), while the rest (triplicates per concentration per day) were stored at 37 °C. No extraction buffer was added to the sample tube before storage. The negative control was obtained by swabbing (triplicates) the surface with the dried food mixture before adding gliadin. For the milk assay, only two types of swabs (polyurethane and cotton) were applied in triplicate with the two milk spiking dilutions for days 0, 1, 2, 3, and 7, using two storage temperatures: 4 (fridge) and 37 °C.

2.5. Validation at an external laboratory

Validation of both assays on gliadin and milk was undertaken at an external laboratory (Mérieux NutriSciences, Crete, IL, USA) with the polyurethane swabs at three storage temperatures for both milk and gluten proteins; experiment was performed on a stainless-steel surface that was pre-cleaned with soap and ethanol. The experiment performed was the same as described above (see section 2.4), except no food was added on the stainless-steel surface and the ELISA analysis was undertaken by external laboratory resources. The concentrations used were 45 and 51 ppm gliadin, and 1.63 and 5.37 ppm milk.

2.6. Statistical analysis

T-tests (comparison between two means) and one-way ANOVA (comparison between more than two means) were applied to compare triplicate means. A threshold of 0.05 was used to identify significant differences between the swab, the storage temperature, and the storage time. All data were analysed using R software (R Core Team, 2013).

3. Results

3.1. Stability of gliadin on the swabs during storage at 37 °C

Food items tested for gliadin were gluten-free, and no gluten was detected on the stainless-steel surface prior to the addition of the spiking solutions. Colony-forming units (CFU) were observed on the total flora medium, but no further microbial identification was performed.

The protein recoveries calculated on day 0 varied between 23.1% (± 14.1%) for rayon and 41.6% (± 0.7%) for cotton for the 80.4 ppm concentration, and from 25.9% (± 2.5%) for polyurethane to 45.5% (± 5%) with cotton for the 40.4 ppm concentration.

Fig. 1 displays the gliadin concentrations over 7 days. The gliadin concentration (mean of triplicates) was above the limit of quantification (LOQ; 5 ppm) with polyurethane, cotton, and rayon when stored at 37 °C for 7 days.

No statistical difference was observed between gliadin concentrations recorded on days 1, 2, 3, and 7 for the five types of swabs, except for rayon with the 80.4 ppm concentration. Hence, no significant decrease was recorded over time, except for the rayon at 80.4 ppm

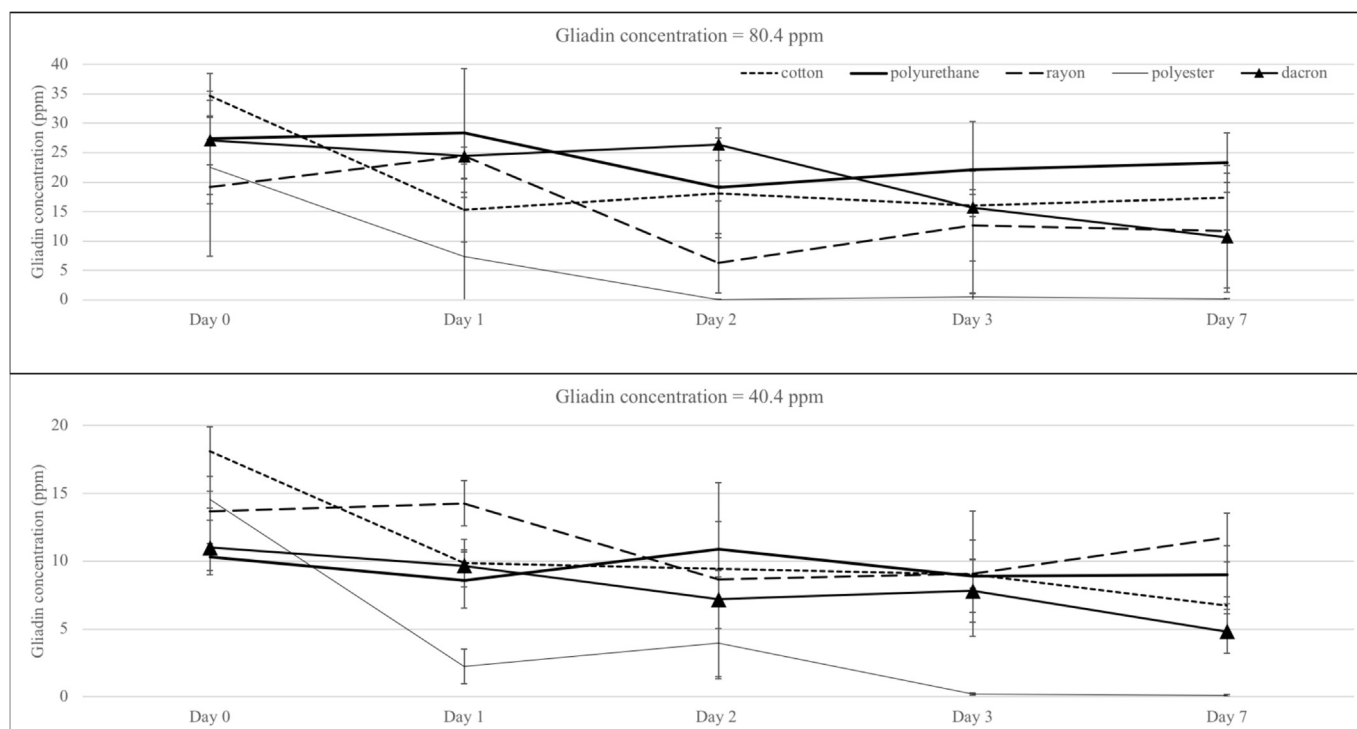


Fig. 1. Recovered gliadin on the swabs during storage at 37 °C with two gliadin spiking concentrations.

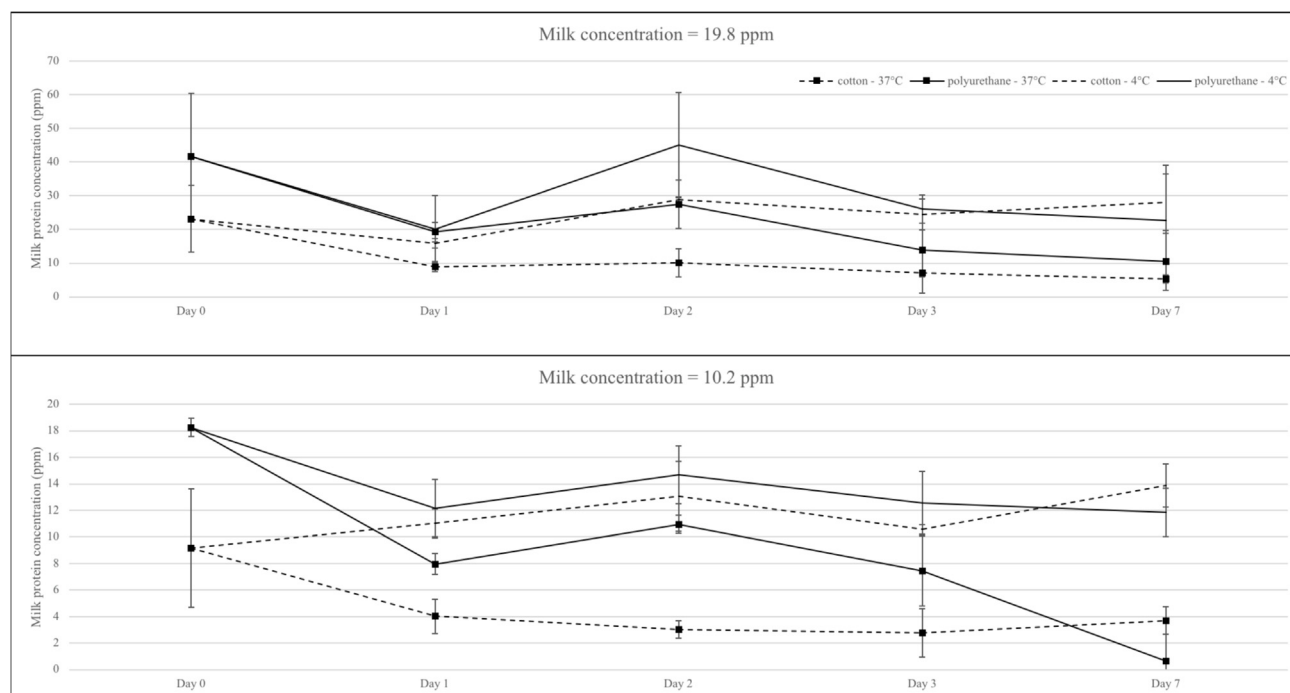


Fig. 2. Recovered milk protein on the swabs during storage at 4 and 37 °C with two milk spiking concentrations. Concentrations are displayed in ppm of milk protein with no dilution factor; 1/20 dilution factor should be applied, as the extraction occurred in 1 ml instead of 20 ml.

($P > 0.05$).

With the polyester swabs, gliadin was detected on day 0, but the measurements on most individual swabs were below both the LOQ and the limit of detection (LOD; 0.5 ppm) on days 1, 2, 3, and 7, using both concentrations.

The gliadin recovered on the Dacron swabs on day 7 using the 40.4 ppm concentration was inferior to the LOQ (4.82 ppm). Besides, for the same swab type, at the 80.4 ppm concentration, at least one

individual swab obtained a value below the LOQ or the LOD within the triplicate group on days 3, 4, 5, 6, and 7 (data not shown).

The same observation was made with the rayon swabs, with at least one individual that resulted in a value below the LOQ or the LOD within the triplicate on days 2, 3, and 7 for either the low or high concentration (data not shown).

The cotton and polyurethane results were constant over time, and no results below the LOQ were recorded with both concentrations.

When comparing all swabs together on days 1 and 7, there was a significant difference between the five swab types with both concentrations. For the 80.4 ppm concentration, on days 1 and 7, the Dacron, rayon, cotton, and polyurethane means were statistically equal and different from the polyester one. For the 40.4 ppm concentration, there was a significant difference between the five swabs on each day.

3.2. Stability of milk on the cotton swabs and the polyurethane swabs during storage at 4 and 37 °C

The cotton swabs and the polyurethane swabs were used for the milk analysis because, for those two swab types, all gliadin concentrations were above the LOQ with no false negatives.

Negative controls confirmed the absence of milk on the stainless-steel surface and in food items used to prepare the mixture. CFU and mould colonies were observed on total flora medium, but no further research was conducted to identify the species. The allergen recoveries at day 0 for the 10.2 and 19.8 ppm concentrations were 12.5% ($\pm 6.91\%$) and 16.3% ($\pm 6.9\%$), respectively, with cotton. For the polyurethane swabs, recoveries were 24.9% ($\pm 0.9\%$) and 29.4% ($\pm 13.2\%$) for the 10.2 and 19.8 ppm concentrations, respectively.

On day 7, regardless of the starting concentrations and the swab type, milk concentration was above the LOQ of 2.5 ppm, if stored at 4 °C. At 37 °C, using the 19.8 ppm concentration, milk allergen was still detected after 3 days when selecting both types of swabs (Fig. 2), but on day 7, one cotton swab and one polyurethane swab had a value below the LOQ. For the 10.2 ppm concentration, at 37 °C, milk concentrations below the LOQ were observed on days 2 and 3 using the cotton swabs, and on day 7 with the polyurethane swabs.

Milk concentration remained constant over time (from day 1–7) at 37 °C, except for the polyurethane swabs for which a statistical difference was recorded from day 3–7 for the 10.2 ppm concentration (one-way ANOVA). Statistical differences were also observed at 4 °C with the two swab types, for the 19.8 ppm concentration, due to the high concentrations recorded on days 1 and 2.

T-Tests were applied to compare the swab types on the same day. At 37 °C, for the concentration 19.8 ppm, there was no significant difference between polyurethane and cotton on days 1, 3, and 7 ($P > 0.05$). For the concentration 10.2 ppm, the authors observed a statistical difference between the swabs on days 1, 2, and 7. When comparing the swabs stored at 4 °C, regardless of the day and concentration, there was no significant difference between both swab types.

Finally, the influence of storage temperature was assessed. For cotton, statistical differences were observed on days 2, 3, and 7 for both concentrations, and on day 1 for the 10.2 ppm concentration. Milk concentration was higher when the cotton swab was stored at 4 °C compared to the 37 °C storage temperature. No statistical difference was observed for polyurethane, except for the 10.2 ppm concentration on day 7 for which the milk concentration was statistically higher for the swabs stored at 4 °C.

3.3. Validation at the external laboratory

3.3.1. Gliadin

The two gliadin spiking solutions were assessed by two different operators. The recoveries for both assays were 23% for operator 1 (51 ppm) and 8.8% for operator 2 (45 ppm). The concentrations of gliadin collected by operator 1 were statistically different from the ones collected by operator 2. The results are displayed in Fig. 3.

For the 51-ppm gliadin concentration, the swabs stored at room temperature and in the fridge remained above the LOQ and were interpreted as positive results for 7 days. There was no significant difference between days 1 and 7 for the swabs stored in the fridge, nor between the swabs tested on day 7 and stored in the fridge and at room temperature. However, for those stored at 37 °C, one swab had a gliadin concentration below the LOQ on day 2. The gliadin concentration fell

below the LOQ and remained as a negative result according to Mérieux interpretation guidelines until the end of the 7 days. For the swabs used to collect the 45-ppm gliadin concentration from the surface, all of them had a gliadin concentration below the LOQ on day 2 and for the rest of the experiment, regardless of the storage temperature.

3.3.2. Milk

The milk concentrations, 1.63 and 5.37 ppm, were tested by the same two operators who analysed for gliadin. The results are reported in Fig. 4. The assay with 5.37 ppm milk showed that milk can still be detected (above the LOQ) and interpreted as a positive result when tested on days 2, 3, 4, and 7, except on day 2 when stored in the fridge. It is worth noting that among all the swabs for the assay with the 5.37 ppm concentration, 8 swabs had a negative result (below LOQ). For this assay, there was no significant difference between days 1 and 7 for the swabs stored in the fridge, although the milk concentrations were higher on days 3 and 4 than on days 2 and 7. For the swabs stored at room temperature and at 37 °C, there was no significant difference between days 2, 3, 4, and 7. Milk concentrations on day 7 were not different if stored in the fridge or room temperature, but there was a difference between the swabs at 4 and 37 °C. Regarding the assay with the 1.63 ppm concentration, the results were below the LOQ, except on day 4 for two swabs (in the triplicate) stored in the fridge. As observed for gliadin, the two operators highlighted a difference in the milk recovery with the swabs: 19% for the 5.37 ppm milk concentration and 35% for the 1.63 ppm milk concentration.

4. Discussion

One way of controlling cross-contamination risks lies in the efficiency of cleaning and preventing the allergen presence on the production site. Even if the cleaning procedures already demonstrated efficiency in removing traces (Stephan et al., 2004), too many gaps in the process persist and should be addressed. Some notable issues include the efficiency of the swabs to capture and release the allergenic proteins and their stability on the pieces of soft material during transportation from a facility to an external laboratory and storage, for example. Due to the limited data about the allergen stability on swabs, the recommendations are to test swabs within 24 h after sampling. Accordingly, the external testing laboratory involved in the study currently processes and tests all swabs within 24 h after receipt.

This work aimed to provide data on milk and gliadin stability on different kinds of swabs over time and under different storage conditions. In addition, one of the objectives was to develop a standard procedure for both gliadin and milk.

Our first observation was the low recoveries obtained with both milk and gliadin. The cotton swabs were the most efficient to capture and release gliadin properly, with 43.5% recovery, but were less efficient with milk proteins (14.4%). Milk protein was better captured and released by the polyurethane swabs, with a recovery of 27.1%, and the gliadin recovery was 29.4% with the same swab type. Gliadin recoveries with Dacron, rayon, and polyester, were around 30% ($\pm 5.1\%$). This result indicates the need to optimise and standardise swabbing procedures to better assess the real concentration of allergens on a surface in the context of a validation procedure and for monitoring.

To study the swab capacity to preserve the allergen protein over time, the authors compared the milk and gliadin concentrations on day 1 with the concentrations of the days that followed. It was shown that for milk and gliadin, there was no statistical difference between days (from 1 to 7) regardless of the swab types, the concentrations and the storage temperature, except for the following:

- rayon with 80.4 ppm gliadin, for which day 1 was different from the three other days, and
- polyurethane with 10.2 ppm milk at 37 °C, for which day 7 was

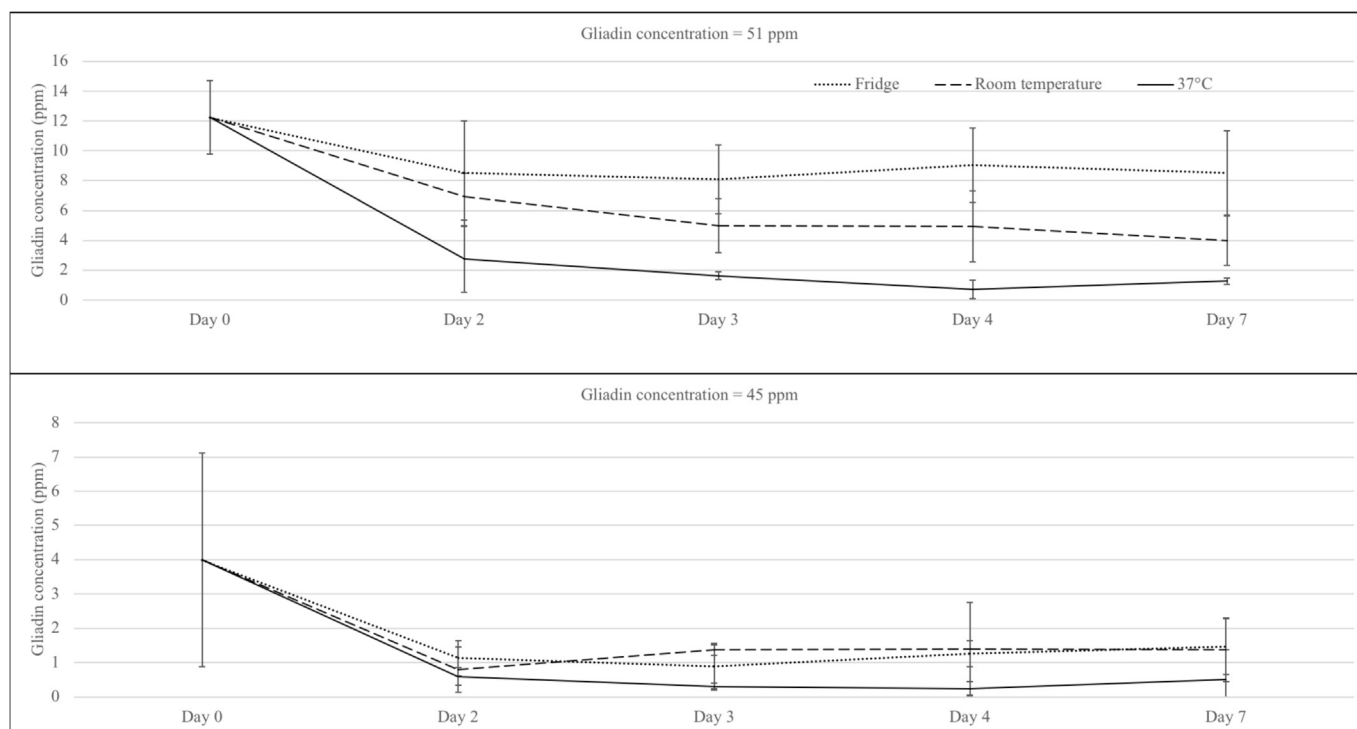


Fig. 3. Recovered gliadin on the polyurethane swabs stored at three different temperatures with two concentrations over time.

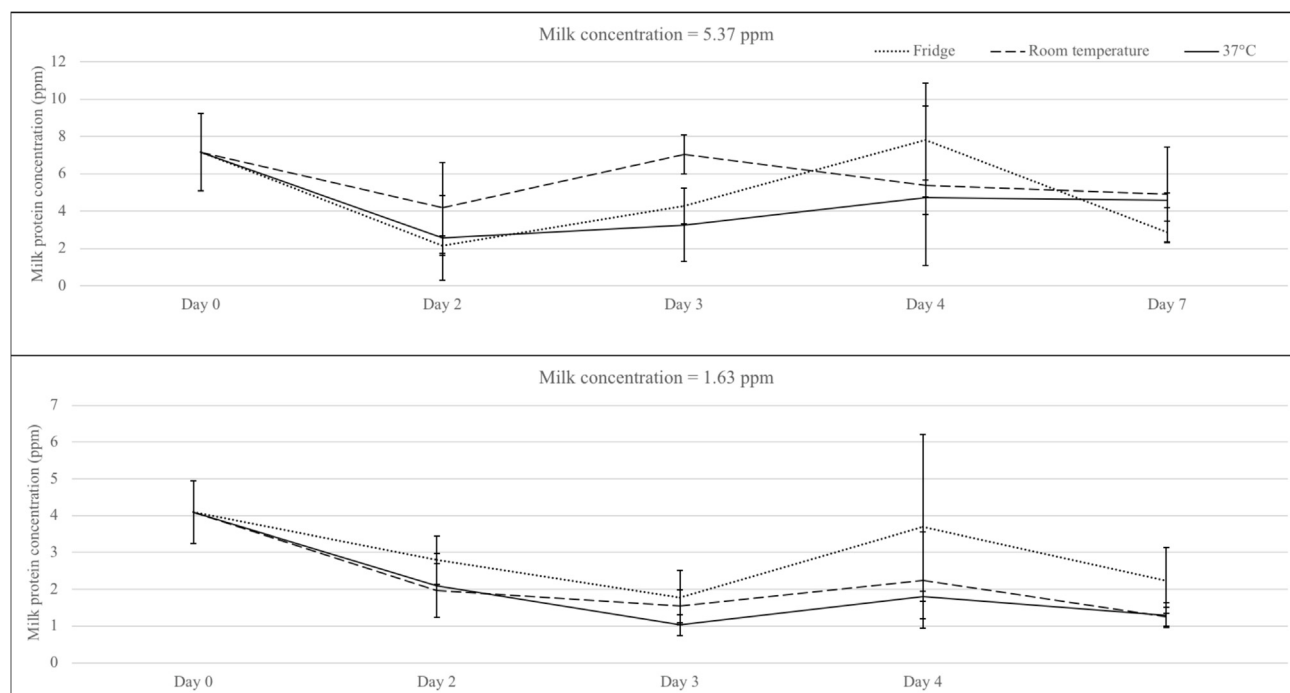


Fig. 4. Recovered milk proteins on the polyurethane swabs stored at three different temperatures with two concentrations over time. Concentrations are displayed in ppm of milk protein with no dilution factor; 1/20 dilution factor should be applied, as the extraction occurred in 1 ml instead of 20 ml.

different from the three other days.

In addition, statistical differences were observed among days for the cotton swabs and the polyurethane swabs at 4 °C with 19.8 ppm milk. However, those differences were due to a high concentration on day 2 for polyurethane, and a low concentration on day 1 for cotton, which did not lead to a significant decrease over time, as observed with the two previous cases. Overall, the milk and gliadin can remain on a swab

for a long time and still be detected after several days. This first observation could initiate a discussion regarding the 24-h testing recommendation, but beforehand, this approach should be confirmed by other studies with the same proteins, and also, with other allergens, like eggs or peanut.

Figs. 1 and 2 show the protein concentrations on day 0. Those data were used to calculate the protein recovery per swab. Overall, day 0 data were statistically equal to that on day 1. The only statistical

difference observed was for cotton and gliadin for both concentrations, for polyester with the 40.4 ppm gliadin concentration, and polyurethane with milk at the 10.2 ppm concentration at 37 °C.

Gliadin was detected with the polyurethane swabs and the cotton swabs for all assays (concentration > LOQ), indicating good stability of gliadin over a long time on the swab types and under poor storage conditions (at 37 °C). With the objective to propose a single procedure for both milk and gliadin, the Dacron and rayon swabs were not applied in the milk experiment after these two types gave inconsistent results with gliadin, as at least one swab within the triplicate group had a value below the LOQ or the LOD, resulting in a false negative. In addition, the polyester piece was also removed from the milk assay because the gliadin concentrations found on this swab type were below the LOQ on the first days of the experiment. Therefore, the cotton swabs and the polyurethane swabs were selected for further analysis. A temperature of 37 °C was chosen to represent the worst-case scenario that might happen during poor transportation/storage conditions, in seasons when this condition could persist for several days in a row. Regarding the microbiological aspect, lactic bacteria are found in numerous food commodities, but those that can digest gluten are less common. However, both the gluten-free oat flour and the potato peels were added to the food mixture for the gliadin study in order to maximise the chances of having bacteria that could degrade the storage protein in grains. The authors have studied milk at both 4 and 37 °C, as it was expected that milk would be degraded by microorganisms and undetectable after a few days at the highest temperature.

Milk stability on swabs was studied over time, with different storage temperatures and with two types of swabs. Overall, from day 1–7, the only difference observed was on day 7, 37 °C, for polyurethane with the 10.2 ppm concentration compared with the previous days. When stored at 4 °C, the cotton swabs and the polyurethane swabs were equivalent to preserve milk proteins. At 37 °C, the statistical analysis indicated that there was no difference between the swabs for the 19.8 ppm concentration (except on day 2), but for the 10.2 ppm concentration, the swabs were not equivalent. Finally, authors compared milk concentrations from the same swab type stored at either 4 or 37 °C and the same day (1, 2, 3, and 7). It was shown that milk concentration is independent of the temperature when collected with the polyurethane swabs, except on day 7 for the 10.2 ppm concentration for which the milk concentration was higher when stored at 4 °C. If the allergen swabbing is made with cotton, the authors found that storing the swabs at 4 °C would be preferable than 37 °C.

The validation performed at Mérieux NutriSciences laboratory supports the conclusions that gliadin and milk proteins can be stable on the swabs presented in this work over time and, consequently, detectable after 24 h following the swabbing. However, gliadin was shown to be less persistent on the polyurethane swabs if stored at 37 °C rather than 4 °C. In addition, 5.37 ppm milk was applied for this experiment and was still detected after storage at room temperature or in the fridge for 7 days. The differences found with gliadin enhances the need for similar studies to be conducted, ideally in a real allergen cleaning validation context. Besides, the assay with 45 ppm gliadin concentration showed that if 4 ppm is collected on the swabs on day 0, testing the swabs later would lead to negative results despite that gliadin was put on the surface. The same observation can be made with milk.

According to the results from both the preliminary study and the validation study, the authors recommend storing the swabs in a cold place to enhance the allergen stability over time. If working with other allergens, like peanut or eggs, a validation procedure should be undertaken to select the right swab type when controlling cleaning activities. Depending on the swab, the target protein of capture and the concentration of a given protein allergen, the recovery and release capacities could greatly differ from one swab to another. In addition, using the same piece, the recovery can differ, depending on the captured protein and its concentration. The pieces of soft material that were tested also had different behaviours. When swabbing with Dacron,

rayon and polyester, the swabs dried quickly compared with the cotton and the polyurethane ones. The high standard deviations observed among the triplicates could be attributed to one or several factors presented above, as well as the operator (if he/she is trained and his/her relative experience), the procedure (the inclination, the angle, the position relative to the covered area) or the ELISA analysis. It was observed that a difference between executions of the protocol by two distinct operators could lead to a variance in the amount of allergen captured. Hence, with the same protocol, a change in the procedure could influence results, enhancing the need for its standardisation. It is suggested here that by doing triplicates, these biases might be reduced. Testing triplicate would decrease this risk even if the conditions of both swabbing and storage are poor. However, food industry operators do not usually execute swabbing in triplicate but, according to our observations, only one swab analysed per area could potentially lead to a false negative.

More research is needed to confirm the conclusions made from this study and, also, to explore the behaviour of other allergens and propose a single standard procedure to swab allergens on a surface. Our data suggest that swabbing both milk and gliadin is feasible with PBS and polyurethane or cotton swabs, but an internal validation should be undertaken. In addition, a standard procedure should describe best practices for swabbing, testing pieces of soft material, and how to relate these results with production in order to support industry in controlling allergen and gluten occurrences in agri-food plants. Food industries and external laboratories could use this study to adapt their protocols and eventually improve the interpretation of allergen swabbing results according to the delay of transportation and treatment by third parties. This same study could be performed with other allergens to enhance the response to questions posed by food industries, test makers, regulators and testing laboratories regarding the allergen swabbing performance. Additionally, the results stemming from those studies could help to support the development of common guidelines for allergen swabbing in food industries and the implementation of training sessions to learn how to swab surfaces to test for allergen presence.

Authors contributions

Virginie Barrere: developed the protocol for milk assay experiments, performed the experiments for milk assay, prepared the experiment design at Mérieux laboratory, prepared the manuscript, submitted the manuscript, handled the reviewers' comments, performed statistics, prepared the new version of the manuscript, re-submitted the manuscript.

Jérémie Theolier: developed the protocol for gliadin assay experiments, performed the experiments for gliadin assay, prepared the experiment design at Mérieux laboratory, prepared the manuscript, analysed statistics, reviewed the manuscript.

Sebastien Lacroix: performed statistics.

Steven Zbylut: Supervised experiments at Mérieux, provided advice on experiment design at Mérieux, provided comments on results, reviewed the manuscript.

Alexcia Valdez: provided advice on experiment design at Mérieux, provided comments on results, reviewed the manuscript.

Nick Collopy: provided advice on experiment design at Mérieux, provided comments on results, reviewed the manuscript.

Brandon Lahey: performed gliadin experiment at Mérieux, provided comments on results, reviewed the manuscript.

Samuel Godefroy: Supervised the experiments at Université Laval, provided comments on results, reviewed the manuscript.

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Declaration of competing interest

The authors have no conflict of interest to declare.

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References

- Canadian Food Inspection Agency. Allergen labelling for industry. Safe food for Canadians regulations. (2018). <https://www.inspection.gc.ca/food/requirements-and-guidance/labelling/industry/eng/1383607266489/1383607344939> Accessed 28.10.19.
- FoodDrinkEurope (2013). Guidance on food allergen management for food manufactures. http://www.fooddrinkurope.eu/uploads/press-releases_documents/temp_file_FINAL_Allergen_A4_web1.pdf, Accessed date: 13 October 2019.
- Galan-Malo, P., Lopez, M., Ortiz, J. C., Perez, M. D., Sanchez, L., Razquin, P., et al. (2017). Detection of egg and milk residues on working surfaces by ELISA and Lateral flow immunoassay tests. *Food Control*, 74, 45–53. <https://doi.org/10.1016/j.foodcont.2016.11.027>.
- Gupta, R. S., Springston, E. E., Warrier, M. R., Smith, B., Kumar, R., Pongracic, J., et al. (2011). The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics*, 128, e9–e17. <https://doi.org/10.1542/peds.2011-0204>.
- Jackson, L. S., Al-Taher, F. M., Moorman, M., DeVries, J. W., Tippet, R., Swanson, K. M., et al. (2008). Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operations. *Journal of Food Protection*, 71, 445–458. <https://doi.org/10.4315/0362-028X-71.2.445>.
- Ortiz, J. C., Galan-Malo, P., Garcia-Galvez, M., Mateos, A., Ortiz-Ramos, M., Razquin, P., et al. (2018). Survey on the occurrence of allergens on food-contact surfaces from school canteen kitchens. *Food Control*, 84, 449–454. <https://doi.org/10.1016/j.foodcont.2017.09.003>.
- R Core Team (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R foundation for Statistical Computing. <http://www.R-project.org/>.
- Röder, M., Ibach, A., Baltruweit, I., Gruyters, H., Janise, A., Suwelack, C., et al. (2008). Pilot plant investigations on cleaning efficiencies to reduce hazelnut cross-contamination in industrial manufacture of cookies. *Journal of Food Protection*, 71, 2263–2271. <https://doi.org/10.4315/0362-028X-71.11.2263>.
- Schalk, K., Lexhaller, B., Koehler, P., & Scherf, K. A. (2017). Isolation and characterization of gluten protein types from wheat, rye, barley and oats for use as reference materials. *PLoS One*, 12, e0172819. <https://doi.org/10.1371/journal.pone.0172819>.
- Schlegel, V. L., Yong, A., & Foo, S. Y. (2007). Development of a direct sampling method for verifying the cleanliness of equipment shared with peanut products. *Food Control*, 18(12), 1494–1500. <https://doi.org/10.1016/j.foodcont.2006.11.008>.
- Soller, L., Ben-Shoshan, M., Harrington, D. W., Fragapane, J., Joseph, L., St Pierre, Y., et al. (2012). Overall prevalence of self-reported food allergy in Canada. *The Journal of Allergy and Clinical Immunology*, 130, 986–988. <https://doi.org/10.1016/j.jaci.2012.06.029>.
- Stephan, O., Weisz, N., Vieths, S., Weiser, T., Rabe, B., & Vatterott, W. (2004). Protein quantification, sandwich ELISA, and real-time PCR used to monitor industrial cleaning procedures for contamination with peanut and celery allergens. *Journal of AOAC International*, 87, 1448–1457.
- Wang, X., Young, O. A., & Karl, D. P. (2010). Evaluation of cleaning procedures for allergen control in a food industry environment. *Journal of Food Science*, 75, T149–T155. <https://doi.org/10.1111/j.1750-3841.2010.01854.x>.