Comparative Evaluation of Analytical Methods for Assessing Allergen Removal in Domestic Laundry

Lisa Schlenker, Dominique Boursillon und Benjamin Eilts

Abstract

This study compares three analytical approaches for quantifying residual allergens on textiles, considering sensitivity, costs and processing time: the Bradford assay, ELISA and qPCR. qPCR shows the highest analytical sensitivity but is limited by inconsistent DNA recovery and does not directly quantify residual allergenic protein. ELISA also offers high sensitivity but is affected by washing-induced structural changes, potentially overestimating allergen removal. In contrast, the Bradford assay detects total protein regardless of conformation and due to its low cost and short processing time, it proves suitable for assessing allergen reduction using a standardised soiling matrix.

Keywords: Allergen removal, allergen detection, domestic laundry, method assessment, textiles

Vergleichende Bewertung analytischer Methoden zur Beurteilung der Allergenentfernung in der Haushaltswäsche

Kurzfassung

Diese Studie vergleicht drei analytische Verfahren zur Quantifizierung von Allergenrückständen auf Textilien hinsichtlich Sensitivität, Kosten und Analysezeit: den Bradford-Assay, ELISA und qPCR. Die qPCR zeigt die höchste Sensitivität, ist jedoch durch inkonsistente DNA-Ausbeute limitiert und quantifiziert verbleibende allergene Proteine nicht direkt. ELISA bietet ebenfalls hohe Sensitivität, reagiert jedoch stark auf waschbedingte Strukturänderungen und kann dadurch die Allergenreduktion überschätzen. Der Bradford-Assay hingegen detektiert Gesamtprotein unabhängig vom Konformationszustand und erweist sich aufgrund der geringen Kosten und kurzen Analysezeit als geeignet zur Bewertung der Allergenreduktion.

Schlagworte: Allergenentfernung, Allergennachweis, Haushaltswäsche, Methodenbewertung, Textilien

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Introduction: Background and scope

Over the past decades, the prevalence of allergies remains constantly high, affecting a large part of the European population, with up to 20 % of patients suffering from severe, debilitating forms (EAACI 2015, Robert Koch Institut 2024). Aeroallergens are ubiquitous in the environment and therefore unavoidable for sensitized individuals. Besides outdoor sources, indoor exposure plays a crucial role, as textiles can act as reservoirs for airborne allergens such as pollen, dust mite particles and animal dander, which can accumulate on fabrics and thereby serve as allergen carriers (ASL & AAFA 2025, Jantunen & Saarinen 2011).

Allergen removal through laundering therefore represents an important aspect of allergy management. However, despite its relevance, no standardized analytical methods currently exist to evaluate the allergen reduction performance of washing machines. Reliable analytical approaches are needed to quantify allergen levels on textiles before and after laundering, enabling evaluation of washing efficiency.

This study aims to compare three analytical approaches for quantifying allergen removal from textiles after laundering: two protein-based assays (Bradford and ELISA) and one DNA-based method (qPCR). Bovine serum albumin (BSA) and soy protein are selected as model allergens to simulate textile contamination. The evaluation of the analytical approaches focuses primarily on their sensitivity and practical applicability, while cost and working time are also considered.

Methodology

Sample preparation

To simulate allergen contamination, cotton fabric carriers are soiled with a protein-mucin-starch matrix containing either BSA or soy protein (from soy milk) and air dried prior to washing. The prepared carriers are stored frozen until use.

Washing procedure

The soiled carriers are added to 5 kg dry base load and washed in a laboratory washing machine (Wascator FOM 71 CLS, Clarus Control, Electrolux) using 28 g IEC 60456 type A base detergent and a pre-programmed wash cycle in accordance with IEC-TS 63429. The 5 kg load consists of 18 towels, 4 pillowcases and 2 bed sheets in accordance with the IEC standard.

Each washing cycle consists of a main wash with 17 L of water for 12 minutes, followed by two rinse cycles (12 L, 3 minutes each). After each rinse, a short extraction step at 500 revolutions per minute (rpm) for 60 seconds is performed. The program concludes with a final extraction at 1100 rpm for 5 minutes. For each washing temperature (20 °C, 30 °, 40 °C or 60 °C), one test run with four carriers is performed.

Protein detection

Bradford

A Bradford assay is performed to quantify the total protein remaining after washing. Carriers are extracted in PBS by ultrasonic treatment. For the assay, 800 µL of sample extract are mixed with 200 µL of 5× Bradford reagent (Serva Electrophoresis GmbH) and incubated for 10 minutes at room temperature before measurement. A standard curve is prepared with BSA, and absorbance is measured at 595 nm.

ELISA

Residual intact soy protein after washing is quantified using a commercial sandwich ELISA kit (InviLisa® Soya ELISA) according to the manufacturer's instructions.

To enhance protein recovery, carriers are sonicated for 5 minutes in the kit-provided extraction buffer prior to extraction. Absorbance is measured at 450 nm. The standard curve is generated using soy flour standards provided with the kit.

DNA detection

DNA detection is performed by duplex real-time qPCR using the InviScreen® Soya Detection Kit (Invitek Diagnostics), which targets soy-specific DNA via a hydrolysis probe–based assay. The InviSorb® Spin Food Kit (Invitek Diagnostics) is used for DNA extraction. Both assays are conducted according to the manufacturer's instructions. A standard curve is prepared by serial dilution of extracted soy-milk DNA of known concentration to enable quantitative analysis.

Determination of reduction factors and detection limits

The allergen reduction is expressed as the logarithmic reduction factor (LR), calculated from the difference between the base 10 logarithms measured before and after washing (Equation 1).

Equation 1: Calculation of the logarithmic reduction factor

$$LR = \log_{10}(C_{Contr.}) - \log_{10}(C_{remain.})$$

The LOD (limit of detection) of the Bradford assay is calculated by the average signal obtained from six unsoiled and untreated carriers. The LOD of the qPCR is determined by analysing serial dilutions of extracted soymilk DNA with known concentrations. The lowest concentration detectable is defined as the LOD. The LOD of the ELISA kit is defined according to the manufacturer's specifications. Based on the LOD of each method, the maximum quantifiable reduction (LR_{max}) is calculated (Equation 2).

Equation 2: Calculation of the maximum quantifiable reduction

$$LR_{max} = \log_{10}(C_{Contr.}) - \log_{10}(C_{LOD.})$$

Values below the LOD are reported as $\geq LR_{max}$. For each washing condition, four carriers are analysed. Due to the small sample size, no statistical testing is applied. Instead, variability is represented by standard deviations.

Results

Sample stability

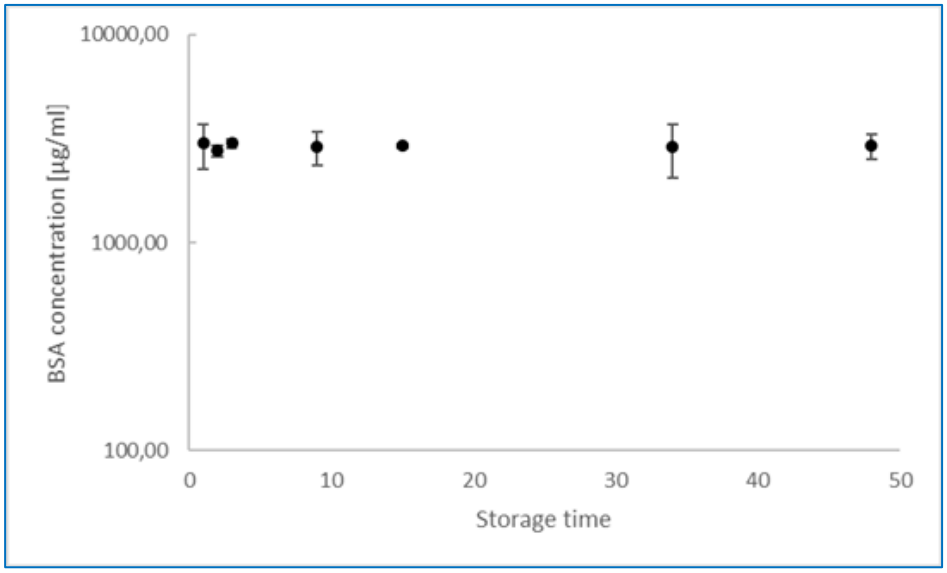

Figure 1: BSA concentration on textile carriers measured over 48 days by the Bradford assay (mean ± SD, n=3 per time point)

Figure 1 shows that BSA concentrations on frozen textile carriers remain stable over 48 days (mean: 2866.4 ± 460.9 µg/mL). No decrease in concentration is observed, only variations within measurement days.

Impact of detergent and temperature on protein removal

Washing with detergent results in a consistently higher reduction of BSA at all tested temperatures compared to washing without detergent (Figure 2).

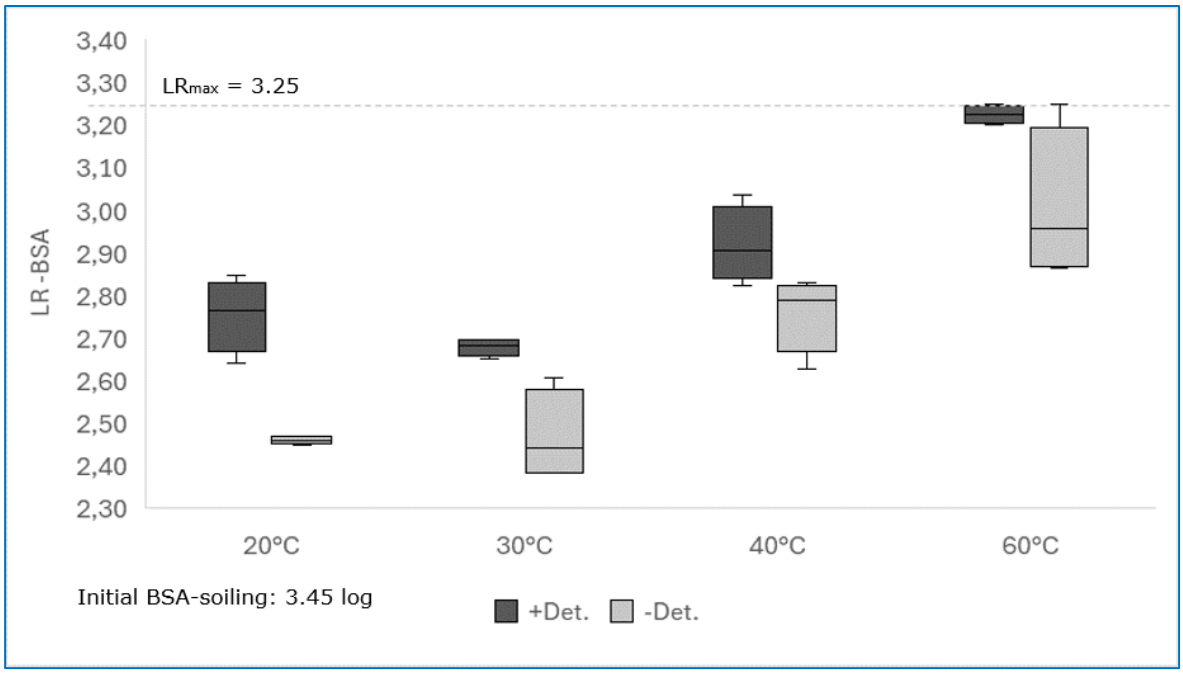

Figure 2: LR of BSA concentration after washing with (+Det.) and without (- Det.) detergent (n=4); initial BSA-soiling: 3.45 log; LR_{max}= 3.25 LR indicated by dashed grey line; values below the LOD are displayed as 3.25 LR

Within both washing conditions, no noticeable difference in protein reduction is observed between 20 °C and 30 °C. At higher temperatures (40 °C and 60 °C) a clear increase in reduction occurs for both washing procedures. At 60 °C without detergent, BSA removal shows considerable variation across replicates (mean LR: 3.00 ± 0.18). In both detergent and non-detergent 60 °C test runs, one out of four replicates exceeds the LR_{max} of 3.25 LR and is therefore reported as ≥3.25 LR.

Comparison of BSA and soy protein removal

At 30 °C, both proteins show lower removal compared to higher washing temperatures. Despite the higher initial protein load of BSA (3.45 log) than soy protein (2.28 log), its removal is consistently higher under all washing conditions (Figure 3). BSA removal increases markedly with temperature and reaches LR_{max} at 60 °C, whereas soy protein removal consistently remains below LR_{max} and shows only minor temperature dependence.

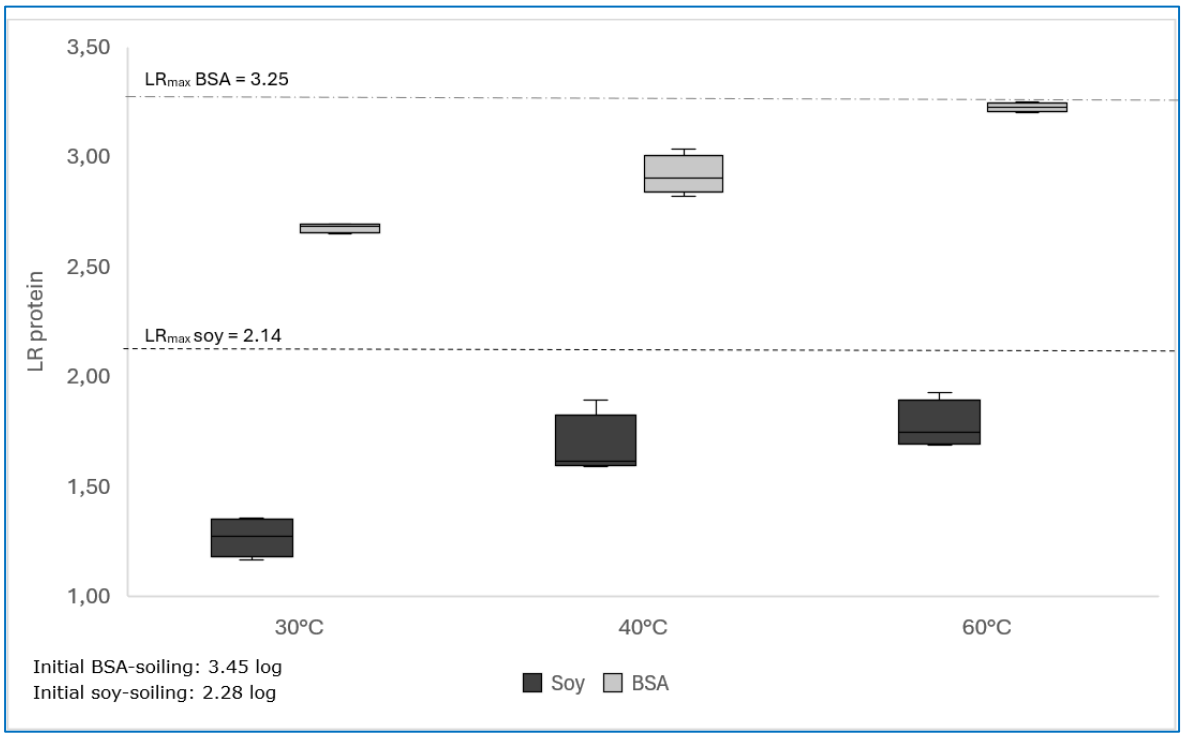

Figure 3: Comparison of BSA and soy protein removal at 30 °C, 40 °C and 60 °C determined by the Bradford assay (n=4); initial soiling: 3.45 log (BSA) and 2.28 log (soy); LR_{max}: (BSA 3.25 LR and soy:2.14 LR) indicated by dashed grey line

Detection of residual soy protein: Bradford and ELISA

To ensure comparability, the carriers are washed in a single test run for each temperature and subsequently analysed by the Bradford assay and the ELISA.

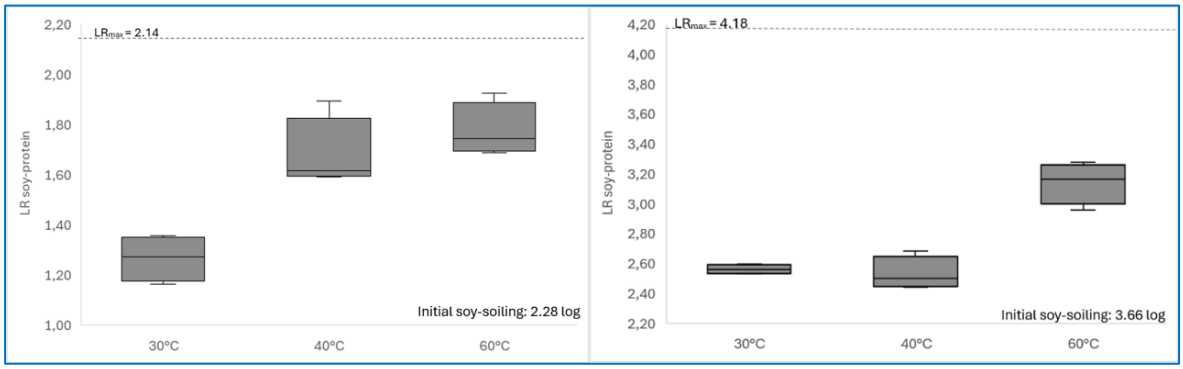

Figure 4: Comparison of soy protein removal detected by the Bradford assay (left) and ELISA (right); initial detected soiling: 2.28 log (Bradford) and 3.66 log (ELISA); LR_{max} (Bradford:2.14 LR and soy: 4.18 LR) indicated by dashed grey line

Despite identical soiling levels (Figure 4), ELISA detects higher initial protein concentrations on the unwashed controls than the Bradford assay. Based on their respective LODs, the Bradford assay can quantify up to 2.14 LR, while ELISA extends the measurable range to 4.18 LR. Bradford results show a moderate increase in protein removal between 30 °C and 40 °C and only a minor change between 40 °C and 60 °C. In contrast, ELISA indicates a stronger temperature effect, with higher apparent protein reduction at 60 °C.

Detection of residual soy DNA: qPCR

The LOD of the qPCR, determined by a dilution series, is 2.77 pg/µL. DNA extraction from carriers contaminated with soy milk already yields variable results (0.52 ± 0.13 ng/µL). For the more complex soy milk–mucin–starch matrix, unwashed control carriers showed a broad concentration range (0.10–0.50 ng/µL).

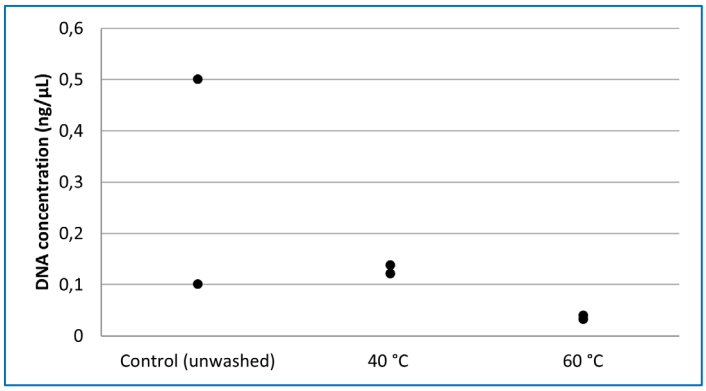

Figure 5: Soy DNA concentrations detected by qPCR on carriers washed at 40 °C and 60 °C compared to unwashed controls (n=2 per condition)

Increasing washing temperature reduces the detectable soy DNA on the carriers (Figure 5). At 40 °C, the mean residual concentration is 0.13 ng/µL, while at 60 °C it decreases to 0.04 ng/µL, corresponding to reductions of 0.36 LR and 0.91 LR, respectively.

Discussion

Sample stability

Substantial variation between replicates was already observed on day 1, indicating inconsistent recovery of BSA from the textile carriers. This suggests that differences in BSA concentration are primarily due to variable recovery from the textile carrier rather than degradation. As all subsequent measurements across storage days remain within this initial variability range, no time-dependent loss of BSA is evident during storage.

Impact of detergent and temperature on protein removal

Comparing BSA removal with and without detergent clearly demonstrates that the use of detergent substantially improves removal efficiency across all tested temperatures. This aligns with the established concept that detergents enhance protein solubilization and facilitate the detachment of protein residues from textiles (Smulders et al. 2002: 11).

The IEC base powder contains nonionic and anionic surfactants, which lower surface and interfacial tension through adsorption at interfaces. Proteins form complexes with anionic surfactants due to polar interactions with charged residues, leading to denaturation and thereby increased solubility. Since dried proteinaceous stains are particularly resistant to removal, enzymatic additives such as proteases enhance cleaning efficiency by hydrolysing peptide bonds in these proteins (Smulders et al. 2002: 204–205). At lower temperatures, removal efficiency shows little difference, whereas a clear improvement is only apparent at higher ones, indicating that washing efficiency is strongly influenced by temperature. This effect can be explained by improved detergent activity, increased protein solubility and reduced solution viscosity at elevated temperatures, which facilitate protein detachment from the carrier material (Smulders et al. 2002: 11).

Since lower temperatures showed little effect on protein removal and high variability was observed at 60 °C without detergent, subsequent experiments with soy-based carriers were conducted only with detergent at 30 °C, 40 °C and 60 °C.

Comparison of BSA and soy protein removal

The comparison between BSA and soy protein reveals considerable differences in washing behaviour. Despite the higher initial load of BSA, its removal is more efficient at all tested temperatures. This can be attributed to molecular properties: BSA is a water-soluble, globular protein with a molecular weight of approximately 66.5 kDa, which may explain its consistently higher removal efficiency (Kajal & Pathania 2021).

In contrast, soy protein consists of heterogeneous storage proteins such as glycinin and β-conglycinin, with denaturation temperatures ranging from 65 °C to 95 °C (Damodaran 2017: 235–351, Liu et al. 2004:815–822) These structural characteristics contribute to its lower removal efficiency and weaker temperature dependence.

These results show that different proteins including potential allergens can exhibit markedly different removal behaviour under identical washing conditions.

Performance comparison of Bradford and ELISA

ELISA detects higher initial protein levels and larger logarithmic reductions than the Bradford assay. These differences reflect their underlying detection principles: Bradford quantifies total protein via dye binding to exposed amino acid residues and is largely unaffected by conformational changes, whereas ELISA relies on epitope recognition and is therefore more sensitive to structural alterations (Fischer 2022: 33–44, Gruber 2023: 21–52, Key 2023: 53–71).

Because heat and detergent can disrupt protein structure, ELISA may overestimate protein removal at higher temperatures. Reduced antibody binding would appear as increased reduction even when unfolded proteins remain present on the textile. This interpretation is supported by the Bradford assay, which is less affected by conformational changes and therefore reflects the remaining protein more reliably under these conditions. It measures total protein, including partially unfolded molecules, since Coomassie dye binding relies on accessible cationic and hydrophobic amino acid side chains rather than on the protein's native tertiary structure (Fischer 2022: 33–44).

Washing with detergent and elevated temperature leads to loss of tertiary structure, resulting in disruption of antibody-recognized epitopes. Although previous studies suggest that allergenic activity is reduced under such conditions, complete loss of allergenicity cannot be assumed (Davis & Williams 1998: 102–105).

Detection of residual soy DNA: qPCR

The qPCR enables sensitive detection of soy DNA even when proteins are structurally altered or degraded by washing. All measured concentrations remain above the assay's LOD, indicating that assay sensitivity is not limiting. However, the method is mainly constrained by inconsistent DNA extraction yields, which introduce substantial variability already in the unwashed control carriers.

In addition, qPCR as a standalone method is limited in its ability to reflect the actual allergen load on textiles, as DNA may remain detectable even when allergenic proteins no longer retain their native conformation. Therefore, further investigations are required to validate whether quantified allergen DNA concentrations correlate with the presence of allergenic protein.

Evaluation of assay performance

Currently, no threshold values exist for allergen residues on textiles, making it difficult to assess the potential risk of residual proteins. In contrast, food allergens are regulated through health-based reference doses such as those defined in the VITAL framework (Allergen Bureau 2024). Because these values are derived from oral exposure and cannot be directly applied to allergen residues on fabrics, this highlights the need for textile-specific threshold values.

In this study, the assessment is therefore limited to evaluating the analytical performance of the applied methods in quantifying allergen reduction, without determining whether the detected residual levels are clinically relevant.

Although no international standards for allergen removal in domestic laundering exist, Allergy Standards Limited (ASL) provides a relevant reference point. Through the "Asthma & Allergy Friendly®" certification, ASL evaluates washing machines based on their ability to reduce allergen levels. Certified machines must demonstrate at least a 95 % reduction in allergen content, maintain temperatures of ≥55 °C for 15 minutes and effectively inactivate dust mites (Allergy Standards Limited n.d., ASL and AAFA 2025).

The present study compares three analytical approaches in terms of their sensitivity and ability to quantify allergen removal from textiles, as well as their hands-on time and cost. Cost estimates are based on single-sample processing following the method used in this study.

The qPCR shows the highest analytical sensitivity within the compared methods. However, its overall performance is predominantly limited by inconsistent DNA extraction yields rather than by the sensitivity of the qPCR itself. This reduces the reliability of the recovered concentrations, particularly at higher DNA loads. It also requires the longest processing time (≈4 h) and is the most expensive method (≈185 € per sample), largely due to the need for separate extraction and qPCR kits. Furthermore, qPCR does not directly quantify residual allergenic protein and therefore cannot provide a direct indication of allergenic potential. Given these limitations, qPCR should be used only as a complementary method alongside protein-based assays when evaluating allergen removal.

The ELISA assay provides high analytical sensitivity but is susceptible to interference from conformational changes of the target protein. Structural alterations induced by washing temperature or detergent can reduce antigen–antibody binding, which may lead to an overestimation of actual protein removal. Consequently, the higher log reductions measured by ELISA may not necessarily reflect true protein elimination, since denatured proteins can remain present despite losing their native structure. Although previous studies suggest that allergenic activity is reduced under such conditions, complete loss of allergenicity cannot be assumed (Davis & Williams 1998: 102–105).

Compared with the Bradford assay, ELISA requires a longer processing time (≈2 h) and results in approximately three times higher costs per sample (≈90 €). The Bradford assay detects proteins regardless of their structural state and therefore reflects the actual amount of protein removed. Due to its low material costs (≈30 €) and short hands-on time (≈1 h), it is well suited for assessing allergen-reduction performance using a standardized soiling matrix. The Bradford assay detects up to 2.14 LR of soy-protein soiling (≈99.3 % removal) in this experimental setup.

This exceeds the >95 % reduction required by ASL certification and demonstrates that the assay is sensitive enough to detect allergen reduction beyond this threshold. These findings highlight the need for standardized analytical protocols for allergen-reduction testing and suggest that the Bradford assay may provide a robust and practical approach for routine evaluation.

Conclusion

This study demonstrates that allergen removal from textiles depends strongly on both the protein type and the washing conditions. While BSA is efficiently removed, soy proteins show greater resistance, particularly at lower temperatures. The comparison of analytical methods revealed clear differences in their suitability: the Bradford assay provides robust and temperature-independent quantification of residual total protein, whereas ELISA is highly sensitive but strongly affected by conformational changes of the target protein. qPCR offers the highest analytical sensitivity but is primarily limited by variable DNA extraction yields and does not directly quantify residual allergenic protein and therefore cannot provide a direct indication of allergenic potential.

Overall, detergent use and higher washing temperatures improved allergen reduction, but the effectiveness and detectability varied depending on the analytical method. Establishing textile-specific threshold values would be essential for assessing whether the remaining residues represent a clinically relevant risk.

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Nach Angaben der Korrespondenzautorin besteht kein Interessenkonflikt. Der vorliegende Beitrag beruht auf der Bachelorarbeit der Autorin Lisa Schlenker mit dem Titel „Evaluation of detection methods for allergen removal in domestic laundry” (Erstgutachter: Prof. Dr. Benjamin Eilts).

Zitation

Schlenker L, Boursillon D, Eilts B (2026): Comparative Evaluation of Analytical Methods for Assessing Allergen Removal in Domestic Laundry. Hauswirtschaft und Wissenschaft 74 (ISSN online 2626-0913) doi: 10.23782/HUW_15_2025