

Airborne exposure to wheat allergens: measurement by human immunoglobulin G4 and rabbit immunoglobulin G immunoassays

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Summary

Background: Exposure to airborne wheat allergens in the bakery trade is associated with a high risk of occupational allergy and asthma. Control and reduction of allergen exposure require relatively simple but reliable monitoring techniques. We developed new rabbit IgG-based enzyme immunoassays (EIA) for wheat allergens, which might be a convenient alternative for the thus far used human IgG4 inhibition assay.

Methods: The reactivity and specificity of rabbit antibodies were assessed by EIA and immunoblotting, and compared with those of IgE from wheat-sensitized bakers, and with the antibodies used in the IgG4 inhibition EIA. An IgG inhibition and a sandwich EIA were developed for analysis of airborne dust samples.

Results: Human IgG4 and rabbit IgG inhibition EIAs had comparable sensitivities, with limits of detection (LOD) between 18 and 88 ng/mL, while the sandwich EIA was much more sensitive (LOD < 0.2 ng/mL). Human IgG4 and rabbit IgG reacted in immunoblotting with most of the IgE-binding wheat proteins, although with quantitative differences. All three assays showed a strong reaction with wheat proteins, and some cross-reactivity with rye and barley, but were further highly specific for cereal flour proteins. Concentrations measured with the three EIAs in 432 airborne dust samples were highly correlated ($r > 0.95$) and their absolute values showed less than 10–20% differences.

Conclusion: The rabbit IgG EIAs are valid substitutes for the human IgG4 inhibition EIA, with important practical advantages. The inhibition EIA is recommended for routine wheat allergen measurements. The sandwich EIA may be used to measure low allergen levels, as in short task-related exposure measurements or in subfractions of airborne dust samples.

Keywords baker's asthma, enzyme immunoassay, exposure assessment, human IgG4, rabbit IgG, wheat flour allergen

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Introduction

Exposure to airborne wheat allergens is a major cause of occupational rhinitis and asthma in bakers and flour mill workers [1–3]. Simple, reliable techniques to measure these allergens are indispensable tools to control allergen exposure levels and reduce the incidence of occupational respiratory disorders. Different measurement methods have been used thus far: a human IgE inhibition RAST [4], a rabbit IgG inhibition radioimmunoassay (RIA) [5] and a human IgG4 inhibition enzyme immunoassay (EIA) [6]. The latter assay has been applied in epidemiologic studies in which dose–response relations were demon-

strated between the levels of allergen exposure and the risk of wheat allergy [7–9]. All assays measure a wide range of water/salt-soluble wheat proteins (albumins and globulins), which are considered to be the most relevant IgE-inducing allergens [10–12]. IgE reactions with water/salt insoluble wheat protein fractions like gliadins and glutenins [13] have also been reported in some studies, but practically only in sera that also reacted at least moderately with the soluble proteins [14, 15]. A monoclonal antibody-based assay has been described, which measures a single wheat flour allergenic protein with MW of 13 kDa and α -amylase inhibitory activity [16].

However, IgE-responses in bakers show a great diversity in the number and the type of recognized wheat proteins [17], and it is questionable whether an assay measuring only one single protein would give a sufficiently valid estimate of the real allergen exposure levels. Instead, less specific assays measuring a broad spectrum of most or all potentially allergenic wheat proteins may be preferred.

No comparative studies for wheat allergen exposure assessment methods have as yet been reported. Studies comparing assays for other allergens – like fungal α -amylase [18] or rodent urinary proteins [19–21] – have shown that, in spite of a usually moderate to high level of correlation between the measured allergen levels, reported values can differ systematically depending on the type of immunoassay and the filter elution method, and the same might be true for airborne wheat allergens.

An obvious disadvantage of assays using human IgE or IgG4 antibodies is the limited availability of suitable sera and the occupational health risks associated with the handling of human blood samples. This is particularly true for IgE inhibition assays, where low absolute titres require the use of practically undiluted sera. IgG4 inhibition assays can be performed with much more diluted sera, but remain dependent on the use of human blood samples with its obvious disadvantages, especially for non-clinical laboratories. Furthermore, tests of different laboratories using their own serum pools may be subject to inter-laboratory and possibly inter-batch differences in allergen recognition profiles.

We have therefore explored the use of polyclonal rabbit antibodies for the measurement of airborne wheat allergen, as an alternative for the human IgG4 inhibition EIA method. Rabbit IgG inhibition and sandwich EIAs were developed and compared with the human IgG4 inhibition EIA. This work was part of the European project MOCALEX (Measurement of Occupational Allergen Exposure), which aimed at evaluation of existing and development of new techniques for the measurement of important occupational allergens, like wheat and soy allergens, enzymes in the food and feed industry and rodent allergens.

Materials and methods

Antigen preparations

Standard wheat antigen preparation was prepared from a batch of wheat flour from an industrial bakery. The flour was extracted as a 10% (w/v) suspension in phosphate-buffered saline (0.01 M phosphate, pH 7.4) by repeated vortexing and sonication, followed by centrifugation (15 min; 5 000 g) [6]. After dialysis, protein was measured with the modified Lowry assay [22, 23] using bovine serum albumin (BSA) as the standard. The extract was stored at -20°C in 30 μL portions for single use as a calibration standard in the EIAs.

Various single (non-blended) commonly used wheat flours ($n = 11$), wheat malt flour and flours of other cereals (rye, barley, maize) were obtained from a flour-producing company. As for the wheat standard, 10% (w/v) suspensions were extracted in phosphate-buffered saline (PBS) by vortexing and sonication and, after centrifugation and dialysis, the supernatants were aliquoted and stored at -20°C . Allergen extracts of barley, oats and rye for skin prick tests (SPT) were also included in the analyses, as well as a series of non-cereal allergen extracts – English rye grass pollen, Timothy grass pollen, yeast, fungi (*Aspergillus niger*, *Aspergillus fumigatus*), potato, latex and milk proteins (all from ALK Benelux, Houten, the Netherlands) and a solution of the 'bakery enzyme' fungal α -amylase (Fungamyl, NOVO Nordisk, Bagsvaerd, Denmark) [24].

Additionally, two water/salt-insoluble wheat protein fractions (gliadin and glutenin) were extracted from commercial wheat flour with ethanol and SDS/ dithiothreitol (DTT), as described by Mittag et al. [25] The reactivity of these fractions was tested by chessboard titration of human IgG4 anti-wheat and rabbit IgG anti-wheat antibodies in microwells coated with albumin/globulin, gliadin and glutenin, at concentrations ranging from 2.5 to 40 $\mu\text{g}/\text{mL}$. Their inhibitory capacity was further tested in the human IgG4 inhibition and rabbit IgG inhibition EIAs for wheat allergens, with the standard water/salt-soluble wheat allergen preparation as the calibration standard.

Human immunoglobulin G4 serum pool

A pool was made of 11 bakers' sera showing strong IgG4 reactions with the standard wheat extract ($\text{OD}_{492} > 1.0$ at 1/600 serum dilution), and stored in 50 μL portions at -20°C . Its reactivity in inhibition EIA and immunoblotting experiments was shown to be very similar to that of the previously used human IgG4 anti-wheat serum pool, made of 59 bakers' sera [6], and results of airborne dust extracts tested in IgG4 inhibition EIAs with the new and the old pool correlated with r values > 0.95 (data not shown).

Human immunoglobulin E serum pool

The sera of eight bakers with work-related wheat flour allergy were mixed to a pool with a high wheat-specific IgE antibody concentration of 54.7 kU/L [26].

Rabbit anti-wheat antibodies

Anti-wheat antiserum was produced in a New Zealand White rabbit by immunization with wheat flour extract (0.5 mg protein) in complete Freund's adjuvant and monthly boosting with the same antigen in incomplete Freund's adjuvant. The immunoglobulin fraction was

isolated by ammonium sulphate precipitation from a mix of strongly positive sera from four bleedings ($OD_{492} > 1.0$ at 1/500 000). Heat-inactivated (30 min, 56 °C) serum was cooled down on ice, and solid ammonium sulphate (0.2 g/mL serum) was slowly added while stirring. Stirring was continued for 2 h, after which the mixture was centrifuged (8 000 g, 20 min). The precipitate was resuspended in PBS, extensively dialysed, filtered through 0.22 µm filters and stored in 50 µL portions at -20 °C. Part of these antibodies were biotinylated [24], dialysed and stored at 4 °C.

Human immunoglobulin G4 inhibition enzyme immunoassay

The human IgG4 inhibition EIA procedure was essentially as previously described [6]. Microtitre plates (Microton, Greiner - Bio One, Alphen aan den Rijn, the Netherlands), coated overnight at 4 °C with 5 µg/mL (200 µL/well) of the wheat allergen standard, were washed with PBS with 0.05% Tween-20 (PBT), and blocked for 60 min at 37 °C with PBT-0.2% gelatin (PBTG), which was also used as a diluent in subsequent steps. Test samples diluted 1/5, 1/10 and 1/20 (or higher, in second tests of strongly positive samples) and wheat standard (six serial dilutions from 1 µg/mL to 31 ng/mL) were added at 0.1 mL/well, after which 0.1 mL of the human IgG4 anti-wheat serum pool diluted 1/1200 was added. On each plate, there were three wells of the 0% inhibition control (PBTG plus the diluted serum pool) and three blanks. After incubation (120 min, 37 °C), IgG4 binding was measured by 1 h incubation with mouse anti-human IgG4-horseradish peroxidase (HRP)(1/1000; Sanquin, Amsterdam, The Netherlands), and finally *o*-phenylenediamine (OPD) (Sigma-Aldrich, St Louis, MO, USA) with 0.015% H₂O₂. The reaction was stopped with 50 µL of 2 M HCl, and the OD_{492} was read. Assay results were calculated from the calibration line made by semi-log curve fitting (Softmax, Molecular Devices Corporation; Menlo Park, CA, USA). Lower and upper limits of detection (LOD) of the assay were defined as the standard concentrations, giving 15% and 85% inhibition, respectively.

Rabbit immunoglobulin G inhibition enzyme immunoassay

The rabbit IgG inhibition EIA was performed exactly as the human IgG4 inhibition EIA, except that rabbit anti-wheat serum was used as the primary antibody (at 1/150 000 dilution), and swine anti-rabbit IgG-HRP (1/2000; DakoCytomation, Glostrup, Denmark) as the detection antibody.

Rabbit immunoglobulin G sandwich enzyme immunoassay

Microtitre plates were coated overnight with rabbit anti-wheat IgG at 5 µg/mL in PBS (0.1 mL/well), and blocked

with PBTG. Wheat antigen standard was added at eight serial dilutions (0.16–20 ng/mL), and test samples at 1/100, 1/300 and 1/900 (0.1 mL/well). After incubation for 60 min, captured wheat proteins were quantified by incubation for 60 min with biotinylated rabbit anti-wheat antibodies (1/250, 0.1 mL/well), followed by 60 min incubation with 0.1 mL of 1/2000 diluted avidin-alkaline phosphatase (AP; Calbiochem, Darmstadt, Germany) and finally 60 min with 0.1 mL of 1 mg/mL paranitrophenylphosphate (Sigma-Aldrich) in 10 mM diethanolamine buffer containing 2 mM MgCl₂ (pH 10.6). The reaction was stopped with 50 µL of 2 M NaOH, and the OD was read at 405 nm. Assay results were calculated with the 4-parameter curve fitting option of the Softmax program. Upper and lower LOD were defined as the standard concentrations, giving OD_{405} values of 3.0 and 0.05 above the mean OD of the assay blanks (PBTG instead of sample or standard), respectively. The lower LOD was chosen as a value that was never exceeded during testing of any diluents or extraction media. The higher cut-off OD_{405} -value of 3.0 was chosen because the dose-response curve tended to level off above that value, thus making reading at that part of the curve inaccurate.

Immunoblotting

Flour extracts and the wheat standard preparation (10 µg protein per lane) were separated by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes (8.3 × 7.3 cm, 0.2 µm pore size), using the Xcell SureLock™ Mini-Cell System (Invitrogen, Carlsbad, CA, USA). After blocking with 1% BSA and 1% polyvinylpyrrolidone 40 in Tris-buffered saline (TBS) [17], 10–15 mL of the human IgG4 anti-wheat serum pool (1/25), the human IgE anti-wheat serum pool (1/20) or rabbit anti-wheat antiserum (1/30 000) in TBS-Tween with 2% BSA were added and incubated for 90 min (IgG4 and IgG) or overnight (IgE) in Petri dishes, with gentle agitation. Human IgG4 binding was detected by incubation (90 min) with 15 mL of mouse anti-human IgG4 (1/1000; Sanquin), 90 min with 15 mL of AP-conjugated goat anti-mouse IgG (H+L) (1/2000; Zymed, Carlsbad, CA, USA) and finally with 15 mL of AP substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP; Sigma-Aldrich) for 5–6 min. Rabbit IgG was visualized by 90 min incubation with 15 mL of AP-conjugated goat anti-rabbit IgG (1/30 000; Sigma-Aldrich), followed by BCIP (5–6 min), and human IgE by 180 min incubation with 15 mL of AP-conjugated goat anti-human IgE (1/1000; Sigma), and 15 mL of BCIP for 10 min.

Airborne dust samples

Airborne wheat flour dust was sampled in bakeries and flour mills in the Netherlands, Germany, Spain and United Kingdom, using a parallel sampling device [27], modified

for simultaneous collection of 10 airborne samples. The sampling time varied from 30 min to 6 h to obtain a range of dust and allergen loads on the filters. The same samples were used to investigate the effects of different extraction procedures on wheat allergen yields (J. Bogdanovic et al., submitted). Briefly, filters were eluted for 60 min by applying one of the investigated extraction procedures in which four parameters varied: (1) elution medium: PBS, PBS+0.05% Tween-20, or PBS+0.5% Tween-20; (2) shaking method: agitation on a high-frequency shaking platform or in an end-over-end-rotator, or intermittent vortexing at 0, 30 and 60 min intervals; (3) type of extraction vial: glass, polystyrene or low protein binding-polyethylene tube; and (4) centrifugation speed: 0 g, 1000 g or 3000 g. Filter eluates were stored at -20°C until analyses.

Results

Specificity of the assays

Dose-response curves for the wheat antigen standard and potentially cross-reacting extracts are presented in Fig. 1. The inhibition assays had comparable sensitivities, with an LOD (for undiluted solution) of 18–52 ng/mL (human IgG4) and 27–88 ng/mL (rabbit IgG), whereas the LOD of the sandwich EIA was much lower, ranging from 31 to 188 pg/mL. Accordingly, calibration curves in the inhibition EIAs ranged from approximately 0.02 to 5 $\mu\text{g/mL}$, and from 0.2 to 20 ng/mL in the sandwich EIA, thus in the latter at > 100-fold lower concentrations.

The three EIAs showed a strong reactivity with wheat flour extracts, and a substantial cross-reactivity with some batches of flour extracts of other Triticeae species (barley and rye). Rye flour obtained from a flour company (rye 1) showed the strongest cross-reactivity. Compared with the wheat antigen standard, rye extract had three to four times lower potency in the inhibition EIAs, and six to seven times lower in the sandwich EIA. The rye flour SPT preparation (rye 2), on the other hand, showed much less cross-reactivity (400–1300 lower potency compared with the wheat standard) in all three assays. Barley SPT allergen (barley 2) was approximately 30 times less potent, while an extract prepared from barley flour (barley 1) was much less reactive (600–1200 times) than the wheat standard. Extracts of oat and maize flours showed much weaker cross-reactivity (10 000 times lower reactivity) with wheat, and essentially no reactivity was found for grass pollen and potato extracts, fungal amylase, *A. niger*, yeast and other cereal-unrelated proteins.

Reactivity of different wheat flour extracts

EIA analyses of 12 wheat flour extracts showed that the antigenicity of wheat preparations varied within a narrow range (shown for six extracts in Fig. 2). The allergen/

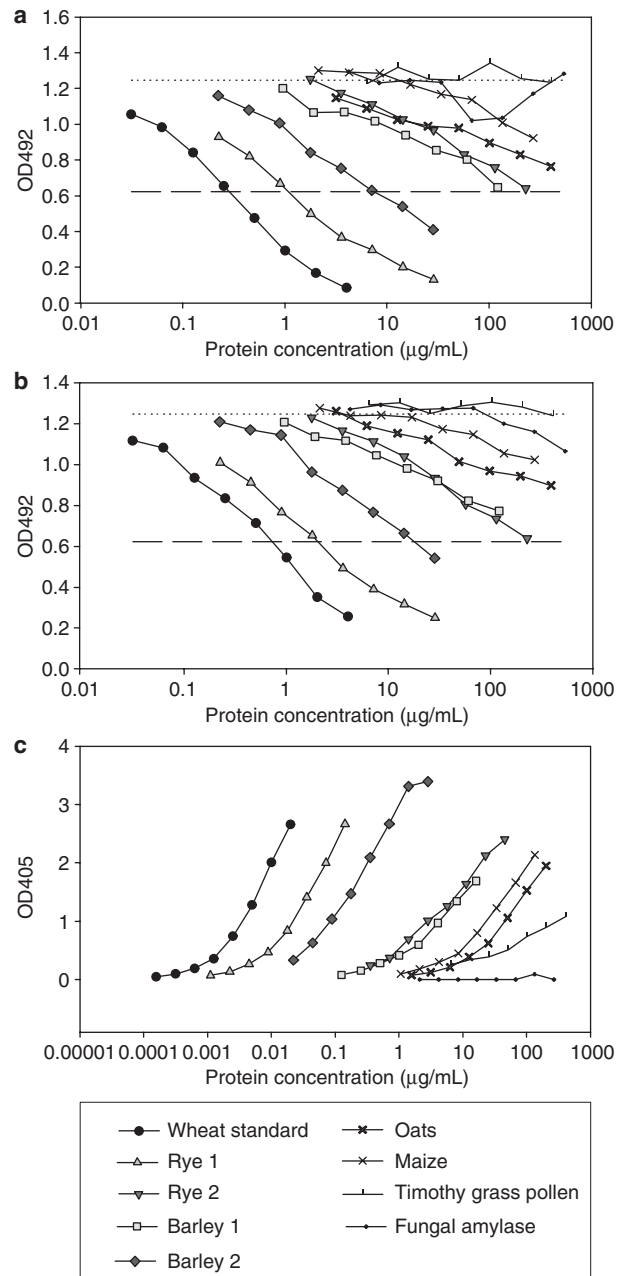


Fig. 1. Specificity of the human IgG4 inhibition enzyme immunoassay (EIA) (a), rabbit IgG inhibition EIA (b) and rabbit IgG sandwich EIA (c), tested with various extracts. The upper and lower horizontal lines in graphs A and B represent 0% and 50% inhibition, respectively.

protein ratios, calculated as the allergen content measured by the various EIAs divided by the protein content measured by Lowry assay, were 1.4 ± 0.4 , 1.5 ± 0.3 and 1.0 ± 0.2 (average \pm SD) in the human IgG4 and rabbit IgG inhibition EIA, and the sandwich EIA, respectively. Immunoblotting confirmed the similarity in protein composition (Fig. 3): hardly any differences were observed between the extracts of the six common baking flours (lanes 1, 2, 3, 5, 6, 7), or between these extracts and the

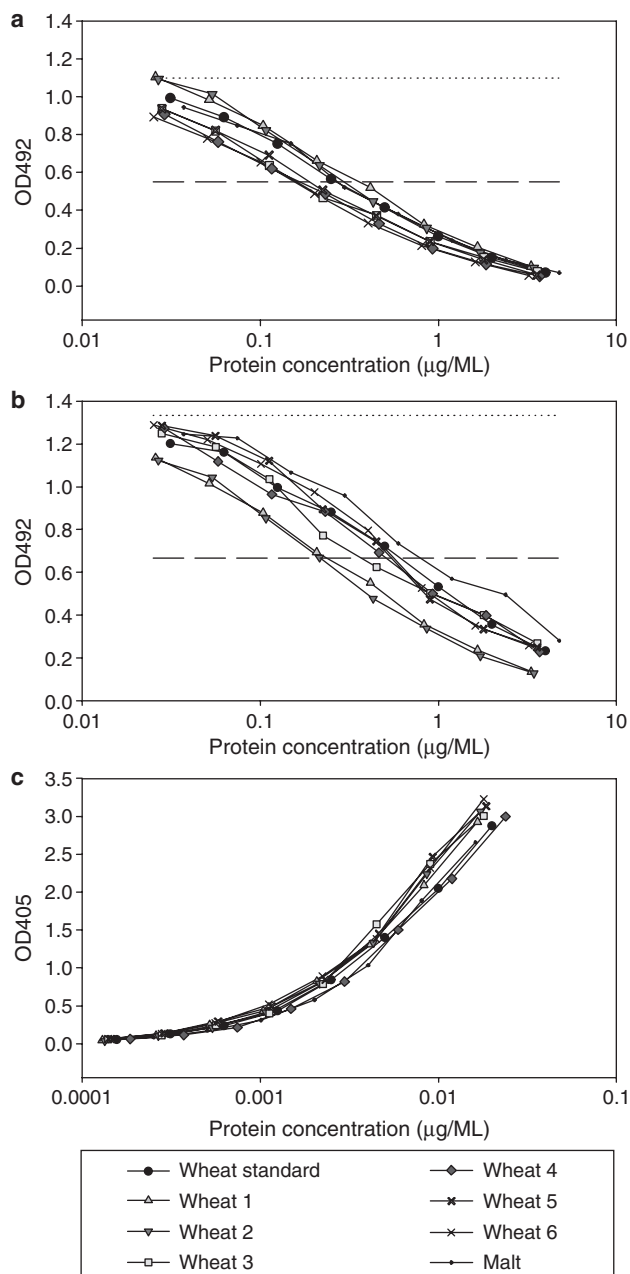


Fig. 2. Comparison of various wheat flour extracts in the human IgG4 inhibition enzyme immunoassay (EIA) (a), rabbit IgG inhibition EIA (b) and rabbit sandwich EIA (c). The upper and lower horizontal lines in graphs A and B represent 0% and 50% inhibition, respectively.

wheat allergen standard (lane 8). Similar results were obtained with Coomassie protein staining of the gels after SDS-PAGE (not shown). In contrast, wheat malt flour (lane 4) showed marked differences in protein and antigen content in immunoblots, especially in its reactions with human IgG4 and human IgE.

Allergen binding by human and rabbit antibodies

Human IgG4, human IgE and the rabbit IgG all reacted with a large number of wheat proteins with molecular

weights (MWs) between 5 and 70 kDa (Fig. 3). The rabbit IgG reactions showed, in general, more diffuse protein bands than human IgG4 and IgE, but the overall patterns showed extensive similarities. Most of the proteins reacted with each of the three reagents, although the relative intensities of the reactions of particular proteins could be different. Thus, human IgG4 showed a much stronger reaction than IgE or rabbit IgG to a protein of approximately 20 kDa, human IgG4 and rabbit IgG reacted more intensively than human IgE to proteins of 28–38 kDa and rabbit IgG reacted much stronger with the malt extract than human IgG4 or IgE. All reagents also reacted with rye proteins, in accordance with its cross-reactivity in the wheat EIAs. Interestingly, the IgE serum pool showed strong reactivity with rye proteins over almost the entire MW range below 100 kDa, whereas human IgG4 reacted most intensively with rye proteins with an MW between 28 and 49 kDa.

Reactivity of the water/salt-insoluble wheat proteins

Titration of the human IgG4 anti-wheat serum pool and rabbit anti-wheat antiserum on parallel coatings of the water/salt-soluble (albumins/globulins) and insoluble (gliadins and glutenins) wheat proteins showed that the antibodies reacted most avidly with albumins/globulins, and then with gliadins and much less with glutenins (not shown). Accordingly, the ethanol-soluble gliadins showed some reactivity in the inhibition assays, with a calculated wheat allergen content of 75 and 229 $\mu\text{g}/\text{mg}$ protein in the human IgG4 inhibition EIA and rabbit IgG inhibition EIA, respectively, while the corresponding values for the SDS/DTT-soluble glutenins were 3 and 15 $\mu\text{g}/\text{mg}$ protein.

Comparison of the assays

Of 432 airborne dust samples, 86% showed detectable wheat allergen levels in all three assays, and 5% were completely negative, even after retesting at the lowest dilutions (1/2, 1/4, 1/8) and in the most sensitive sandwich assay, with an LOD nearly two orders of magnitude lower than that of the two inhibition EIAs (Fig. 1). Intra-assay coefficients of variation (derived from values found at different sample dilutions) were lower in the sandwich assay (6%) than in the inhibition assays (17%), while the inter-day/inter-assay variation – determined by retesting 30 samples – was higher for the sandwich EIA (39%) than for the inhibition assays (15–16%). Correlations between concentrations found in the three assays were high (Fig. 4), with Pearson correlation coefficients for ln-transformed values > 0.95. Ratios of allergen levels measured in the different assays were close to 1 (see Fig. 4), indicating that there is also a good agreement in the reported absolute values, although relative differences

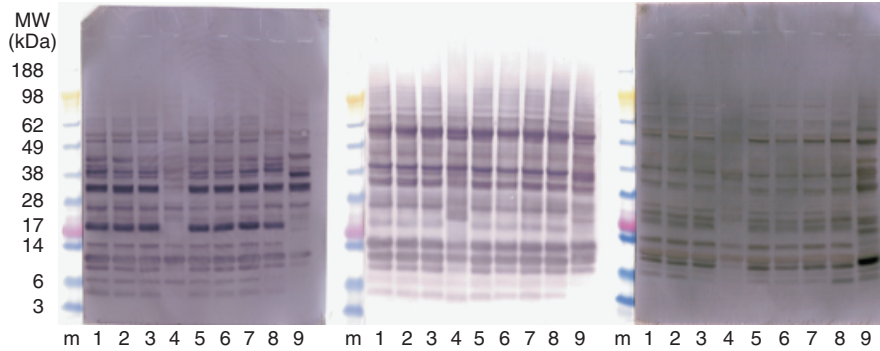


Fig. 3. Wheat and rye immunoblots obtained with, from left to the right, human IgG4 serum pool, rabbit IgG serum and human IgE serum pool. The first lane from the left (m) represents the marker, lanes 1, 2, 3, 5, 6, 7 six common baking flours, lane 4 malt flour, lane 8 wheat standard extract and lane 9 rye extract.

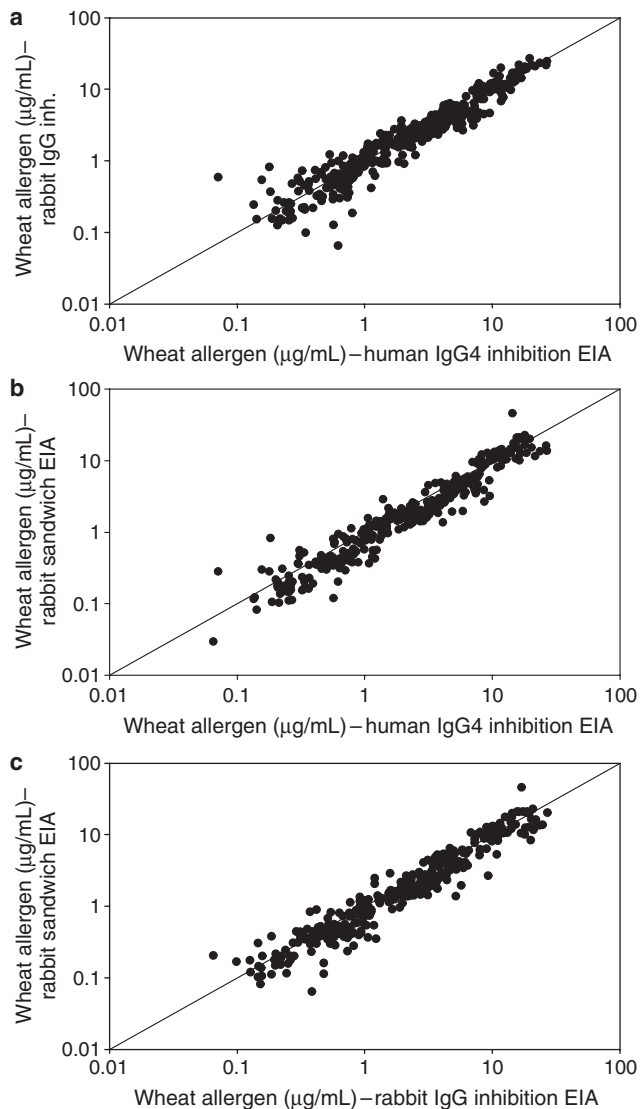


Fig. 4. Scatter-plots showing correlation between results obtained in human IgG4 inhibition enzyme immunoassay (EIA), rabbit IgG inhibition EIA and rabbit IgG sandwich EIA. Only samples positive in both assays are presented ($n = 372$, 375 and 373 , respectively).

appeared to be higher at lower allergen levels. The allergen levels measured with the sandwich EIA tended to be lower, with geometric mean ratios of 0.80 and 0.84 compared with levels measured in the human IgG4 and the rabbit IgG inhibition EIA, respectively.

Discussion

Three assays for the measurement of wheat proteins were compared in order to optimize and standardize methods for airborne wheat allergen exposure assessment. The assays were shown to be largely specific for flour proteins from the Triticeae species, with strong reactions with wheat, and variable but substantial cross-reactivity with rye and barley proteins. Owing to a large number of closely related proteins in different cereal species [28, 29], cross-reactivity in wheat assays can hardly be avoided. This, however, should not represent an important limitation, as IgE from most flour-sensitized bakers reacts strongly both with wheat and rye flours [30, 31], and a highly specific assay may even underestimate the levels of relevant allergenic proteins. Interestingly, different batches of rye and barley extracts – freshly prepared from flours at our laboratory or purchased as SPT preparations – differed markedly in their reactivity in the wheat allergen assays. This suggests ‘natural’ heterogeneity in their content of cross-reactive proteins, or may reflect an effect of differences in preparation procedures leading to variations in protein composition. In contrast, the wheat allergen standard and extracts of a series of commonly used wheat flours showed highly comparable antigenic/allergenic reactivity, and a very similar composition, as shown by EIA analyses and immunoblotting. This confirmed that the thus far used wheat extract is a representative standard reagent, and may, if necessary, be replaced by extracts of other common baking flours without significant changes in EIA results.

Use of IgE from allergic patients might be considered as a ‘golden standard’ for allergen measurements, as it would

imply measurement of the actual agents causing sensitization and allergic reactions. It has been shown previously that a pool of IgG4 anti-wheat antibodies reacts with most IgE-binding wheat proteins [6]. In our present study, we confirmed this similarity in reaction profiles, although the obvious differences in band intensities suggest significant quantitative and possibly qualitative differences (Fig. 3). In addition, we showed that the large majority of IgE-binding proteins in wheat extracts were also recognized by the rabbit IgG anti-wheat antibodies, induced by active immunization.

Our human IgG4- and rabbit IgG-based EIAs were shown to measure predominantly water/salt-soluble wheat flour proteins (albumins and globulins) and, with much less efficiency, the ethanol-soluble gliadins and SDS/DTT-soluble glutenins. Measurement of primarily water/salt-soluble proteins should not affect the validity of allergen exposure measurements as albumins/globulins are undoubtedly highly, if not the most relevant wheat protein fraction involved in the aetiology of baker's allergy and asthma [10–12]. Some recent studies suggest that IgE-positive bakers' sera react either with albumins/globulins alone, or both with albumins/globulins and gliadins or glutenins [25], but there is little evidence of IgE-reactivity with gliadins or glutenins in the absence of at least moderate reactivity to albumins/globulins [15, 25].

The comparison of results for a large series of airborne dust samples tested in the three assays in the same laboratory showed high levels of correlation (Pearson correlation coefficients > 0.95) and a good agreement in reported absolute values. The sandwich EIA was > 100 -fold more sensitive than the inhibition assays, which makes this assay particularly suitable for analysis of samples from short-term airborne measurements [32], low-exposure environments, or analyses of subfractions of airborne dust separated by size-selective sampling methods [33]. The reproducibility of the sandwich assay results was, however, lower. This was partly due to the additional high dilution factors required for eluates of 'conventional' samples in this highly sensitive assay. Also, clearly higher CV values were observed for the samples with only one result value (out of three tested dilutions) in the measurement range, than for those whose results were calculated as averages from two or three values in the range.

A major advantage of the rabbit IgG inhibition EIA may be the use of serum at very high dilutions. While the human IgG4 anti-wheat serum pool was used at 1/1200, the rabbit antiserum could be used at 1/150 000. The rabbit antiserum also appeared to be a very stable source of reagents after more than 10 years of storage at -20°C , and the antibodies also retained their reactivity for more than a year when stored prediluted (1/100 in PBTG) at -20°C in small aliquots for single use. In this way, an

amount of less than 0.05–0.1 mL antiserum would be sufficient for testing $> 10\,000$ airborne dust samples.

To summarize, we have introduced two assays for the measurement of airborne wheat allergen based on rabbit polyclonal IgG antibodies – an inhibition EIA and a sandwich EIA – each of them being a valid alternative for the human IgG4 [6] or human IgE inhibition assay [4]. The new assays are sensitive, measure allergens of clinical relevance, show high correlations in results and have obvious practical advantages compared with assays using human sera. The rabbit IgG inhibition EIA can be recommended as the most convenient assay for routine measurements of full-shift airborne wheat samples from a medium- to high-exposure bakery or flour mill environment, while for analysis of samples with expected low amounts of wheat allergen, the highly sensitive wheat sandwich EIA is to be preferred.

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