



Research paper

Development of a two-site enzyme immunoassay based on monoclonal antibodies to measure airborne exposure to (1→3)-β-D-glucan

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ABSTRACT

(1→3)-β-D-glucan is found in cell walls of some fungi, bacteria and plants. It plays a crucial role in bioaerosol-induced inflammatory reactions. To estimate the level of airborne (1→3)-β-D-glucan exposure, a monoclonal antibody-based two-site enzyme immunoassay (mAb-EIA) was developed. The results obtained with the mAb-EIA were compared with the results of a *Limulus* amoebocyte lysate-based assay for (1→3)-β-D-glucan.

Three mAbs produced by mouse immunization with bovine serum albumin-conjugated laminarin were enriched by *in vitro* production in a modular minifermenter and affinity purified. Two mAbs were selected for the development of a two-site EIA specific for (1→3)-β-D-glucan. Different polysaccharides, fungal and plant seed extracts, and airborne inhalable dust from workplaces (poultry farms, pig stables, grain storage houses, and a laboratory animal facility) were sampled with portable pumps and measured with both the mAb-EIA and GlucateLL® assay.

Using carboxymethylated curdlan as a standard, the mAb-EIA gave a steep dose-response curve for concentrations between 0.36–15 ng/ml. The mAb-EIA was specific for (1→3)-β-D-glucan and was sufficiently sensitive to detect (1→3)-β-D-glucan in airborne dust samples. In comparing the EIA results to the values obtained with the GlucateLL® assay, the correlation was found to be high (coefficient of correlation $r^2=0.91$), and the mean ratio of the values was 1.7. Depending on the dust source, either the GlucateLL® assay or the mAb-EIA gave higher results. The mAb-EIA is sensitive enough to detect (1→3)-β-D-glucan in airborne dust samples collected with portable pumps. Thus, the assay is suited for the investigation of the health effects induced by exposure to this class of biologically active molecules.

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1. Introduction

(1→3)-β-D-glucan, a glucose polymer with variable molecular weight and degrees of branching, is found in the cell walls of fungi, some bacteria, and plants. (1→3)-β-D-glucan affects the human immune system. On one hand, (1→3)-β-D-glucan may be associated with a blunting effect of allergic

symptoms and sensitization frequencies (Schram-Bijkerk et al., 2005; Iossifova et al., 2007; Yamada et al., 2007). On the other hand, it is believed to play a role in bio-aerosol-induced inflammatory reactions, respiratory symptoms, and sick-building syndrome (Thorn et al., 1998; Thorn and Rylander, 1998; Rylander, 1999; Douwes et al., 2000b; Beijer et al., 2002; Douwes, 2005). Several studies show a relationship between bio-aerosol-associated symptoms and a history of dampness or flooding of buildings, as these conditions favor the growth of fungi and certain bacteria (Meklin et al., 2005; Shoemaker and House, 2006; Cho et al., 2006; Rao et al., 2007). These microorganisms contain endotoxin, mycotoxins, (1→3)-β-D-glucan, and different allergens that can cause a variety of toxicologic and immunologic effects (Husman, 1996). Although there is debate about which agents

Abbreviations: mAb, Monoclonal antibody; EIA, Enzyme immunoassay; OD, Optical density; PBS, Phosphate-buffered saline; PBST, PBS containing 0.05% Tween 20; BSA, Bovine serum albumin.

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are the most relevant for the effects observed after microbial exposure, in most cases, viability of microorganisms is not required for the elicited toxicologic and immunologic health effects. Therefore, risk estimations based on the viability of microorganisms may be inaccurate, and indicators of cell biomass or measures of the specific toxic agent should be used (Rylander, 1999). To quantify (1→3)- β -D-glucan, two basically different methods have been used. The first is based on the reactivity of coagulation factor G from the horseshoe crab coagulation cascade to (1→3)- β -D-glucan (Aketagawa et al., 1993). Using the chromogenic substrate Boc-Leu-Gly-Arg-p-nitroanilide, very sensitive amebocyte lysate assays, like Fungitic-G (Seikagaku, Japan) and GlucateLL® (Associates of Cape Cod, USA), were developed to diagnose invasive fungal infections. In these assays, a few picograms of (1→3)- β -D-glucan can be detected (Obayashi et al., 1995; Odabasi et al., 2004). The Fungitec-G (Thorn et al., 1998; Thorn and Rylander, 1998; Rylander, 1999; Beijer et al., 2003; Rylander and Carvalheiro, 2006) and the GlucateLL® test (Iossifova et al., 2007), and a modification of the Limulus amebocyte lysate assay (Foto et al., 2004; Miller et al., 2007) have also been used for environmental and occupational exposure assessment. The second method to measure (1→3)- β -D-glucan in environmental samples is based on specific antibodies. Milton et al. (2001) developed an enzyme immunoassay based on the high affinity receptor galactosyl ceramide. In this assay, galactosyl ceramide is the capture reagent and a monoclonal antibody specific for (1→6)-branched-(1→3)- β -D-glucans is the detector. Rao et al. (2007) used a (1→3)(1→6)- β -D-glucan-specific assay based on a combination of monoclonal and polyclonal antibodies. Douwes et al. (1996) developed an inhibition assay based on polyclonal antibodies specific for (1→3)- β -D-glucan. This assay has a detection range of 40–3000 ng/ml laminarin, the (1→3)- β -D-glucan which is used as the standard; the assay has been used in several studies for exposure assessment (Douwes et al., 2000a; Douwes et al., 2000b; Douwes et al., 2000c; Gehring et al., 2001; Schram et al., 2005; Schram-Bijkerk et al., 2005; Douwes et al., 2006; Wouters et al., 2006). While the amebocyte lysate assays have a lower detection limit than the antibody inhibition assay, the high cost and the variability of the horseshoe crab material makes it disadvantageous for large environmental studies and assay standardization. It was the aim of this study to generate (1→3)- β -D-glucan-specific monoclonal antibodies (mAb), and to develop a (1→3)- β -D-glucan immunoassay that is cost effective, sensitive, and easily standardized, thus making it suitable for large environmental and occupational exposure studies.

2. Methods

Airborne dust was sampled in stables of a poultry farm and a chicken stable (nine samples), in bird houses (pigeons and parakeets, three samples), in pig and piglet stables (twelve samples), in two grain storage houses (six samples), in a laboratory animal facility with mice (three samples), and outside an office building (two samples) using GSP sampling heads (BGIA, St. Augustin, Germany) and Gilian pumps (air flow 3.5 l/min) on Teflon filters (diameter 3.7 cm, pores 1 μ m, Millipore, Schwalbach, Germany). The GSP sampling heads had been found to adequately sample (Kenny et al., 1997) with

respect to the inhalable sampling convention of the Comité Européen de Normalisation (CEN). The sampling heads, routinely used for personal air sampling in Germany, were fixed in our study between 1.2–1.70 m above the floor. Sampling time varied between 1 and 8 h.

2.1. Extraction of polysaccharides, plant seeds, moulds and filter samples

CM-pachyman and CM-curdlan (Megazym, Wicklow, Ireland) were dissolved as described by the manufacturer. Pachyman, lichenan, oat β -D-glucan, pullulan, curdlan, (Megazym), pustulan (Calbiochem, La Jolla, CA), paramylon, laminarin and glucan from baker's yeast (Fluka AG, Buchs, Switzerland), and dextran, mannan, and β -D-glucan from barley (Sigma, Taufkirchen, Germany) were dissolved by autoclaving for 1 h at 120 °C (10⁵Pa). After centrifugation at 1000 $\times$ g, the resulting supernatant was frozen in aliquots at –20 °C.

Fungi (*Aspergillus niger*, *A. oryzae*, *A. fumigatus*, *A. versicolor*, *Penicillium chrysogenum*, *Aureobasidium* (*Pullularia*) *pullulans*, *Phomae betae*, *Trichoderma viride*, and *Saccharomyces cerevisiae* (Allergon, Ängelholm, Sweden)), crushed seeds (soy bean, millet, sesame, buckwheat, spelt wheat, amaranth, oat, and barley), flours (rye and wheat), and filters with sampled airborne dust were extracted in 5 ml phosphate buffered saline (PBS, pH 7.4) containing 0.05% Tween 20 (PBST) by end-over-end rotation for 1 h, autoclaving for 1 h at 120 °C (10⁵Pa), vortexing, and centrifugation at 3000 $\times$ g. The resulting supernatants were frozen in aliquots at –20 °C. Before measurement, each aliquot was mixed, heated to 95 °C for 10 min and diluted in PBS.

2.2. Immunization and production of monoclonal antibodies

Oxidized laminarin was conjugated to bovine serum albumin as described by Douwes et al. (1996), and used for the immunization of eight BALB/c mice using TiterMax™Gold as an adjuvant. Four mice produced antibodies to laminarin. Fusion of antibody-secreting spleen cells with myeloma cells (NSO-cells) and screening for laminarin-binding resulted in nine hybridoma cell lines producing antibodies to laminarin. To assure the long-term survival of single cell clones, three hybridoma cell lines were generated. These lines all produced mAbs binding to laminarin but differed in pustulan binding ability. The lines were subcloned by the limited dilution technique, and cultivated in modular minifermenters (Mini-Perm®, In vitro systems & services, Göttingen, Germany) to obtain high-density cultures as described previously (Sander et al., 1997). The mAbs were named according to the mouse, subcloning procedure, and well of the microtiter plate with the mAb-producing single cell clone (e.g., 3200 3F12: mouse 3, second subcloning procedure, well 3F12).

2.3. Affinity chromatography of mAbs

Anti(1→3)- β -D-glucan mAbs from miniPERM® culture supernatant were loaded via FPLC (Pharmacia, Uppsala, Sweden) on a HiTrap rProtein A column (GE Healthcare, München, Germany), washed with phosphate-buffer (20 mM, pH 7), and eluted with citrate-buffer (pH 3.0). Eluted fractions

were neutralized directly with a 1:10 volume of 1 M Tris–HCl-buffer (pH 9), pooled and dialysed.

2.4. Biotinylation of mAb

Purified monoclonal antibodies (1 mg/ml in 10 mM carbonate buffer, pH 8.5) were biotinylated by mixing with a 33-fold molar excess of dimethylsulfoxide dissolved biotin-N-hydroxysuccinimide-ester (Boehringer Mannheim GmbH, Mannheim, Germany) during continuous agitation for 3 h at 20 °C. The biotinylated antibodies were dialysed extensively and stored in aliquots at –20 °C.

2.5. Antigen-binding Enzyme Immunoassay (EIA)

Microtiter plates (MaxiSorp™ StarWell™, Nunc, Wiesbaden, Germany) were coated with autoclaved laminarin by incubating 100 µl solution (1.6 µg/well) in PBS overnight at 4 °C. The wells were then blocked with 0.5% (w/v) gelatine in PBS for 2 h and washed with PBST. For the detection of IgG antibody binding, supernatants (100 µl/well) of hybridoma cell cultures were incubated for 2 h at 37 °C in the microtiter plates. After washing three times with PBST, the bound antibodies were detected with alkaline phosphatase-conjugated goat anti-mouse IgG (A1047, Sigma, Taufkirchen, Germany), diluted 1:5000 in PBST with 0.5% (w/v) gelatine. The substrate p-nitrophenyl phosphate (Hycor Biomedicals, Kassel, Germany) was added and optical density (OD) at 405 nm was determined using an ELISA plate reader.

2.6. Two-site monoclonal antibody-based EIA

Purified mAb 2100 3C9 (1 µg protein/ml carbonate/bicarbonate buffer, pH 9.6) was coated on MaxiSorp™ (Nunc) microtiter plates overnight at 4 °C. The wells were blocked with casein buffer (product code PHRPDILX; RDI, NJ, USA) at 37 °C for 2 h and washed three times with PBST. Nine standards with concentrations ranging from 2 ng to 50 ng/ml CM-curdlan, were incubated in 0.1 ml PBS/well for 1 h at 37 °C on each plate in duplicate and parallel to the diluted samples. Each sample was tested repeatedly in a two-fold dilution series of at least three steps until the values fitted to the measuring range. After washing 3 times with PBST the biotinylated mAb 3200 3F12 diluted 1:3000 in PBST (0.3 µg/ml) was added. Ninety minutes later, all wells were washed 3 times and then incubated for 1 h with 0.1 ml horseradish-peroxidase/streptavidin conjugate (Bio-source, Solingen, Germany, diluted 1:4000 in PBS). After washing three times with PBST and two times with PBS, the assay was developed with 100 µl 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS, Sigma, Germany, Taufkirchen) and activated with H₂O₂ according to manufacturer's instructions. The enzyme reaction was stopped after 10–20 min by adding 100 µl 0.32% NaF. Sample concentrations were read by interpolation of OD values (OD₄₁₄ nm) on a log–log fit of the standard preparation, using Softmax Pro 4.7.1 from Molecular Devices (Sunnyvale, CA, USA). The lower detection limit of the assay was defined by adding 0.2 OD units (rounded 10-fold mean of the standard deviation of double estimated background values from five plates) to the background value of each plate. Only

glucan amounts above that value were considered positive. The upper detection limit was set to OD 2.

2.7. Glucatell® assay

The Glucatell® assay (Pyroquant, Mörfelden, Germany) contains lysed amebocytes from the hemolymph of *Limulus polyphemus* that had been processed to remove Factor C, making the lysate specific for (1→3)-β-D-glucan. In addition, the processed lysate contains the chromogenic substrate Boc-Leu-Gly-Arg-p-nitroaniline which is cleaved after (1→3)-β-D-glucan in the test sample (or standard) activates Factor G, which then activates the proclotting enzyme of the *Limulus* protease activation pathway. The accumulation of free p-nitroaniline at 37 °C after mixing 25 µl diluted filter extracts of airborne samples with 100 µl of the processed lysate was measured every 20 sec for 1 h at 405 nm with a Spectramax plus photometer (Molecular Devices). The onset time until the OD reached 0.03 was used to calculate the content of (1→3)-β-D-glucan in the filter extracts with Softmax Pro 4.7.1 by a log–log fit of the standard dilution series (Pachyman from Pyroquant, 3.125–100 µg/ml). The detection limit for the Glucatell® assay is 10 µg/ml.

2.8. Statistical analysis

Correlation analyses of data (two-tailed *p*-value and Pearson coefficient) were performed with GraphPad Prism (GraphPad Software Inc., San Diego, CA USA). The graphs were created with SigmaPlot 8 (Systat Software Inc., San Jose, CA USA).

3. Results

The laminarin-binding mAbs produced by three different hybridoma cell lines were characterized by laminarin binding in the presences of competitively inhibiting polysaccharides in an antigen-binding EIA (Fig. 1). While the (1→3)-β-D-glucans laminarin and curdlan inhibited binding in all cases, the α-D-glucans dextran and mannan did not inhibit binding to laminarin. Pustulan, a (1→6)-β-D-glucan which has some β(1→3)-glucosidic structures (Douwes et al., 1996; Milton et al., 2001), inhibited the binding of antibodies 2100 3F4 and 3200 3F12 to laminarin, but not of mAb 2100 3C9, if used in a concentration of up to 1 mg/ml. For development of a two-site immunoassay, the mAb 3200 3F12 was purified, biotinylated and used as the detection antibody and combined with the purified mAb 2100 3C9 as the coating antibody. The (1→3)-β-D-glucan CM-curdlan was used as a standard in the two-site immunoassay because laminarin was not detectable in this assay (Table 1). In comparison to curdlan, CM-curdlan gave less variation when the solution procedure was repeated. The concentrations of both mAbs were crucial for the specific detection of CM-curdlan. Excess concentrations of mAbs increased the background of the assay, insufficient concentrations led to a loss of sensitivity. The optimized concentrations were 0.15 µg/well of capture antibody and a 1:3000 dilution of the detection antibody (~0.030 µg/well). In this combination the assay had a mean detection range of 0.36 ng to 15 ng/ml.

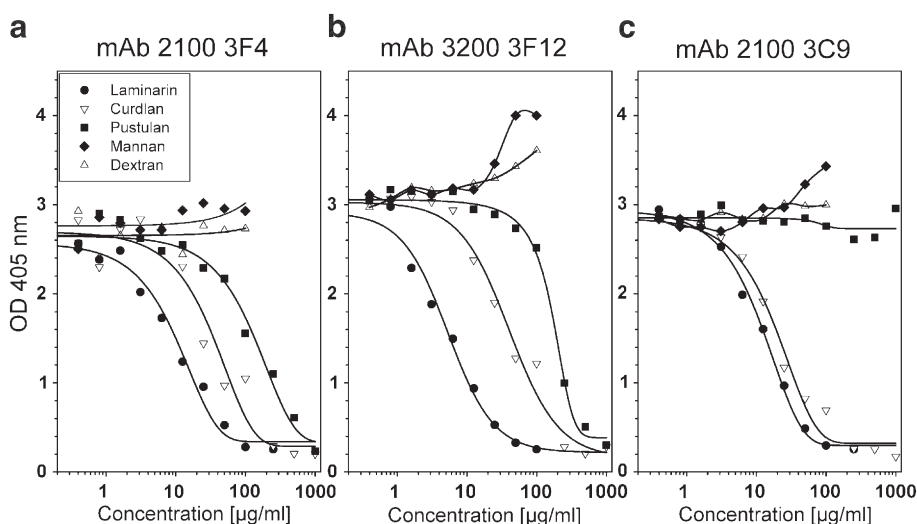


Fig. 1. Binding of mAbs 2100 3F4 (a), 3200 3F12 (b), and 2100 3c9 (c) to laminarin-coated wells. Laminarin binding was inhibited by addition of different concentrations of polysaccharides.

Solutions of (1→3)-β-D-glucans with different molecular weights and branching, other polysaccharides (Table 1), and extracts of fungi (Fig. 2a) or seeds (Fig. 2b) were used to verify the characteristics and specificity of the EIA. The assay did not react to dextran, mannan, pullulan, and laminarin. It was most sensitive for curdlan, CM-curdlan, pachyman, and CM-pachyman (Table 1). In addition, yeast, barley and oat glucan, lichenan, pustulan, paramylon and all tested fungi and plant seed extracts were detectable. The dilution curves (Fig. 2) were parallel to the standard curve allowing for the calculation of (1→3)-β-D-glucan content per weight. Fungal extracts had a higher concentration of measurable (1→3)-β-D-glucans than plant seed extracts.

The two-site EIA was used to measure airborne dust from different workplaces and environments (35 samples). For air sampling, portable pumps with GSP sampling heads for inhalable dust were used, which is the equipment routinely used for personal inhalable dust exposure measurements in Ger-

many. With the exception of four samples from the grain storage houses (5.7–49 mg dust/filter), all filters had a dust load below 2 mg. In EIA, all filter extracts contained measurable amounts of (1→3)-β-D-glucan. Since airborne inhalable dust was sampled at a flow rate of 3.5 l/min, and the detection limit per filter was 1.8 ng (1→3)-β-D-glucan (0.36 ng/ml × 5 ml), the resulting detection limit for airborne inhalable dust was approximately 1.1 ng/m³ for 8 h sampling time. The mAb-EIA-measured (1→3)-β-D-glucan concentration was between 2 ng/m³ and 406 µg/m³. Highest concentrations were measured in the grain storage houses (1.8–406 µg/m³), in the poultry farm stables the concentration ranged from 2–972 ng/m³, in the pig stables from 33–410 ng/m³, in the bird houses from 14–52 ng/m³, in the animal facility from 16–38 ng/m³, and outside an office the concentration ranged from 13–26 ng/m³.

The EIA results of the filter extracts were compared with the results of the GlucateLL[®] assay (Fig. 3). The correlation of (1→3)-β-D-glucan values was significant ($p < 0.0001$, Pearson

Table 1
Specificity of the mAb-based enzyme immunoassay

(1→3)-β-d-glucan and related polysaccharides	Source	Main linkage ^a		Mol WT ^a	Limit of detection (ng/ml)
Laminarin	<i>Laminaria digitata</i>	(algae)	(1→3)(1→6)-β-d-glucan (LSB)	5850	ND ^b
CM-curdlan	<i>Alcaligenes faecalis</i>	(bacteria)	(1→3)-β-d-glucan (L, homogeneous)	>95,000	0.36
Curdlan	<i>Alcaligenes faecalis</i>	(bacteria)	(1→3)-β-d-glucan (L, homogeneous)	>95,000	0.36
CM-pachyman	<i>Poria cocos</i>	(fungi)	(1→3)-β-d-glucan (L, homogeneous)	80,000	0.4
Pachyman	<i>Poria cocos</i>	(fungi)	(1→3)-β-d-glucan (L, homogeneous)	80,000	0.9
Paramylon	<i>Euglena gracilis</i>	(protist)	(1→3)-β-d-glucan (L)	>118,000	20
Lichenan	<i>Cetraria islandica</i>	(lichen)	(1→3)(1→4)-β-d-glucan (L)	33,000	200
Pustulan	<i>Umbilicaria papulosa</i>	(lichen)	(1→6)-β-d-glucan (L)	20,000	200
Yeast glucan	<i>Saccharomyces cerevisiae</i>	(fungi)	(1→6)(1→3)-β-d-glucan (B-on-B)	17,200	15
Barley glucan	<i>Hordeum vulgare</i>	(plant)	(1→3)(1→4)-β-d-glucan (L)	>23,100	2,000
Oat glucan	<i>Avena sativa</i>	(plant)	(1→3)(1→4)-β-d-glucan (B)	>100,000	40,000
Dextran	<i>Leuconostoc mesenteroides</i>	(bacteria)	(1→6)-α-d-glucan (L)	513,000	ND ^b
Mannan	<i>Saccharomyces cerevisiae</i>	(fungi)	(1→2)(1→3)(1→6)-α-d-glucan (B)	34,500	ND ^c
Pullulan	<i>Aureobasidium pullulans</i>	(fungi)	(1→4)(1→6)-α-d-glucan (L)	>170,000	ND ^d

ND, not detected.

^a L, Linear; B-on-B, branch-on-branch, data taken from Ref. (Milton et al., 2001; Odabasi et al., 2004).

^b Maximum concentration tested, 400 µg/ml.

^c Maximum concentration tested, 200 µg/ml.

^d Maximum concentration tested, 1,000 µg/ml.

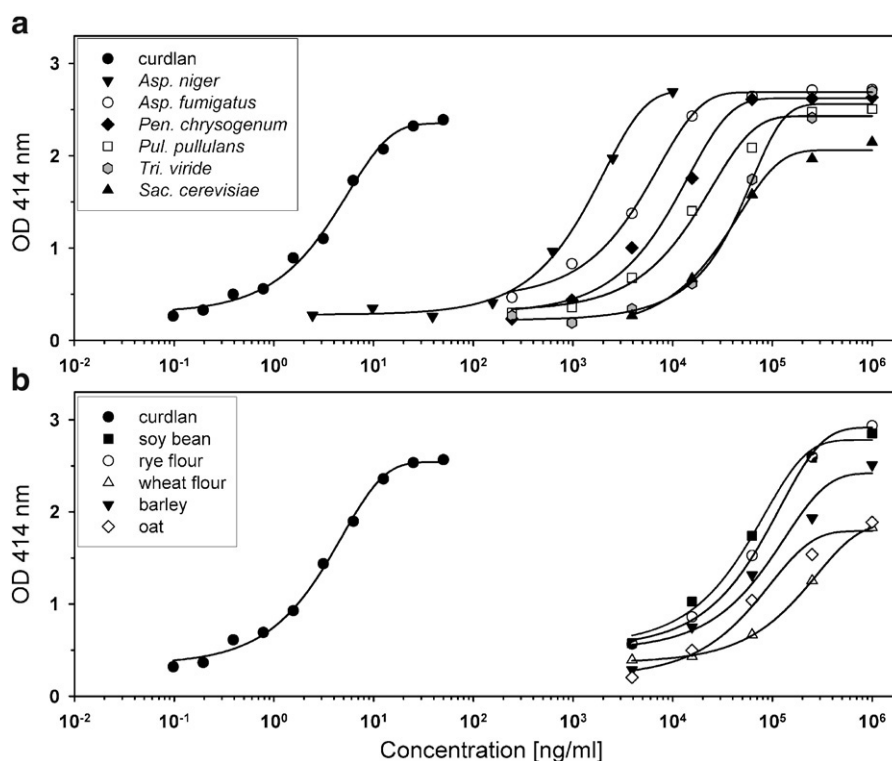


Fig. 2. Dose-response curves of curdlan and fungal (a) or seed extracts (b) with the mAb-based sandwich EIA specific to (1→3)-β-D-glucan.

coefficient of correlation $r^2=0.91$, coefficient of log-transformed values $r^2=0.86$, Fig. 3a). The arithmetic mean ratio (value in EIA/value in GlucateLL[®] assay) was 1.7, the geometric mean ratio was 1.1, and the median ratio was 1.8. The Bland–Altman analyses (Fig. 3b) revealed that the ratio of assays is not influenced by the amount of (1→3)-β-D-glucan on the filters in general, but from the place of sampling. Depending on the place of sampling, either the EIA or the GlucateLL[®] assay tends to give higher values. Most samples from poultry farms showed higher values in the GlucateLL[®] assay (range 13–5000 ng/m³) while nearly all samples from pig stables (range in GlucateLL[®] assay 18–96 ng/m³) and all samples from grain storage houses (range in GlucateLL[®] assay 0.82–121 μg/m³) had higher values in the EIA. To compare the (1→3)-β-D-glucan content of different moulds and seeds, extracts were measured with both assays (Table 2). As a rule, the measuring values of cereals were higher in the GlucateLL[®] assay than in the mAb-EIA, especially rye, oat and barley achieved values from 970–1900 ng/mg in the GlucateLL[®] assay in contrast to values between 6 and 26 ng/mg in the mAb-EIA.

4. Discussion

The mAb-based sandwich EIA is specific for (1→3)-β-D-glucans, including curdlan, pachyman, lichenan, yeast glucan, barley glucan, and paramylon. The linear (1→3)-β-D-glucan, laminarin, from the algae *Laminaria digitata*, which has been used as the antigen for the production of the mAbs, could not be detected by the mAb-based sandwich EIA. As both mAbs used for the sandwich EIA bind to laminarin in the direct EIA and inhibition EIA, we assume that the small molecule

laminarin (~6 kDa) does not allow simultaneous binding of the two antibodies. Pustulan, although mainly consisting of (1→6)-β-D-glucans, can be detected by the sandwich EIA. On a weight basis, it was 500 times less reactive than CM-curdlan or pachyman. According to data by Douwes et al. (1996) and Milton et al. (2001), pustulan consists of some (1→3)-β-D-glucosidic structures. In the inhibition EIA specific for (1→3)-β-D-glucans developed by Douwes et al. (1996), pustulan was 10 times less reactive than laminarin, and 3–5 times less reactive than CM-curdlan. In comparison to this inhibition EIA, in which antibodies were derived by immunization with the same laminarin conjugate used as the mAb-EIA, the detection limit of the mAb-EIA is nominally more than 100 times lower. The mAb-EIA detects CM-curdlan in a concentration of 0.36 ng/ml, while the inhibition EIA detects laminarin in a concentration of 40 ng/ml and CM-curdlan in a concentration of about 200 ng/ml. The binding pattern of the newly developed mAb EIA is clearly different from the assay of Milton et al. (2001) for (1→6) branched (1→3)-β-D-glucans, mainly yeast glucans, which does not bind curdlan and barley glucan.

In comparison to the *Limulus* lysate-based GlucateLL[®] assay, the detection limit of the mAb-EIA for pachyman is 40 times higher. Sensitivity of the GlucateLL[®] assay for pachyman (10 pg/ml) was the same as for curdlan and CM-curdlan (Odabasi et al., 2004). These (1→3)-β-D-glucans were also detected with similar sensitivity (0.4 ng/ml) in the mAb-EIA. Thus, the different standards used in these tests (pachyman for GlucateLL[®], CM-curdlan for the mAb-EIA) should not have influenced the values and comparisons. However, some other polysaccharides show remarkably different reactivities

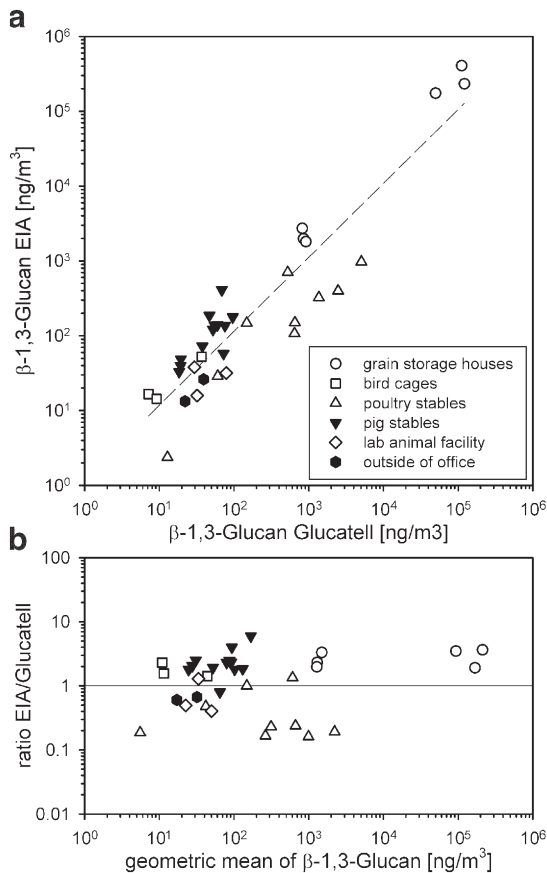


Fig. 3. Scatter-plots (a) of (1→3)-β-D-glucan values of airborne dust samples from different workplaces and environments measured by the mAb-based sandwich EIA and GlucateLL[®] assay. Relative difference (ratio) of the same values plotted against their geometric mean (b).

compared to the standard glucans. In comparison to CM-curdlan, the detection limit for oat (1→3)(1→4)-β-D-glucan was more than 100,000-fold higher, and for barley (1→3)(1→4)-β-D-glucans it was 5500-fold higher in the mAb-EIA (Table 1). In contrast, the detection limit for barley glucan was similar to CM-curdlan, curdlan and pachyman in the GlucateLL[®] assay according to the data of Odabasi et al. (2004). Therefore, the much higher (1→3)-β-D-glucan values measured by the GlucateLL[®] assay in barley and oat extracts, in comparison to the mAb-EIA (Table 2), are coherent.

The degree of branching, chain length and conformation of (1→3)-β-D-glucan influences the detection in the assays. For the amebocyte lysate based assays, (1→3)-β-D-glucans with a single helical conformation have been found to be more effective than those taking a triple helical conformation (Saitô et al., 1991; Aketagawa et al., 1993). For the newly developed mAb-EIA it was found that mould extracts yielded higher measurable (1→3)-β-D-glucan values than plant extracts (Fig. 2), and the molecular weight of the (1→3)-β-D-glucan seems to play a role. The largest discrepancies between the mAb-EIA and the GlucateLL[®] assay were observed when measuring *Penicillium* and barley or oat extracts (Table 2). However, to date how (1→3)-β-D-glucans from different spe-

cies differ in their impact on inhalation toxicology is not well characterized.

For the comparison of airborne (1→3)-β-D-glucan exposure, 35 stationary inhalable dust samples with varying sampling times were taken from poultry, bird and pig stables, a laboratory animal facility, two grain storage houses, and, as an example of environmental (1→3)-β-D-glucan exposure, outside our office building. With the exception of the grain storage houses, the sampling places had a low dust concentration. However, all samples were measurable with the newly developed EIA. The highest (1→3)-β-D-glucan concentrations were found in the grain storage houses followed by the poultry farm and pig stables. In poultry houses and pig stables, exposure to (1→3)-β-D-glucan has been previously measured (Douwes et al., 1996; Rylander and Carvalho, 2006). Rylander and Carvalho (2006) found 4–870 ng/m³ of non-water-soluble (1→3)-β-D-glucan in poultry houses as measured by the Fungitec G test. We observed a similar range of 2–972 ng/m³ (1→3)-β-D-glucan in poultry stables, although the extraction procedure and the quantification method (sandwich mAb-EIA) differ. In pig stables, the values measured with the inhibition EIA of Douwes et al. (1996) (<200–38,490 ng/m³) were considerably higher than the 33–410 ng/m³ measured by mAb-EIA in our study. The extraction procedure was similar; in both cases (1→3)-β-D-glucan was solved by autoclaving. Extraction of (1→3)-β-D-glucan by autoclaving was the preferred extraction procedure for the mAb-EIA measurements. The alternative alkaline extraction procedure, shaking in 0.3 N sodium hydroxide, used by Rylander's group (Rylander, 1997; Thorn et al., 1998; Thorn and Rylander, 1998; Rylander, 1999; Rylander and Lin, 2000; Thorn et al., 2001; Beijer et al., 2002; Rylander and Carvalho, 2006), or shaking in 0.5 N (Foto et al., 2004) or 0.6 N sodium hydroxide (Iossifova et al., 2007) was not used to avoid high sodium hydroxide concentrations that could potentially inhibit antibody binding in samples with low (1→3)-β-D-glucan content that could not be highly diluted.

Table 2
(1→3)-β-D-glucan content of mould and seed extracts measured by mAb-EIA and GlucateLL[®] assay

	GlucateLL [®] assay(1→3)-β-D-glucan [ng/mg]	mAb EIA(1→3)-β-D-glucan [ng/mg]
Moulds		
<i>A. niger</i>	4214	5610
<i>A. oryzae</i>	788	549
<i>A. fumigatus</i>	1237	1379
<i>A. versicolor</i>	2696	2173
<i>Trichoderma viride</i>	367	68
<i>Penicillium chrysogenum</i>	2230	511
<i>Phoma betae</i>	1610	128
Seeds		
Soy bean flour	28	26
Sesame	21	19
Buckwheat	10	5
Amaranth	25	11
Millet	12	35
Cereals		
Wheat flour	42	6
Barley	1904	17
Oat	1694	6
Spelt wheat	76	5
Rye flour	971	26

All filters in our study were heat-extracted, and aliquots of same extracts were tested in both the Glucate[®] and the newly developed mAb-EIA. The correlation and concordance between the two assays was significantly high, and both assays gave values on the same order of magnitude. However, while the samples from grain storage houses, the pig stables and the bird cages (pigeon and parakeet) gave higher values in the mAb-EIA, in poultry stables, the laboratory animal facility and outside the office, the Glucate[®] assay values were higher. That indicates a characteristically different dust and (1→3)-β-D-glucan composition at the workplaces. However, the dust at the grain storage houses and in the pig stables does not seem to consist primarily of (1→3)-β-D-glucans from barley and oat, otherwise the values in the Glucate[®] assay would have been expected to be much higher than with the mAb-EIA.

Altogether, the new developed mAb-EIA is particularly suitable for airborne dust samples. The detection limit is low enough to measure (1→3)-β-D-glucans even in low exposure areas, which is an advantage over the inhibition assay from Douwes et al. (1996). Further, the values are comparable to the *Limulus* amoebocyte lysate based Glucate[®] assay. As the mAb-EIA is based on exclusively on monoclonal antibodies, it is especially suited for standardisation. This fact, and the much lower price of components in comparison to the Glucate[®] assay, may contribute to the relevance and potential usage of the mAb-EIA. The assay can be used to quantify inhalable (1→3)-β-D-glucans to assess either the adverse or protective health effects of this immunologically active polysaccharide.

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