Asia Pacific Environmental and Occupational Health Journal (ISSN 2462 -2214), Vol 6 (1): 13 - 24, 2020
Published Online © 2020 Environmental and Occupational Health Society

A Rapid Quantitation of Occupational Aeroallergens by Magnetic Immuno-Chromatographic Test (MICT)

Dilek Inekci[1], Nicklas Skjoldager[1], Merete Simonsen[1], André Bergman[2], Fan Dong[2], and Bjarne Vincents[1]

[1]Novozymes A/S, Bagsvaerd, Denmark
[2]MagnaBioAnalytics LLC, San Diego, CA, United States

Corresponding author: Dilek Inekci, PhD. Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd Denmark.
Mobile: +45 30773335. E-mail: dkie@novozymes.com

ABSTRACT

Objective: Quantitative point-of-use assay platforms for the simple, rapid and accurate detection of aeroallergens in non-laboratory settings are fundamental to investigate their aerodynamics and implement preventive interventions for aeroallergen-associated diseases. Here, we demonstrate the application of the novel assay platform Magnetic Immuno-Chromatographic Test (MICT) for the quantitation of fungal alpha-amylase in one step and with a total assay time of 30 minutes. MICT is a magnetic-based lateral flow immunoassay (LFIA) integrated into a lab-on-a-chip format and here developed with a novel built-in hook alert function.

Method: The superparamagnetic nanoparticle probes and the magnetic detection technology makes MICT superior over traditional chromophore-based LFIAs and allow a sensitivity and accuracy comparable with central laboratory immunoassays. The alpha-amylase MICT was adjusted to a dynamic range of 0.1 to 22.3 ng/mL showing an accuracy at 99.6% (%CV 9.5). The strong analytical performance of MICT in aeroallergen studies was demonstrated by spiking air sampling filters and performing air sampling trials and the results were compared with ELISA.

Result: The principal setup shows a novel, transferable and flexible approach for quantitation of various occupational allergens. The MICT offers the required functionalities to become the new versatile lab-on-a-chip tool for hazard prevention and control of occupational allergy and asthma by allowing continuous surveillance of the allergen levels in the working environment.

Keywords: *Aeroallergens; Enzyme exposure assessment; Immunochemical quantitation; Magnetic Immuno-Chromatographic Test (MICT); Occupational hygiene*

1. Introduction

Occupational allergic respiratory diseases and asthma are disorders caused by exposure to certain airborne particles at the workplace [1–8]. Development of respiratory allergy follows a two-step process: (1) Continuous exposure to the aeroallergen leading to sensitization of the individual and upon repeated exposure (2) induction of allergy with accompanying symptoms like sneezing, runny nose, watery eyes and cough, which eventually could lead to development of asthma [9,10]. Aeroallergen exposure studies and industrial hygiene surveillance programs are essential to elucidate the exposure problems and implement hazard controls [11,12]. These include regular monitoring of the occupational air quality by air sampling, filter extraction and measurement by bioanalytical assays. While the conventional enzyme-linked immunosorbent assay (ELISA) is used in some specialized laboratories,

there is no standardized bioanalytical platform for occupational aeroallergen exposure assessments, limiting the comparability between routine occupational hygiene surveys and epidemiological studies [13]. Moreover, the time-consuming and labour-intensive process of ELISA and the necessity of professional expertise and advanced laboratory facilities, obstacle the efficacy and point-of-use needs of the exposure assessment analyses.

Lateral flow immunoassays (LFIAs) merge the technology of ELISA and chromatography and provide a sensitive, simple and rapid point-of-use bioanalytical platform [14,15]. LFIAs have the advantage of simple instrumentation, portability, low operational cost, rapidity and user friendliness. The assay platform has a broad range of applications in analysis of various analytes and, indeed, the variety of clinical and non-clinical commercial applications of LFIA-based devices is still increasing [16]. Compared with ELISA, LFIA is flexible and can be tailored to meet the needs of a specific application and testing environment with no need of infrastructure. Moreover, the ongoing advances in materials and facilitative technologies have moved LFIA towards a versatile technology with an analytical performance comparable to ELISA [17].

Magnetic Immuno-Chromatographic Test (MICT) is a LFIA-based lab-on-a-chip assay using superparamagnetic nanoparticles (SPNP) for signal generation and detection, which has been demonstrated to provide higher analytical sensitivity and quantitative capacity when compared with other common chromophore-based LFIA systems [16,18–21]. In addition, the MICT is here developed with a build-in alert function rejecting results from antigen overload. In this study, we developed and validated a MICT for the quantitative analysis of the occupational aeroallergen, fungal alpha-amylase. Alpha-amylase has long been used as a supplement in flour in several baking products to improve the dough quality. Various studies have shown that exposure to flour and flour supplements in bakeries is a risk factor for the development of symptoms of occupational allergic respiratory diseases and asthma. These symptoms have commonly been attributed to wheat flour proteins and fungal alpha-amylase but other components of flour dust may also play a role in respiratory symptoms [22–24].

Based on the present knowledge, continuous monitoring of alpha-amylase exposures by MICT would aid in the control of the risk of sensitization and development of respiratory symptoms. Although a few assays have been reported in the literature, the alpha-amylase MICT is the first commercially available point-of-use assay for the detection and quantitation of alpha-amylase exposure [25,26]. In addition, to distinguish between endogenous alpha-amylases and supplemented alpha-amylases, the assay cannot be activity-based and only immuno-based methods are applicable. Here, the performance of MICT was compared to that of a specific alpha-amylase ELISA by using spiked filter samples and air samples from a controlled exposure trial. The simplicity and rapidity of the assay combined with the high accuracy and sensitivity promise an analytical tool for quantitative analysis of various occupational aeroallergens in non-laboratory environments and has the potential to be a key enabling platform in industrial hygiene surveillance programs.

2. Materials and Method

2.1 Anti-alpha-amylase IgG for the immunoassays

A fungal alpha-amylase was recombinantly expressed and purified by Novozymes A/S, Denmark. The concentration of purified alpha-amylase was calculated from the enzyme activity and its specific activity. Polyclonal anti-alpha-amylase IgGs were produced by Dako, Denmark. Four rabbits and two goats were immunized by subcutaneous injection with 0.1 mL emulsion of 0.1 – 0.2 mg purified alpha-amylase protein mixed with Freund's incomplete adjuvant in equal volumes. Four consecutive immunizations were performed with 2-week intervals and the animals were bled 14 days after the last immunization. After week 4, injections were given at 4-week intervals until stable titer levels were reached and the animals were bled 14 days after each immunization. Serum titers were assessed by direct ELISA and Mancini's radial immunodiffusion test. The rabbit and goat IgGs were enriched by ammonium sulphate precipitation and purified by ion exchange chromatography. The rabbit IgGs were further purified on a Protein A column (Thermo Fischer Scientific, USA). The purified IgGs were dialysed against 0.01 M PBS and stored at 4 °C upon addition of 0.02% (w/v) sodium azide (Sigma, USA).

2.2 Preparation of IgG-SPNP conjugates

SPNPs were conjugated to the rabbit anti-alpha-amylase IgG for the test conjugate and goat IgG (Arista Biologicals, USA) for the control conjugate using cross-linking chemistries as previously described16,18,27. Briefly, carboxylated SPNPs (300 nm, Ademtech, Pessac, France) were activated with N-hydroxy-succinimide (NHS) and 1-ethyl-3-[3-

dimethylaminopropyl]carbodiimide (EDC) in MES buffered saline, pH 4.7, to form an amine reactive sulfo-NHS ester. Anti-alpha-amylase IgGs and control IgG were added to the activated SPNPs in 50 mM borate buffer, pH 8.5, resulting in an amide bond between the IgGs and the SPNPs. Residual active coupling sites were blocked by addition of 5% amicase for 30 min at 37 ºC followed by washing. The rabbit anti-alpha-amylase conjugate was mixed with goat IgG conjugate at appropriate ratio and lyophilized in a buffer containing 3% sucrose, 1% trehalose, 2% BSA, 1% Tween 20 in 10 mM Tris-HCL, pH 8 in microtubes. The conjugate tubes were stored in desiccated condition until further packaging into assay device.

2.3 Fabrication of alpha-amylase MICT cassette

The MICT devices were fabricated as described [16,18,27]. Briefly, rabbit anti-alpha-amylase IgG, 1.5 mg/mL, and donkey anti-goat IgG, 0.75 mg/mL, (BiosPacific, USA) in 10 mM potassium phosphate buffer were dispensed separately onto a nitrocellulose membrane (Hi Flow plus HF75; Millipore, MA, US) to constitute the test line (T) and control line (C) using a Biodot RR4500 liquid dispenser (Biodot Inc., Irvine, CA, USA).

Purified alpha-amylase antigen was also dispensed on the same membrane as a hook alert line (H) to prevent high dose hook effect causing reporting errors28. The membrane was dried at 45 ºC, followed by blocking as previously described to reduce nonspecific particle binding on membrane [18]. The dried nitrocellulose membrane was laminated onto a MICT base card (US 20070287198A1) with microfluidic flow channel, adsorbent pad and cover tape. The fully assembled card was cut to 5 mm strips using a customized Biodot CM4000 cutter (Biodot, CA, US) and incorporated into a plastic cassette (MagnaBioAnalytics, CA, US). The positions of T, C and H lines on the fully assembled MICT test strip is illustrated in Fig. 1.

2.4 Calibration of MICT device

The MICT alpha-amylase device was calibrated using alpha-amylase calibration standard in 0.01 M PBS, 0.023% Brij 30 (v/v), 0.5% BSA (w/v). Alpha-amylase calibrators covering a range of 0 – 750 ng/mL were tested in three to eight replicates in a single MICT reader over three days. Regression analysis of data from 0 – 22.3 ng/mL alpha-amylase were performed using average results from three MICT readers to generate standard curve coefficients for reporting alpha-amylase concentration based on measured T line and C line signals. Data from calibrators with alpha-amylase concentrations greater than 50 ng/mL were used to determine the cut-off value for reporting extremely high alpha-amylase concentrations. All calibration information was encoded in a 2D barcode on the test cassette as illustrated in Fig. 1.

2.5 MICT reader

To achieve a quantitative measure, a Magnetic Assay Reader (MAR) technology was used (US 6275031B1, US 6607922B2 and US 7547557B2). The MAR system uses two sensors which are placed on the surfaces of two magnetic pole pieces containing a high frequency alternating magnetic field (Fig. 2). The strip of the MICT cassette fits between these pole pieces and between the magnetic sensors. The sensors are configured as a gradiometer designed to produce an electric signal when an inhomogeneous magnetic field is detected.

As the SPNPs immobilized on the reaction lines of the strip pass through the magnetic field and over the sensors, the superparamagnetic properties of the particles cause them to align with the magnetic field and disrupt the field passing through the sensors, thus creating an inhomogeneity that is proportional to the total mass of the particles. This creates a signal in the sensors that is directly proportional to the mass of the particles trapped in the reaction lines and is reported as Relative Magnetic Units (RMU). By quantitating the RMU signals produced by each line on the assay device, the reader can use the ratio of the signals produced by both the T line and the C line (T/C) as well as the H line and C line (H/C) as independent variables in computing the dependent concentration values. This reduces the test-to-test variations associated with systemic variables such as sample volume, temperature and magnetic calibration. Each test cassette incorporates a 2D barcode that contains the information needed by the reader to locate and quantitate the signals produced by the reaction lines and contains the lot-based information needed to convert these to analyte concentrations along with the limits of detection for the given assay.

2.6 Assay Procedure

Samples (100 µL) were prepared in 0.01M PBS, 0.023% Brij 30 (v/v) (Sigma, USA), 0.5% BSA (w/v) (Sigma, USA), added to the microtube containing lyophilized SPNP-conjugated rabbit anti-alpha-amylase IgG and SPNP-conjugated goat control IgG and mixed by pipetting until the pellet was fully dissolved. The entire conjugate/sample content was transferred to the sample pad of the MICT cassette and incubated for 30

minutes at RT. The T, C and H lines of the assay were read by a MAR (MagnaBioscience, LLC).

2.7 Technical validation

The accuracy and reproducibility of the alpha-amylase MICT assay were tested by measurement of six replicates of seven calibrator samples with concentration levels covering the dynamic range of the calibration curve (0.1 – 22.3 ng/mL) on three days. The position of the MICT hook point was verified by measuring of alpha-amylase calibrator samples in concentrations up to 750 ng/mL.

2.8 Alpha-amylase ELISA

A 96-well microplate was coated with rabbit-anti-alpha-amylase IgGs in 0.05 M carbonate buffer (pH 9.6) by incubation at 5 ºC overnight. The wells were blocked with 2% BSA, 0.01 M PBS at RT for 1 h. Samples were serially two-fold diluted in 0.01 M PBS, 0.023% Brij 30 (v/v) (Sigma, USA), 0.5% BSA (w/v) (Sigma, USA) and incubated at RT for 1 h. Goat-anti-alpha-amylase IgG in 0.01 M PBS, 0.023% Brij 30 (v/v), 0.5% BSA (w/v) buffer was added and incubated for another 1 h at RT. Subsequently, bovine-anti-goat IgG-HRP conjugate (Jackson ImmunoResearch, UK) was added and incubated at 30 min at 37 °C. The optical densities (ODs) were measured at 490/620 nm with a microplate reader after colour development with OPD (o-Phenylenediamine dihydrochloride) solution (Sigma, USA) and termination with 1 M H2SO4. During the steps an ELISA washer (BioTek Elx405, BioTek Instruments, Winooski, VT, USA) with 0.01 M PBS, 0.05% Tween 20 (v/v) wash buffer was used to remove unbound antigens or antibodies. A calibration curve was performed by a 9-point dilution of the purified alpha-amylase at 0, 0.11, 0.22, 0.45, 0.89, 1.06, 1.30, 1.58, 1.86, 2.23 ng/mL.

2.9 Spiking of filters with known enzyme amount

Teflon filters (37 mm, 1 µm pore size, Millipore) were spiked 200 µL buffer solution (0.01 M PBS, 0.023% Brij 30 (v/v), 0.5% BSA (w/v)) containing alpha-amylase [Fungamyl 800L, Novozymes A/S], in three different concentrations, low, medium and high, giving a final enzyme load of 12, 46 and 185 ng/filter, respectively. The filters were dried O/N at RT, thus spiking the surface of the filters with enzyme. The spiked filters (n=4) were eluted in 5 mL 0.01M PBS, 0.023% Brij 30 (v/v), 0.5% BSA (w/v) buffer for 30 min with magnetic stirring. The eluates were analysed in four replicates by MICT and in duplicate over a six 2-fold dilution series for ELISA.

2.10 Exposure with aerosolized enzyme

To simulate the exposure to alpha-amylase in occupational settings, a controlled exposure trial was conducted by adopting a standardized experimental set-up29 (Suppl. Fig. 1). Alpha-amylase, 10 µg/mL, was formulated with stabilizing agents (3.33 g/L Model detergent A liquor containing 12% LAS, 11% AEO Biosoft N25-7 (NI), 5% AEOS (SLES), 6% MPG (mono propylene glycol), 3% ethanol, 3% TEA (triethanolamine), 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 2% sodium hydroxide, 2% sodium citrate, 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (propenoic acid)) and applied with a spray bottle to generate aeroallergen particles.

Briefly, in a closed room with no ventilation each spray (n=4) was applied to a vertical target surface of framed fabric by executing five trigger-spray events with a frequency of 1 event (~1 g) per second at a distance of 15 cm while air was sampled in four replicates using air sampling pumps (25 L/min) and air-sampling heads mounted with teflon filters (37 mm, 1 µm pore size, Millipore) over a period of 11 min where the air-sampling was started 1 min prior to the applications and stopped 10 min after (Suppl. Fig. 1). The room was ventilated between each spray bottle replicate and background monitoring was conducted prior to each cycle. The collected air-sampling filters were eluted in 5 mL 0.01 M PBS, 0.023% Brij 30 (v/v), 0.5% BSA (w/v) buffer for 30 min with magnetic stirring. The eluates were analysed in four replicates by MICT and in duplicate over a six 2-fold dilution series for ELISA.

2.11 Data analysis

All data were analysed by JMP® Software 14.0 from SAS Institute. The agreement between MICT and ELISA was assessed by constructing Bland-Altman plots with the mean differences between MICT and ELISA measurements and the 95% limits of agreement (LOA)30. A standard least squares regression analysis was used to analyze the method comparison for the dataset with air filters spiked with a known enzyme amount. A p-value of < 0.05 was deemed statistically significant.

3. Results

3.1 Principle of MICT

The alpha-amylase MICT is a two-site sandwich immunoassay in a direct flow format, part microfluidic and part capillary membrane (Fig. 3). In the first step SPNP-conjugated anti-alpha-amylase IgG specifically bind to the target molecule in a liquid sample. Upon application of the SPNP-IgG-alpha-amylase complex to the cassette, the complex migrates along the strip by lateral flow, entering the nitrocellulose membrane and is captured by the alpha-amylase-specific IgG immobilized on the T line.

The residual SPNP-antibody conjugates continue migrating along the membrane and are detected on the hook line. A SPNP-goat IgG conjugate is applied together with the specific conjugate as a built-in procedural positive control and is detected by donkey anti-goat IgG on the C line. Data is reported as the intensity of the magnetic signal which is proportional to the amount of SPNP-antibody-antigen complex formed in the T line normalized with intensity from the C line (T/C). The intensity of the hook effect alert line is inversely proportional with alpha-amylase concentrations allowing detection of alpha-amylase concentrations significantly greater than 50 ng/mL.

3.2 Evaluation of technical parameters

Several assay parameters were optimized before evaluation of the technical performance of the assay, including the concentration of IgGs, conjugate volume and different buffering solutions. The dynamic range of the alpha-amylase MICT was set to be 0.1 – 22.3 ng/mL. The assay accuracy was defined as percent relative to target concentration and determined as 99.6% with a %CV of 9.5 (Suppl. Table 1).

The MICT dose-response curve shows a hook effect at high concentrations of the analyte, giving a falsely low result (Fig. 4) [28,31,32]. The high-dose hook effect occurs when a large excess of analyte binds to both the capture and detection antibody and prevents the formation of immune complexes, resulting in a decrease in assay signal. Therefore, an analyte excess alert function is programmed into the MICT software. In case of an analyte concentration exceeding the dynamic range of the assay, the operator is warned and must re-test at higher dilution. The MICT dose-response curve shows increasing signals proportional to the analyte amount loaded from 0.1 ng/mL to 150 ng/mL, where the signal starts to level off. The hook threshold of the alpha-amylase MICT was set as > 50 ng/mL corresponding to three times lower than the observed value.

3.3 Comparison between ELISA and MICT

A method-comparison study was conducted to compare the analytical performance of the alpha-amylase MICT against an established fungal alpha-amylase ELISA. Two sets of air sample filters were prepared and analysed in both MICT and ELISA. The first set of samples were prepared by direct spiking of air filters with three known alpha-amylase concentrations at low, medium and high levels (Fig. 5). The agreement between the fungal alpha-amylase MICT and ELISA was evaluated by a standard least squares regression analysis showing no significant difference between the measurements of the two methods ($p < 0.05$) at any of the tested concentrations.

The comparison of MICT and ELISA was further assessed by construction of Bland-Altman plots showing a strong agreement and verifying the interchangeability of the two methods with a mean difference of 0.02 – 1.59 ng/filter ($p > 0.05$) (Fig. 6, Suppl. Table 2). The ELISA and MICT measurements are giving somewhat lower values than the activity-based pre-adsorption amount, which most likely is a consequence of loss of surface structures when the alpha-amylase is adsorbed to the filters or loss of protein during filter elution.

The second set of air filter samples were collected from an aeroallergen exposure trial, simulating an intensive presence of airborne fungal alpha-amylase in an occupational setting. A previously described standardized lab-scale exposure set-up was used to create conditions simulating human inhalation of an aeroallergen [29]. The alpha-amylase was aerosolized by sprayers, and the enzyme aerosols were trapped on filters connected to a sampler simulating a nasal airflow. The Bland-Altman difference plots showed a good agreement in alpha-amylase levels measured by MICT and ELISA supporting the interchangeability of the two methods (Fig. 7, Suppl. Table 3).

Of unidentified reasons one of the sprayers showed a significant negative bias ($p < 0.03$), with a mean of 29.33 ng/mL or 16% in MICT compared with the ELISA method (Fig. 7.D). Overall, the MICT measurements of filters from the exposure trial were found to be slightly below the ELISA method. The absence of a gold standard method and the fact that the analyte concentration in the air is unknown, could imply a systematic underestimation of alpha-amylase in the MICT

method. However, the strong method agreement in the spike trial, where three known concentrations were tested, excludes a systematic discrepancy between the two methods. In seek of an explanation, it could be speculated if the stabilizing agents in the spray formulation could impact either of the assays in spite of substantial dilution during the filter elution.

4. Discussion

Alpha-amylase and other enzymes of bacterial and fungal origin have numerous applications as biocatalysts in industrial products and have a long history of safe use. They have the advantages of being biodegradable, water soluble and non-toxic. Enzymes, like other proteins, are aeroallergens and may induce sensitization and development of respiratory allergy in workers upon inhalation of airborne enzymes at occupational settings [1].

The first incidences of enzyme-related occupational allergy were seen during the 1960s because of introduction of subtilisin in the detergent industry [33]. Decades of preventative approaches like encapsulation of processes, ventilation and use of personal protection equipment as well as improvements of enzyme products towards safer formulations have minimized the exposure rates among workers [34,35]. However, there are still processes in some industrial settings that put employees at risk for enzyme exposure, emphasizing the importance of routine occupational hygiene surveys and epidemiological studies. Despite the decades-long knowledge of the risk with enzymes in occupational allergy, there is still no standardized analysis tool for measuring occupational airborne exposures to enzymes. The lack of a standardized analysis tool hampers the industry-wide implementation of routine occupational hygiene programs and limits the comparison between aeroallergen exposure studies.

This study investigated the potential of the MICT platform as the first standardized tool for aeroallergen exposure studies. To our knowledge, this is the first commercially available assay for aeroallergen analysis, facilitating standardization in the field. In contrast to laborious central laboratory immunoassays, the simplicity of the one-step MICT assay will allow rapid on-site analysis, wide implementation of exposure assessments and comparison of data from different studies.

In the lack of a gold standard method, the analytical performance of MICT was compared with ELISA. The alpha-amylase MICT was shown to be just as reliable fit for quantitative detection as ELISA. Moreover, MICT offered a broader dynamic range of ELISA, a total assay time of only 30 minutes and a built-in hook alert to adjust for assay overload that could otherwise result in skewed results. The analytical performance combined with rapidity and simplicity makes the MICT platform ideal for the analyses of various samples at non-laboratory settings with very limited access to infrastructure and expert personnel such as for occupational safety analyses, food safety analyses and quality tests. Future work will further explore the field applications of MICT by investigating real-case-scenarios for the use of the method.

Acknowledgement

The authors acknowledge the technical assistance from the laboratories of Application Safety, Novozymes A/S and MagnaBioAnalytics LLC. We thank Ditte Sidelmann Brinch, Nomi Skovgaard and Kristoffer E. A. Kristensen for comments for improving the manuscript.

Conflicts of Interest

Dilek Inekci, Nicklas Skjoldager, Merete Simonsen and Bjarne Vincents are employed by Novozymes A/S. André Bergman is the General Manager of MagnaBioAnalytics LLC. Fan Dong is employed by MagnaBioAnalytics LLC.

References

1. Larsen, A. I. et al. Allergy Development in Adulthood: An Occupational Cohort Study of the Manufacturing of Industrial Enzymes. J. Allergy Clin. Immunol. Pract. (2020) doi:10.1016/j.jaip.2019.06.007.

2. Karjalainen, A., Kurppa, K., Virtanen, S., Keskinen, H. & Nordman, H. Incidence of occupational asthma by occupation and industry in Finland. Am. J. Ind. Med. (2000) doi:10.1002/(SICI)1097-0274(200005)37:5<451::AID-AJIM1>3.0.CO;2-U.

3. McDonald, J. C., Keynes, H. L. & Meredith, S. K. Reported incidence of occupational asthma in the United Kingdom, 1989-1997. Occup. Environ. Med. (2000) doi:10.1136/oem.57.12.823.

4. Cathcart, M. et al. Enzyme exposure, smoking and lung function in employees in the detergent industry over 20 years. Occup. Med. (Chic. Ill). (1997) doi:10.1093/occmed/47.8.473.

5. Johnsen, C. R. et al. Allergy risk in an enzyme producing plant: A retrospective follow up study. Occup. Environ. Med. (1997) doi:10.1136/oem.54.9.671.

6. Larsen, A. I., Johnsen, C. R., Frickmann, J. & Mikkelsen, S. Incidence of respiratory sensitisation and allergy to enzymes among employees in an enzyme producing plant and the relation to exposure and host factors. Occup. Environ. Med. (2007) doi:10.1136/oem.2005.025304.

7. Schweigert, M. K., Mackenzie, D. P. & Sarlo, K. Occupational asthma and allergy associated with the use of enzymes in the detergent industry - A review of the epidemiology, toxicology and methods of prevention. Clinical and Experimental Allergy (2000) doi:10.1046/j.1365-2222.2000.00893.x.

8. Cullinan, P. et al. An outbreak of asthma in a modern detergent factory. Lancet (2000) doi:10.1016/S0140-6736(00)03264-5.

9. Platts-Mills, T. A. E., Vervloet, D., Thomas, W. R., Aalberse, R. C. & Chapman, M. D. Indoor allergens and asthma: Report of the Third International Workshop. J. Allergy Clin. Immunol. (1997) doi:10.1016/S0091-6749(97)70292-6.

10. Custovic, A. & Simpson, A. The role of inhalant allergens in allergic airways disease. Journal of Investigational Allergology and Clinical Immunology (2012).

11. Larsen, A. I. et al. Allergy Development in Adulthood: An Occupational Cohort Study of the Manufacturing of Industrial Enzymes. J. Allergy Clin. Immunol. Pract. (2019) doi:10.1016/j.jaip.2019.06.007.

12. Sarlo, K. Control of occupational asthma and allergy in the detergent industry. in Annals of Allergy, Asthma and Immunology (2003). doi:10.1016/S1081-1206(10)61646-8.

13. Raulf, M. et al. Monitoring of occupational and environmental aeroallergens - EAACI Position Paper. Allergy (2014) doi:10.1111/all.12456.

14. Urusov, Zherdev & Dzantiev. Towards Lateral Flow Quantitative Assays: Detection Approaches. Biosensors (2019) doi:10.3390/bios9030089.

15. Sajid, M., Kawde, A. N. & Daud, M. Designs, formats and applications of lateral flow assay: A literature review. J. Saudi Chem. Soc. (2015) doi:10.1016/j.jscs.2014.09.001.

16. Connolly, R. & O' Kennedy, R. Magnetic lateral flow immunoassay test strip development – Considerations for proof of concept evaluation. Methods (2017) doi:10.1016/j.ymeth.2017.02.002.

17. O'Farrell, B. Lateral Flow Immunoassay Systems: Evolution from the Current State of the Art to the Next Generation of Highly Sensitive, Quantitative Rapid Assays. in The Immunoassay Handbook (2013). doi:10.1016/B978-0-08-097037-0.00007-5.

18. Workman, S. et al. Rapid detection of HIV-1 p24 antigen using magnetic immuno-chromatography (MICT). J. Virol. Methods (2009) doi:10.1016/j.jviromet.2009.04.003.

19. Peck, R. B. et al. A magnetic immunochromatographic strip test for detection of human papillomavirus 16 E6. Clin. Chem. (2006) doi:10.1373/clinchem.2006.072884.

20. Hong, L. et al. High performance immunochromatographic assay for simultaneous quantitative detection of multiplex cardiac markers based on magnetic nanobeads. Theranostics (2018) doi:10.7150/thno.29070.

21. Moyano, A. et al. Magnetic immunochromatographic test for histamine detection in wine. Anal. Bioanal. Chem. (2019) doi:10.1007/s00216-019-02031-6.

22. Vanhanen, M. et al. Enzyme exposure and enzyme sensitisation in the baking industry. Occup. Environ. Med. (1996) doi:10.1136/oem.53.10.670.

23. Houba, R., Van Run, P., Doekes, G., Heederik, D. & Spithoven, J. Airborne levels of α-amylase allergens in bakeries. J. Allergy Clin. Immunol. 99, 286–292 (1997).

24. Marraccini, P. et al. Bakery flour dust exposure causes non-allergic inflammation and enhances allergic airway inflammation in mice. Clin. Exp. Allergy (2008) doi:10.1111/j.1365-2222.2008.03038.x.

25. Koets, M., Sander, I., Bogdanovic, J., Doekes, G. & Amerongen, A. Van. A rapid lateral flow immunoassay for the detection of fungal alpha-amylase at the workplace. J. Environ. Monit. (2006) doi:10.1039/b605389k.

26. Bogdanovic, J. et al. Rapid detection of fungal α-amylase in the work environment with a lateral flow immunoassay. J. Allergy Clin. Immunol. (2006) doi:10.1016/j.jaci.2006.07.004.

27. Handali, S. et al. Development and evaluation of a magnetic immunochromatographic test to detect taenia solium, which causes taeniasis and neurocysticercosis in humans. Clin. Vaccine Immunol. (2010) doi:10.1128/CVI.00511-09.

28. Amarasiri Fernando, S. & Wilson, G. S. Studies of the 'hook' effect in the one-step sandwich immunoassay. J. Immunol. Methods (1992) doi:10.1016/0022-1759(92)90104-2.

29. Weeks, J. A., Harper, R. A., Simon, R. A. & Burdick, J. D. Assessment of sensitization risk of a laundry pre-spotter containing protease. Cutan. Ocul. Toxicol. (2011) doi:10.3109/15569527.2011.565010.

30. Bland, J. M. & Altman, D. G. Measuring agreement in method comparison studies. Stat. Methods Med. Res. (1999) doi:10.1177/096228029900800204.

31. Selby, C. Interference in immunoassay. Ann. Clin. Biochem. 36, 704–721 (1999).

32. Hoffman, K. L., Parsons, G. H., Allerdt, L. J., Brooks, J. M. & Miles, L. E. Elimination of 'hook-effect' in two-site immunoradiometric assays by kinetic rate analysis. Clin. Chem. (1984).

33. Flindt, M. L. Pulmonary disease due to inhalation of derivatives of Bacillus subtilis containing proteolytic enzyme. Lancet (1969) doi:10.1016/s0140-6736(69)92165-5.

34. Basketter, D. A. et al. Managing the Risk of Occupational Allergy in the Enzyme Detergent Industry. J. Occup. Environ. Hyg. (2015) doi:10.1080/15459624.2015.1011741.

35. Basketter, D., Berg, N., Kruszewski, F. H., Sarlo, K. & Concoby, B. Relevance of sensitization to occupational allergy and asthma in the detergent industry. J. Immunotoxicol. (2012) doi:10.3109/1547691X.2012.656855.

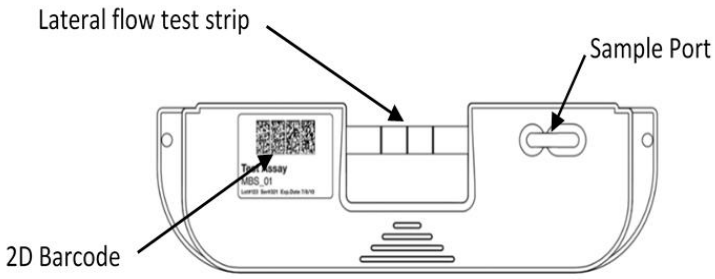

Fig. 1. The assembled MICT cassette (11.5 x 3 x 0.5 cm) containing the test strip and a 2D barcode of assay specifications needed by the Magnetic Assay Reader to quantitate the concentration of the analyte. The vertical lines on the lateral flow test strip indicates the T, C and H control lines, respectively from the left.

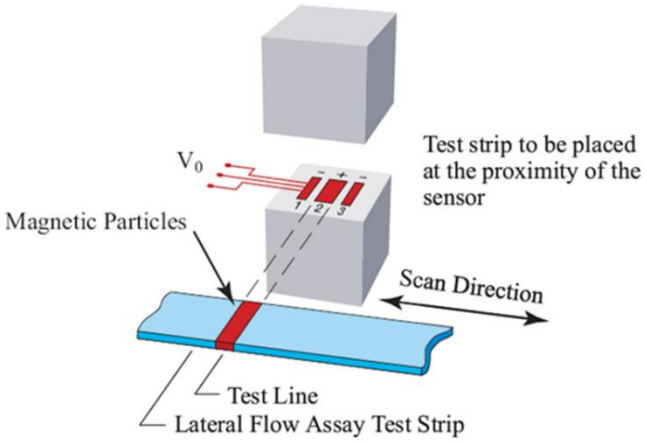

Fig. 2. Illustration of the MAR technology using an array of thin film coil sensors arranged as a gradiometer. The sensors measure the magnetic field induced in the SPNPs immobilized in the reaction lines and compare to a master curve using numerical curve-fitting methods. The amplitude of magnetic signal is directly proportional to the amount of SPNP conjugates immobilized in the reaction lines

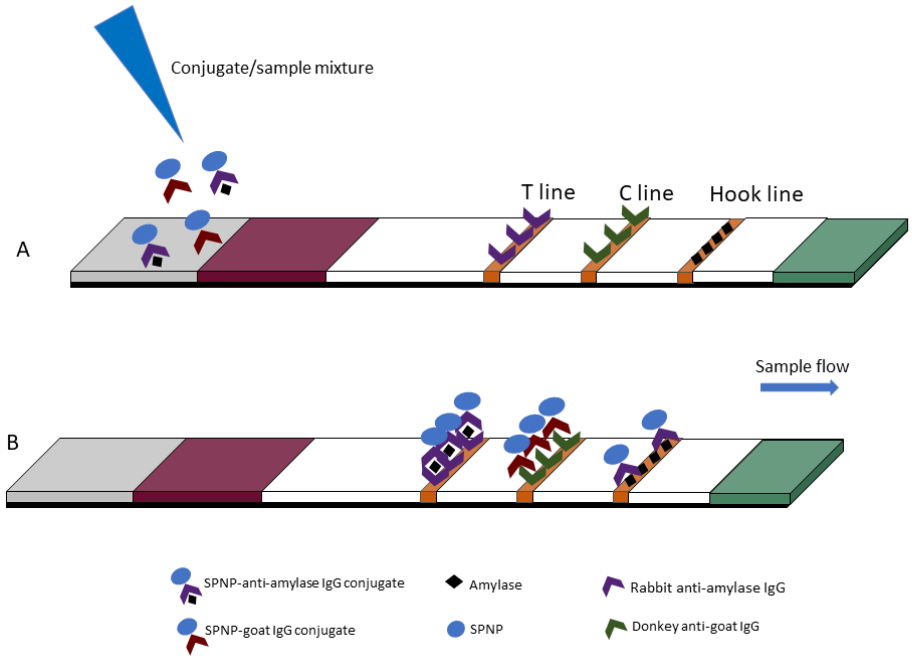

Fig. 3. Schematic illustration of the MICT assay. (A) The MICT strip, consisting of a sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad. Sample with alpha-amylase is mixed with SPNP-anti-alpha-amylase conjugate and the mixture is transferred to the sample pad of the strip. (B) The SPNP-anti-alpha-amylase IgGs complexed with alpha-amylase migrate along the strip and bind to the immobilized anti-alpha-amylase antibody on the T line to form a sandwich complex. SPNP-non-specific IgG conjugate is captured by the anti-IgG antibody on the C line. The residual SPNP-anti-alpha-amylase conjugates continue migrating along the membrane and are detected on the H line which prevents invalid results caused by the hook effect at high analyte concentrations.

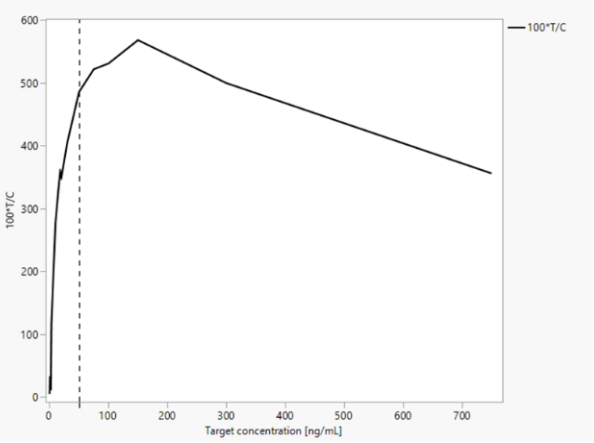

Fig. 4. The MICT dose-response curve. The assay signal levels off at approximately 150 ng/mL showing a hook effect for concentrations above 150 ng/mL. The dotted line indicates the applied hook cut-off threshold of the alpha-amylase MICT (50 ng/mL).

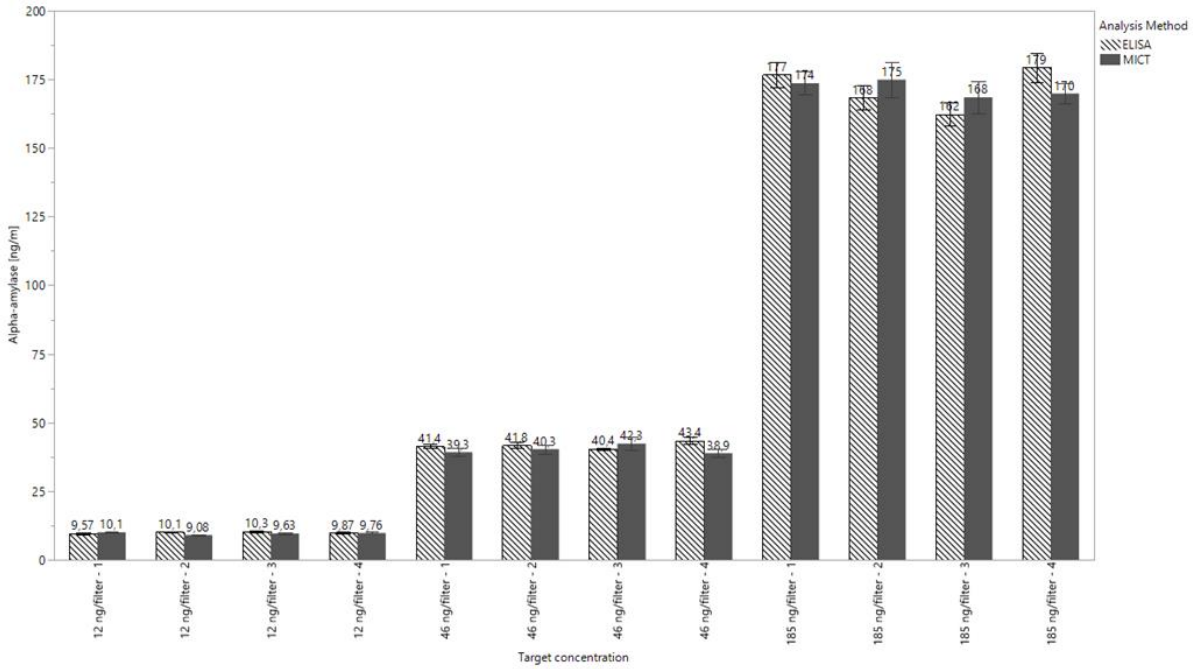

Fig. 5. Alpha-amylase levels measured by MICT and ELISA in air filters spiked with a low (n=4), medium (n=4) and high (n=4) amount of alpha-amylase, respectively. Each bar represents a mean of four replicates for MICT and two to eight replicates for ELISA. The error bars represent the standard error.

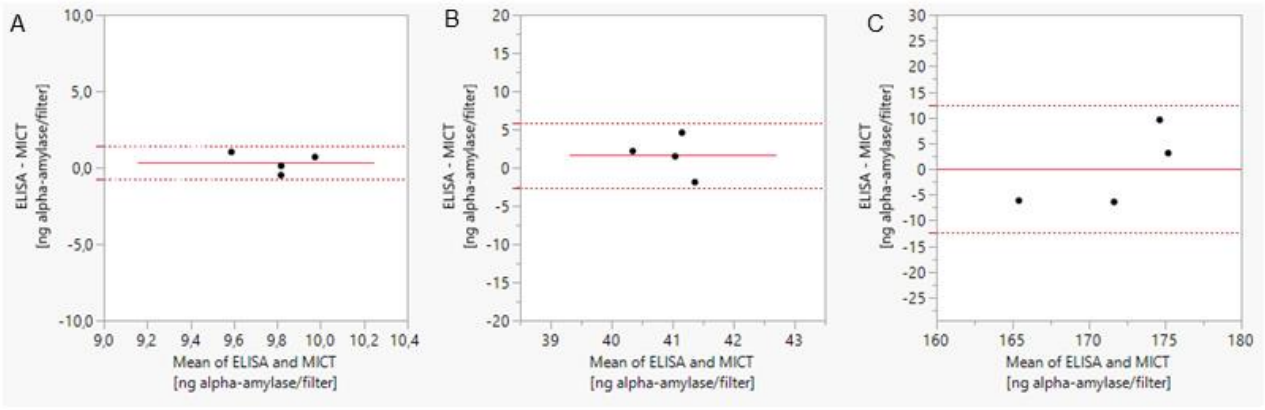

Fig. 6. Bland-Altman plots illustrating the agreement between MICT and ELISA measurements of fungal alpha-amylase levels in air filter samples spiked with a known amount of enzyme (A: low, B: medium and C: high). The difference between the measurement of MICT and ELISA is plotted as a function of the mean of the two values. The solid and dotted lines represent the bias and LOAs, respectively. The Bland-Altman plot was calculated based on mean measurements for MICT and ELISA, which were based on four filter samples for each tested concentration (high, medium and low) measured in four replicates for MICT and two to eight replicates for ELISA.

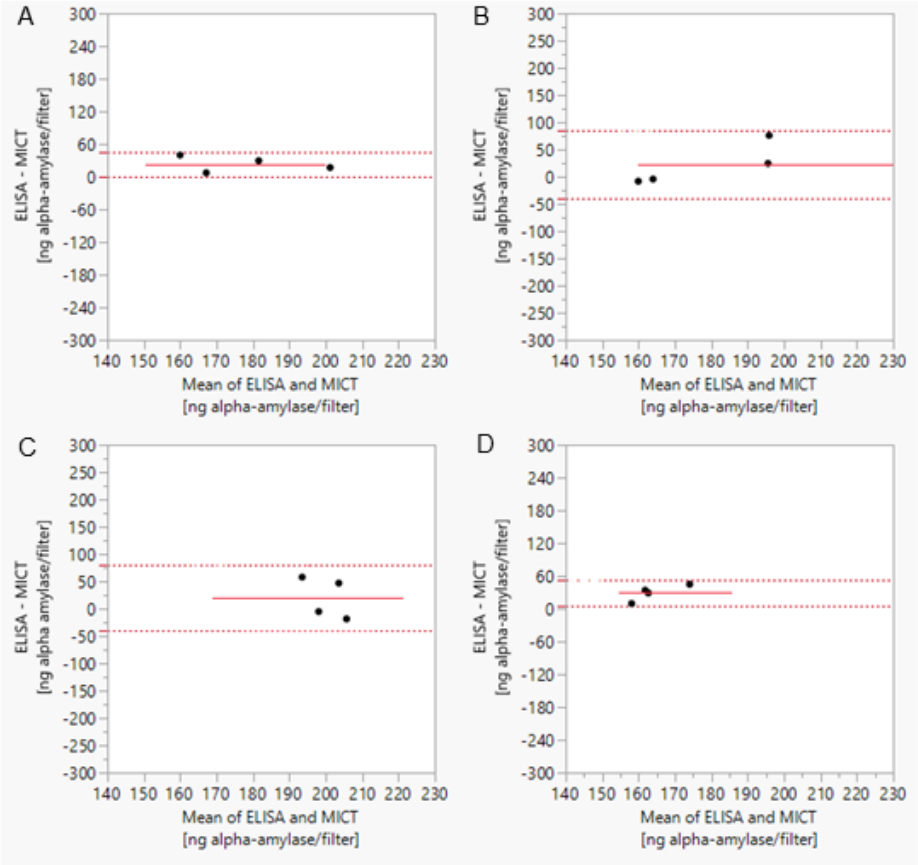

Fig. 7. Bland-Altman plots illustrating the agreement between MICT and ELISA measurements of fungal alpha-amylase in air filter samples collected during an exposure trial. The difference between the measurement of MICT and ELISA is plotted as a function of the mean of the two values. The solid and dotted lines represent the bias and LOAs, respectively. The exposure trial was repeated by using four sprayers (A, B, C and D) and four air filters were collected for each, which were measured in four replicates for MICT and two to eight replicates for ELISA.