

# INDOOR EXPOSURE TO MOULD ALLERGENS

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Humid indoor environments may be colonised by allergenic filamentous microfungi (moulds), *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Alternaria* spp. in particular. Mould-induced respiratory diseases are a worldwide problem. In the last two decades, mould allergens and glucans have been used as markers of indoor exposure to moulds. Recently, mould allergens Alt a 1 (*Alternaria alternata*) and Asp f 1 (*Aspergillus fumigatus*) have been analysed in various environments (residential and occupational) with enzyme-linked immunosorbent assays, which use monoclonal or polyclonal antibodies. Household Alt a 1 and Asp f 1 levels were usually under the limit of the method detection. By contrast, higher levels of mould allergens were found in environments with high levels of bioaerosols such as poultry farms and sawmills. Data on allergen Alt a 1 and Asp f 1 levels in agricultural settings may provide information on possible colonisation of respective moulds and point out to mould-related diseases in occupants.

**KEY WORDS:** *Alt a 1, Asp f 1, A. alternata, A. fumigatus, ELISA, occupational exposure*

Humans continuously inhale spores and fragments of allergenic moulds. Exposure to fungal allergens in outdoor and indoor environments might result in respiratory diseases in sensitive individuals (1). However, indoor exposure to mould allergens has not received due attention. This article gives a brief review of the findings of Alt a 1 (*Alternaria alternata*) and Asp f 1 (*Aspergillus fumigatus*) as markers of mould exposure in various environments reported by us and other researchers.

## DISTRIBUTION OF MOULDS

### *Outdoor environments*

Generally, *Cladosporium* spp. and *Alternaria* spp. are the most common moulds outdoors worldwide (2-4). Fungal ecology is influenced seasonally, geographically, and by the diurnal cycle (2, 5). In Zagreb, Croatia, *Alternaria* spp. and *Cladosporium*

spp. spores are detectable from May to November, peaking in the late summer (6). A similar seasonal pattern has been reported in other temperate European climates (7). On 17 days of August 2003, Zagreb air concentration of *Alternaria* spores exceeded the limit value of 100 spores per m<sup>3</sup>, which is associated with increased risk of allergy (8). This concentration of airborne moulds was influenced by meteorological conditions (temperature, humidity, solar radiation, wind, and rainfall). In the United Kingdom, *Alternaria*-related asthma has been reported in association with thunderstorms in the summer of 2002 (9). *Alternaria* spores can get dispersed over hundreds of miles from the source, especially in dry seasons. In addition, human activities, especially in agricultural settings can considerably contribute to the mould load in outdoor environments (10, 11).

### *Indoor environments*

Healthy houses and buildings with low indoor humidity do not favour indoor fungal growth (10).

Generally, the composition of indoor airborne fungi in healthy, non-mouldy houses resembles the composition of outdoor fungi (*Alternaria* and *Cladosporium*) (2, 12, 13-15). Table 1 shows a marked difference between the distribution of moulds in healthy and sick/mouldy dwellings. It also shows currently thresholds for moulds in homes. In healthy indoor environments, indoor-to-outdoor mould ratio (I:O) is <1 (2, 10, 16). Shelton et al. (2) reported a median I:O in a US building of 0.16. In contrast, in sick buildings the I:O ratio was >1, which points to considerable indoor sources of moulds. In damp buildings, moulds can grow and sporulate on the surface of the building material and in settled dust (15). *Aspergillus versicolor* is one of the most common moulds in damp buildings in European countries, the United States, and Canada (17). Pei-Chih et al. (13) reported an extremely high I:O ratio of 9.6 for *Aspergillus* in a suburban area of Taiwan. In indoor environments heavily contaminated with fungi, mould concentrations are usually up to three orders of magnitude higher than in healthy houses (Table 1). The highest fungal load has been detected in mould-contaminated houses in New Orleans after Hurricane Katrina (18, 19). Based on previous studies, moulds are suggested as bioindicators of indoor air quality (10).

#### Mould spores and fragments

The aerodynamic size of the spores is typical for the species. The conidia of the *Aspergillus* spp. and *Penicillium* spp. (mostly 2 µm to 5 µm in diameter) are relatively small compared to the spores of *Alternaria* spp. (10 µm to 40 µm in diameter) (1, 20). During sporulation, mould spores and hyphae are released into the air in a large number and inhaled (21). Generally, airborne mould spores with the aerodynamic diameter of >10 µm are deposited in the

upper respiratory tract. They are associated with nasal and ocular symptoms (hay fever) (22, 23). On the other hand, particles in the range of 2 µm to 10 µm, especially those <5 µm, are respirable and can trigger allergic reaction. Laboratory-based studies have shown that moulds release into the environment large quantities of particles far smaller than spores (0.03 µm to 2 µm in diameter) (24-27). This fragmentation of spores is caused by various physical and biological processes (21-25, 28, 29). In two school buildings, the highest concentration of airborne fungal fragments was in the size range of 1.1 µm to 4.7 µm. This indicates that smaller particles are very common, especially in damp buildings as opposed to healthy ones (30). Similarly, Gorny et al. (24) and Reponen et al. (29) demonstrated considerably higher levels of mould fragments than of intact spores in mould-contaminated buildings. Inhaled ultrafine particles (<1 µm) can penetrate deeply into the lung alveoli and make the respiratory symptoms and diseases even worse (28-30).

#### ALLERGENS OF *A. FUMIGATUS* AND *A. ALTERNATA*

Most fungal allergens have been identified from their conidia, hyphae, and small mycelial fragments (24, 31, 32). All these particles contribute to exposure and allergic sensitisation in humans. Fungal allergens have been investigated systematically only in *A. fumigatus*, *A. alternata*, and *Cladosporium herbarum* (22, 33). Among more than 100 genera of fungal aeroallergens, *A. fumigatus* produces more allergenic molecules than other moulds (31-34). Table 2 lists *A. fumigatus* allergens recognised by World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-

**Table 1** Mould aerosols in healthy, sick, and flooded buildings

| Building             | I:O <sup>a</sup> | Dominant mould                             | Total fungi /<br>cfu m <sup>-3</sup>    | Spores<br>per m <sup>3</sup>                | Mycotoxin       | Stachybotrys | Ref.      |
|----------------------|------------------|--|---|---|-----------------|--------------|-----------|
| Healthy <sup>b</sup> | <1               | <i>Cladosporium</i>                        | 10 to 10 <sup>3</sup>                   | <1000                                       | nd <sup>c</sup> | nd           | 2, 10     |
| Sick                 | ≥1               | <i>Penicillium</i> ,<br><i>Aspergillus</i> | >10 <sup>3</sup>                        | >1300                                       | detected        | detected     | 2, 91, 92 |
| Flood-<br>affected   | >1               | <i>Penicillium</i> ,<br><i>Aspergillus</i> | 22x10 <sup>3</sup> to 5x10 <sup>5</sup> | 82x10 <sup>3</sup> to<br>63x10 <sup>4</sup> | detected        | detected     | 18, 19    |

<sup>a</sup>Indoor-outdoor mould ratio

<sup>b</sup>non-mouldy

<sup>c</sup>not detected

committee. Currently, the list refers to 23 molecules (35). Asp f 1 (ribotoxin) is the main allergen and a critical factor in provoking allergic response (type I hypersensitivity) and bronchopulmonary aspergillosis (36-40). Asp f 1 is a conserved protein, highly specific to *A. fumigatus* and not present in other members of the genus (35, 36, 41). Recently, Asp f 34 has also been identified as a major allergen associated with sensitisation to *Aspergillus* (42). With the exception of Asp f 1 and Asp f 5 (metalloprotease), *A. fumigatus* allergens are homologous to the allergens of *A. nidulans*, *A. oryzae*, and other fungal species (35, 41). In addition, some allergens such as Asp f 11 and Asp f 27 (cyclophilins), and Asp f 28 and Asp f 29 (thioredoxins) are homologous to human antigens (cross-reactive pan-allergens) (38). Allergens from other aspergilli has been less investigated. Beendorf et al. (43) identified seven allergens in *A. versicolor* spores. Recently, Liang et al. (17) identified two proteins (with molecular mass of 43 kDa and 41 kDa) in *A. versicolor*, which could be specific for this mould. *A. niger* and *A. oryzae* used in industrial

fermentations have been recognised as important occupational allergens in baker's asthma (33).

Table 3 shows *A. alternata* allergens. They include the following proteins: aldehyde dehydrogenase, heat shock protein, enolase, ribosomal protein P1 and P2, as well as other related uncharacterised proteins (33, 45). Alt a 1 is the major allergen of *A. alternata*, as more than 80 % of patients allergic to *Alternaria* spp are sensitised to it (45, 46). Recent studies have found homologues of Alt a 1 in the phylogenetically related *Stemphylium* and *Ulocladium* species. This suggests that Alt a 1 may not be species-specific (47, 48). Several other *A. alternata* allergens have shown significant cross-reactivity with a closely related mould *C. herbarum* and with non-fungal species (47). Other worldwide-occurring moulds such as *C. herbarum* and *Penicilium* spp. are also important sources of allergens for mould-allergic patients (33). Cross-reactivity between the most common allergenic moulds has been presented elsewhere (47-51).

Several studies have shown that germination of *Alternaria* and *Aspergillus* spores plays a significant role in allergen release in the environment, including the major respective allergens Alt a 1 and Asp f 1 (32, 52, 53).

**Table 2** Allergens of *A. fumigatus*

| Allergen         | Nature of allergen                               |
|------------------|--|
| Asp f 1          | Ribotoxin, major allergen                        |
| Asp f 2          | Fibrinogen binding protein, major allergen       |
| Asp f 3          | Peroxisomal membrane protein                     |
| Asp f 4          | Unknown  |
| Asp f 5          | Metalloproteinase, protein degradation           |
| Asp f 6          | Mn-superoxide dismutase, pan-allergen            |
| Asp f 7          | Unknown  |
| Asp f 8          | Acidic ribosomal protein P2                      |
| Asp f 9/<br>Crfl | Cell wall glucanase                              |
| Asp f 10         | Aspartic endopeptidase, protein degradation      |
| Asp f 11         | Cyclophilin, cross-reactive pan-allergen         |
| Asp f 12         | Heat-shock protein, hsp 90                       |
| Asp f 13         | Alkaline serine protease                         |
| Asp f 15         | Alkaline serine protease                         |
| Asp f 16         | Putative glycosylhydrolase                       |
| Asp f 17         | Galactomannoprotein                              |
| Asp f 18         | Vacuolar serine protease                         |
| Asp f 22         | Enolase  |
| Asp f 23         | L 3 ribosomal protein                            |
| Asp f 27         | Cyclophilin, cross-reactive pan-allergen         |
| Asp f 28         | Thioredoxin, cross-reactive pan-allergen         |
| Asp f 29         | Thioredoxin, cross-reactive pan-allergen         |
| Asp f 34         | PhiA cell wall protein, protease, major allergen |

## DETECTION OF MOULDS

### *Environmental sampling*

Sample collection is an important step in the analysis of indoor moulds. However, optimal sampling methods and standard protocols for routine assessment of mould exposure have not yet been established (54, 55). Generally, there are two techniques for

**Table 3** Allergens of *A. alternata*

| Allergen | Biological activity                    |
|----------|--|
| Alt a 1  | unknown, major allergen                |
| Alt a 2  | Aldehyde dehydrogenase, major allergen |
| Alt a 3  | Heat shock 70 protein                  |
| Alt a 4  | Protein disulfide isomerase            |
| Alt a 5  | P2 ribosomal protein                   |
| Alt a 6  | Enolase                                |
| Alt a 7  | YCP4 protein                           |
| Alt a 8  | Mannitol dehydrogenase                 |
| Alt a 10 | Aldehyde dehydrogenase                 |
| Alt a 12 | P1 ribosomal protein                   |
| GP70     | Major allergen                         |

bioparticulates: air and surface sampling (56). Air sampling is suitable for determining spore count and viable mould parts (1, 54). Settling of bioaerosols depends on the diameter of a particle, and environmental and physical factors (15). Settled dust can be a reservoir of mould spores, their components (conidiophores, hyphae, fine fragments, allergens, glucans), and their metabolites (mycotoxins) accumulated over time (57, 58). While air sampling may be a good proxy for recent mould exposure associated with acute symptoms, dust sampling may be a good proxy for long-term exposures, especially in epidemiologic studies (14, 59). However, dust sampling does not give information about the potential of contaminants to aerosolise (57).

### Methods

Traditional methods (culture-based and microscopy) are the primary laboratory tools for mould detection and identification (55). However, these methods have serious limitations. Cultivation-based methods do not identify dead or dormant mould fragments, which also may act as antigens (5). Furthermore, microscopic spore count does not identify smaller fungal fragments and hyphae. Germinated hyphae have higher allergenic activity than fungal conidia; therefore, hyphae significantly affect the air quality of mould-contaminated buildings (24, 53, 57). Gorny et al. (24) suggested that spore count should be extended to fungal fragments (24).

In contrast to the traditional methods, molecular and immunological tests may detect all types of mould particles (60). Molecular methods (rDNA and qPCR) do not rely on the viability of organisms and can detect very small quantities of genetic material, which is especially valuable in assessing *Aspergillus* levels in hospitals (60-62). Pitkäranta et al. (62) suggested that a combination of traditional and molecular tests could give a more comprehensive picture of the fungal flora than any of the methods alone.

Several immunoassays (immunoblotting, Halogen immunoassay, and enzyme-linked immunosorbent assay - ELISA) have been used to identify mould allergens (31, 63). The halogen immunoassay is more common in experimental settings to demonstrate how aerosolised fungal fragments and hyphae act as aeroallergens, while the capture ELISA has found more practical application as a tool for detecting mould antigens in real-life (occupational or other) settings (64, 65). Commercial ELISAs are available for specific allergens Asp f 1 and Alt a 1 and for *A.*

*versicolor* and *S. chartarum* antigens. Due to cross-reactive antigens among mould species, species-specific tests are recommended for the detection of moulds in various environments (49, 65, 66). The advantages of immunoassays are the speed, analytical sensitivity, and the possibility to detect a relatively large number of samples in several hours.

### ELISA for mould allergens

The mass fraction of Alt a 1 and Asp f 1 is usually measured in dust samples. The protocols for the mAb-based Alt a 1 ELISA and mAb/pAb-based assay for Asp f 1 have been described elsewhere (32, 67-69). The limit of detection (LOD) for Asp f 1 is 3.6 ng g<sup>-1</sup> to 5.0 ng g<sup>-1</sup> and for Alt a 1 0.12 µg g<sup>-1</sup> (68-70). So far, no guideline or limit values for these major allergens in dust extracts have been suggested. Salo et al. (64) has proposed a cut-off point of 7 µg g<sup>-1</sup> for overall *Alternaria* spp. antigens in household dust.

## DETECTION OF ALT A 1 AND ASP F 1

### House dust

Table 4 shows the levels of Alt a 1 and Asp f 1 in different environments. Neither allergen has been detected in settled dust in urban damp-free homes in Zagreb, Croatia (68, 69). Similar results were reported for Alt a 1 and Asp f 1 in several US studies (32, 70, 71) and in Canada (72). Sporik et al. (32), detected Asp f 1 in only 5 % of households (32). These very low frequencies of Alt a 1 and Asp f 1 in household environments may be associated with a relatively low spore count typically found in healthy, non-mouldy houses (71).

### Occupational environments

Unlike households, agricultural settings are usually associated with high exposure to moulds and allergic alveolitis (11). In our recent studies, we detected Alt a 1 and Asp f 1 in 100 % and 62 % of poultry farm dust samples, respectively (68, 69). Exposure to Alt a 1 (median Alt a 10.37 µg g<sup>-1</sup>) in poultry farms was much higher than to Asp f 1 (median Asp f 117.9 ng g<sup>-1</sup>) (Table 4). Both exposures are relatively low, but we also observed wide ranges of mass fractions of both allergens. This suggests that reservoir dust in poultry farms may become a hazardous secondary source of occupational exposure to these allergens.



**Table 4** *Alt a 1 and Asp f 1 in settled dust*

| Source         | Median                       |                              | Reference |
|----------------|------------------------------|------------------------------|-----------|
|                | Alt a 1 / µg g <sup>-1</sup> | Asp f 1 / ng g <sup>-1</sup> |           |
| Homes, offices | <LOD                         | <LOD                         | 68-72     |
| Poultry farms  | 0.37                         | 17.9                         | 68, 69    |
| Sawmill        | <LOD                         | >LOD <sup>a</sup>            | 68        |

<sup>a</sup>data not presented  
 LOD - limit of detection

There is little information on mould allergens in other occupational environments. Forestry and wood industry-related workplaces are characterised by high levels of bioaerosols containing moulds. Dillon et al. (56) detected Asp f 1 in air samples taken from a wood chipping facility and suggested it as a suitable marker of exposure to *A. fumigatus* (56). This study showed that Asp f 1 ELISA was sensitive enough to detect airborne Asp f 1 in a sawmill. To my knowledge, fungal allergens have not been measured in other occupational settings.

#### OTHER INDICATORS OF INDOOR MOULDS

Mould constituents such as beta-1,3-glucan (57, 73-75), ergosterol (74), and extracellular polysaccharides from *Aspergillus* and *Penicillium* (EPS-Asp/Pen) (75) have been used as general markers of fungal biomass. They have been used for exposure assessment to moulds in low-exposure environments and in damp buildings (73-76). In contrast to specific allergens, these indicators do not identify a specific mould.

#### HEALTH EFFECTS OF MOULDS

Generally, moulds can affect human health through allergic reactions, infections, and toxic responses (Table 5) (77, 78). Among them, sensitisation and allergic diseases are most commonly associated with inhalation of mould antigens. The major asthma-related aeroallergen in children and adults worldwide is the genus *Alternaria* (79-81). Exposure to *Cladosporium* is associated with allergic respiratory symptoms in children (14, 82). The prevalence of mould allergy in atopic individuals is about 20 % to 30 % and up to 6 % in the general population (83). A GA<sup>2</sup>LEN network survey by Heinzerling et al. (84) showed regional differences in sensitisation to mould allergens, which was higher in the UK and northern Europe than in the Mediterranean countries. Recent studies (24, 43, 85-87) have extensively investigated the role of indoor fungi such as *Aspergillus* and *Penicillium* in the pathogenesis of allergic diseases. *A. fumigatus* is the cause of 80 % of *Aspergillus*-related diseases in humans (78). *A. fumigatus*-related allergies include rhinitis, allergic asthma, sinusitis, and allergic bronchopulmonary aspergillosis (ABPA) (Table 5). ABPA is a severe complication related to

**Table 5** *Human diseases and symptoms caused by indoor moulds*

| Category   | Diseases  | Moulds  | Reference      |
|--|---|---|----------------|
| Hypersensitivity                                 | Asthma, allergic sinusitis and rhinitis   | <i>Alternaria</i> ,<br><i>Aspergillus</i> ,<br><i>Penicillium</i> | 10, 14, 48, 77 |
|  | <sup>a</sup> ABPA, allergic alveolitis  | <i>Aspergillus</i>  | 11, 88         |
| Infection  | Aspergilloma  | <i>Aspergillus</i>  | 77             |
| Invasive infection                               | Invasive pulmonary aspergillosis,<br>invasive rhinosinusitis  | <i>A. fumigatus</i>   | 35, 78         |
| Toxic  | Mycotoxicosis (toxins such as aflatoxins,<br>trichothecenes, gliotoxin, ochratoxin)                                   | <i>Stachybotrys</i> ,<br><i>Aspergillus</i>                       | 91-93          |
| Nonspecific symptoms<br>(sick building syndrome) | Nasal congestion, itchy and watering eyes,<br>headache, fatigue, generalised malaise, airway<br>infections, skin rash | various moulds  | 59, 85, 94-97  |

<sup>a</sup>Allergic bronchopulmonary aspergillosis

*A. fumigatus* sensitisation and colonisation in the lung that affects patients with severe asthma (35, 39, 88, 89). It is a frequent complication in cystic fibrosis patients (90). ABPA patients showed a marked increase in IgG and IgE antibodies against special *A. fumigatus* allergens (35). While recombinant allergens Asp f 4 and Asp f 6 were used in serological diagnosis of ABPA (88), rAsp f 4 was efficient in diagnosis of ABPA in patients with cystic fibrosis (90).

Fungal infection poses a particular risk for immunodeficient patients. Inhaled conidia of *A. fumigatus* may not be efficiently eliminated by immune mechanisms of these patients (38) and may cause life-threatening aggressive infections. Invasive aspergillosis is associated with the colonisation of the lung environment with *A. fumigatus* in immunocompromised patients (leukaemia and bone marrow transplant patients, individuals infected by HIV) (35, 78). Hospitals should therefore take particular care to control exposure of immunocompromised patients to this opportunistic mould.

#### OTHER HEALTH EFFECTS OF INHALED INDOOR MOULDS

Indoor moulds produce mycotoxins that pose a health risk (10, 27, 91). The production of mycotoxins by building-associated moulds such as *Stachybotrys* spp., *Aspergillus* spp., and *Penicillium* spp. has been well documented, particularly in environments with high concentrations of airborne spores, including farms and compost-handling facilities (11). These mould species produce several mycotoxins such as aflatoxins, trichothecenes, gliotoxin, and ochratoxin (Table 5) (92). Similar to allergens, airborne trichothecene mycotoxins are associated with both spores and small (<2 µm) respirable mould particles (26, 27). Long-term exposure to living or dead particles containing fungal mycotoxins has been associated with immunosuppression in healthy people (11) and with haematotoxicity, inflammation, and immunosuppression in laboratory animals (93). Jarvis and Miller (92) reported pulmonary mycotoxicosis in farmers exposed to very high quantities of mycotoxins at work.

Table 5 also shows the symptoms associated with poor indoor air quality and sick building syndrome (SBS). The aetiology of SBS is still not clear. Many studies have shown the association between dampness (94), fungal contamination (95), volatile organic

compounds (11, 85), and building-related illnesses. There is evidence that *Penicillium* spp. and *Aspergillus* spp. may play a significant role in SBS (96, 97). Furthermore, inhalation of beta-1,3-glucan from indoor moulds could be associated with non-allergic respiratory symptoms (98). However, Noss et al. (73) suggested that glucan exposure in early childhood can protect against the development of allergy (73). As potent immunological activators, beta-glucans have been recognised to possess a potential to act against several diseases (cancer, diabetes, infectious diseases) and to help patients to recover from chemotherapy and radiotherapy (99).

Further investigations are necessary to find out the role of indoor moulds in aetiology of SBS and other diseases.

#### CONCLUSION

Monitoring of mould allergens in reservoir dust may be useful in assessing environmental contamination with moulds. However, due to high cross-reactivity among fungi, only several species-specific mould allergens (Alt a 1 and Asp f 1) have been identified and used in environmental studies. In low-exposure environments determination of dust-borne Alt a 1 and Asp f 1 using ELISA seems to be limited. In bioaerosol-rich environments such as agricultural or wood-industry environments, however, determination of dust-borne Alt a 1 and Asp f 1 may be quite useful in assessing exposure to moulds. Further research should focus on determining mould allergens in air.

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**Sažetak****IZLOŽENOST ALERGENIMA PLIJESNI U UNUTARNJEM OKOLIŠU**

Vlažni, unutarnji prostori mogu biti kolonizirani alergogenim, filamentoznim mikrogljivicama (plijesni) uglavnom rodova *Aspergillus*, *Penicillium*, *Cladosporium* i *Alternaria*. Respiratorne bolesti uzrokovane plijesnima zdravstveni su problem diljem svijeta. U posljednja dva desetljeća, neki sastavni dijelovi plijesni kao alergeni i glukan rabe se kao pokazatelji izloženosti plijesni u unutarnjem okolišu. Nedavno su alergeni plijesni Alt a 1 (*Alternaria alternata*) i Asp f 1 (*Aspergillus fumigatus*) određivani u različitom okolišu (kućnom i profesionalnom) enzim-imunokemijskom metodom koja rabi monoklonska ili poliklonska antitijela. Razina Alt a 1 i Asp f 1 u kućnoj prašini ispod je granice detekcije. Nasuprot tomu, alergeni plijesni su određeni u okolišu s visokom razinom bioaerosola kao peradarnici i pilane. Razine alergena Alt a 1 i Asp f 1 u nekim poljoprivrednim objektima pružaju informaciju o mogućoj kolonizaciji plijesnima, što upućuje na moguće zdravstvene učinke kod zaposlenika.

**KLJUČNE RIJEČI:** *Alt a 1*, *Asp f 1*, *A. alternata*, *A. fumigatus*, *ELISA*, *profesionalna izloženost*

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