

# TOXIPILEX BASIC

A direct mycotoxin detection  
assay for human serum/plasma

**Kunal Garg**

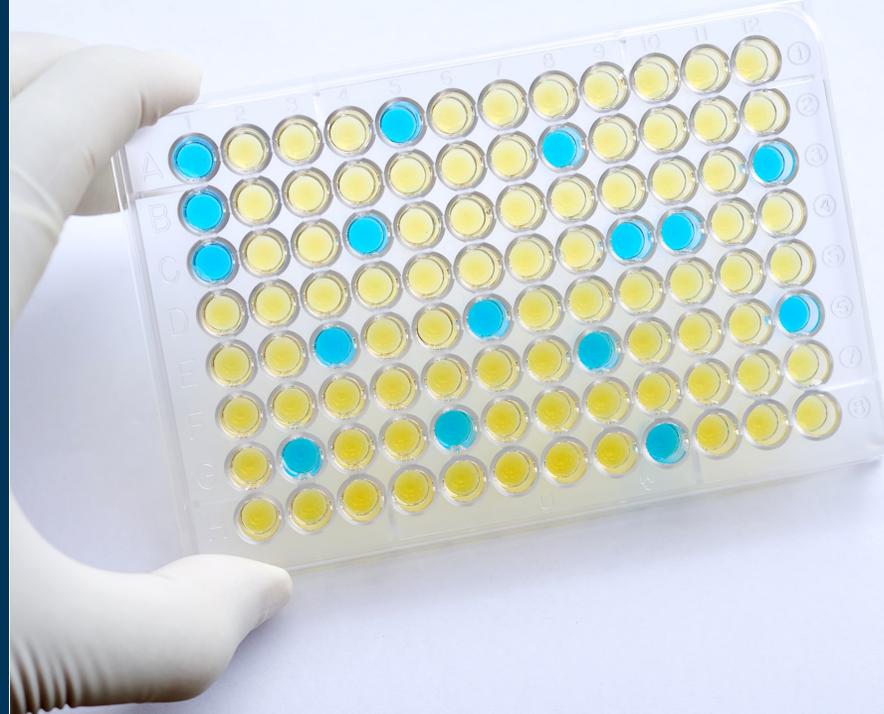
Chief Technology Officer

Tezted Ltd

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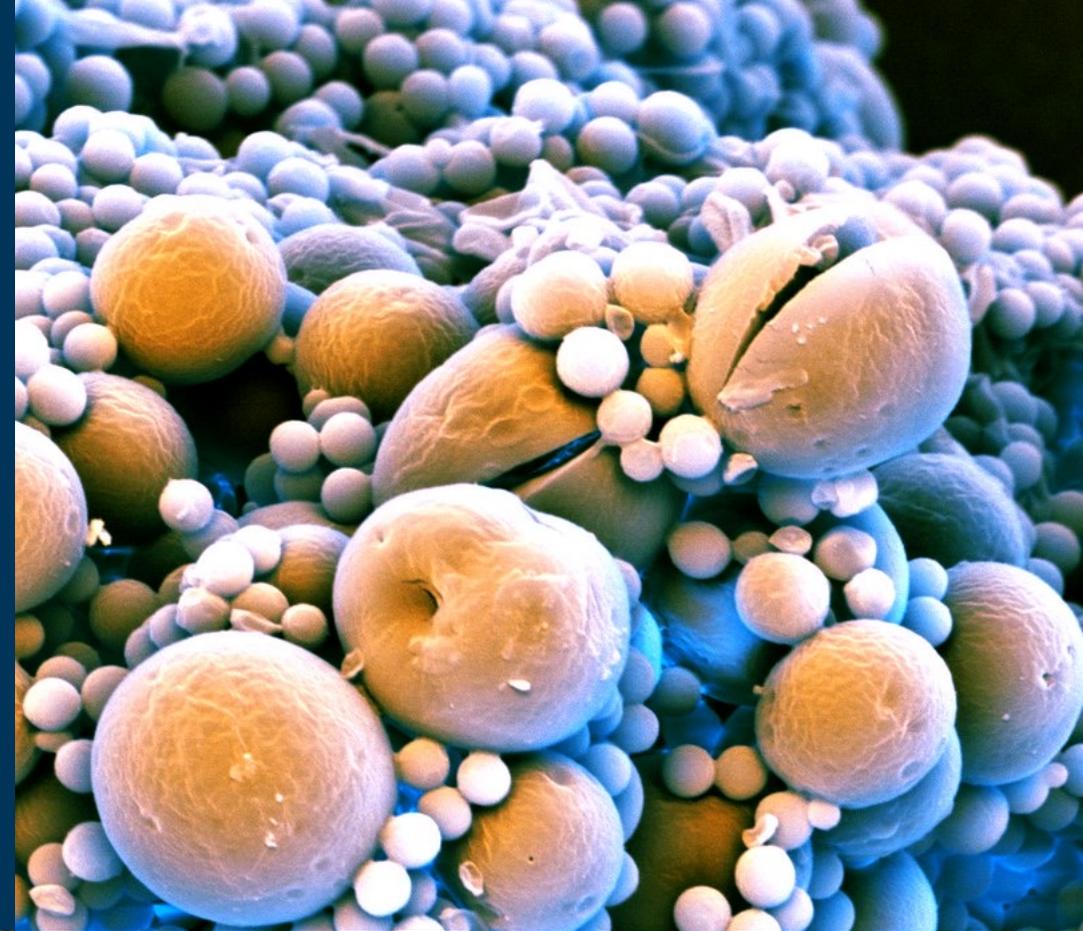


# Agenda



1. Modes of mycotoxin exposure and impact on health
2. Current methods to detect mycotoxins
3. Mycotoxin detection with TOXIPLEX BASIC
4. Summary

# Modes of mycotoxin exposure and impact on health



# Exposure to mycotoxins through foodstuffs

“ Exposure to mycotoxins can happen either directly by **eating infected food** or indirectly from animals that are fed contaminated feed, in particular from milk.

[World Health Organization](#)



As an individual consumer, you generally cannot control the presence of mycotoxins in your food. The fungi that produce mycotoxins generally grow during crop production and storage – steps in the food supply chain that the FDA regulates and monitors to ensure the food available for you to buy is not contaminated. The mycotoxins in human food that the FDA currently focuses on are [aflatoxins](#), [deoxynivalenol](#), [fumonisin](#), [patulin](#), and [ochratoxin A](#).

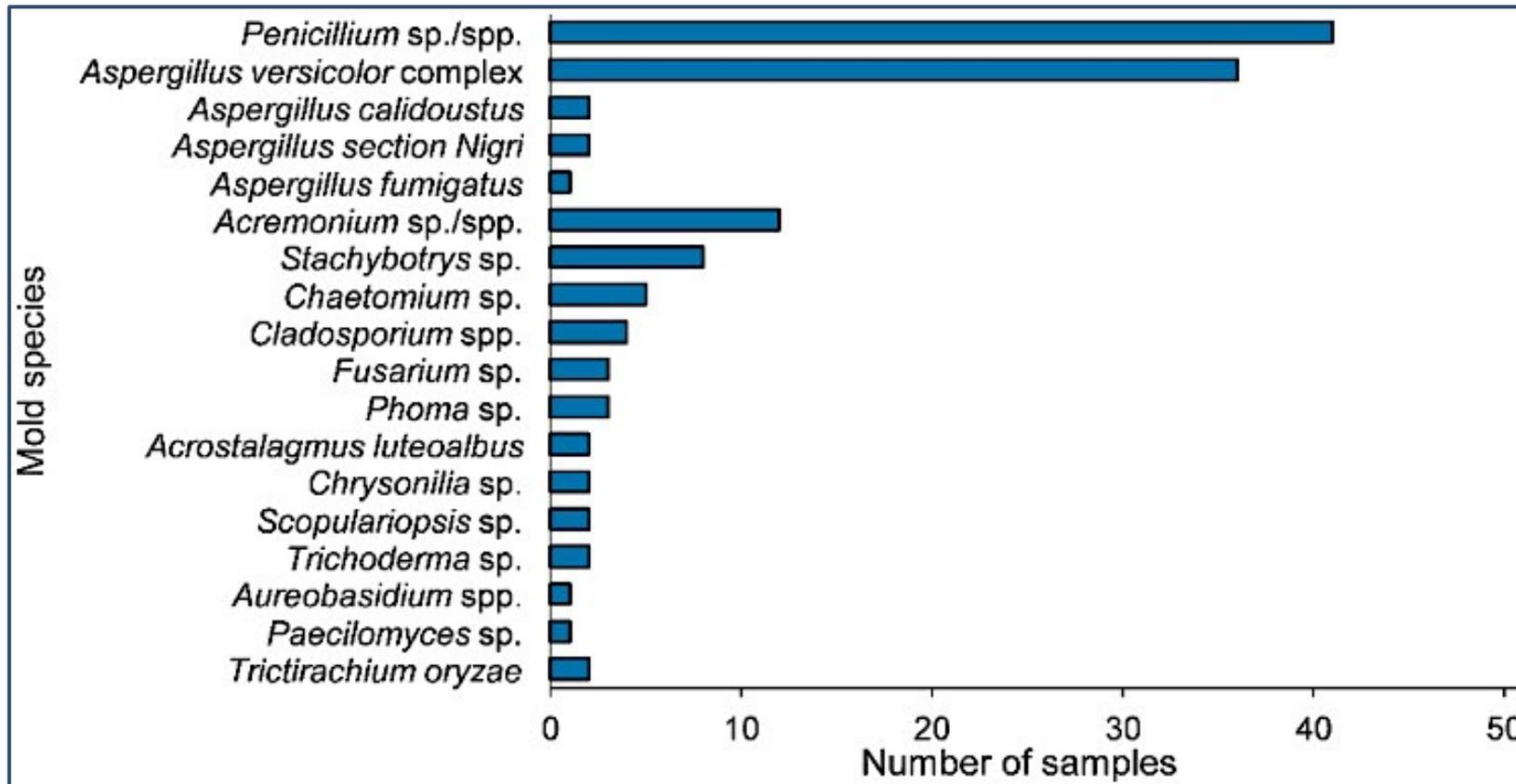


The mycotoxins of most concern from a food safety perspective include:

- aflatoxins (B1, B2, G1, G2 and M1)
- ochratoxin A
- patulin toxins produced by Fusarium moulds, including fumonisins (B1, B2 and B3)
- trichothecenes (principally nivalenol, deoxynivalenol, T-2 and HT-2 toxin)
- zearalenone
- ergot alkaloids, citrinin, sterigmatocystin and alternaria toxins

# Exposure to mycotoxins through indoor environments

Lindemann et al. (2022) tested mold-infested samples ( $n=51$ ) from 24 households in northern Germany. Building materials included wallpapers, plasters, wood, isolation materials like Styrofoam, and glass wool

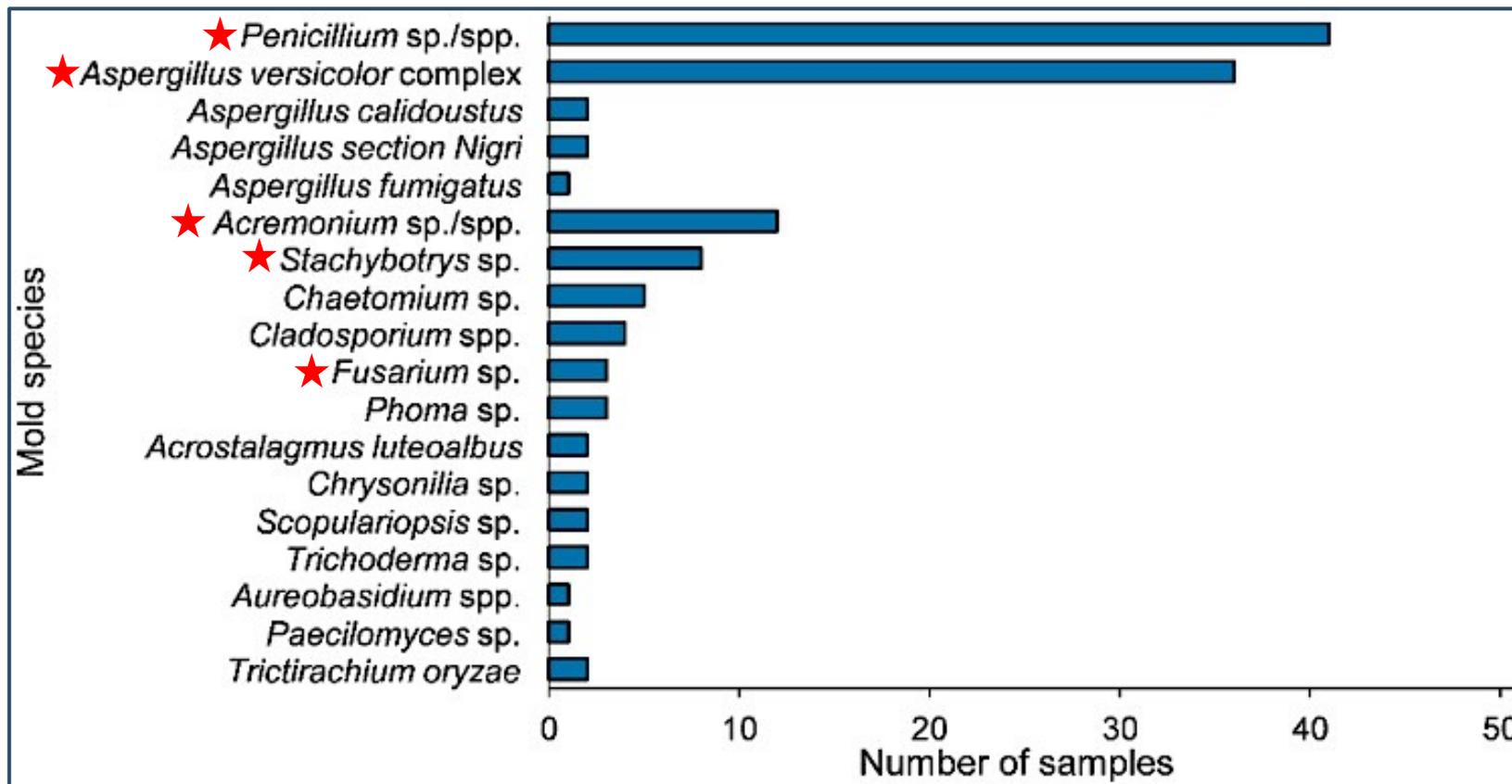


Modified from Lindemann et al, 2022

Lindemann et al, 2022  
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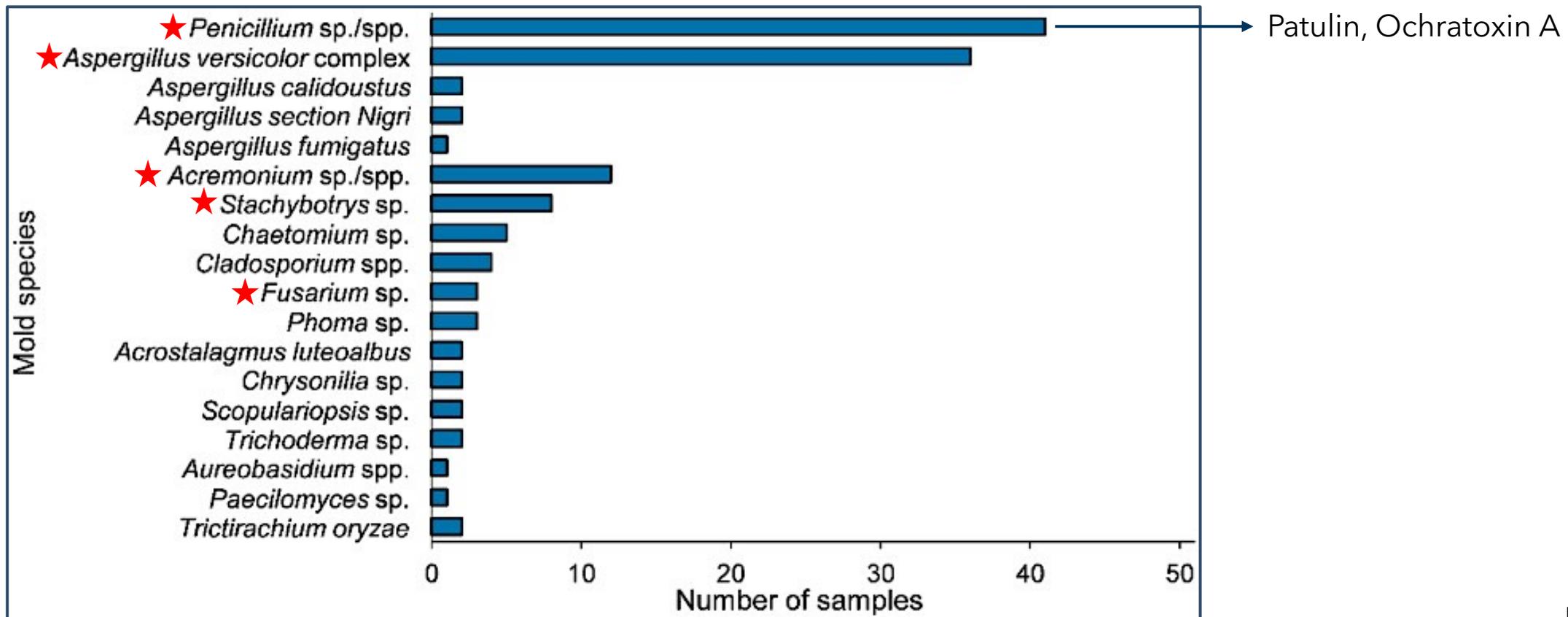


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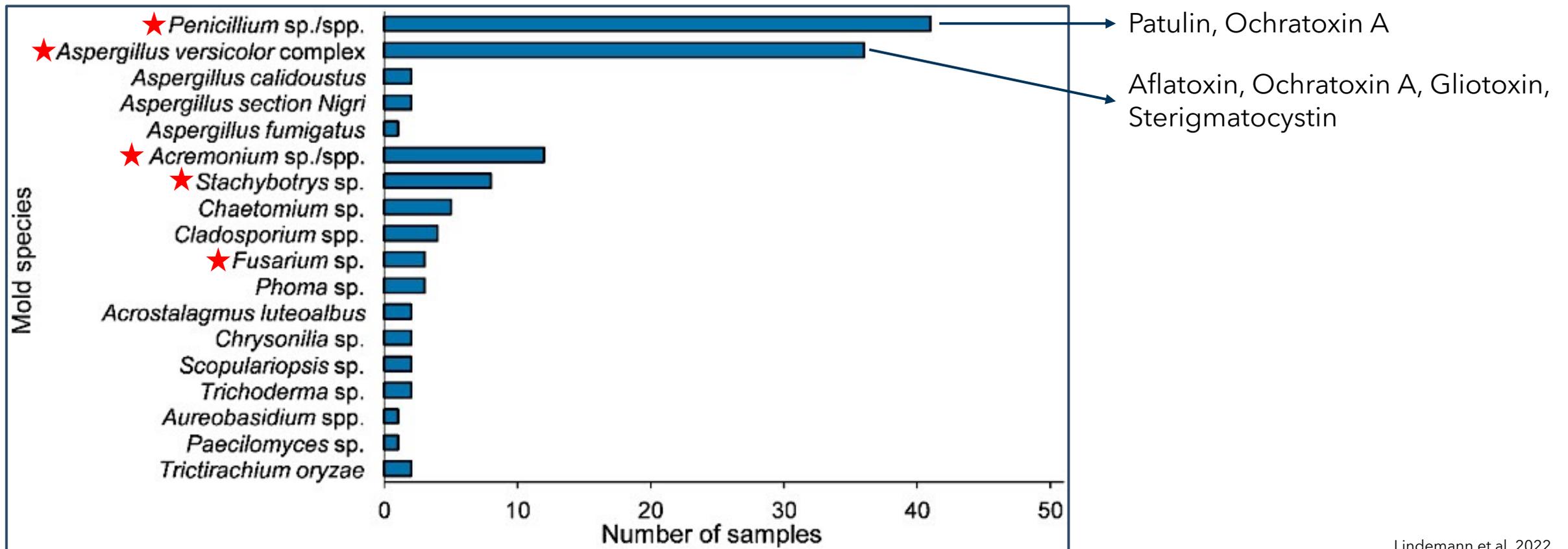


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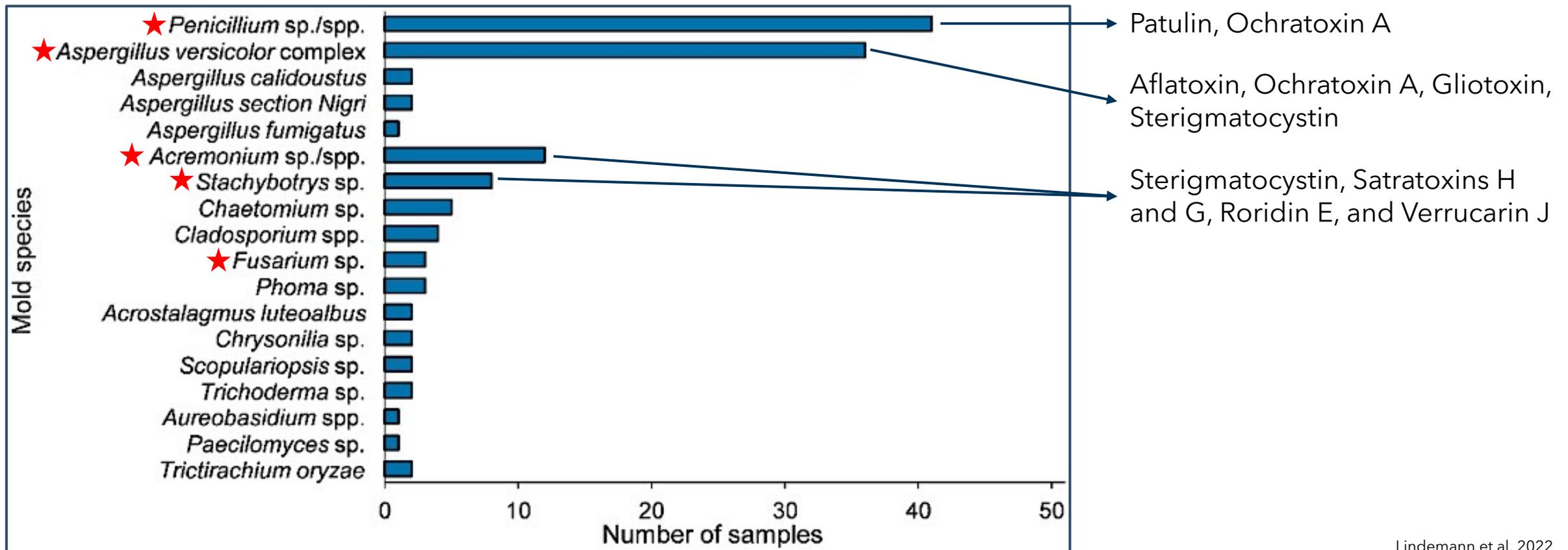


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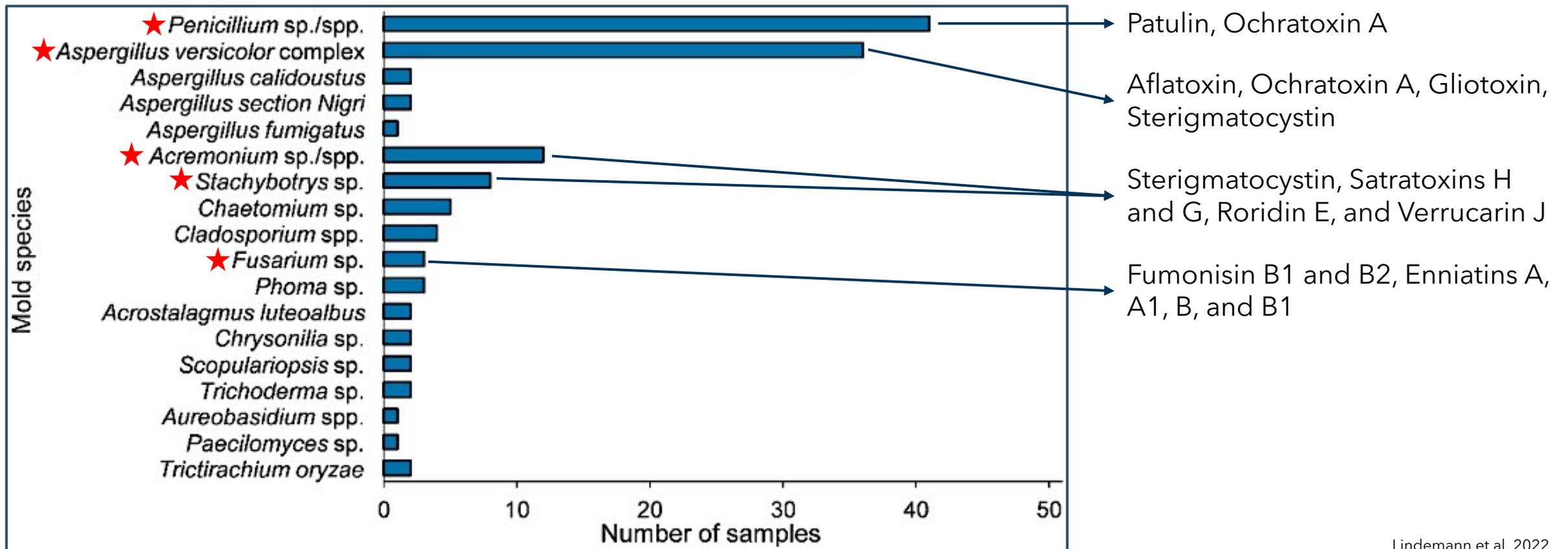


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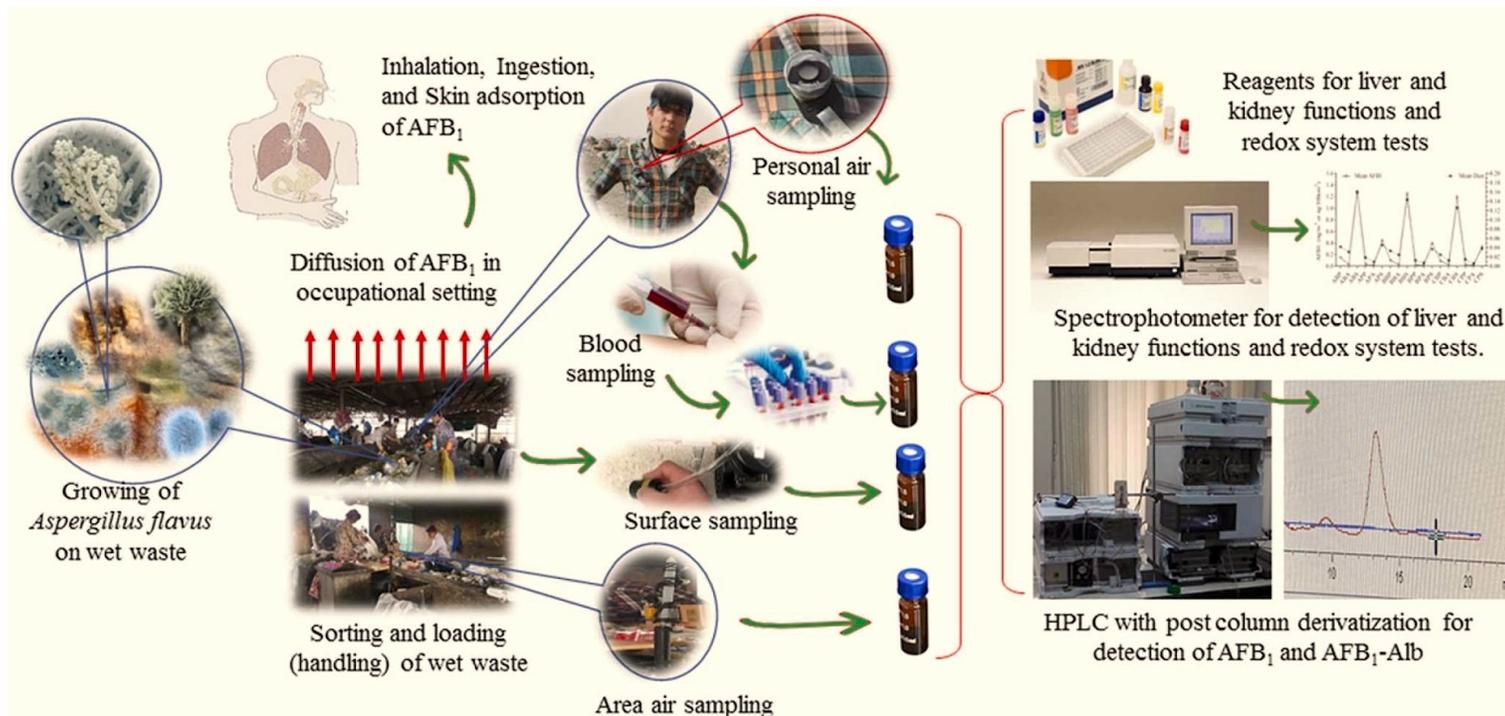


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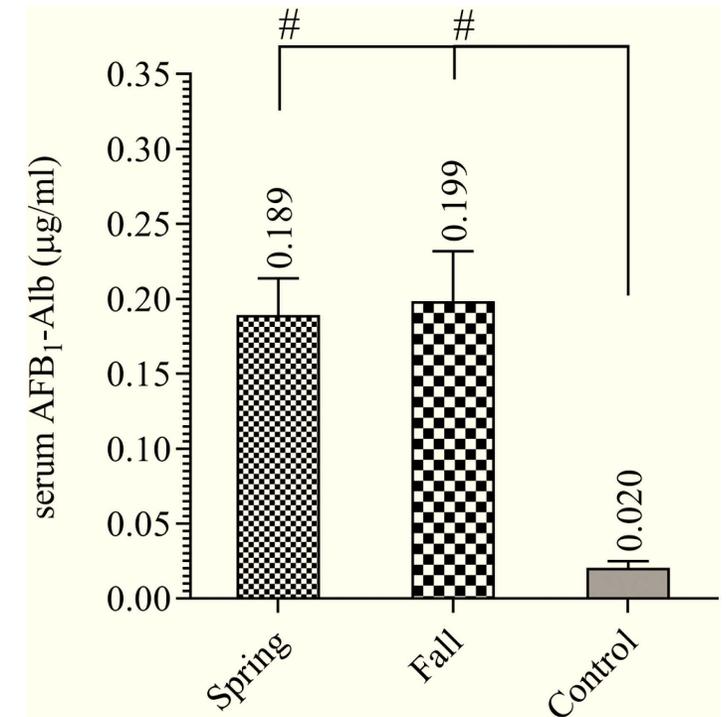
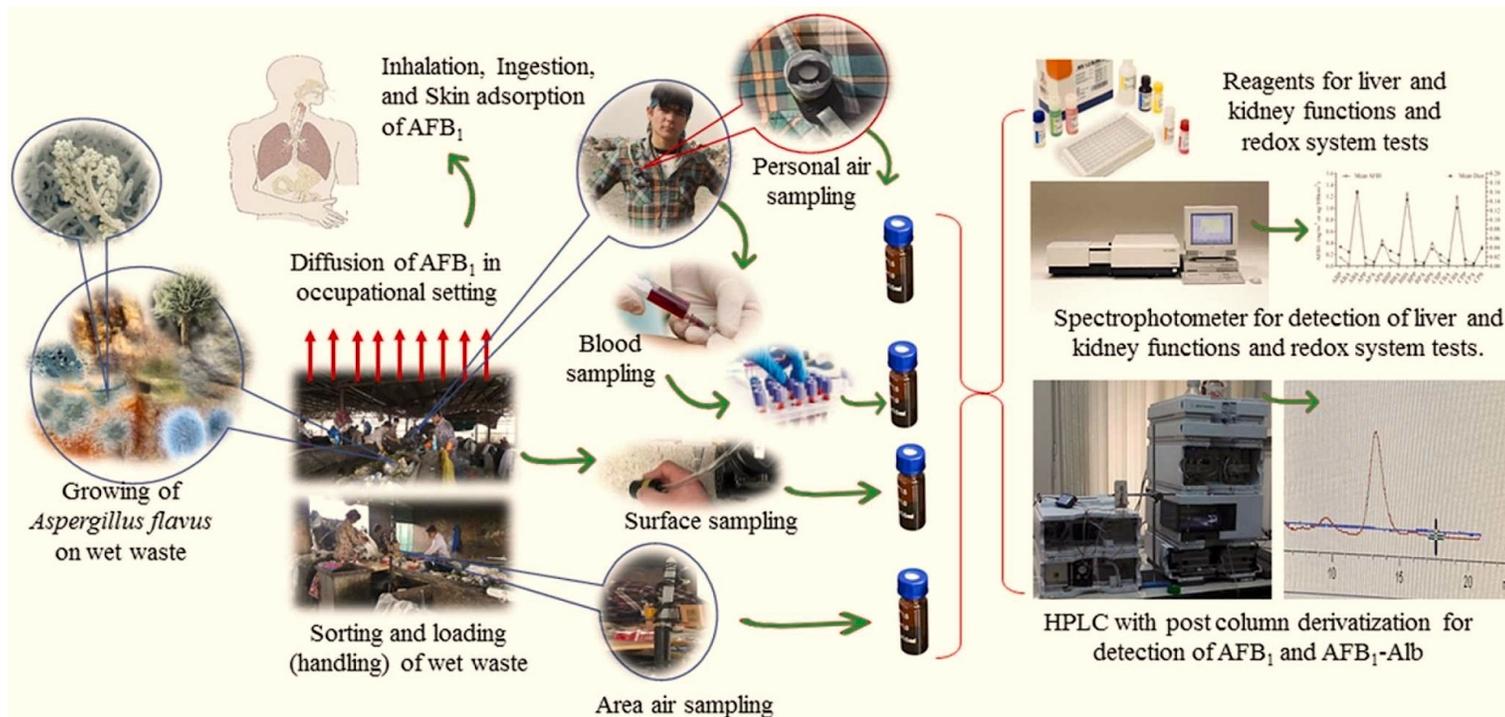
# Exposure to mycotoxins through indoor environments

Aflatoxin B1 (AFB<sub>1</sub>) and its albumin derivative were measured in wet waste management air, surface, and human serum ( $n = 60$ ) in Tehran, Iran, indicating exposure to mycotoxins like AFB<sub>1</sub> as an occupational hazard



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# Exposure to mycotoxins through indoor environments

<b>Mycotoxin</b>	<b>Reference</b>	<b>Summary</b>
Aflatoxin B1 (AFB1)	Polizzi et al, 2009	Found in wallpaper, mycelium, or silicone of water-damaged buildings
Deoxynivalenol (DON)	Tuomi et al, 2000	Found in wallpaper, cardboard, wood, plywood, plasterboard, etc., from the moldy interiors of buildings with moisture issues
Fumonisin B1/B2 (FUM)	Shoemaker et al, 2021	Found in the urine of humans and animals in various indoor settings
Ochratoxin A (OTA)	Polizzi et al, 2009	Found in wallpaper, mycelium, or silicone of water-damaged buildings
Zearalenone (ZEA)	Palmgren et al, 1983	Found on corn dust in grain elevator in an industrial setting

# Common mycotoxins and their impact on human health

Mycotoxins in TOXIPLEX BASIC	Exposure through		Impact on human health
	Food	Indoor	
Aflatoxin B1 (AFB1)	Yes	Yes	Targets liver which can result in hepatocellular carcinoma

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Fumonisin B1/B2 (FUM)	Yes	Yes	Inhibits the maturation of dendritic cells and cytokine secretion

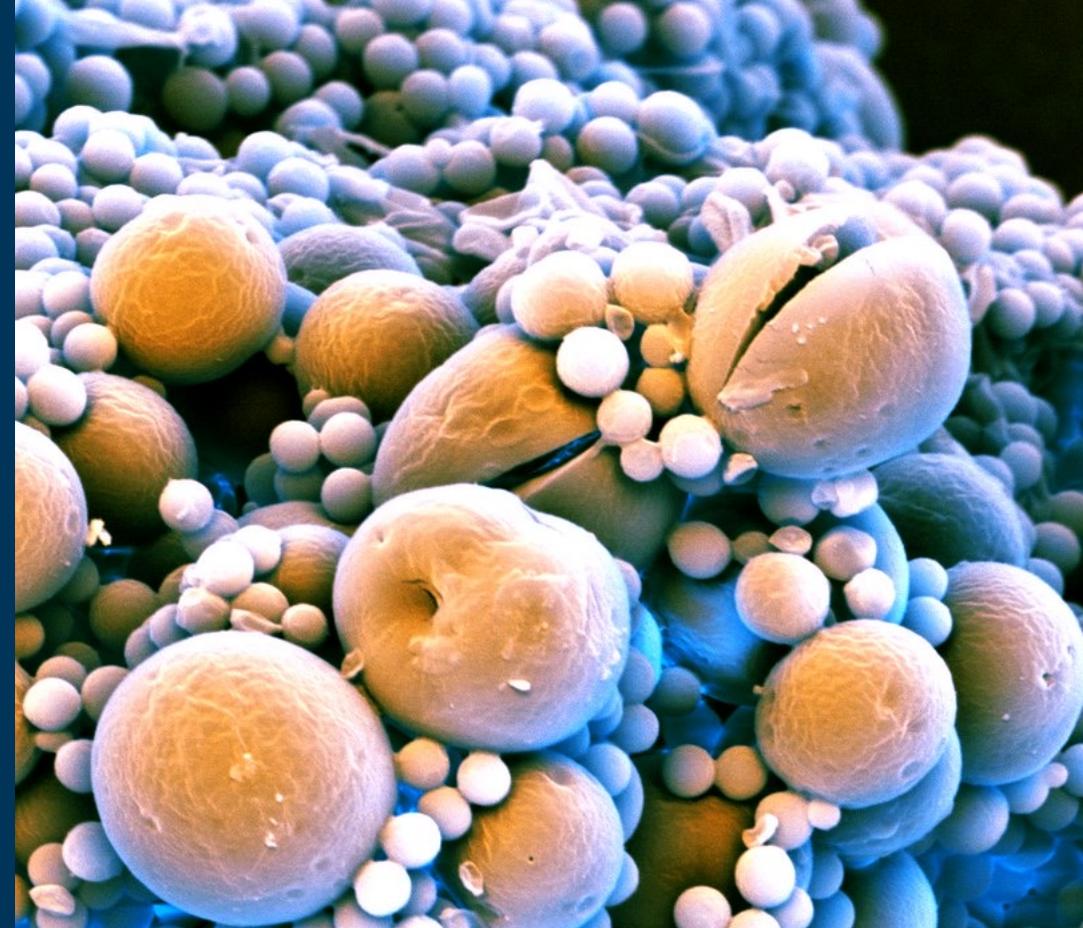
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Ochratoxin A (OTA)	Yes	Yes	Causes nephrotoxicity and pro-inflammatory cytokine secretion

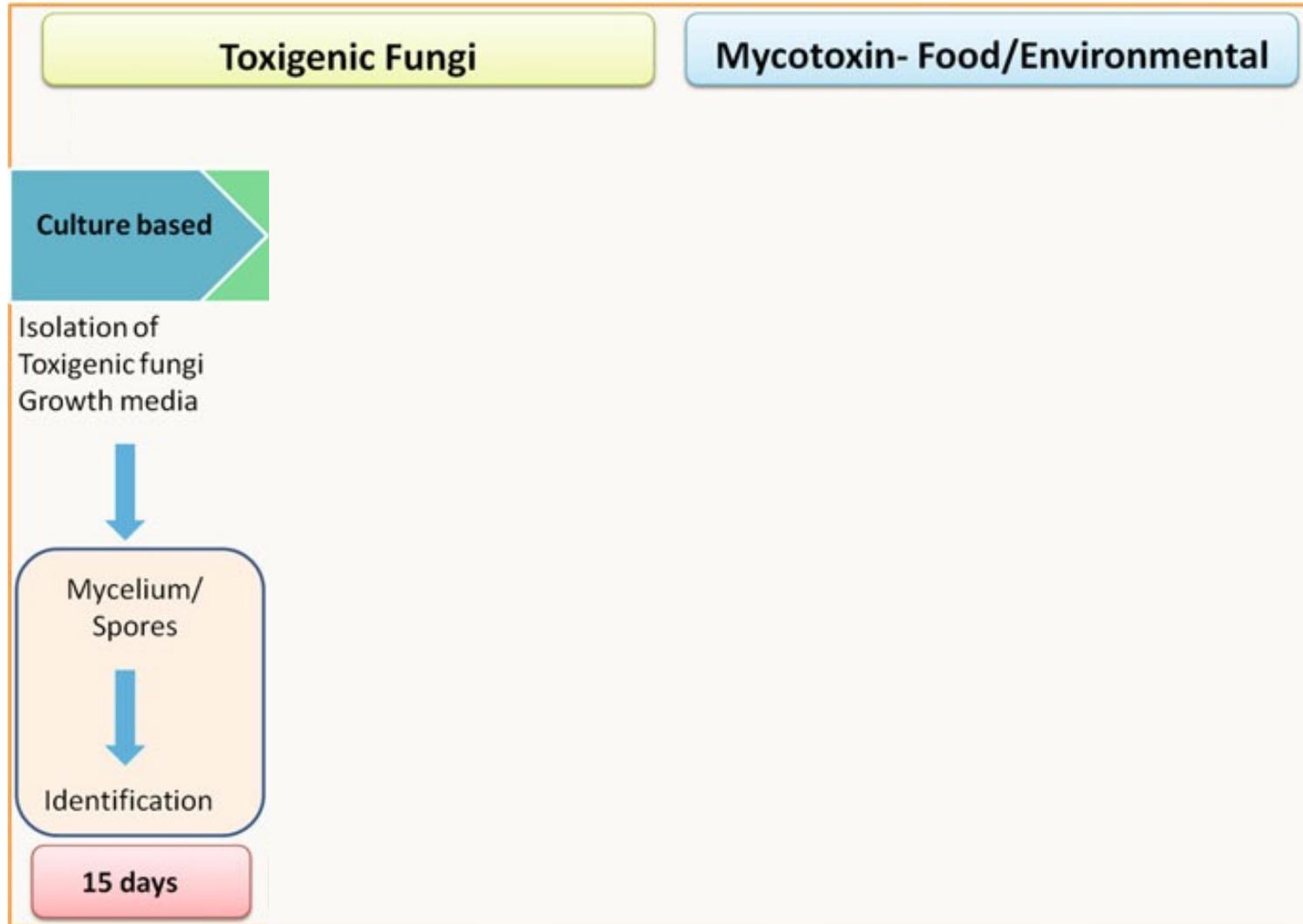
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Zearalenone (ZEA)	Yes	Yes	Induces macrophage apoptosis and inhibits protein/DNA synthesis

# Current methods to detect mycotoxins



# Overview of mycotoxin detection methods



## Culture-based method

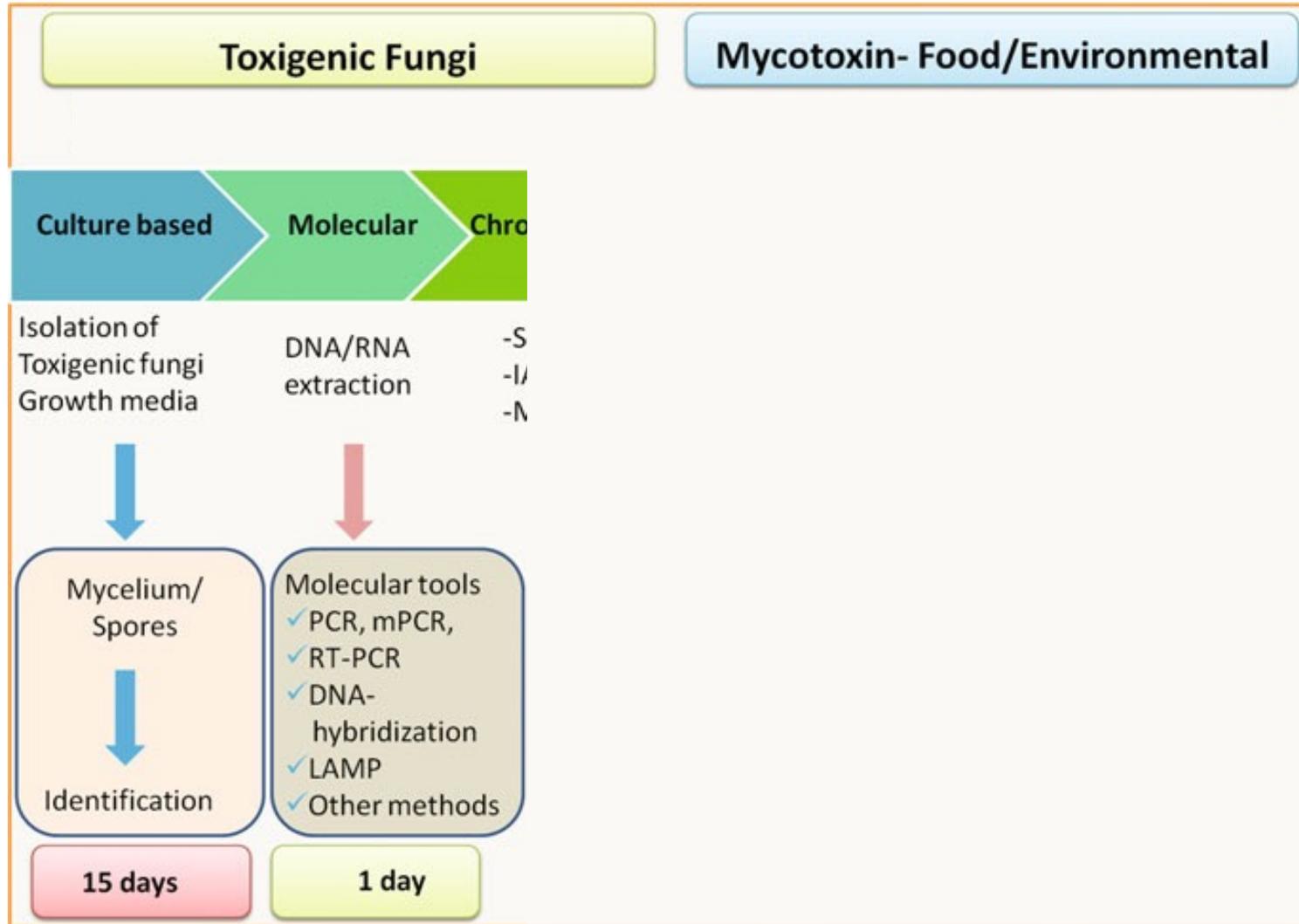
### Advantages

1. The gold standard for fungal infections
2. Yields specific etiological agent
3. Allows susceptibility testing

### Disadvantages

1. May miss over 50% of documented cases
2. Low positivity rate for early diagnosis
3. Low sample recovery (10% - 60 %)
4. 24-72 hrs required for fungi identification
5. Requires high-level expertise
6. Mycotoxin detection is not possible

# Overview of mycotoxin detection methods



## Molecular-based method

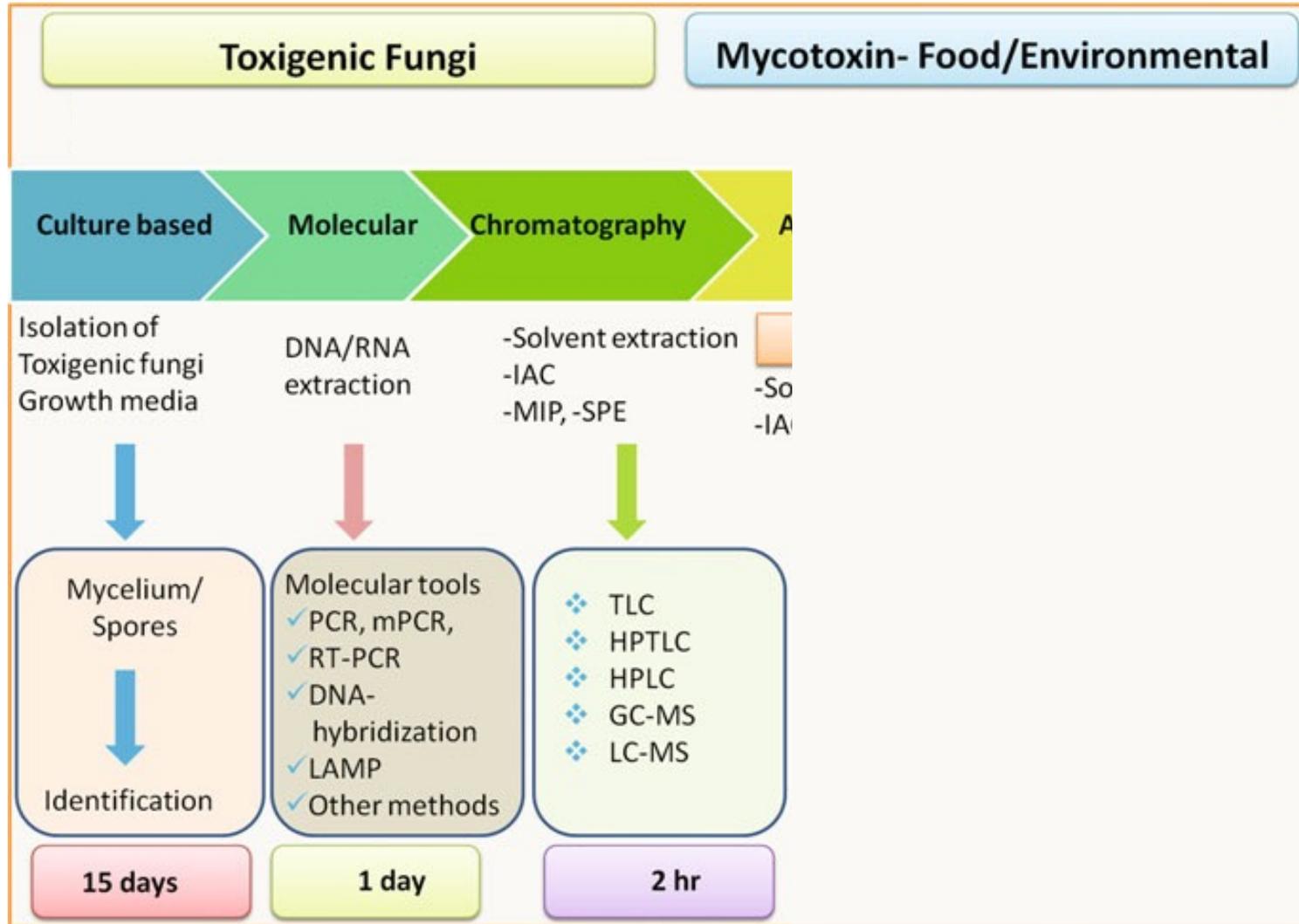
### Advantages

1. It does not need live fungal cells
2. Species-specific detection is possible
3. Specimen contaminated with human tissue is not an issue for PCR-based reaction on positive blood cultures

### Disadvantages

1. Wide intra and inter-lab variation
2. Immunosuppressed patients may manifest rare fungal infection
3. Mycotoxin detection is not possible

# Overview of mycotoxin detection methods



## Chromatography method

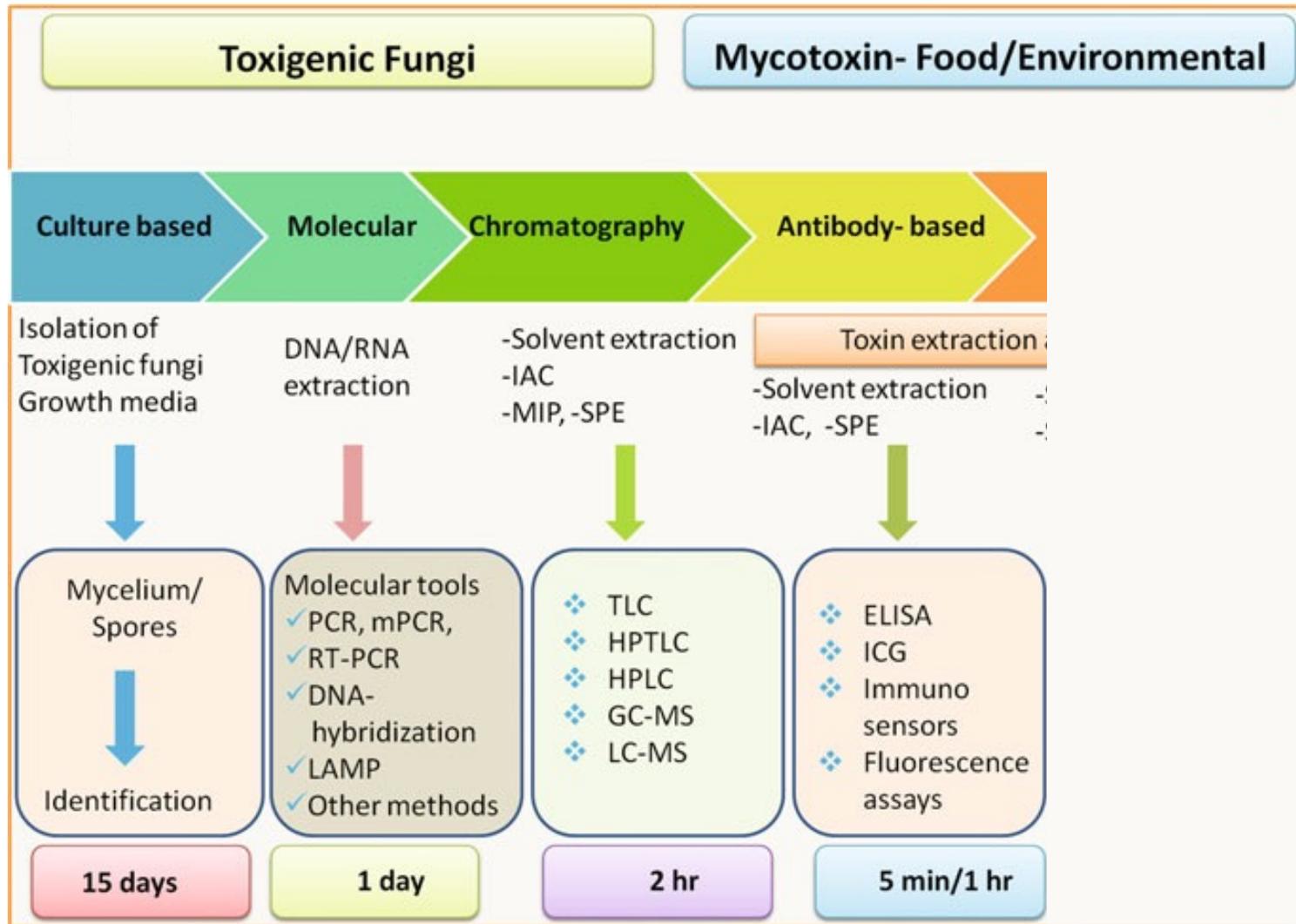
### Advantages

1. Gold standard for mycotoxin detection
2. Simultaneous multi-mycotoxin detection
3. High accuracy and reproducibility

### Disadvantages

1. Expensive machinery and maintenance
2. Requires high-level expertise
3. Ionization method dictates sensitivity

# Overview of mycotoxin detection methods



## Antibody-based method

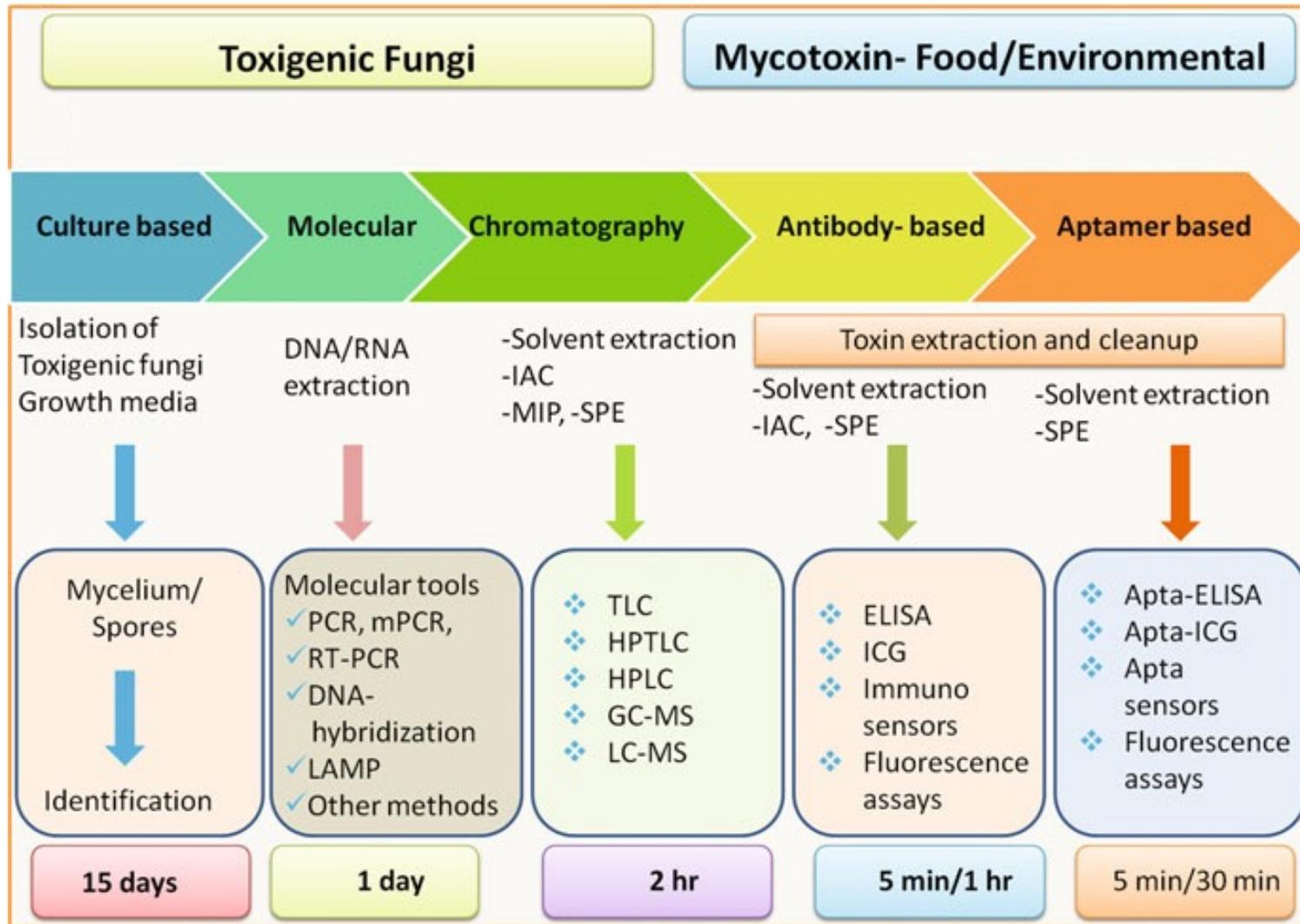
### Advantages

1. A rapid mycotoxin screening method
2. High accuracy and reproducibility
3. Easily accessible for all sizes of labs
4. It does not require high-level expertise

### Disadvantages

1. Prone to cross-reaction
2. Direct ELISA relies on non-linear calibration curves
3. The indirect immunoassay format is unreliable (i.e., measuring IgG, IgE, etc.)

# Overview of mycotoxin detection methods



## Aptamer-based method

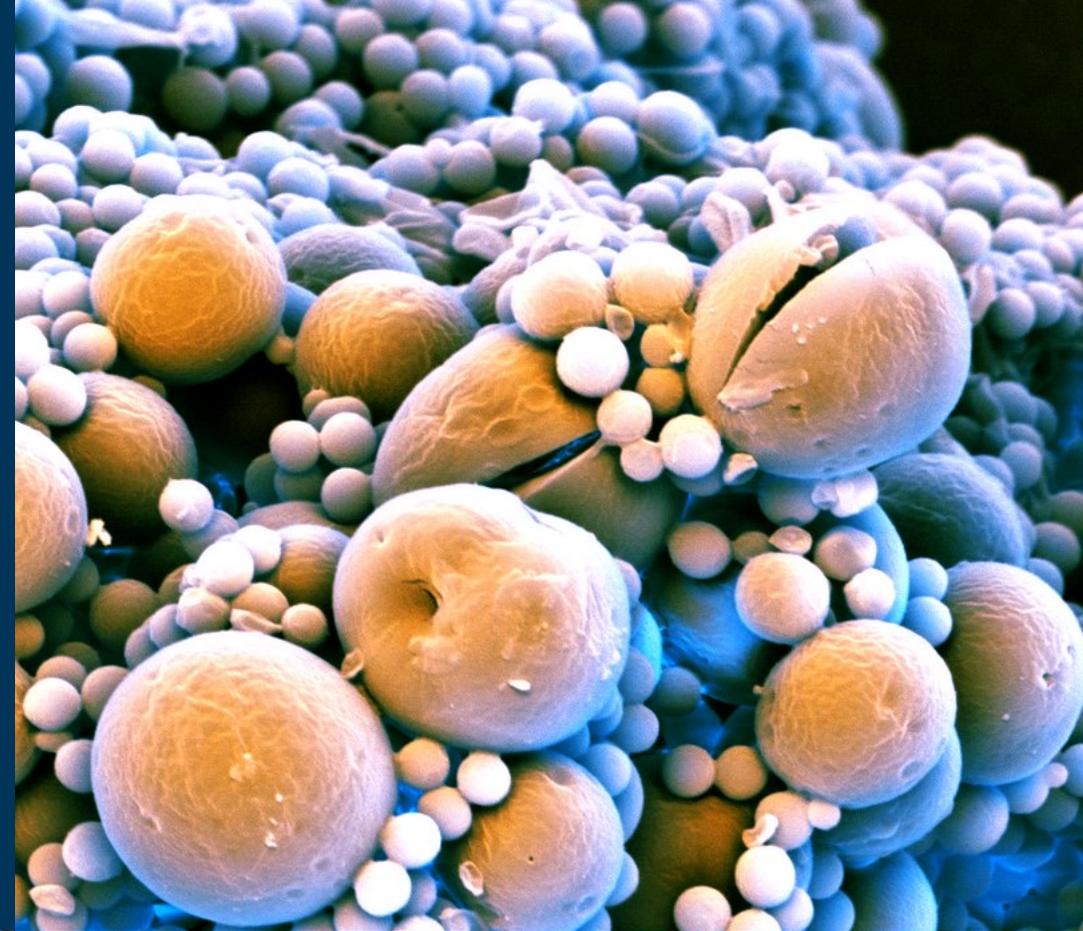
### Advantages

1. A rapid mycotoxin screening method
2. High accuracy and sensitivity

### Disadvantages

1. Prone to cross-reaction
2. Commercially unavailable and not viable
3. The SELEX process requires high-level expertise and expensive machinery

# Mycotoxin detection with TOXIPLEX BASIC



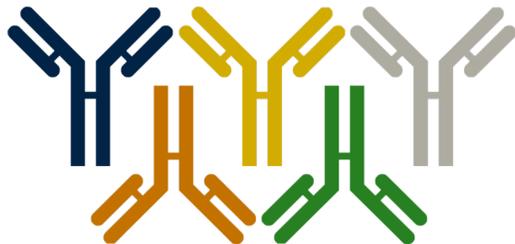
# Direct immunochemical detection of multiple mycotoxins



TOXIPLEX BASIC **DIRECTLY** detects Aflatoxin B1 (AFB1), Deoxynivalenol (DON), Fumonisin (FUM), Ochratoxin A (OTA), and Zearalenone (ZEA).



TOXIPLEX BASIC **DOES NOT** detect human antibody responses (IgA, IgG, IgE, etc.) against AFB1, DON, FUM, OTA, and ZEA.



TOXIPLEX BASIC **DOES NOT** measure mycotoxins in human urine because,

1. The use of human plasma or serum is five times more common than urine in literature (PubMed)
2. Variation in urine volume requires creatinine normalization
3. Daily mycotoxin intake variation demands 24hr sampling

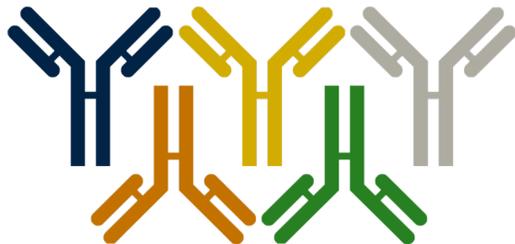
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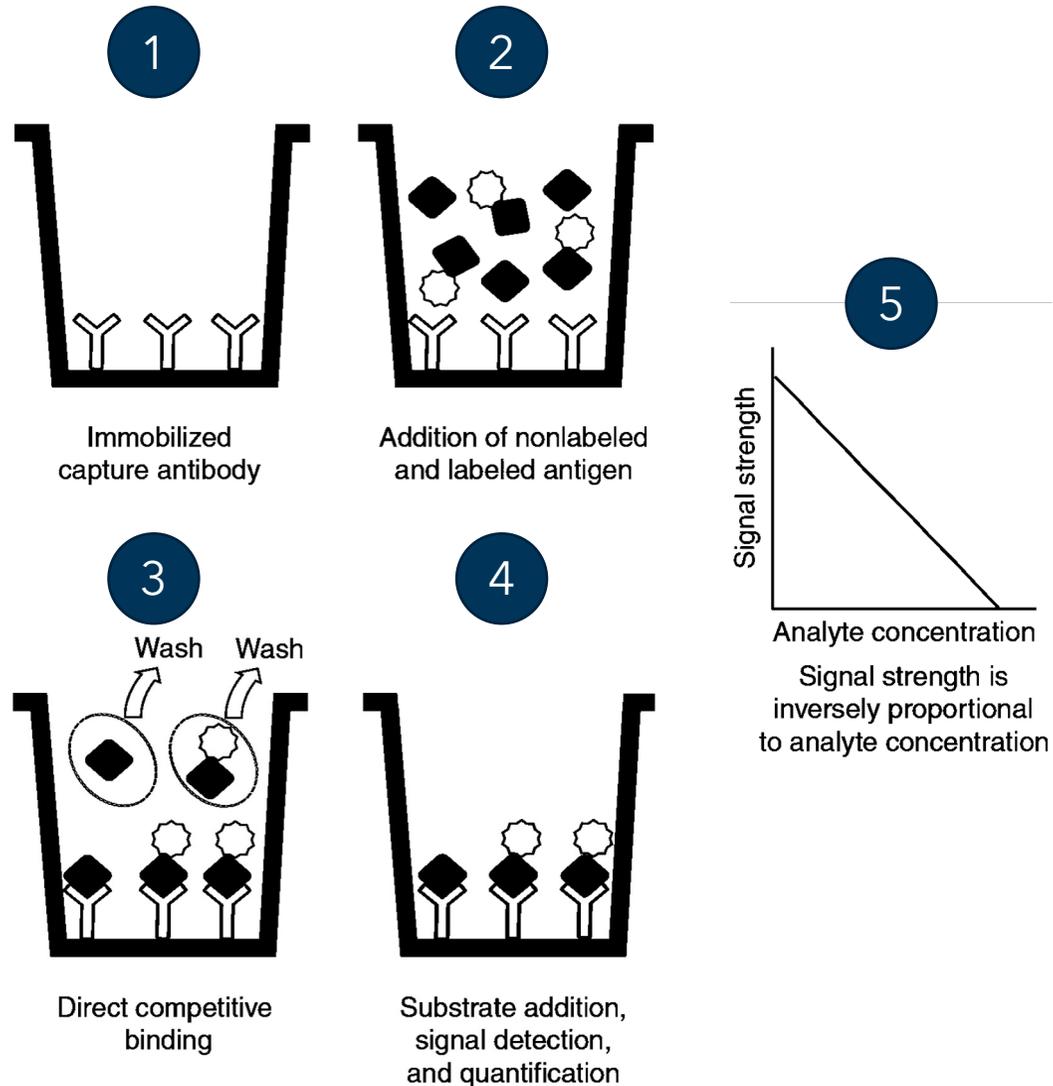
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# TOXIPLEX BASIC assay principle and performance



TOXIPLEX BASIC aids in the detection of five mycotoxins that threaten human health

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- Deoxynivalenol (DON)
- Fumonisin (FUM)
- Ochratoxin A (OTA)
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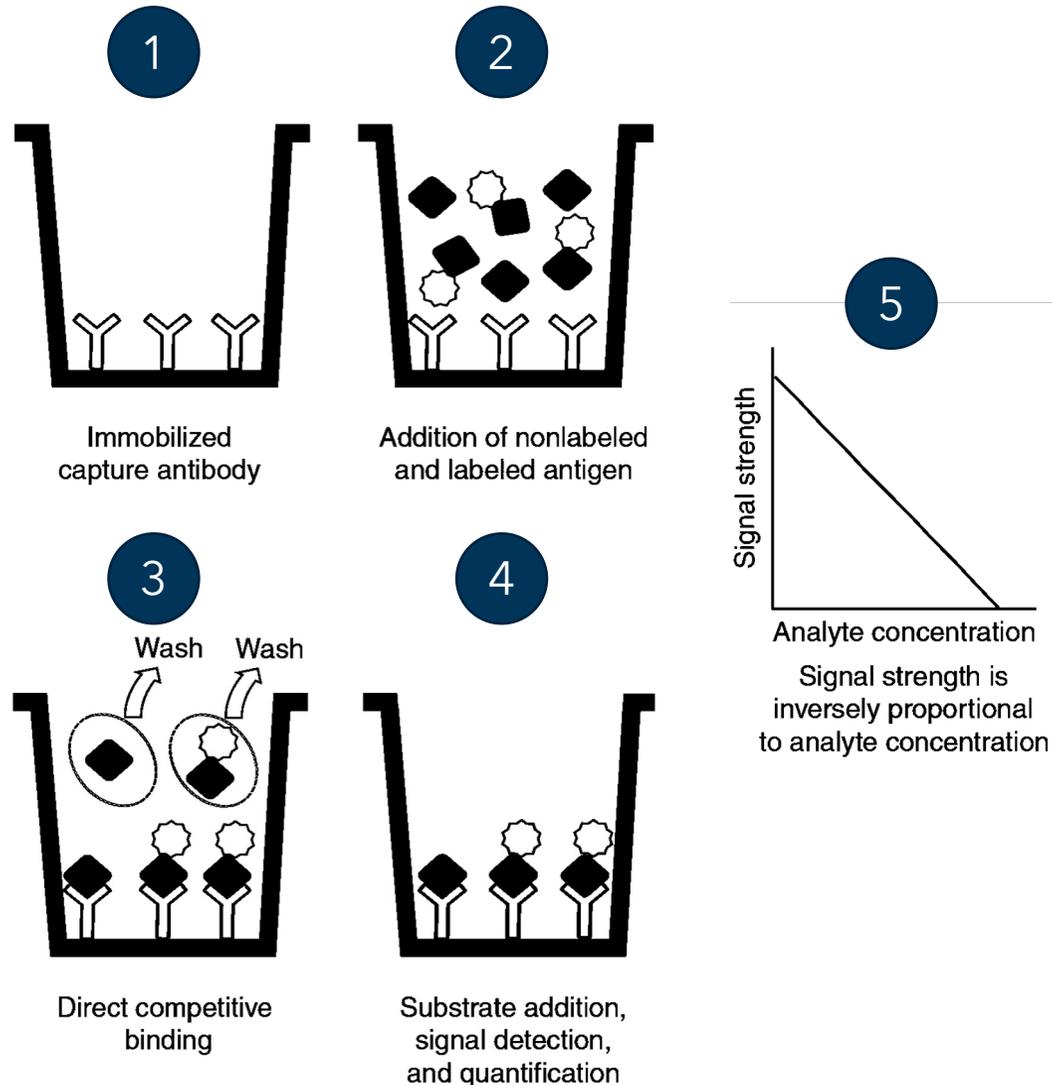
First-of-its kind quantitative and qualitative ELISA

High accuracy (93%), precision, and specificity

Low analytical detection limit (0.15 - 19.53 ppb)

Results available within days at an affordable price

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*Article*

## **Analytical Validation of a Direct Competitive ELISA for Multiple Mycotoxin Detection in Human Serum**

Kunal Garg <sup>1,\*</sup> , Fausto Villavicencio-Aguilar <sup>2</sup>, Flora Solano-Rivera <sup>2</sup> and Leona Gilbert <sup>1,\*</sup> 

<sup>1</sup> Tezted Ltd., Mattilaniemi 6-8, 40100 Jyväskylä, Finland

<sup>2</sup> Sanoviv Medical Institute, KM 39 Carretera Libre Tijuana-Ensenada s/n Interior 6, Playas de Rosarito, Baja 11 California, Rosarito 22710, Mexico

\* Correspondence: kunal.garg@teztet.com (K.G.); leona.gilbert@teztet.com (L.G.)

# TOXIPLEX BASIC offers a low limit of detection

Parameters or Studies for Comparison		AFB1 (ppb)	DON (ppb)	FUM (ppb)	OTA (ppb)	ZEA (ppb)
Lowest EU guidance levels for food °		2 ◊	500 ◊	800 ◊	2 ◊	50 ◊
LLOQ from the present study *		0.61	19.53	4.88	19.53	0.15
<b>Commercial tests for food</b>	Cusabio (ELISA) §	1.5	100	30	1.5	30
	Elabscience (ELISA) ⊗	0.6	150	20	5	6
	Helica™ (ELISA) ⊗	4	500	100	1	NI
	AgraQuant® (ELISA) #	2	250	250	2	25
	VICAM (LFIA) ^	2	250	200	2.5	100
<b>Emerging technology for food</b>	Wu et al. 2020 (LFIA) ∅	0.1	NA	4	0.2	0.8
	Xing et al. 2020 (LFIA)	4	200	20	NA	40
	Charlarmroj et al. 2021 (LFIA) ∅	5	10	0.5	NA	10
	Joshi et al. 2016 (SPR) ∅	3	26	10	13	16
	Wie et al. 2019 (SPR) ∅	0.9	5.3	NA	1.9	10.3

TOXIPLEX BASIC LLOQ compared with antibody tests for foodstuffs because direct ELISA for human serum/plasma did not exist in the literature

# TOXIPLEX BASIC demonstrates low cross-reactivity

		<b>Mycotoxin standards</b>					0	Cross reactivity %
		AFB1	DON	FUM	OTA	ZEA		
<b>Monoclonal antibodies against mycotoxin</b>	Anti-AFB1	100	2	2	2	2	20	
	Anti-DON	10	100	8	11	11	40	
	Anti-FUM	4	4	100	4	4	60	
	Anti-OTA	11	10	8	100	11	80	
	Anti-ZEA	4	4	3	3	100	100	

# TOXIPLEX BASIC example result report

ArminLabs | MVZ für Integrative Diagnostik und Medizin GmbH - branch practice - Zirbelstr. 58 2nd floor - 86154 Augsburg - Germany

MVZ für Integrative Diagnostik  
und Medizin GmbH  
Zirbelstr. 58

D 86154 Augsburg

Patient :

Date of Birth:

**Final report**

Order-ID : Page 1 / 1

Date of Reception/Report :

Analysis	Result	Units	Reference Range	Chart
<b>ToxiPlex</b>				
6 Aflatoxin B1	negative		negative	
6 Deoxynivalenol	62.4		negative	
6 Fumonisin (B1+B2)	negative		negative	
6 Ochratoxin A	312.5		negative	
6 Zearalenone	negative		negative	

Mycotoxin type	Detected (YES / NO)	Calculated concentration (ppb)
Aflatoxin B1 (AFB1)	NO	< 0.61
Deoxynivalenol (DON)	YES	62.4
Fumonisin (FUM)	NO	< 4.88
Ochratoxin A (OTA)	YES	> 312.5
Zearalenone (ZEA)	NO	< 0.15

Plate controls	
Positive	PASS
Negative	PASS

Serologically evidence of an immune reaction against the Deoxynivalenol and Ochratoxin A by TOXIPLX BASIC test.

»Deoxynivalenol: Belonging to the mycotoxin family of trichothecenes, this is found mainly in cereals, such as wheat and beans, as well as in spices. It can also be found in homes, basements, on the filters of air conditioners in cars or triggered through moisture or water damage.

Because it is metabolised rapidly, short-term symptoms may include nausea, vomiting, abdominal pain, headache, dizziness, and fever. Effects at the cellular level are due to binding to ribosomal subunits and inhibition of protein synthesis. Membrane function is thought to be altered due to lipid peroxidation, and intercellular communication and deregulation of calcium homeostasis may be affected.

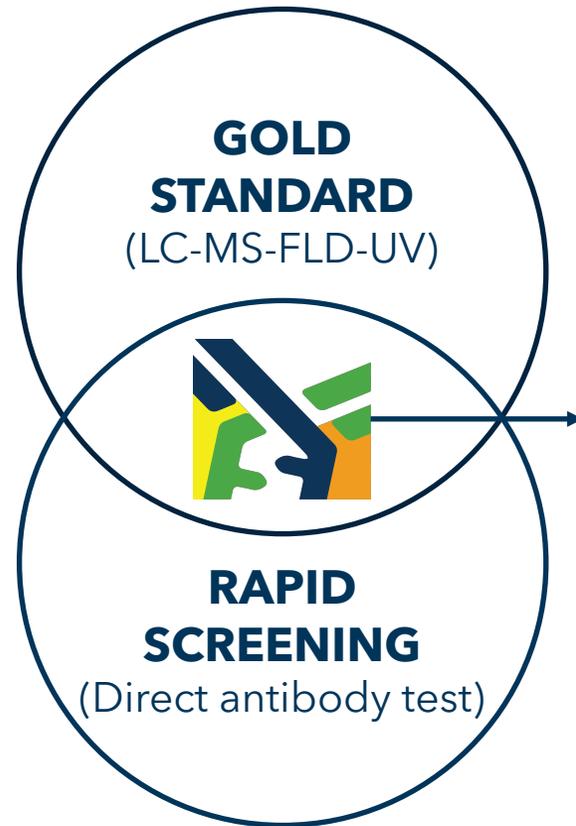
»Ochratoxin A: A mycotoxin found primarily in cereals especially wheat and barley, as well as their products such as dried fruit, spices, licorice, coffee beans, wine, grape juice and roots. It can also occur in meat from animals that have consumed contaminated grains. It is mainly produced by fungi in the genera *Aspergillus* and *Penicillium*. Ochratoxin A has also been detected in dust samples of airborne particulates in relevant environments. Ochratoxin A is associated with endemic nephropathy in humans, as well as urinary tract tumours. There are numerous records of nephrotoxic, hepatotoxic, teratogenic, and immunotoxic damage from this mycotoxin in livestock, but only a few reports of human health effects, which is why IARC classifies it as a Group 2B possible human carcinogen.

validated by

Dr. Armin Schwarzbach, AL Dr. Mihail Pruteanu

# Summary

We are exposed to mycotoxins through contaminated



## TOXIPLEX BASIC

1. Test multiple mycotoxins
2. High accuracy
3. High sensitivity
4. Easily accessible for patient

# HOW TO ORDER

## AONM/ArminLabs Order Form



TICKPLEX ANTIBODY SCREENING		
74	Tickplex Basic IgG/IgM antibodies (Borrelia)	Serum
<input type="checkbox"/>	75 Tickplex Plus IgG/IgM antibodies (Borrelia, Bartonella, Babesia, Ehrlichia, Coxsackie, EBV, Parvovirus B19, Mycoplasma fermentans/pneumoniae, Rickettsia)	Serum
YEASTS & MOULDS		
<input type="checkbox"/>	338 ToxiPlex Basic (Mycotoxins: Aflatoxin B1, Deoxynivalenol, Fumonisin (B1&B2), Ochratoxin A, Zearalenone)	Serum
<input type="checkbox"/>	70a Candida albicans Elispot	CPD
<input type="checkbox"/>	-- Candida albicans iSpot	CPD
<input type="checkbox"/>	70 Candida IgG/IgA/IgM antibodies	Serum
<input type="checkbox"/>	72a Aspergillus Peptide Mix 1&2 Elispot	CPD
<input type="checkbox"/>	Aspergillus Peptide Mix 1&2 iSpot	CPD
AONM TEST PANELS		
<input type="checkbox"/>	12 Standard Virus Panel	Serum

arminlabs

ACADEMY OF NUTRITIONAL MEDICINE

## ORDER AN AONM ARMINLABS TEST KIT

1. **VISIT** [www.aonm.org/mycotoxin-testing/](http://www.aonm.org/mycotoxin-testing/)
2. **CALL** AONM UK Helpline: 03331 210 305
3. **EMAIL** [info@eonm.org](mailto:info@eonm.org)



# Thank you

**Kunal Garg**

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