Flow Cytometry Based Myco-Epidemiology has Potential Impact on Mycotoxin outbreaks and Health: A Largely Ignored Global Concern
A Harmonized Approach to Preventive Medicine

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Abstract:

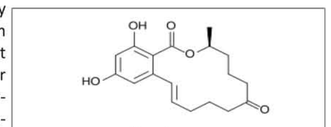
The European Union (EU) is a multi-national cooperative arrangement where surveillance of food-borne mycotoxins must be effective. EU's grain supply is susceptible to fungal infections which in turn can produce a variety of mycotoxins including 6 that pose danger. The 6 mycotoxins as individual or as co-contaminants, represent worldwide health threats. Recently, a competitive immunoassay kit that detects up to 6 mycotoxins based on 2-color flow cytometry (FC) has been introduced as monitoring assay to support the food industries. In the near future, FC may take a commanding role in reducing the risk of diseases associated with mycotoxin in food.
Adverse health effects caused by ingested food is often by mycotoxins, metabolites of fungi. They can be carcinogenic, neurotoxic, immunotoxic, hepatotoxic, & nephrotoxic. So far only risk assessment and risk management (establishment of regulating with maximum tolerable limits) strategies have been deployed in industrialized countries. To reduce health risk, agriculture and health ministries must work in concert. A new tool could provide synergy if harmonization of two administrative branches of a government are successful. Myco-epidemiological alimentation surveillance technology (MEAST) is the new tool. With MEAST, it is possible to collect scientific evidence required to prevent the movement and/or resale of contaminated food. Portable, FC with automated 96 well processing front end can be cost effective. The stage to combat food-borne mycotoxin contamination is set. Implementation is possible with already available FC and mycotoxin multiplexed kits to detect the mycotoxins.

Introduction:

Mycotoxins are produced by fungi found in both animal feedstuff and some human food commodities. These naturally occurring toxins can cause kidney and liver damage, cancer, suppress the immune system, introduce malnutrition, and cause disruption of the endocrine system: hyperoestrogenism.

The Challenge:

Flow cytometry had an amazing clinical impact in the early 1980's, it was used to monitor CD4 T-cells to stratify people suffering with the AIDS pandemic. Can multiplexed flow cytometry, a well established biomedical platform have similar impact on the management of environmental disasters such as poly-mycotoxic outbreaks? Pre-emptive systemic characterization of what is going on throughout the food chain will help to prevent outbreaks of animal and human disease rather than merely explaining them in post disaster assessment. To offer a comprehensive monitoring platform for both food commodities and for already exposed individual's, toxic exposure levels must be monitored. A flow cytometry based poly-mycotoxin assay is introduced as a tool for epidemiologists. The second flow cytometry based component that will detect poly-myotoxins in blood or urine is still under development. Together they will form a pre-emptive platform to set-off alarms when needed to prevent environmental disasters related to mycotoxins.



Zearalenone
(a toxin of Fusarium molds) targets the reproductive organs, because it is similar in chemical structure to estrogens.

Detection Limits USA and EU

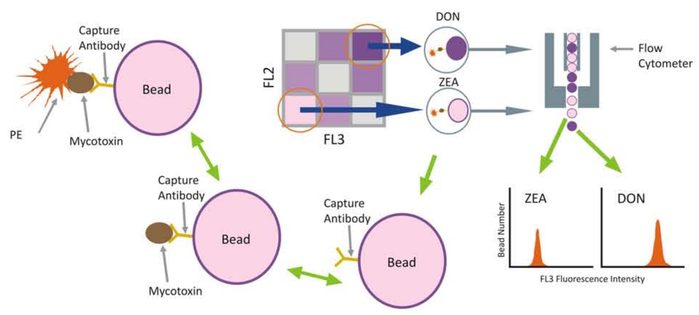
Mycotoxins	*Aflatoxin B1*	*Ochratoxin A*	*Fumonisins*	*T-2 toxin*	*Zearalenone*	*Deoxynivalenol (Vomitoxin)*
EU limits (µg/kg)	2	5	2000	300	100	1250
USA limits (µg/kg)	20	20	5000	500	300	2000

ELISA	Fungi-Plex
1. Mix the components	1. Mix the components
2. Incubate	2. Incubate
3. Wash	3. Measure and analyze
4. Add substrate	
5. Incubate	
6. Add stopping reagent	
7. Measure and analyze	

Methods:

To establish the utility of the multiplexed flow cytometry based competitive immunoassay for mycotoxins, a side by side comparison was performed with ELISA technology for two of the six monitored mycotoxins. The demonstration was to illustrate the flexibility of the multiplexed technology. A consolidated one step extraction method was implemented.

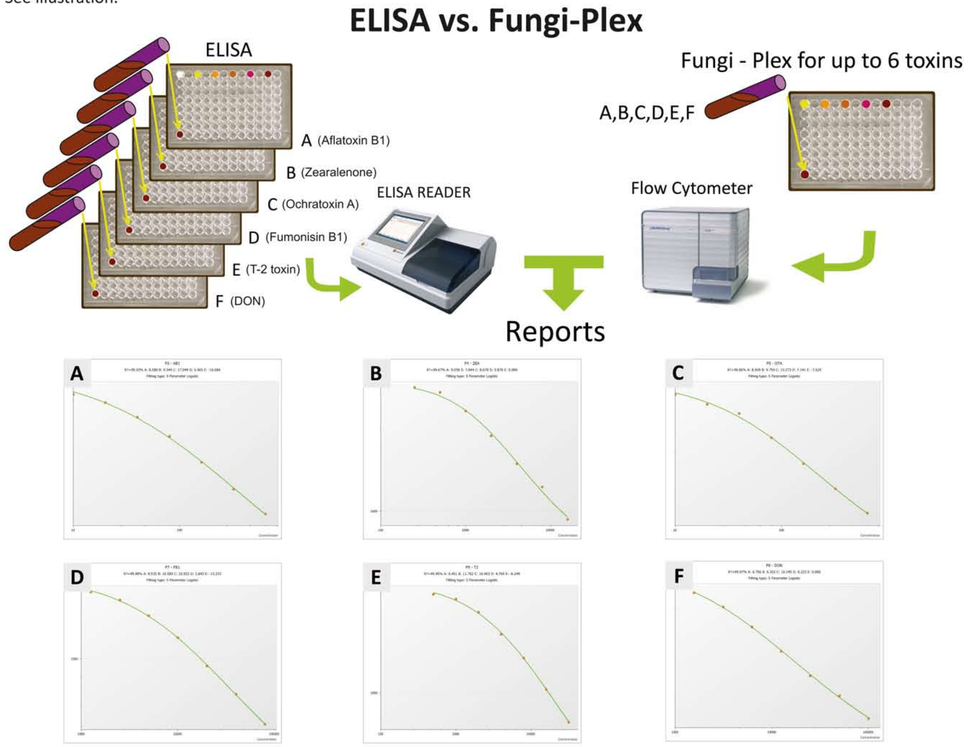
Fungi-Plex for DON and ZEA



Methods:

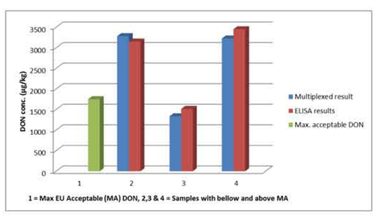
A Multiplexed Competitive Immunoassay Panel for Mycotoxins.
See illustration.

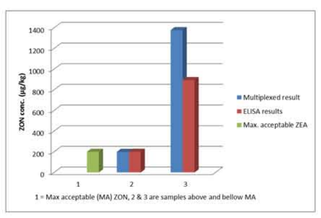
ELISA vs. Fungi-Plex

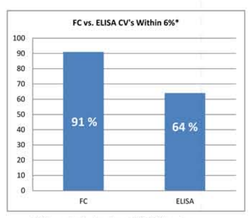


Results:

As illustrated in the tables the results for DON and ZEA compared well with the ELISA result.
Both recovery and reproducibility are excellent.







*When duplicates have CV's 6% or less

Conclusion:

It is possible to develop a pre-emptive platform based on multiplexed flow cytometry that can monitor both primary and secondary poly-mycotoxin characterization. It will help to prevent outbreaks in animal and human populations and thus reduce disease burden. A comprehensive monitoring platform for food commodities was demonstrated. The platform needs to be further developed for monitoring exposed individual's toxin levels. The flow cytometry based poly-mycotoxin assay is a tool for myco-epidemiologists

References

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