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The Comparation of Limits of Detection of Lateral Flow Immunochromatographic Strips of Different Types of Mycotoxins

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***Abstract*—**Mycotoxins are secondary metabolic products of fungi. These are poisonous, carcinogens and mutagens in nature and pose a serious health threat to both humans and animals, causing severe illnesses and even deaths. The rapid, simple and cheap detection methods of mycotoxins are of immense importance and in great demand in the food and beverage industry as well as in agriculture and environmental monitoring. Lateral flow immunochromatographic strips (ICSTs) have been widely used in food safety, environment monitoring. 46 papers were identified and reviewed on Google Scholar and Scopus for their limit of detection and nanomaterial on Lateral flow ICSTs on different types of mycotoxins. The papers were dated 2001-2021. 25 papers were compared to identify the lowest limit of detection of among different mycotoxins (Aflatoxin B1: 10, Zearalenone: 5, Fumonisin B1: 5, Trichothecene-A: 5). Most of these highly sensitive strips are competitive. Sandwich structures are usually used in large scale detection. In conclusion, the limit of detection of Aflatoxin B1 is the lowest among these mycotoxins. Gold-nanoparticle based immunochromatographic test strips have the lowest limit of detection. Five papers involve smartphone detection and they all detect aflatoxin B1 with gold nanoparticles.

***Keywords*—**Aflatoxin B1, limit of detection, gold nanoparticle, lateral flow immunochromatographic strips, mycotoxins, smartphone.

I. Introduction

MYCOTOXINS are toxic natural secondary metabolites produced by fungus, for example Aspergillus and Fusarium, on agricultural commodities in the field or during storage. These toxins cause food-and feed-borne intoxication, and many are cytotoxic, carcinogenic, mutagenic, or immunosuppressive [1], [2]. Aflatoxin is mainly produced by *Aspergillus* (A.) *flavus* and *A. parasiticus*, has four major types: B1, B2, G1, and G2 [3], [4]. Fusarium toxins are produced by Fusarium, can be divided three major groups: Zearalenone (ZEN), Fumonisins, Trichothecenes [5]. ZEN is a non-steroidal estrogenic compound with a toxic oestrogen effect, destroying the reproductive system of animals, such as the oestrogen syndrome in pigs, despite its low toxicity after oral administration [6]. Fumonisins have strong structural similarity to sphinganine and toxic oestrogen effect which lead can lead to cancer. Fumonisins can be divided into A, B, C, P main subgroups, and a subtype in fumonisins B- fumonisin B1 (FB1) is the most toxic and abundant of all the fumonisins [7]. Trichothecenes are a class of sesquiterpenes and also have toxic oestrogen effect on reproductive performance of animals and humans. Trichothecenes have four main subgroups: A, B, C, D and a type in Trichothecenes A (T- 2) is the most common one hat present a potential hazard to health worldwide [8].

25% of the world's crop harvests are contaminated with mycotoxins. Crops that are frequently affected (especially in warm and humid conditions) include maize, sorghum, wheat, oats, rice, soybean, sunflower, cotton seeds, chili peppers, black pepper, coriander, turmeric, ginger, peanut, pistachio, almond, walnut, coconut, Brazil nut, dry vine fruits, wine and grape juice and rice liquor [9]. Mycotoxins arise in foods such as grain if it is invaded by fungi, which happens mainly due to moisture content, temperature, or humidity being too high for safe storage. Field drying is an accepted practice since commercial farming began, but it depends on sun and wind, and mechanical drying is often needed, to get a crop to 12-14% moisture. For qualitative test methods there are no general validation procedures available. Solely the cut off level is defined as the concentration threshold below which positive identification becomes unreliable [10]. The moisture of crops will be measure in due time with period time with concentration of mycotoxin. The threshold of time will be identified in risk assessment to provide quantitative data for human health.

Due to the health risks for humans and animals, authorities such as the European Commission or the Grain Inspection, Packers and Stockyards Administration (GIPSA) has addressed the mycotoxin problem by adopting regulatory limits. Regulations are in force for, e.g., aflatoxins, ochratoxin A, and Fusarium toxins in selected foodstuffs (EC 1881/2006) [11], and there are recommendations for maximum levels of mycotoxins in feed (EC (2006/576/EC)) [12]. The action levels or advisory levels are in force for, e.g., aflatoxins and deoxynivalenol, respectively. Among aflatoxins, AFB1 is the most toxic as a potent carcinogen [13]. Most countries have implemented the maximum residue limits (MRLs) of ZEN in cereal foods and feeds. For instance, the MRL of ZEN in cereals and cereal products is 2 mg/kg, in corn by-products is 3 mg/kg, and 0.1 mg/kg in compound feeds for piglets and young sows, based on the EU guidelines [2]. In Italy, the MRL of ZEN in cereals and cereal products is 0.1 mg/kg [14] and 0.05 mg/kg in Australia [15]. The current ZEN MRL theme in China is "GB 2761-2017 Food Mycotoxin Limit", strictly showing that the MRL of ZEN in wheat, wheat flour, corn, and corn flour is 0.06 mg/kg [13]. Among all fusarium toxins, the ZENs, which is the

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most strongly associated with chronic and fatal toxic effects in animals and humans [5], [6].

Currently rapid portable testing platforms for the detection of potentially dangerous mycotoxins in food and beverage production are very limited. The most used techniques for detecting AFLs are thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). However, these methods require extensive sample preparation, expensive instruments and professional operation. Alternatively, enzyme-linked immunosorbent assay (e.g., ELISA) has been successfully developed for AFLs [16]. However, ELISA also requires incubation and washing steps which are mainly confined to laboratories.

Technologies such as microplate readers are based on 'non-traceable' approximated methodologies and rely on time-consuming laboratory sample preparation and cleaning procedures. Reliable existing fungi testing techniques such as immune-based and molecular assays, e.g., enzyme-linked immunosorbent assay and polymerase chain reaction approaches provide very accurate and sensitive detection of fungi. However, these methods are labour and time intensive (normally take many hours), do not account for fungi mutations, are unclear regarding persistence of infection and/or require prior knowledge of the strains. Alternative faster methods are not accurate and often give false positive/negative results. Thus, there is a need for fast, cost-effective, accurate testing techniques for definitive fingerprint detection of disease and environmental markers at low concentrations.

Microfluidics, Lab-on-a-Chip, Smart Nanospectroscopic, and the now emerging Lab-in-a-Fiber, as well as, sensor technologies are the key technological interfaces that must be overpassed to depart from micro and finally reach nano-volume spectroscopy. Microfluidic/Optofluidic lab-on-a-chip technologies are a commonly used solution in the growing area of diagnostics, particularly point-of-care testing; integrating functional modules commonly used in laboratories, into a small chip, for biomarkers testing. The technology has a unique characteristic of high surface area to volume ratio for a fast analysis time enabling complex diagnostic assays. However, 'interconnect and read-out bottleneck' and heat removal are two widely acknowledged challenges limiting performance, also hindering the implementation of relevant devices in smart needles and endoscopes.

Lateral flow ICSTs have received increasing attention for qualitative and quantitative analysis in different areas [17]. ICSTs has been widely used in food safety, environment monitoring, and precision medicine [18]. Delmulle et al. developed an ICSTs for the detection of AFB1 in Pig Feed in 2005 [1]. Xie et al. have made the greatest effort to investigate the effect of the core-shell silver-gold nanocomposites on the properties of ICSTs [19]. However, this detection method can only provide qualitative results (positive or negative) or semi-quantitative information on analyte concentration, thus the ICSTs could not satisfy requirements for practical applications. Therefore, many devices providing quantitative analyte concentration for testing ICSTs have been developed [20]. A biotin-modified aptamer and fluorescent cyanine 5 have been used as a dot assay on a lateral flow immunoassay test strip for detection of AFB1 by Lu et al. [21]. The intensity of the fluorescent dot was collected in the fluorescence apparatus, desktop computer or laptop, which possessed rapid processing speeds and stable performances. However, due to these bulky and heavy devices, they have been limited to personal users [22]. Obviously, the strip reader which is based on a mobile device is advantageous, as it satisfies the requirement of being portable and feature-rich testing. As a matter of fact, the mobile health market is rapidly developing and portable diagnostics tools provide an opportunity to increase the availability of healthcare and decrease costs [23]. Therefore, various strip readers based on mobile devices have been developed. This paper will evaluate the limits of detection of different mycotoxins.

II. Research Approach

Sandwich structures are usually used in large scale for mycotoxins detection. For the purpose of present study, information was collected using Google Scholar and Science Direct search engine. Scientific papers dated from 2001-2021 were analysed. 25 papers were compared to identify lowest limit of detection among different mycotoxins (AFB1: 10, ZEN: 5, FB1: 5, T-2: 5) (Fig. 1).

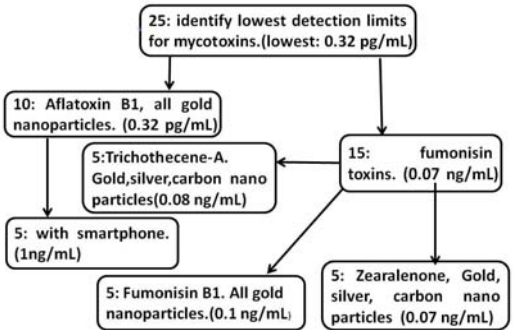

Fig. 1 Construction of Methodology

III. Results

The information of limit of detections on papers for data mining is outlined in Table I.

IV. Discussion

Most of the high sensitive strips are competitive. Most of the papers with low limit of detection have involved gold nanoparticles (other few were silver and carbon ones) (Table I, Fig. 2). In addition, the average detection of limit of gold is much less than silver and carbon ones. Therefore, gold nanoparticle is a great choice for immunochromatographic test strip. We can also find that limit of detection of AFB1 is much lower than those toxins, such as, FB1, ZEN, T-2. The limit of detection with smartphone can lower than the average limits of AFB1 without using smartphone. The smartphone app is still a promising method used in the detection of quantitative concentration of mycotoxins by Lateral flow ICST.

TABLE I

PAPERS WITH LIMIT OF DETECTION OF MYCOTOXINS

Mycotoxin	Limit of Detection (ng/mL)	Nanoparticle	Reference
AFB1	0.00032	Gold	[24]
AFB1	0.03	Gold	[25]
AFB1	0.1	Gold	[10]
AFB1	1	Gold+phone	[20]
AFB1	1.2	Gold+phone	[28]
AFB1	1.5	Gold+phone	[29]
AFB1	2	Gold	[26]
AFB1	2.5	Gold+phone	[30]
AFB1	2.5	Gold	[27]
AFB1	2.8	Gold+phone	[31]
FB1	0.1	Gold	[32]
FB1	0.12	Gold	[33]
FB1	2.5	Gold	[34]
FB1	5	Gold	[35]
FB1	5	Gold	[36]
ZEN	0.07	Gold	[37]
ZEN	0.2	Gold	[38]
ZEN	0.25	Silver	[39]
ZEN	0.58	Silver	[40]
ZEN	12	Carbon	[41]
T-2	0.08	Gold	[42]
T-2	0.1	Gold	[43]
T-2	0.9	Silver	[44]
T-2	5	Silver	[45]
T-2	13	Carbon	[46]

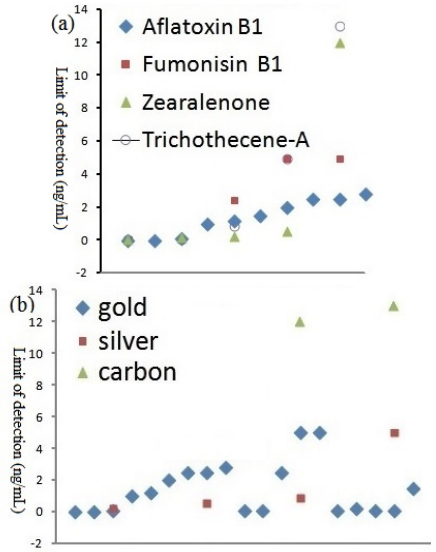

Fig. 2 Distribution of limit of detection on (a) mycotoxins and (b) nanoparticles

V. CONCLUSION

In conclusion, the limit of detection of Aflatoxin B1 is the lowest among these mycotoxins. Gold-nanoparticle based immunochromatographic test strips have the lowest detect limit. Some papers involve smartphone detection and they all detect AFB1 with gold nanoparticles. Quantitative concentration results can be obtained when the user uploads the photograph of test lines using the smartphone application.

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