A smartphone-addressable aptamer-based lateral flow biosensor for ochratoxin A

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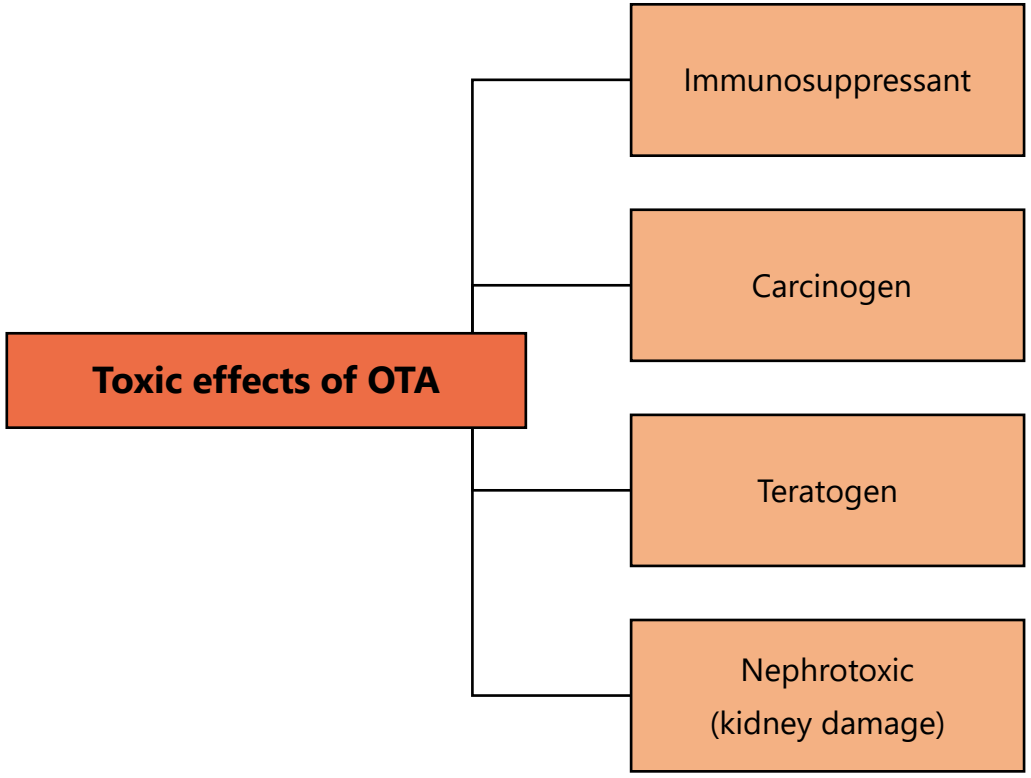
Ochratoxins

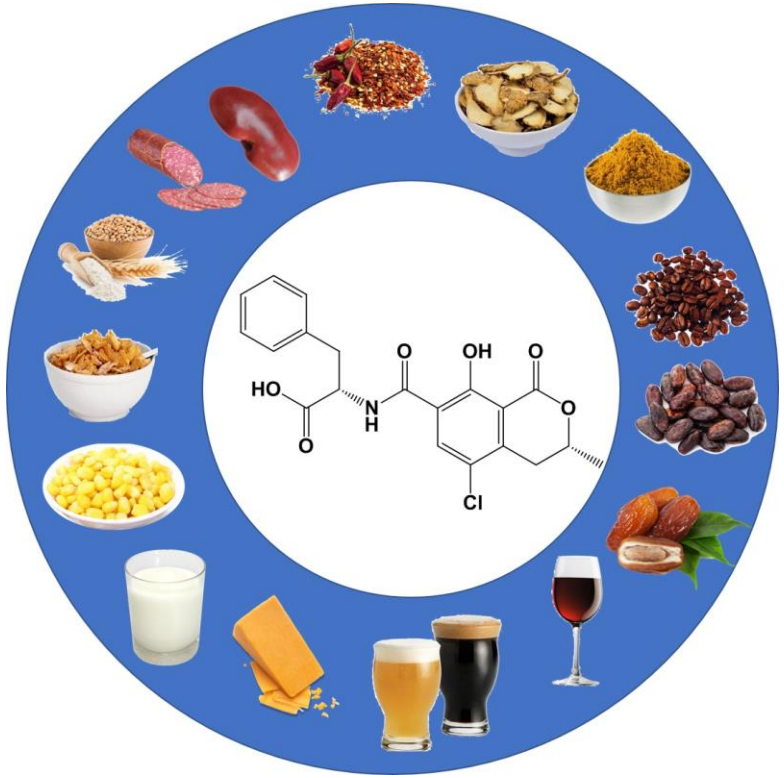
- Metabolites of many different species of *Aspergillus* and *Penicillium*

Ochratoxin A:

✓ Found in contaminated food and crops

✓ Recognized as potentially harmful to humans and animals.





*Source: Xianjiang Li, Wen Ma, Zhiyong Ma, Qinghe Zhang,Hongmei Li,The Occurrence and Contamination Level of Ochratoxin A in Plant and Animal-Derived Food Commodities, *Molecules,* **2021**, 26, 6928

Toxicity of Ochratoxin A:

Group 2B (potential **carcinogenic** to humans)

***IARC (International Agency for Research on Cancer) 1993**

Maximum Levels of OTA in Foodstuffs

Foodstuffs	Maximum levels (µg/kg)*
Unprocessed cereals	**5.0**
Roasted coffee	**3.0**
Wine	**2.0**
Processed cereal-based foods for infants and young children and baby foods	**0.5**
Seeds	**5.0**
Cocoa powder	**3.0**

*REGULATION (EC) No 1881/2006 of 19 December 2006 (setting maximum levels for certain contaminants in foodstuffs)
**REGULATION (EU) 2022/1370 of 5 August 2022 (amending Regulation (EC) No 1881/2006 as regards maximum levels of ochratoxin A in certain foodstuffs)

Analytical Methods for Ochratoxin A detection

	+	-
Chromatographic Techniques (thin-layer chromatography, liquid chromatography, gas chromatography, LC-MS/MS)	▪ high accuracy ▪ repeatability ▪ sensitivity	▪ expensive equipment ▪ time-consuming sample preparation ▪ trained personnel
Enzyme-Linked Immunosorbent Assays (**ELISA**) **Immunochromatographic assay**	▪ convenient ▪ sensitive ▪ easy to operate	▪ cross reactivity ▪ requirement of expensive and limited stability antibodies
Biosensors (Optical, Electrochemical)	▪ small size equipment ▪ sensitivity ▪ simultaneous analysis ▪ portable devices	▪ integration of all optical and electrical components on the same chip

Aptamer-based sensors

Aptamers are short chain oligonucleotides that exhibit binding affinity to selected target analytes

Advantages of aptamers

- ✓ high stability
- ✓ easily synthesized
- ✓ commercially available
- ✓ thermoresistant
- ✓ potential of modification with a variety of chemical groups such as *biotin, thiols*, *enzymes*

Gold nanoparticles

AuNPs react with the **sulfhydryl** or **amino** groups of aptamers to form **Au–S** or **Au–N** bonds.

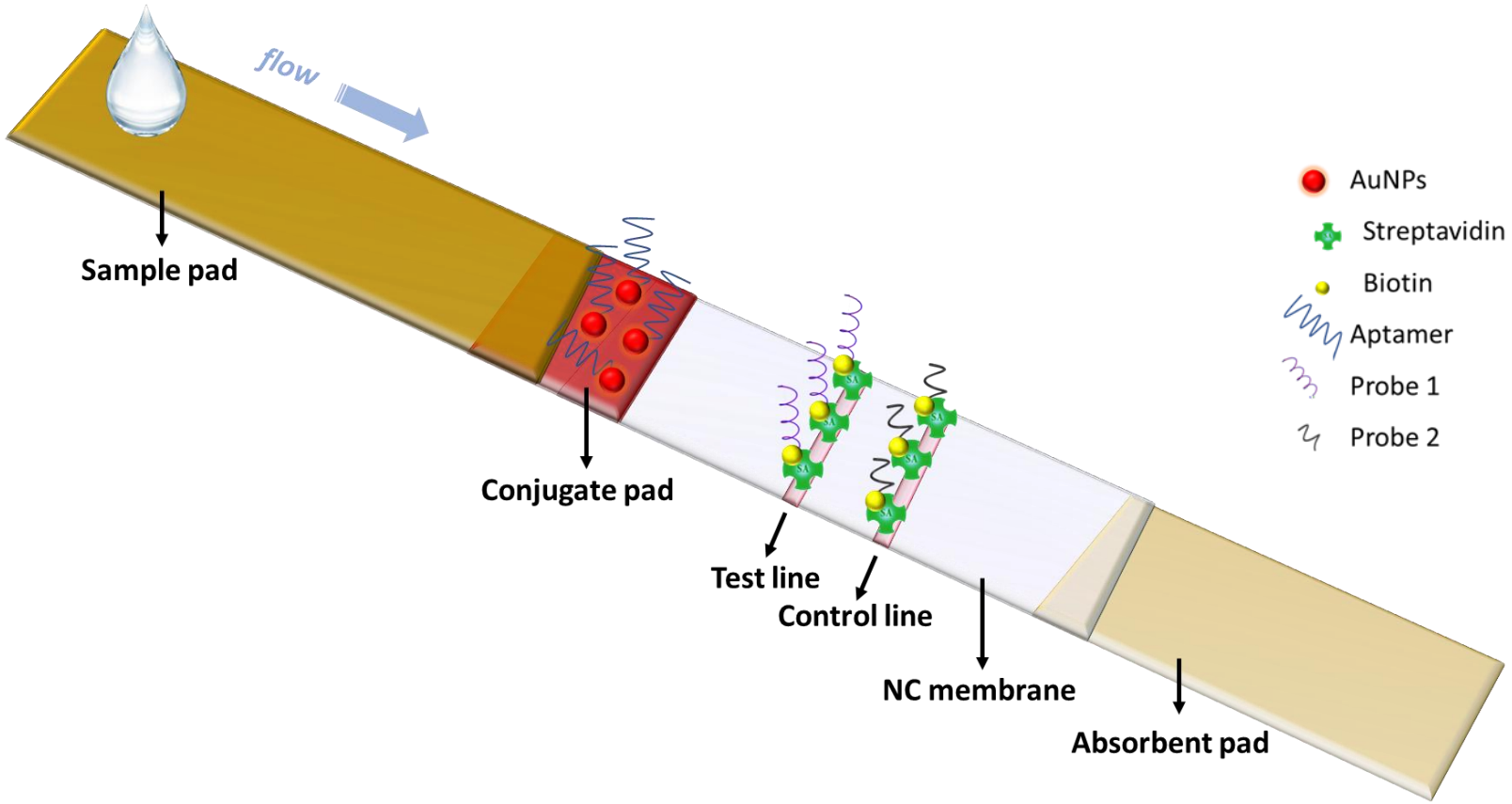
AuNPs aptasensors for OTA determination are classified into:

- ❑ fluorescence aptasensors,
- ❑ electrochemical aptasensors,
- ❑ colorimetric aptasensors and
- ❑ chemiluminescence aptasensors.

Fabrication of the aptamer-based biosensor strip

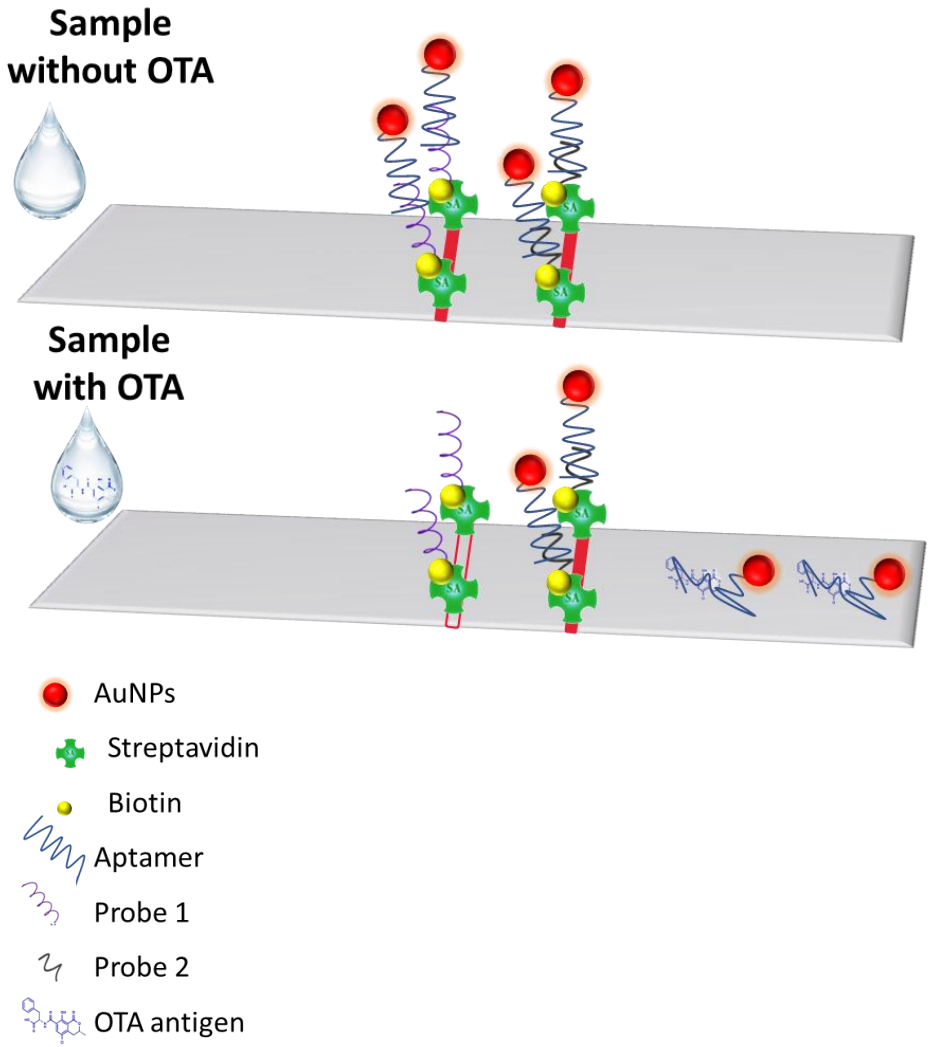
The lateral flow strip consists of: **a)** sample pad, **b)** conjugate pad, **c)** nitrocellulose (NC) membrane, **d)** absorbent pad

Nitrocellulose membrane ⟶ Test-line and control lines



Based on a lateral flow assay using **conjugates of OTA-specific aptamer** with **gold nanoparticles (AuNPs)**

Working Principle of the Assay for OTA Detection



Test line: **streptavidin–DNA probe 1** conjugate on NC

Control line: streptavidin–DNA probe 2 conjugate on NC

Strips are dried and stored at **4 ◦C** until use

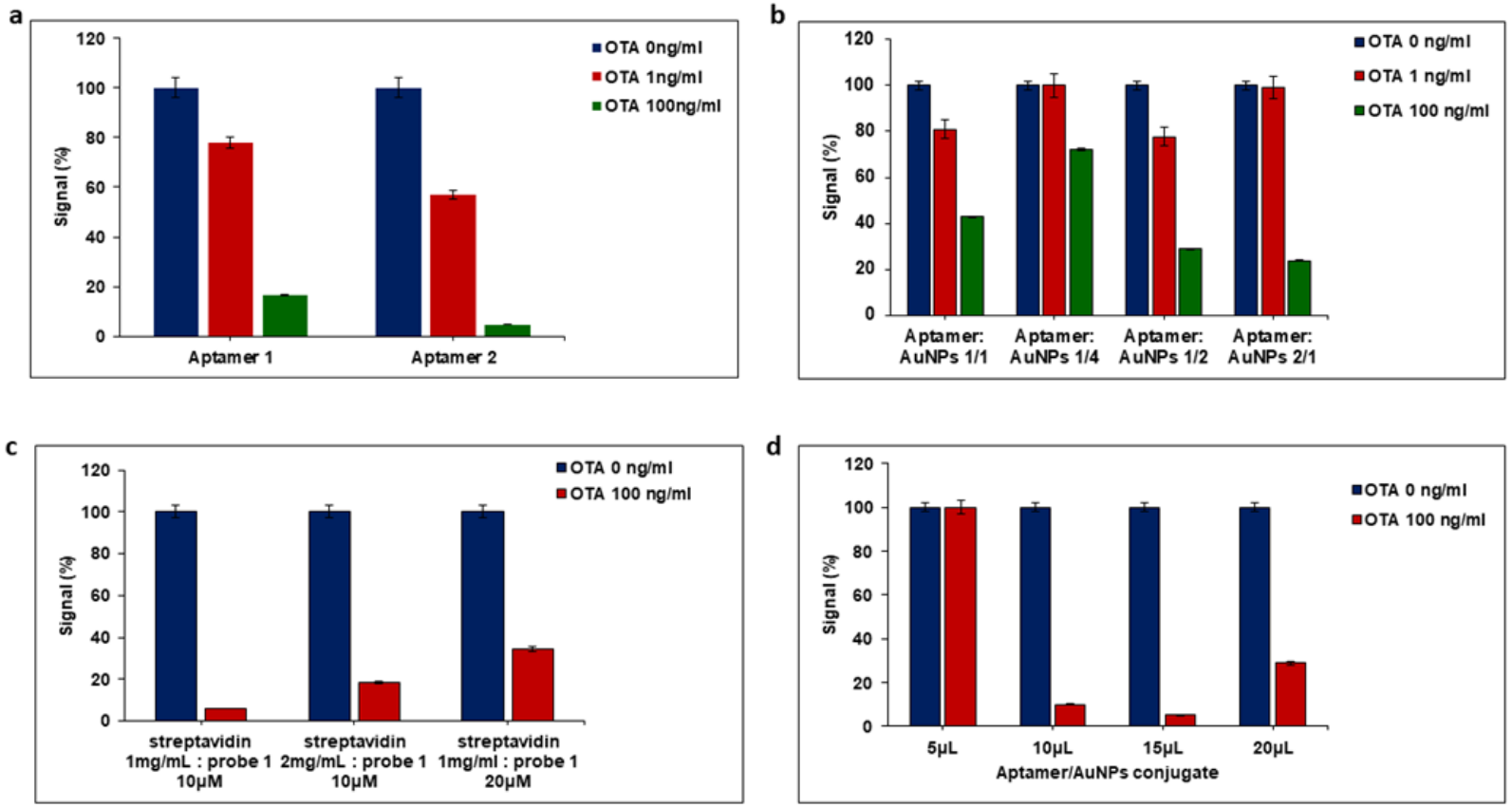
Aptamer probe/AuNPs: added to the conjugate pad

Sample: added to sample pad and move across the strip

Running buffer: added to the sample pad in order to wash the strip

Result in **30 minutes**

Optimization of Experimental Parameters



Testing:

a) **Two OTA specific aptamers (Aptamer 1 and Aptamer 2)**

Aptamer 1: 5`-C6 S-S-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA AAA AAA AAA AAA AAA AAA-3`; Aptamer 2: 5`-C6 S-S- AAA AAA AAA AAA AAA AAA GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3`

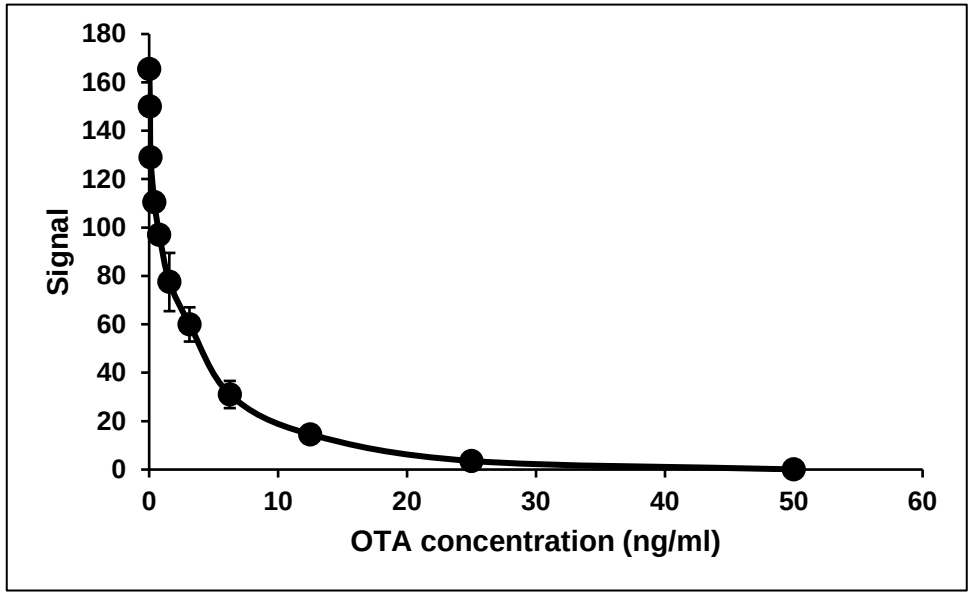
b) **the volume ratio of the aptamer to AuNPs for the formation of OTA aptamer-AuNPs conjugate,**

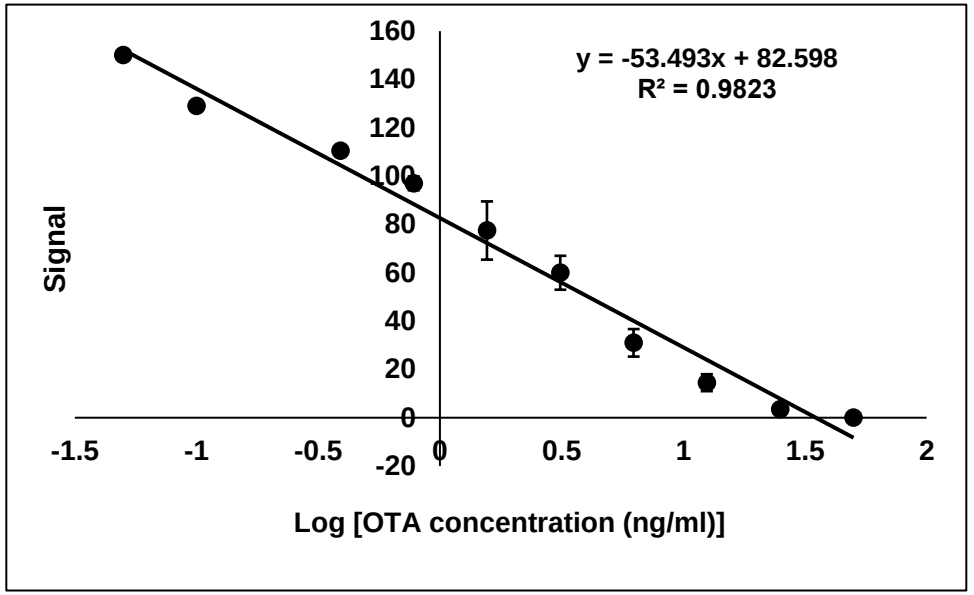
c) **the concentration of streptavidin and probe 1 for the test line,**

d) **the volume of the OTA aptamer/AuNPs conjugate applied on conjugate pad.**

Analytical Performance

Visual LOD
0.05 ng/ml





Semi-quantitative LOD
0.04 ng/ml

Conclusions

A colorimetric lateral flow assay, based on aptamer-AuNPs conjugates, was developed for OTA.

- linear range for OTA from 0.05 to 50 ng/mL
- limit of detection 0.04 ng/mL.
- assay time 30 min
- high sensitivity
- simple
- rapid
- low cost

Future prospects

The application of this assay is ongoing in our laboratory for OTA detection in real samples and similar approaches are under way for selected antibiotics.