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Phycotoxin contamination of fish seafood and water. Development of rapid immune tests and their evaluation

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Phycotoxins as toxic food contaminants

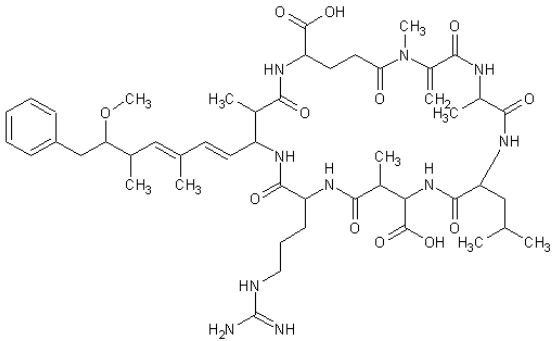
***Phycotoxins* – highly dangerous metabolites of microalgae and cyanobacteria**

Development of the global food market

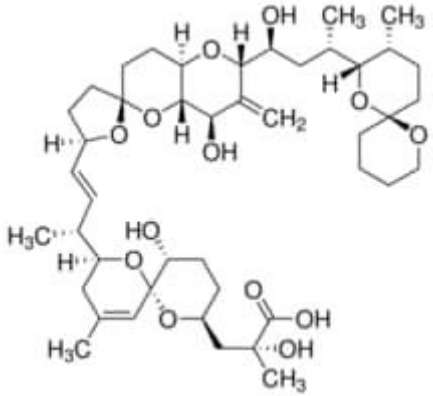
Importance of rapid monitoring of food contamination at different stages of its production and consumption

Reliable and sensitive control of toxic compounds that may contaminate raw materials and finished food products

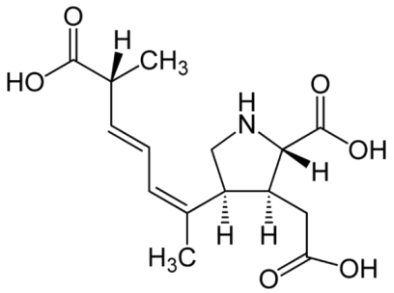
Microcystin-LR (MC-LR)



Okadaic acid (OA)



Domoic acid (DA)



Immunoassays (Ag + Ab = Ag:Ab)

Enzyme-**L**inked **I**mmuno**S**orbent **A**ssay (ELISA)

Lateral Flow Immunoassay (strip-test)

Immunosensors

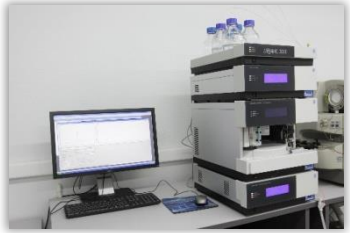
Fluorescence **P**olarization **I**mmunoassay (FPIA)

Traditional methods of phycotoxins' detection

- **Instrumental analytical techniques including HPLC and MS**

+ highly specific and sensitive;

– require expensive equipment, complex procedures of sample preparation before analysis, can be implemented only in laboratory conditions by a qualified personnel



Immunoanalytical methods of phycotoxins' detection

- **Enzyme-linked immunosorbent assay (ELISA)**

+ highly sensitive and specific

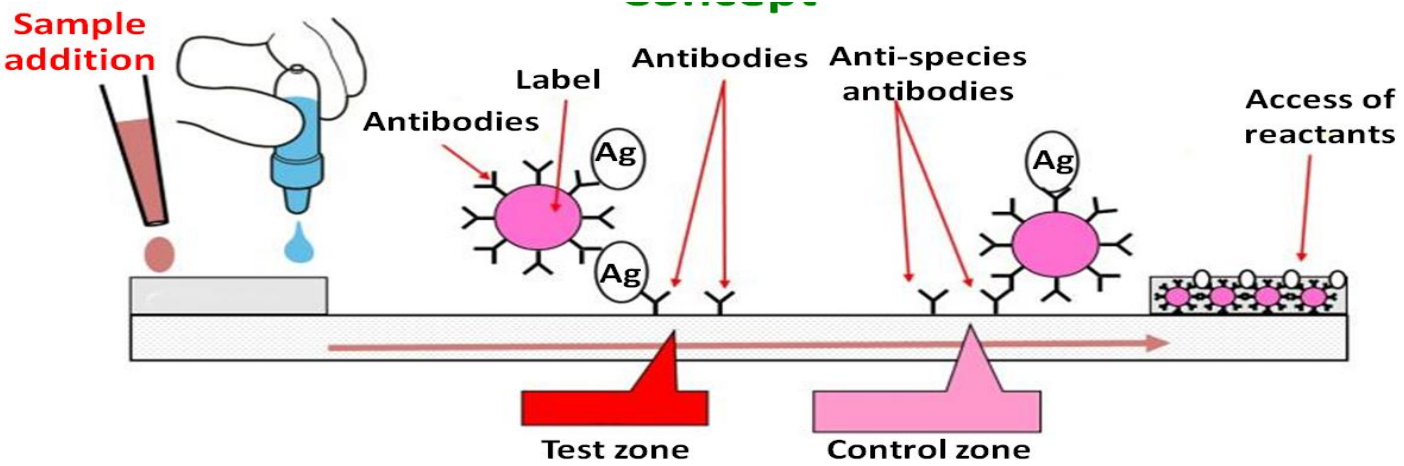
– time-consuming (2–3 hours)

- **Immunochromatographic analysis (lateral flow immunoassay)**

+ highly sensitive and specific, rapid (10–20 min), simple, cheap, and stable, suitable for point-of-care usage without any additional equipment

Lateral flow immunoassay (immunochromatography) (strip-test)

Concept



Advantag

- Test-strip contains all reactants for the assay
- Contact of sample and test-strip initiates all processes of the assay and signal generation
- The assay can be implemented without additional stages
- The assay results may be estimated without additional equipment (visually)

Lateral flow immunoassay

Production of test systems

1. Obtaining the immunoreagents

2. Appling of the components on the membrane carriers

3. Assembling of a multimembrane composite and cutting it into individual test strips

- ❑ All reactants are applied on membranes before the assay
- ❑ Contact of a sample and a test strip initiates all further processes
- ❑ The assay can be carried out without any additional reactants and manipulations
- ❑ The assay results may be estimated visually without any additional equipment

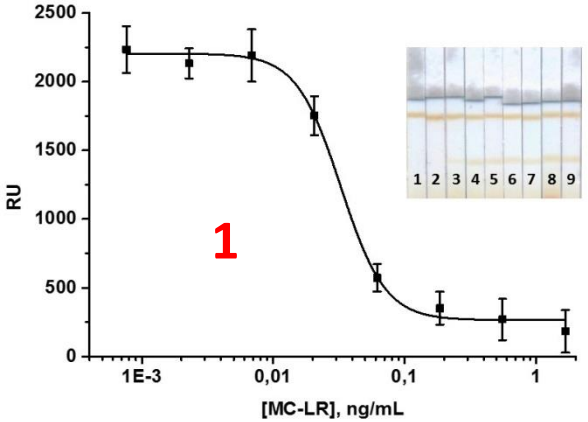
Methods for increasing LFIA sensitivity

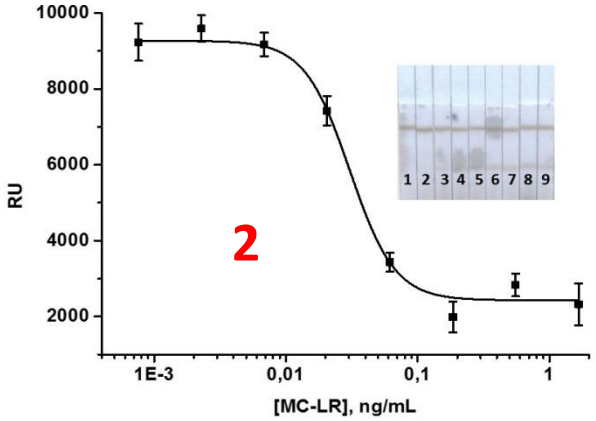
1. Variations in the physicochemical properties of labels (composition, shape, size, etc.)
2. Increasing the amount of a label in test strip zones

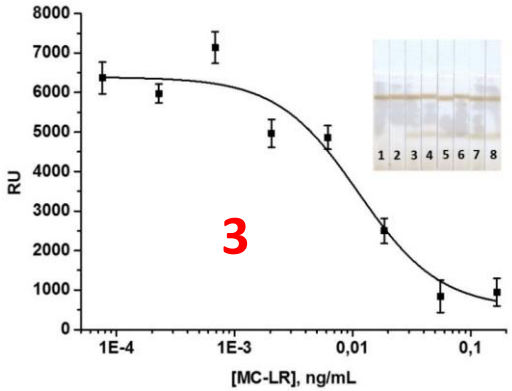
Original approach proposed in our study

The analysis of MC-LR is implemented in the indirect competitive format based on the use of magnetic particles (MPs) as a colored label for secondary antibodies and, at the same time, a carrier for peroxidase tag immobilized on their surface to enhance colorimetric analytical signal by the colored product of the enzymatic reaction

Gain in the LFIA sensitivity with the use of peroxidase enhancement







LFIA formats:

1 – LFIA without amplification

2 – LFIA with peroxidase amplification

3 – LFIA with peroxidase amplification and reducing concentrations of immonpreagents

Analytical parameters of the test systems:

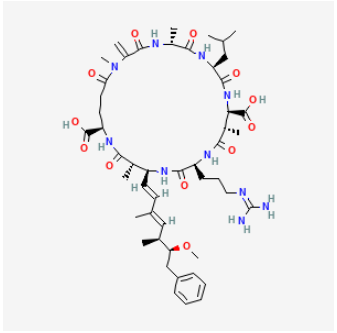
1 – instrumental LOD/cutoff = **13/550 pg/mL**; signal amplitude = **2250 RU**

2 – instrumental LOD/cutoff = **14/550 pg/mL**; signal amplitude = **9200 RU**

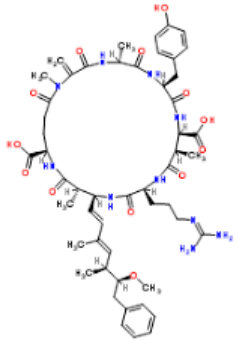
3 – instrumental LOD/cutoff = **2/50 pg/mL**; signal amplitude = **7500 RU**

The assay duration was 17 min

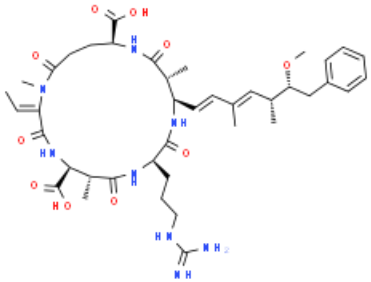
LFIA specificity



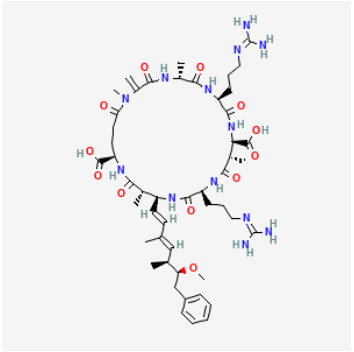
MC-LR



MC-YR



Nodularin



MC-RR

Cross-reactivity values obtained in the LFIA of MC-LR structural analogs

№	Phycotoxin	IC_{50}, ng/mL	Cross-reactivity, %
1	MC-LR	0.01	100
2	MC-RR	–	<0.1
3	MC-YR	–	<0.1
4	Nodularin	–	<0.1

Sample preparation before the analysis

Sample homogenization

Extraction of MC-LR with acetic acid

Centrifugation

Supernatants evaporation

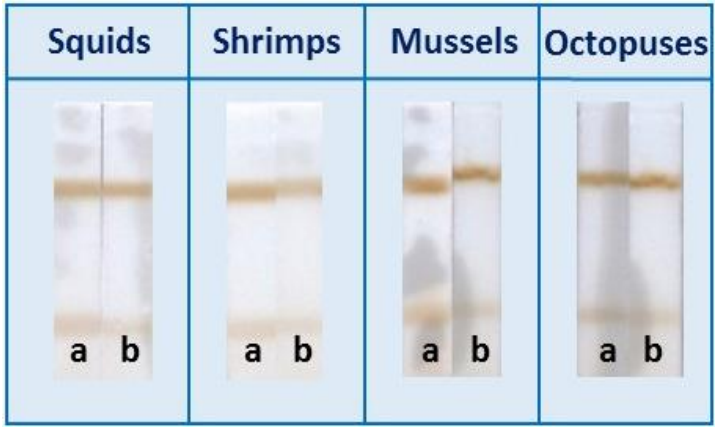
Dissolution of the residues in buffer

LFIA

Tested samples:

- ❑ shrimps
- ❑ squids
- ❑ mussels
- ❑ octopuses

Detection of microcystin-LR in seafood



Scans of test strips
after the determination of MC-LR in seafood
a – 0.01 ng/g; b – 0.02 ng/g of MC-LR

Recoveries of MC-LR from seafood samples

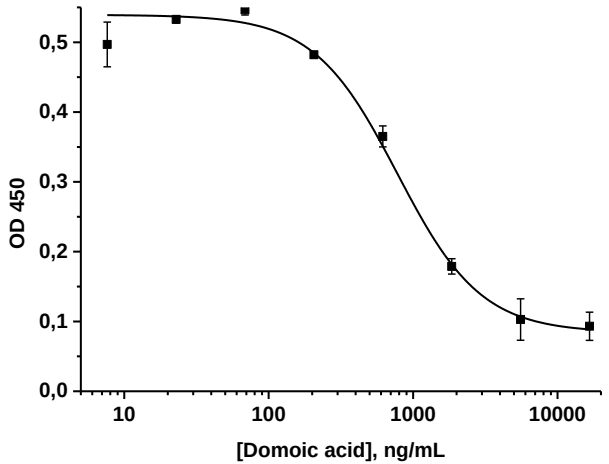
Added MC-LR (ng/g)	Detected MC-LR ± SD (ng/r)/Recovery ± SD (%)			
	Mussels	Shrimps	Octopuses	Squids
0.02	0.021 ± 0.003/ 101 ± 15	0.023 ± 0.001/ 115 ± 5.0	0.022 ± 0.001/ 110 ± 5.0	0.016 ± 0.002/ 80 ± 10
0.01	0.0071 ± 0.0015/ 71 ± 15	0.011 ± 0.001/ 110 ± 10	0.0093 ± 0.001/ 93 ± 10	0.0092 ± 0.0015/ 92 ± 15

Preparation and characterization of the specific reagents for the LFIA of domoic acid

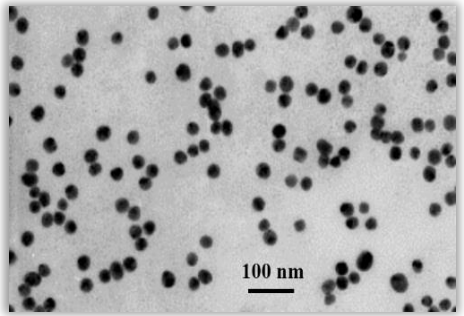
Characteristics of the produced anti-DA monoclonal antibodies

Clone of anti-DA monoclonal antibodies	Ig sub-isotypes	Titer in the ELISA, ng/mL	DA LOD in the ELISA, ng/mL
Dom B5	IgG1	5.9	6000
Dom C1	IgG1	7.9	>10.000
Dom C9	IgG1	12.5	>10.000
Dom D3	IgG1	14.3	180
Dom E4	IgG2b	3.1	9000
Dom F12	IgG2b	1.7	>10.000
Dom G6	IgG2a	11.6	370
Dom G10	n/d	92.9	>10.000
Dom H2	IgG1	205	>10.000

Calibration curve of DA in the ELISA with the use of clone Dom D3

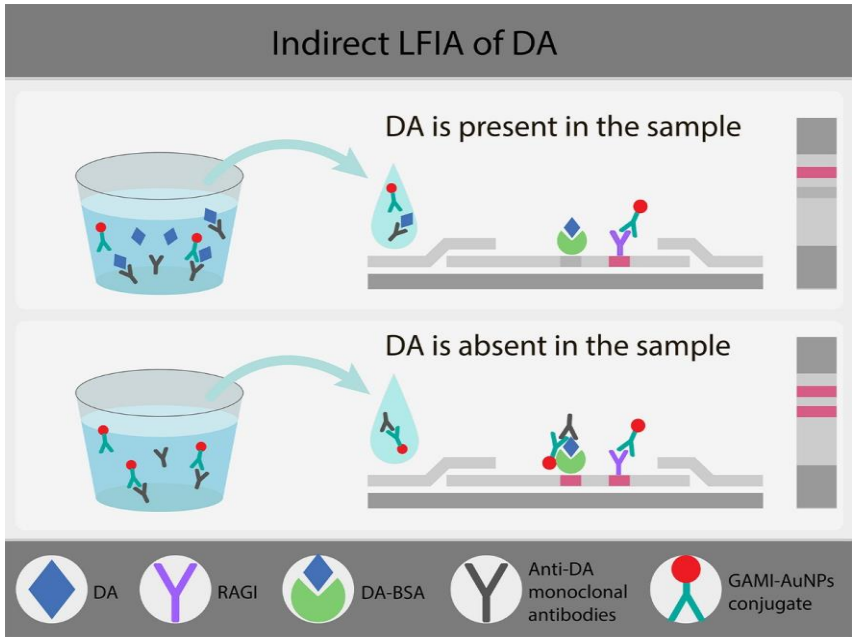


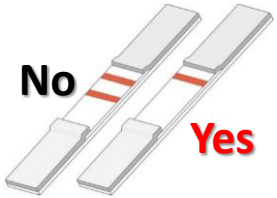
$HAuCl_4 + e^- = Au^0$



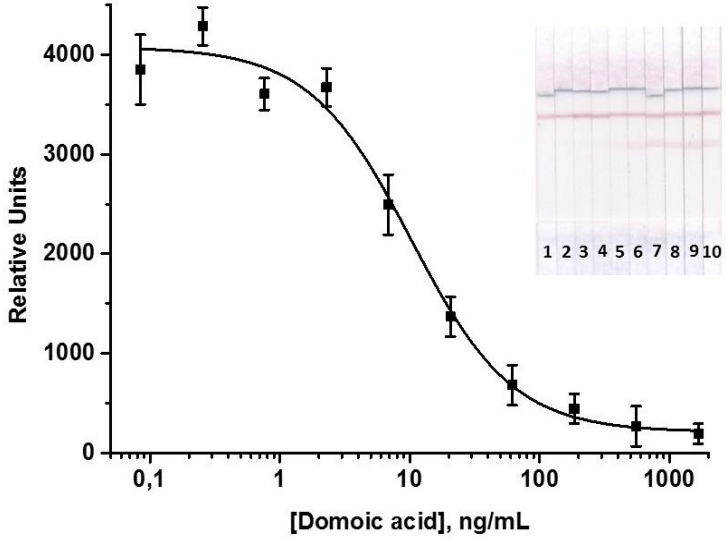
Microphotograph of gold nanoparticles used in the study

LFIA of domoic acid





Calibration curve of DA in the LFIA



Analytical parameters of the test system:

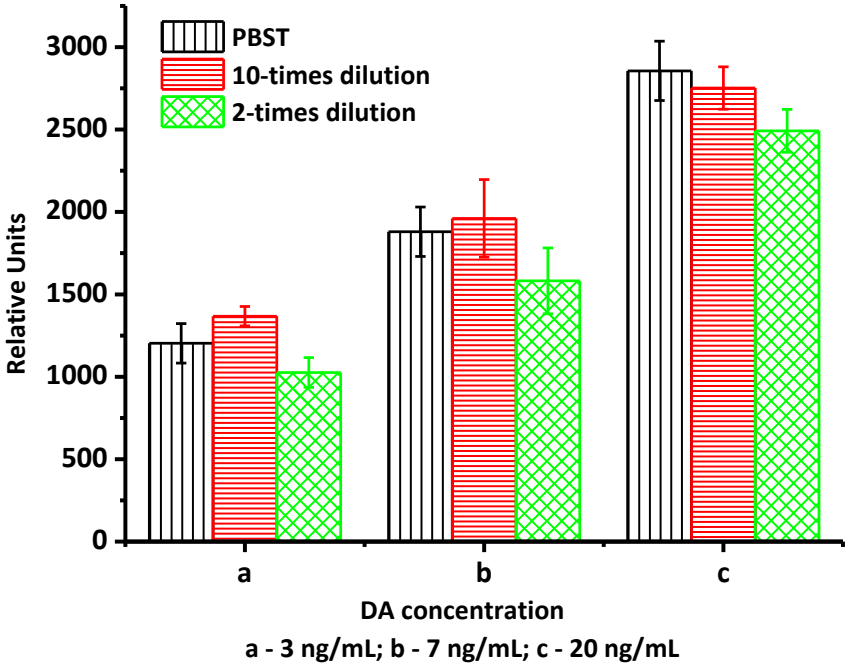
Instrumental LOD = 1.4 ng/mL

Cutoff = 60 ng/mL

The working range = 2.9–35.8 ng/mL

The assay duration = 15 min

Detection of domoic acid in seawater

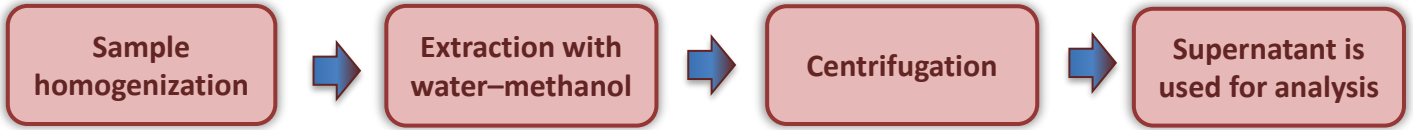


Recoveries of DA from seawater

SEAWATER	
Added DA, ng/mL	Detected DA ± SD (ng/mL)/Recovery ± SD (%)
3	3.6 ± 0.45/120 ±15
7	7.1 ± 0.84/101 ± 12
20	17.7 ± 2.8/88.5 ± 14

Detection of domoic acid in seafood

Sample preparation before the analysis: short 15-min procedure



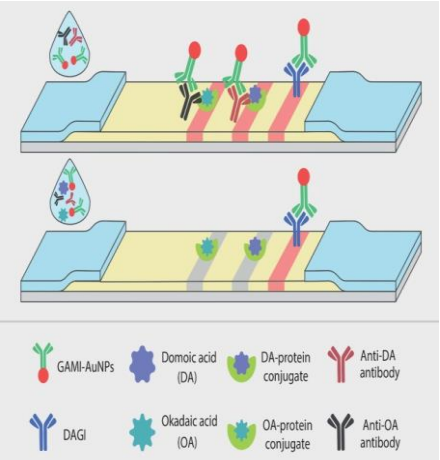
Tested samples:

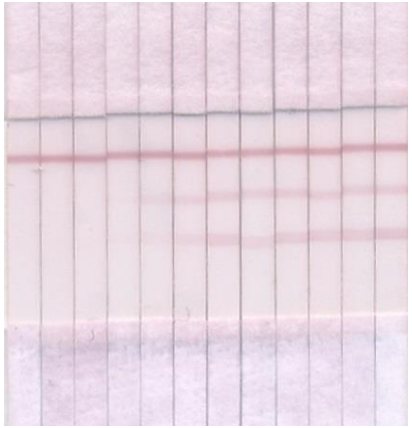
- ❑ shrimps
- ❑ mussels
- ❑ octopuses

SEAFOOD			
Added DA, µg/g	Detected DA ± SD (ng/g)/Recovery ± SD (%)		
	Shrimps	Octopuses	Mussels
0.5	0.62 ± 0.09/ 124 ± 18	0.48 ± 0.10/ 96 ± 20	0.58 ± 0.08/ 116 ± 16
1	1.24 ± 0.16/ 124 ± 16	1.08 ± 0.15/ 108 ± 15	1.1 ± 0.13/ 110 ± 13
2	1.86 ± 0.36/ 93 ± 18	1.78 ± 0.50/ 89 ± 25	2.24 ± 0.44/ 112 ± 22

Simultanious detection of Domoic Acid & Okadaic Acid

Double strip-test





Olga D. Hendrickson, Elena A. Zvereva, Olga N. Solopova, Anatoly V. Zherdev, Peter G. Sveshnikov, Sergei A. Eremin, Boris B. Dzantiev. Double Immunochromatographic Test System for Sensitive Detection of Phycotoxins Domoic Acid and Okadaic Acid in Seawater and Seafood. Micromachines 2022, 13, 1506. https://doi.org/10.3390/mi13091506

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Domoic Acid

Matrix	Added Quantity (μg/g)	Measured Quantity ± SD (μg/g)	Recovery ± [1] SD (%)
Scallops	20	17.4 ± 1.3	87.4 ± 6.4
	8	6.7 ± 0.7	83.4 ± 9.2
	3.2	2.8 ± 0.2	88.8 ± 7.3
Tiger shrimps	20	23.2 ± 1.6	116.2 ± 7.8
	8	9.2 ± 0.4	114.7 ± 5.3
	3.2	2.8 ± 0.3	115.1 ± 8.8
Whelks	20	18.2 ± 1.2	91.0 ± 5.8
	8	7.1 ± 1.1	88.8 ± 13.9
	3.2	2.6 ± 0.2	80.8 ± 5.0
Octopuses	20	20.6 ± 0.9	102.9 ± 4.5
	8	9.9 ± 0.2	123.3 ± 2.7
	3.2	3.7 ± 0.3	116.4 ± 8.2
Mussels	20	22.1 ± 0.3	110.6 ± 1.7
	8	9.6 ± 0.3	119.8 ± 3.2
	3.2	4.0 ± 0.3	124.1 ± 7.8
Mussels	20	22.9 ± 3.0	114.5 ± 15.0
	8	8.9 ± 1.2	111.9 ± 14.7
	3.2	3.8 ± 0.1	120.0 ± 3.1

Okadaic acid

	Added quantity (ng/g)	Measured quantity ± SD (ng/g)	Recovery ± [1] SD (%)
Scallops	320	265.3 ± 32.0	82.9 ± 10
	160	130.7 ± 6.2	81.7 ± 3.9
	80	71.9 ± 5.2	89.9 ± 6.5
Tiger shrimps	320	295.7 ± 46.4	92.4 ± 14.5
	160	165.3 ± 9.1	103.3 ± 5.7
	80	89.3 ± 1.9	111.6 ± 2.4
Whelks	320	291.5 ± 25.6	91.1 ± 8.0
	160	171.8 ± 9.3	107.4 ± 5.8
	80	66.2 ± 6.1	82.7 ± 7.6
Octopuses	320	376.6 ± 48	117.7 ± 15.0
	160	190.2 ± 22.9	118.9 ± 14.3
	80	88.4 ± 7.4	110.5 ± 9.3
Mussels	320	389.4 ± 18.2	121.7 ± 5.7
	160	184.3 ± 2.4	115.2 ± 1.5
	80	97.4 ± 3.7	121.8 ± 4.6
Crabs	320	394.9 ± 13.4	123.4 ± [illegible]
	160	162.4 ± 18.9	101.5 ± [illegible]
	80	84.2 ± 10.1	105.2 ± 12.6

FPIA

Fluorescence

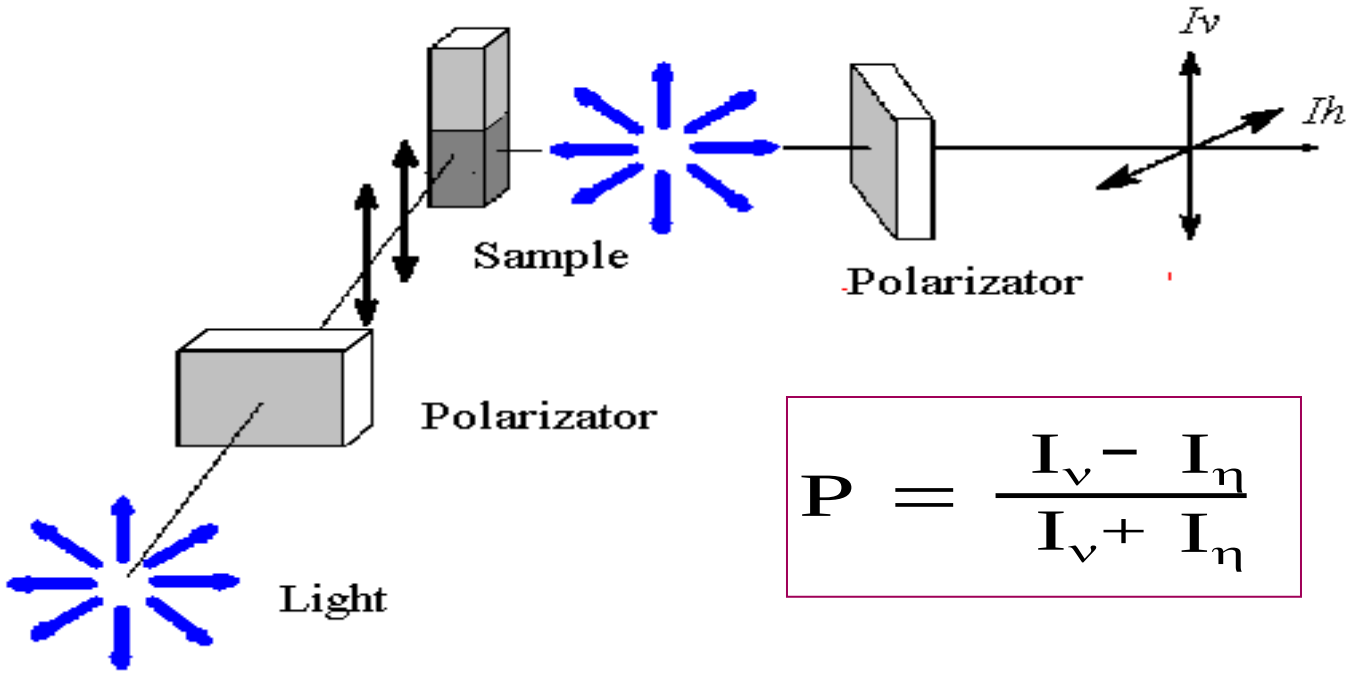
Polarization

Immuno

Assay

Among immunoassay techniques, fluorescence polarization immunoassay (FPIA) is the most extensively used homogeneous technique, which meets the requirements of a simple, reliable, fast and cost-effective analysis

Detection of Fluorescence Polarization



$$P = \frac{I_v - I_h}{I_v + I_h}$$

F. Perrin, *J. Phys. Radium*, 7 (1926) 390-401.

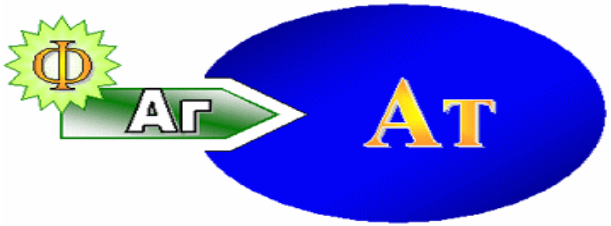
[1] Perrin (8) showed in his equation that the P value can be expressed as a function of such factors as temperature and viscosity together with molecular parameters as follows.

$$\frac{1}{P} = \frac{1}{P_0} + \left(\frac{1}{P_0} - \frac{1}{3}\right) \times \left(\frac{RT}{V}\right) \frac{\tau}{\eta} \qquad (2)$$

P	observed value of fluorescence polarization
Po	a constant (maximal value of P obtained in a rigid medium)
R	gas constant
T	absolute temperature
η	viscosity (poise)
r	relaxation time of fluorescence excitation(s)
V	molecular volume

Principle of Fluorescence Polarization

$$P = \frac{I_v - I_h}{I_v + I_h}$$

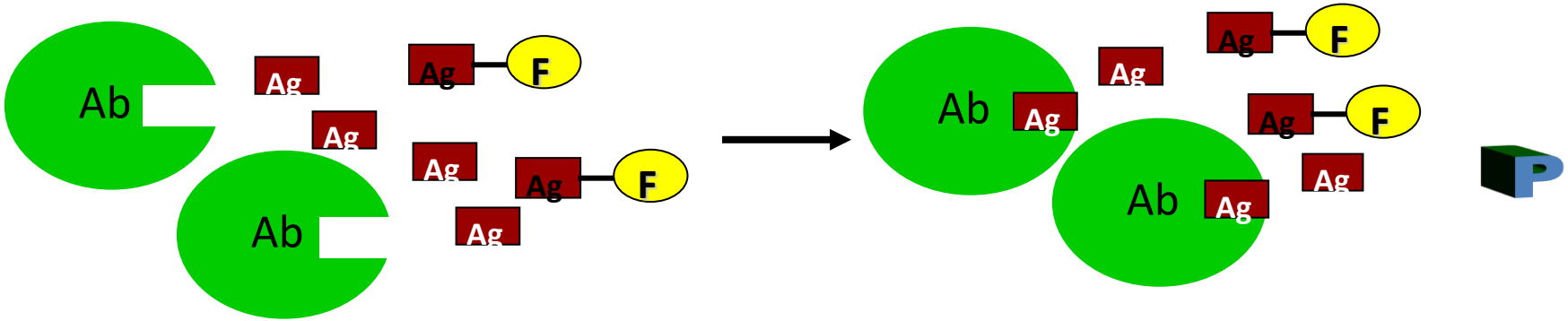


Fast rotation
Low Fluorescence Polarization

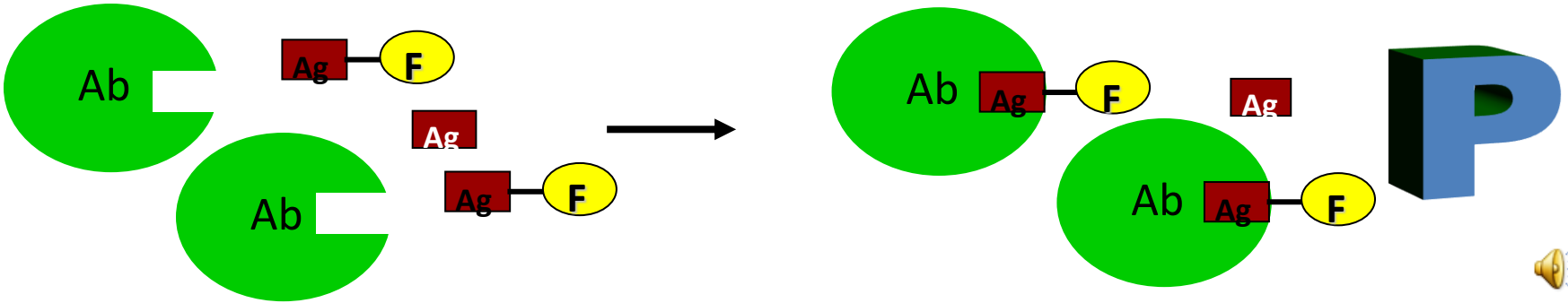
Slow rotation
High Fluorescence Polarization

FPIA – competitive immunoassay

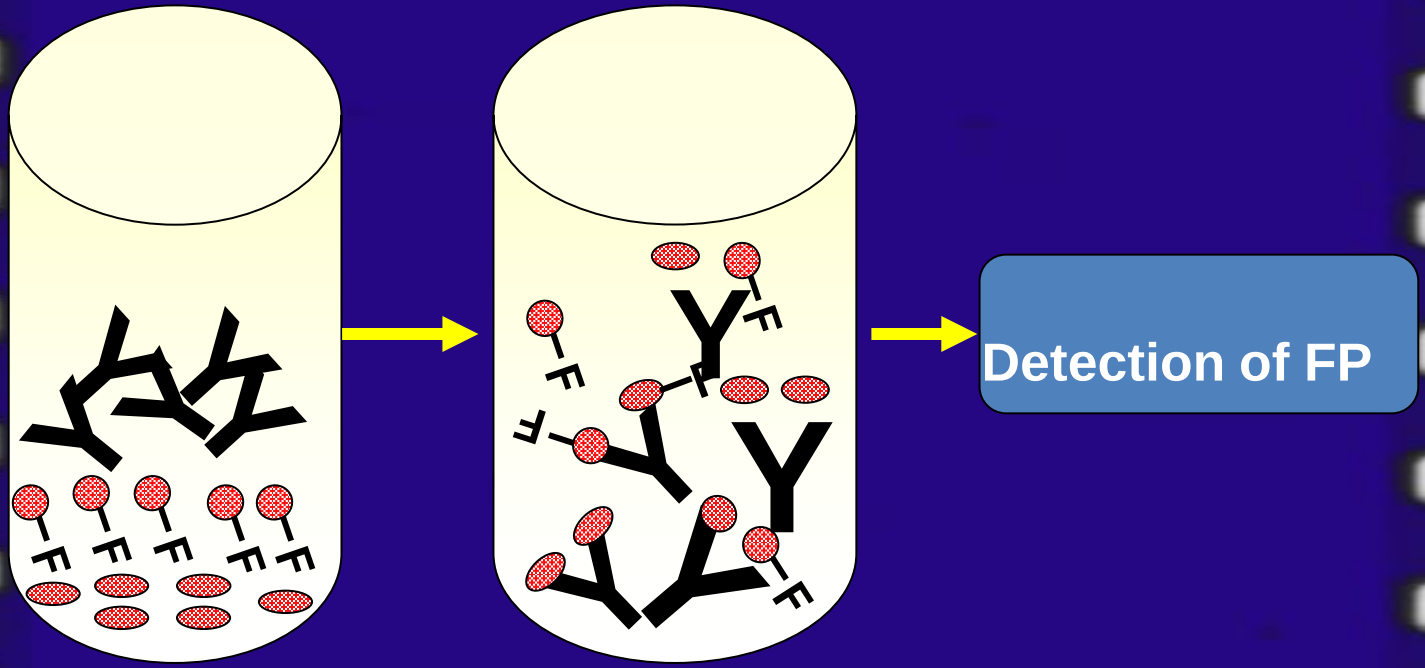
- **HIGH concentration of Ag → LOW fluorescence polarization (mP)**



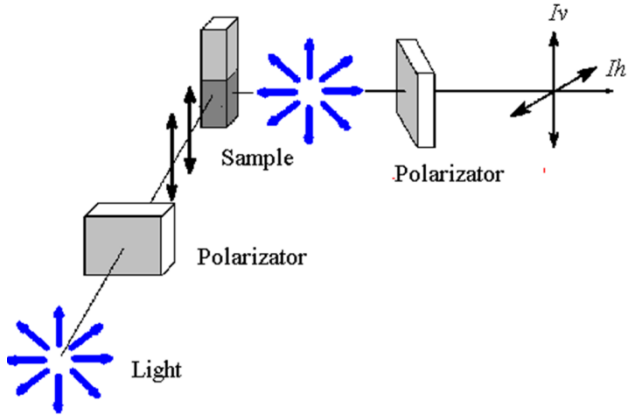
- **LOW concentration of Ag → HIGH fluorescence polarization (mP)**



Performance of FPIA



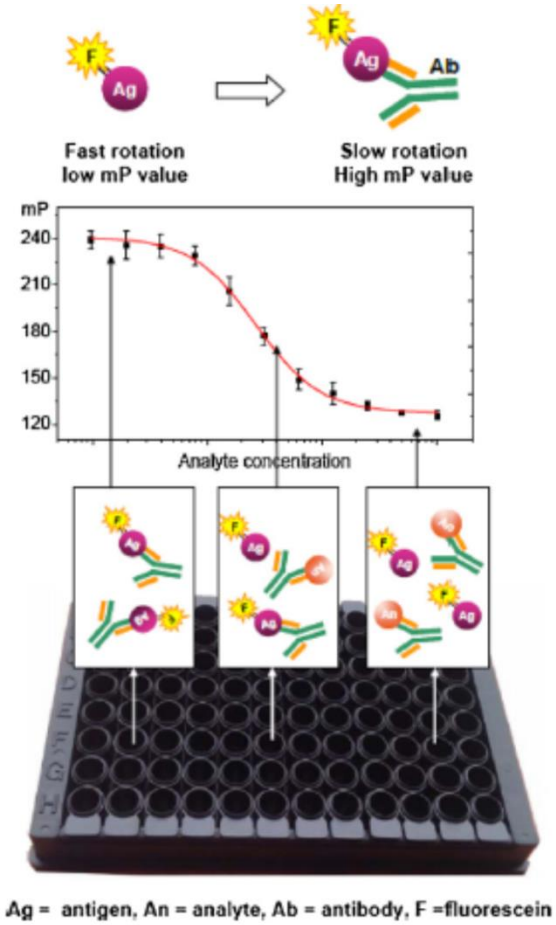
FPIA – Fluorescence Polarization ImmunoAssay



Fast rotation
Low Fluorescence
Polarization

Slow rotation
High Fluorescence
Polarization

$$P = \frac{I_v - I_h}{I_v + I_h}$$



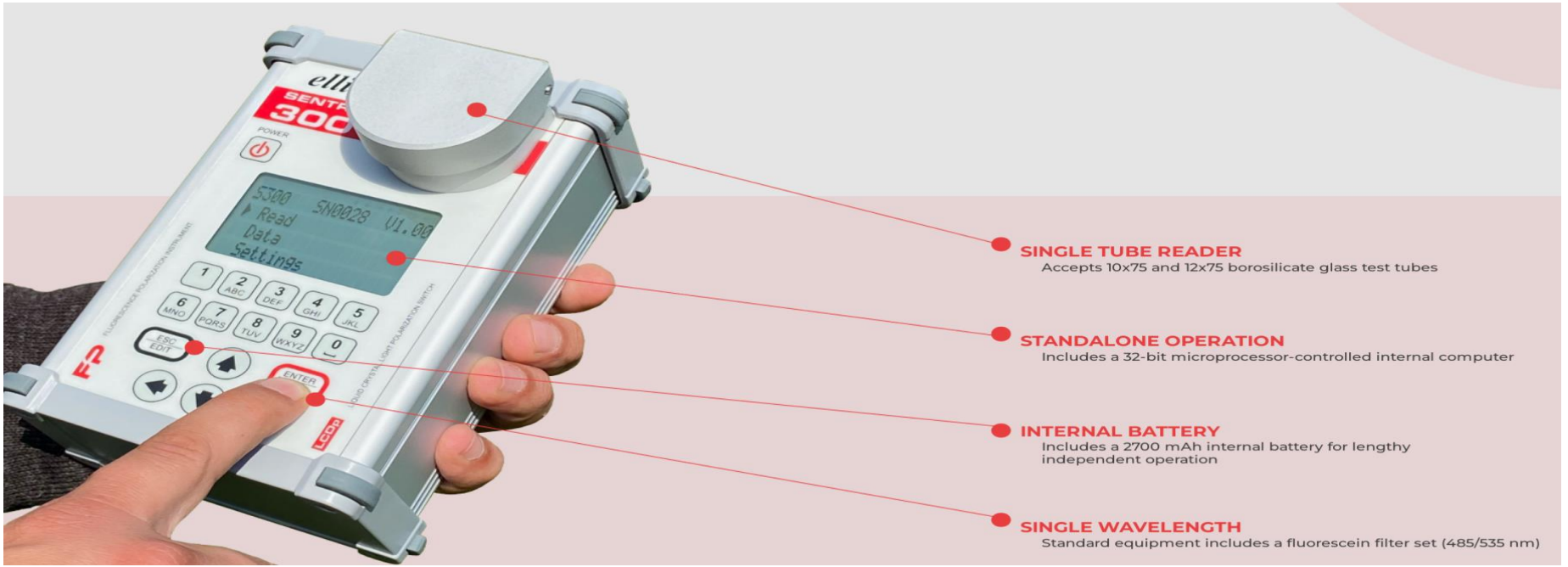
Load:
10 uL sample
0.1 mL tracer solution
0.1 mL antibody
mix
and measure mP

Time for assay <10 min

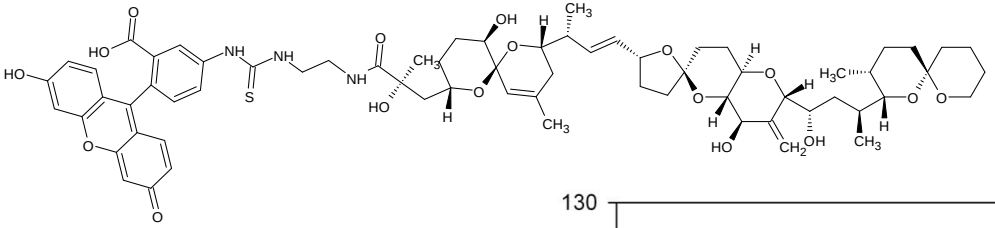
No separation steps
No washing steps
Simple and quick
Cost effective
Stable tracer
High precision
Stability of the
standard curve

new FP instrument – Sentry-300

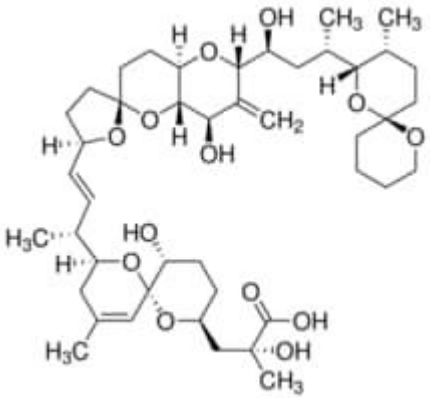
https://ellielab.com/sentry-300/



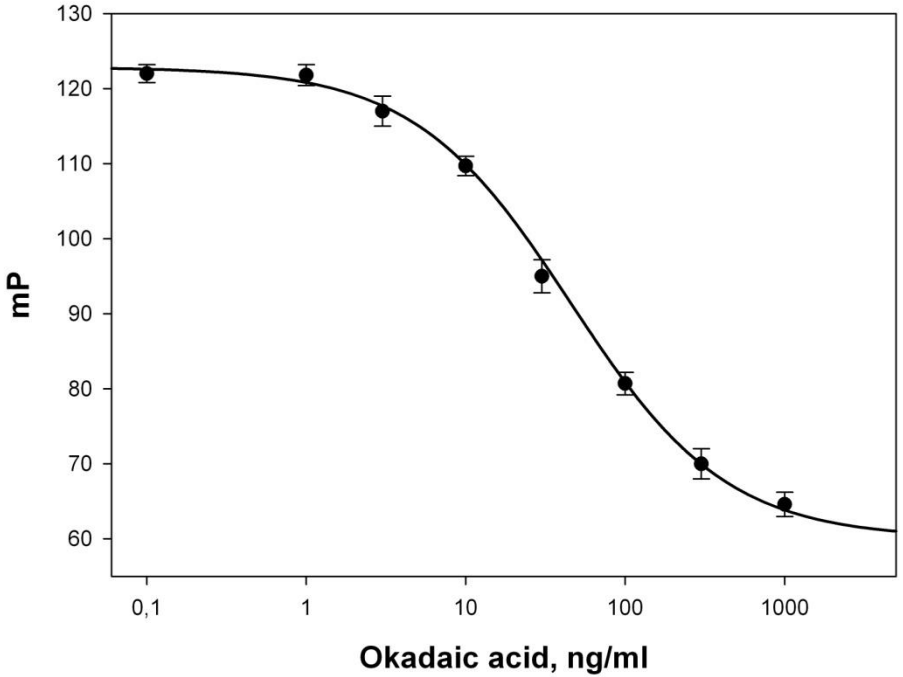
Fluorescence Polarization ImmunoAssay for Okadaic Acid



EDF-OKA tracer



OKA



Method:

50 uL sample

0.5 mL tracer solution

0.5 mL antibody solution

Mix and measure mP

Conclusions

- A sensitive and rapid indirect LFIA for the detection of MC-LR, OA and DA with AuNPs-based colorimetric detection of the assay results was developed.
- Simple and quick quantitative FPIA for OA was developed.
- The analytical characteristics of developed methods not only meet official regulations but also exceed the requirements for the sensitivity. The methods; universality makes the proposed techniques promising bioanalytical platforms for the monitoring of various food contaminants.

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