International Workshop of Pesticide Risk Assessment Model Building
Xi'an, China
October 16-18, 2017

Bio(immuno)analytical techniques for pesticides detection: Existing tools and trends in development

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OUTLINES

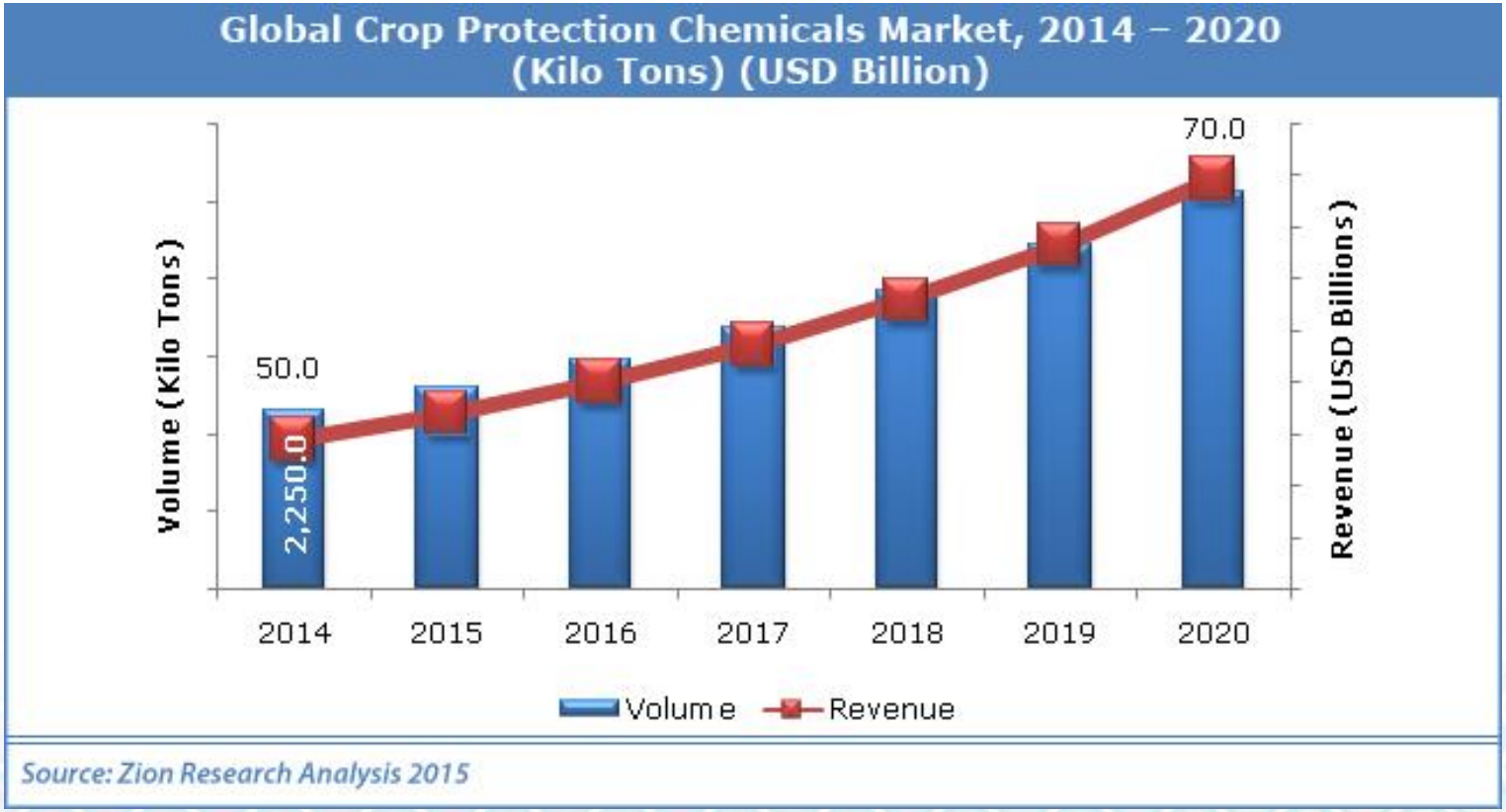
1. Actual tasks of pesticides monitoring
2. Place and variety of immunotechniques
3. Efforts for rapid ELISA
4. Efforts for high-sensitive LFIA
5. Efforts for efficiency of whole assay procedures

1. Actual tasks of pesticides monitoring

PESTICIDES: COMPANIONS IN A LONG JOURNEY

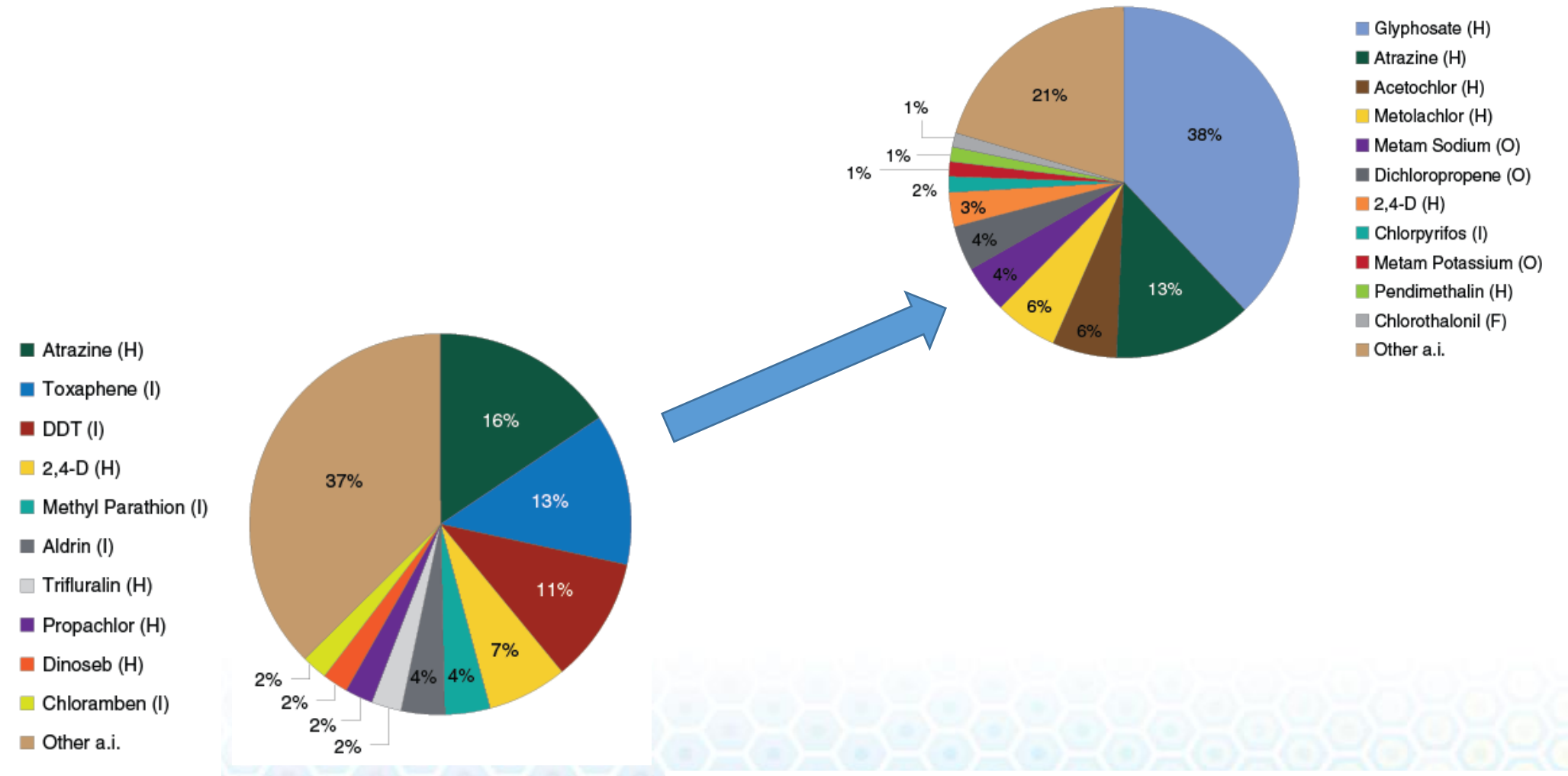
- New generation of pesticides?
- Biopesticides?
- Infection-resistant crops?
- Organic agriculture?
- …?

All factors are important, but the main trend of intense use of pesticides in agriculture is still stable



PESTICIDES: CHANGING COMPANIONS

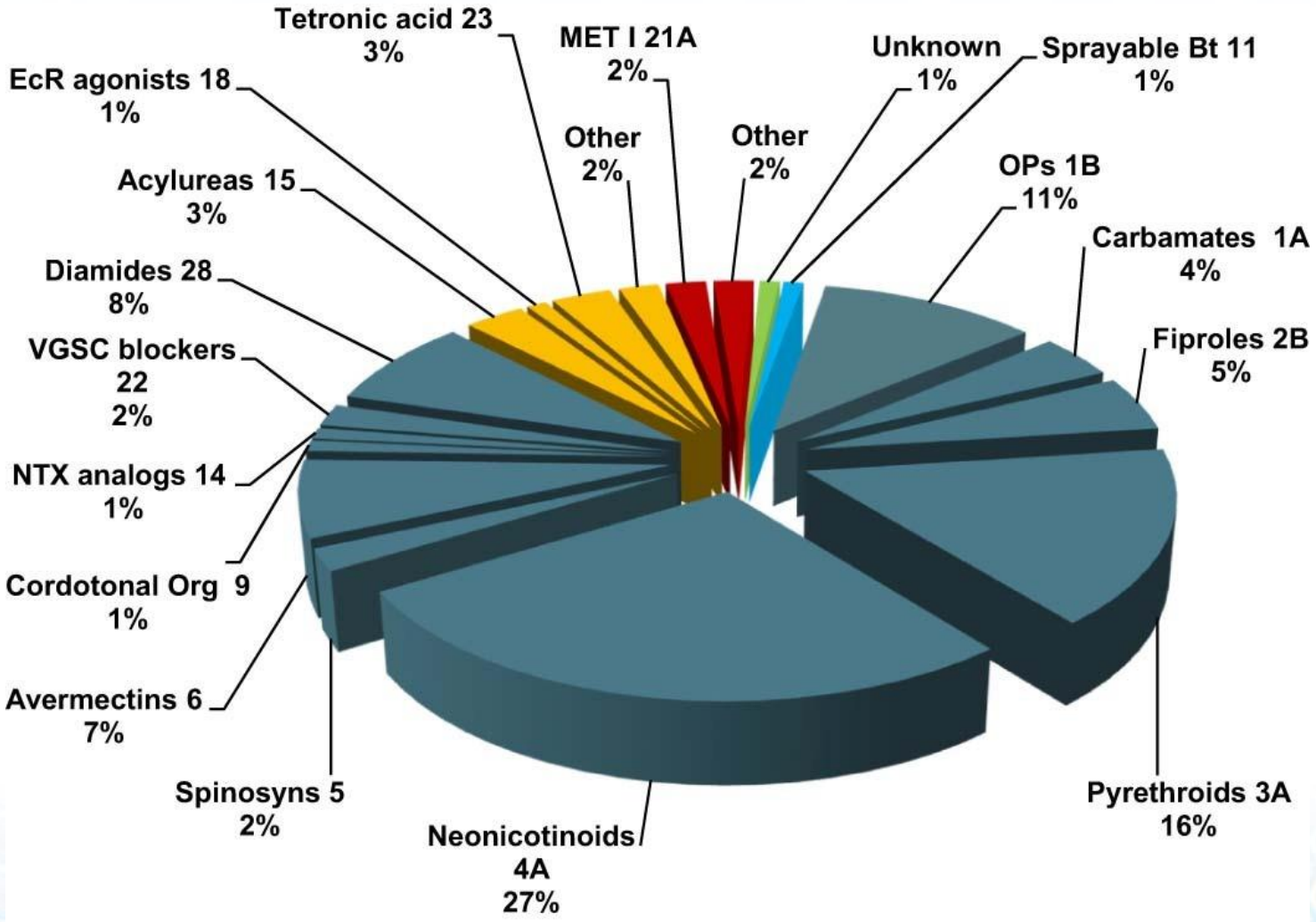
Most heavility used pesticides 50 years ago and now



Note: H = herbicide, I = insecticide, F = fungicide, and O = other.

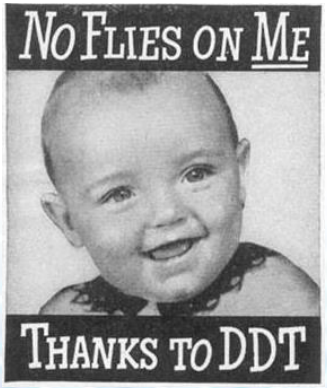
Source: USDA, Economic Research Service using USDA, National Agricultural Statistics Service and proprietary data.

WORLDWIDE MARKET OF PESTICIDES (BY CHEMICAL CLASSES)



REQUESTED CONTROL OF PESTICIDES

1. **Environmental contamination**
2. **Statement for cultivated areas**
3. **Safety of agricultural production**



We are not so optimistic, as previous generations

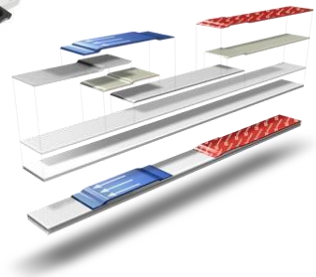
2. Place and variety of immunotechniques

ANALYTICAL SYSTEMS AND PLACE OF IMMUNO-TECHNIQUES AMONG THEM

BASIC DILEMMA:

SENSITIVITY versus **RAPIDITY**

GC-MS | LC-MS | HPLC | ELISA | LFIA



Stationery equipment for specialized laboratories

- High sensitivity
- Unified procedures
- Grounded confirmation
- Responsible conclusion

Small devices for wide testing

- Rapid testing
- Simple procedures
- Low cost
- Wide screening

CHROMATOGRAPHY VS IMMUNOASSAY

CHROMATOGRAPHY

IMMUNOASSAY

Compounds of tested samples are separated and identified individually	Target compounds bind specific receptor and the formed complex is detected
Selectivity is determined by separation technique	Selectivity is determined by receptor's properties
Sample preparation and separation of the extract to be tested are necessary	Minimal sample preparation, dilution of the sample may be sufficient
Structurally similar compounds may be detected individually	Some integrated parameter is registered for structurally similar compounds
Difficulties in identification of bound forms of the analyte	Both free and bound forms of the analyte may be detected

IMMUNOASSAYS AVAILABLE

ELISA

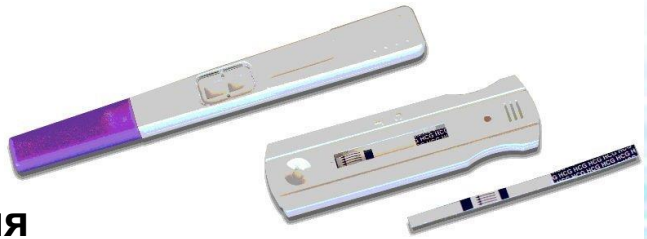
PFIA

- Kits of reactants
- Devices for assaying
- Detectors for results registration

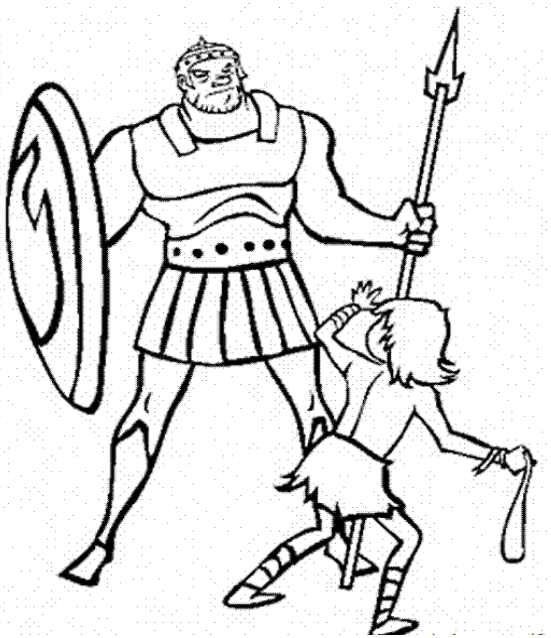
DELFIA

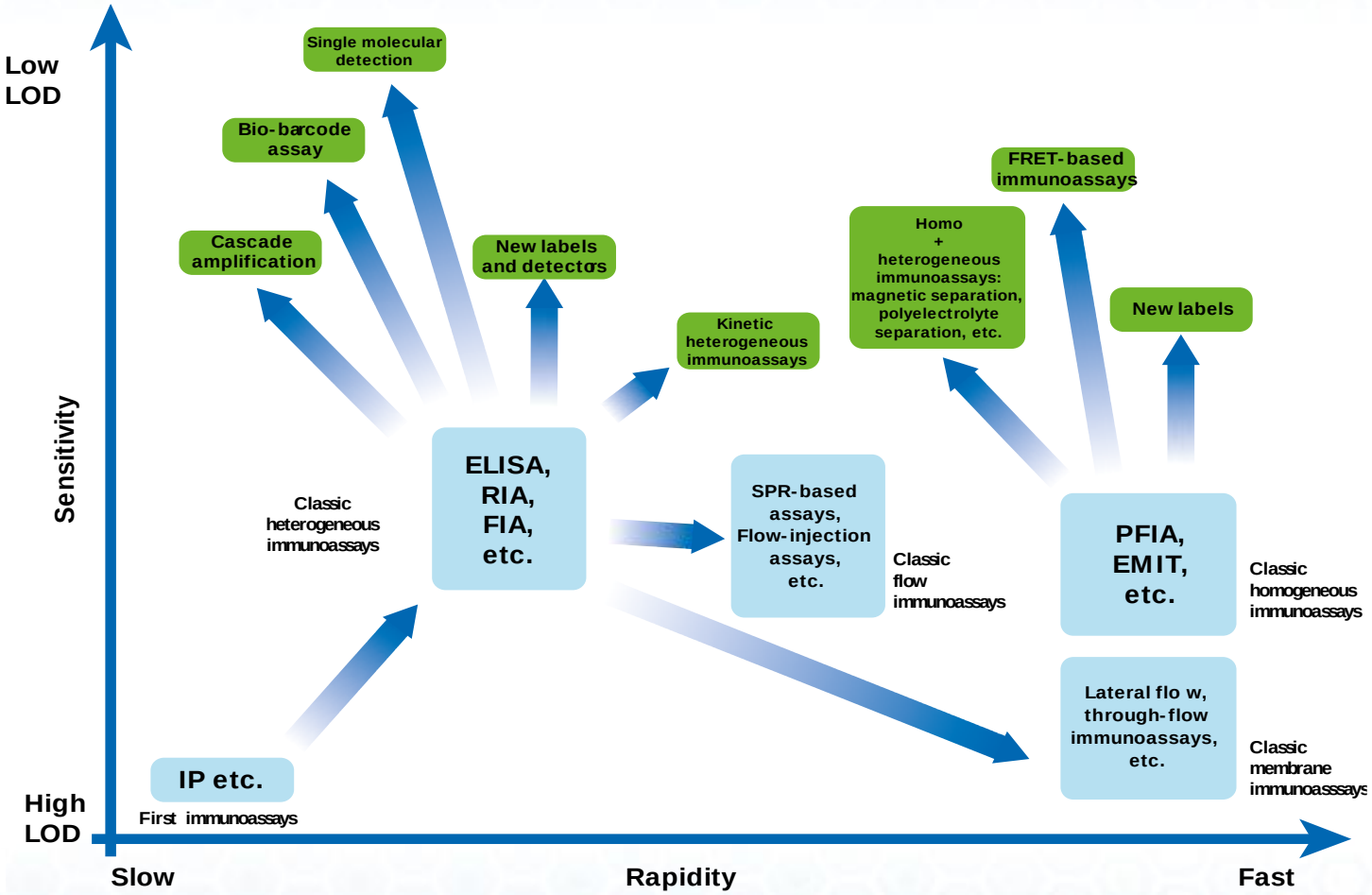
Immunoarrays (immunochips)

Иммуно-
хроматография



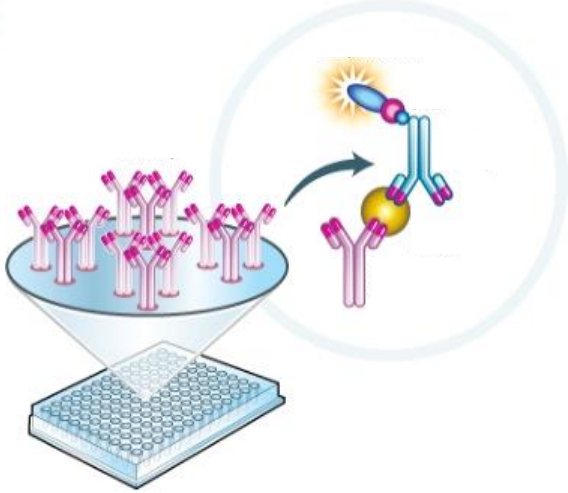
MAP OF IMMUNOASSAYS DEVELOPMEMT





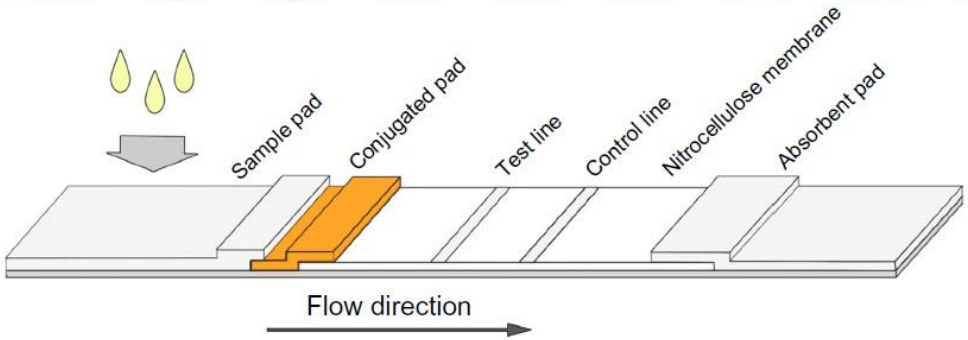
Dzantiev et al.
Biotechnologia Acta,
2013, 6 (4), 94-104.

IMMUNOENZYME ASSAY (ELISA)



- Duration – 1-2 hours
- Several dozens of samples in parallel
- Special registering devices
- High sensitivity

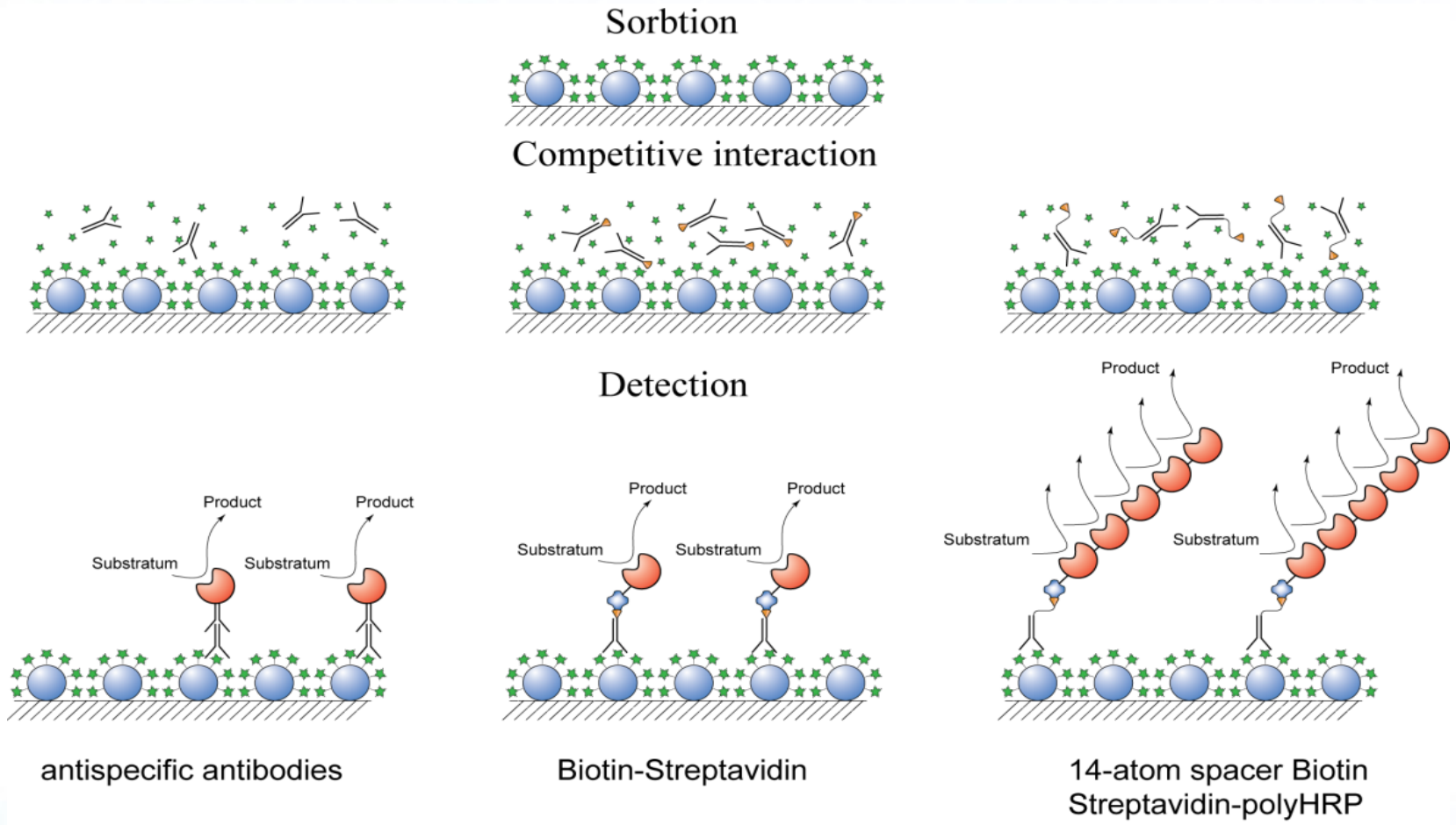
IMMUNOCHROMATO-GRAPHY (LFIA)



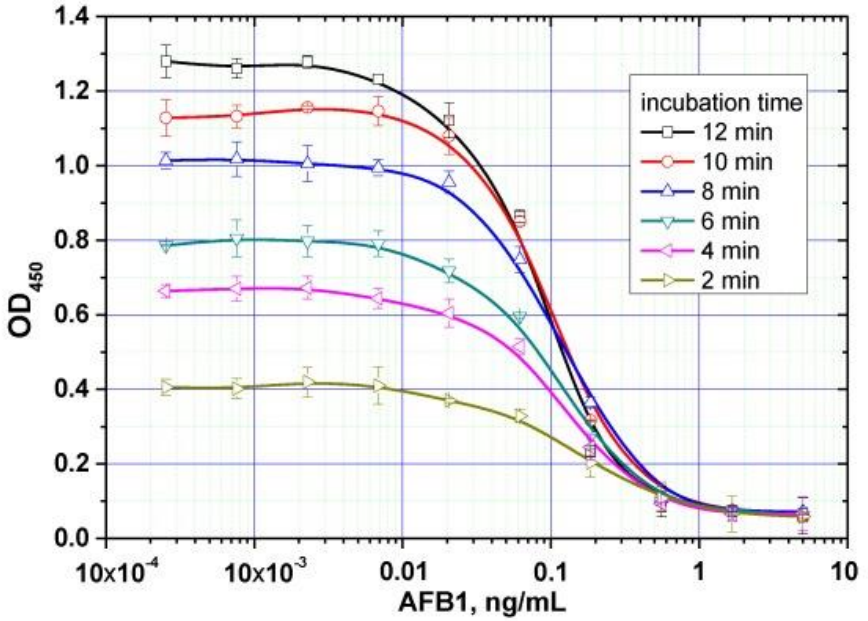
- Duration – 5-15 min
- Simple manipulations
- Possibility of on-site testing
- Visual assessment of results
- Higher detection limits

3. Efforts for rapid ELISA

REDUCTION OF STAGES IN TRADITIONAL ELISA



KINETIC DEPENDENCES FOR TRADITIONAL ELISA



(A1)

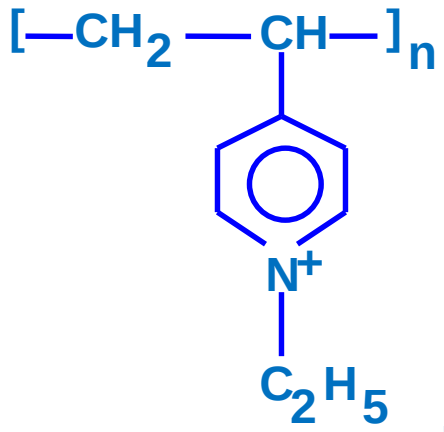
Time, min	IC_{50}, ng/mL	IC_{10}, ng/mL	OD_{max}
12	0.083	0.013	1.29
10	0.102	0.023	1.17
8	0.123	0.030	1.04
6	0.109	0.028	0.83
4	0.155	0.031	0.66
2	0.183	0.071	0.42

(A2)

POLYELECTROLYTE CARRIERS FOR IMMUNOASSAYS

Polycation

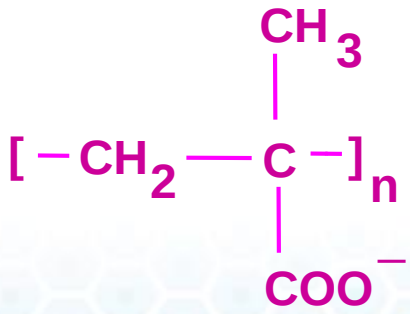
poly-N-ethyl-4-vynilpyridinium



M.W. 2000 kDa

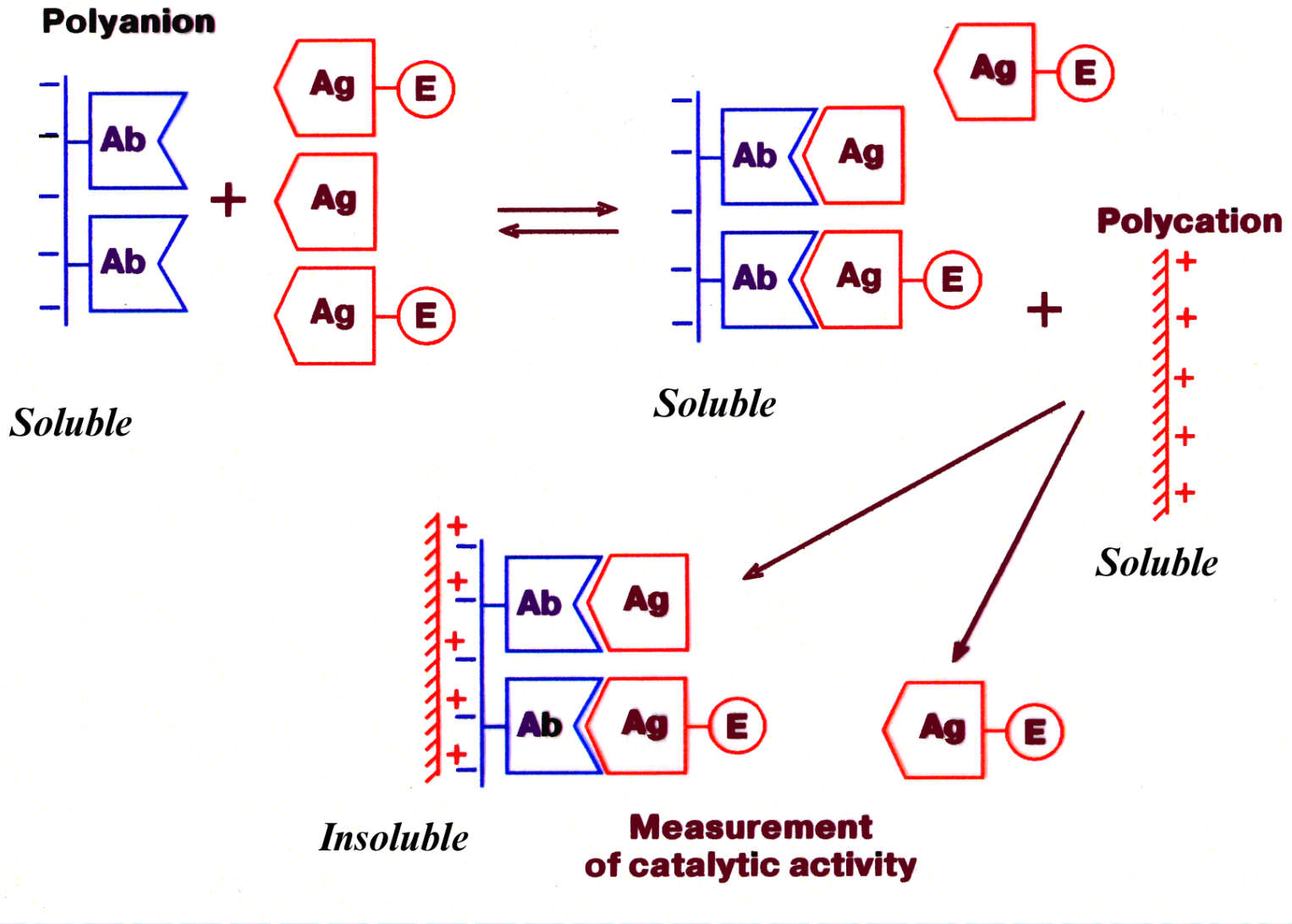
Polyanion

polymethacrylate

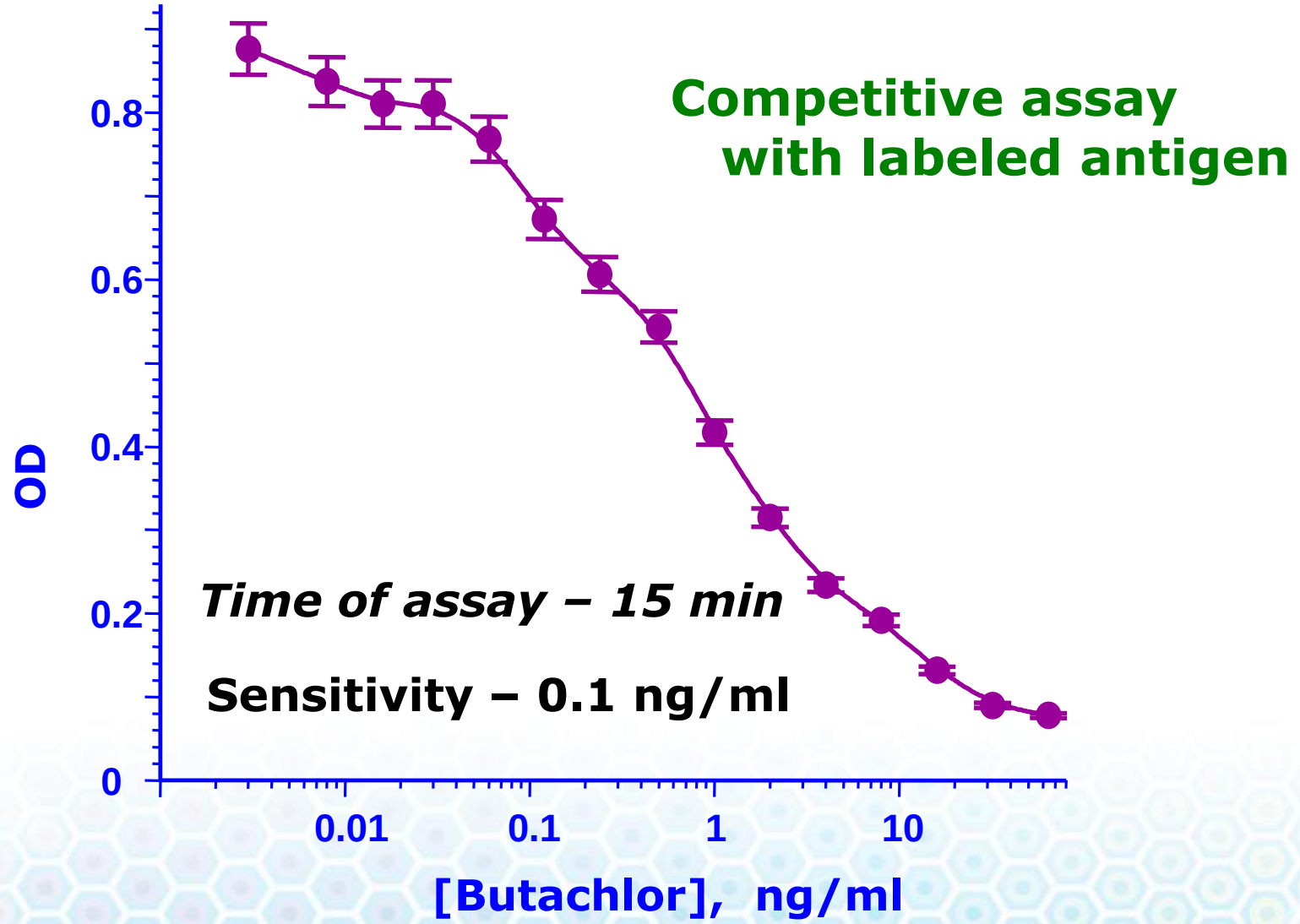


M.W. 260 kDa

ELISA WITH THE POLYELECTROLYTES USE



POLYELECTROLYTE ELISA OF BUTACHLOR



MAGNETIC CARRIERS FOR ELISA

Magnetic particles (MP)

Fe **Co** **Ni**

iron oxide

magnetite Fe_3O_4

ADVANTAGES :

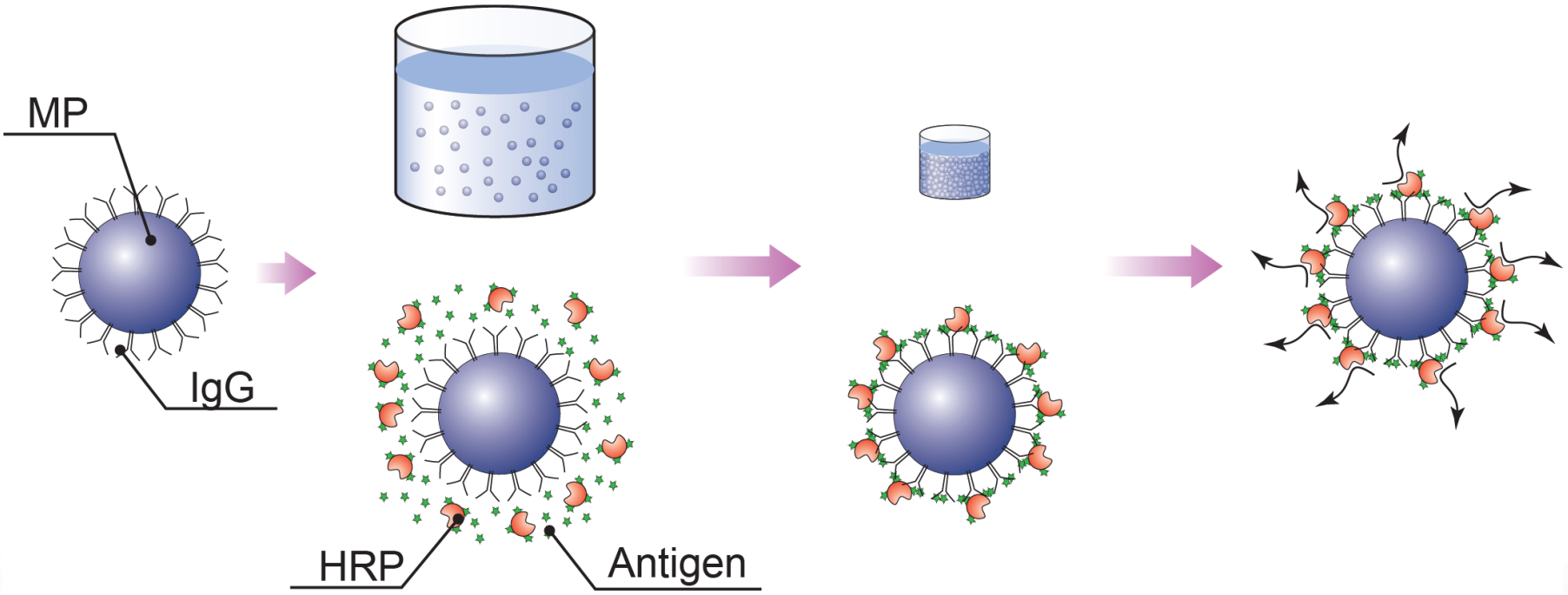
- Large the surface sorption capacity
- The ability to perform analysis directly in the whole volume of the reaction mixture
- Simple and rapid separation of the mixture components

COMPETITIVE ELISA USING MP

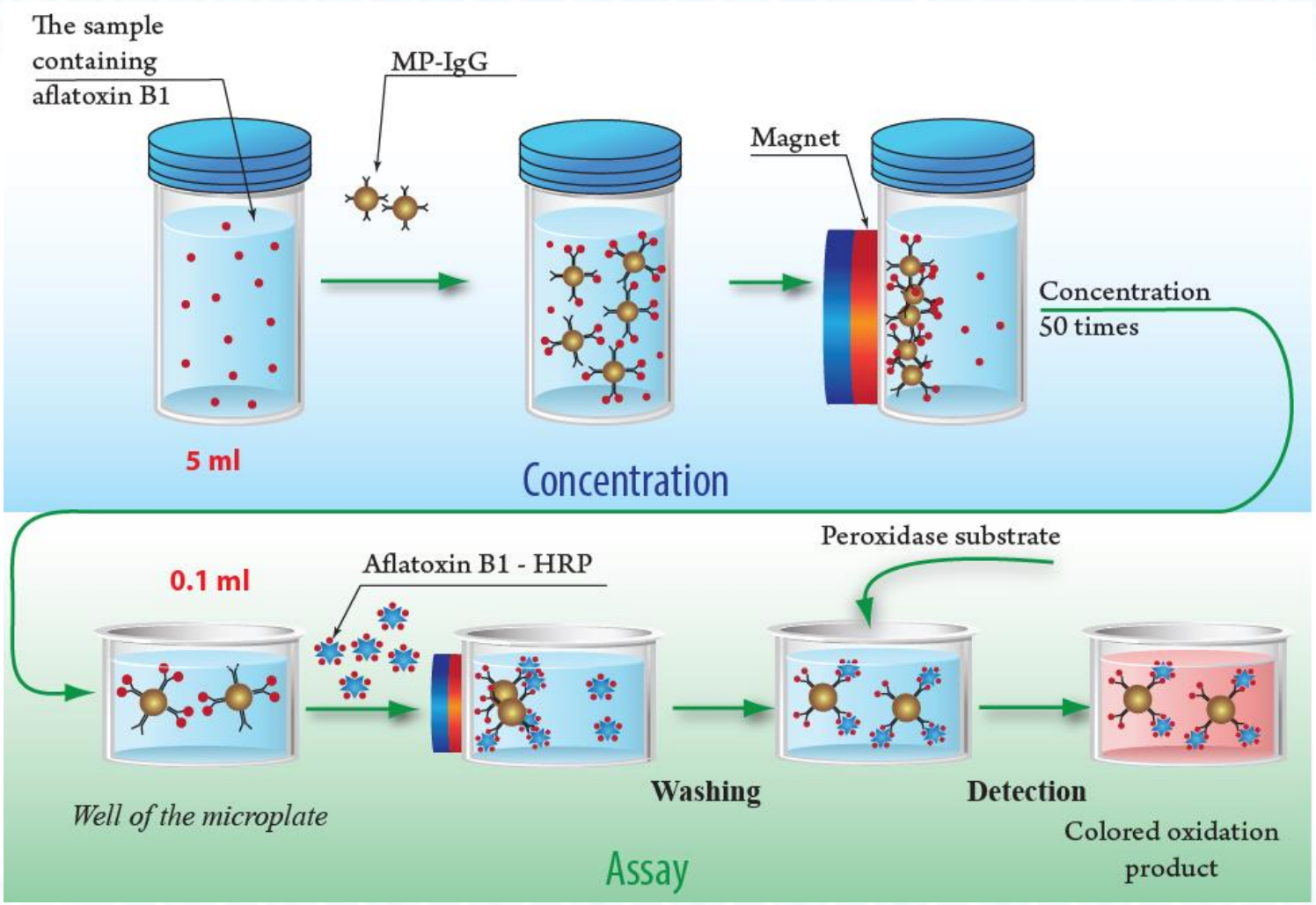
Mixing with the sample and the antigen-HRP conjugate

Incubation, magnetic separation, washing and concentration

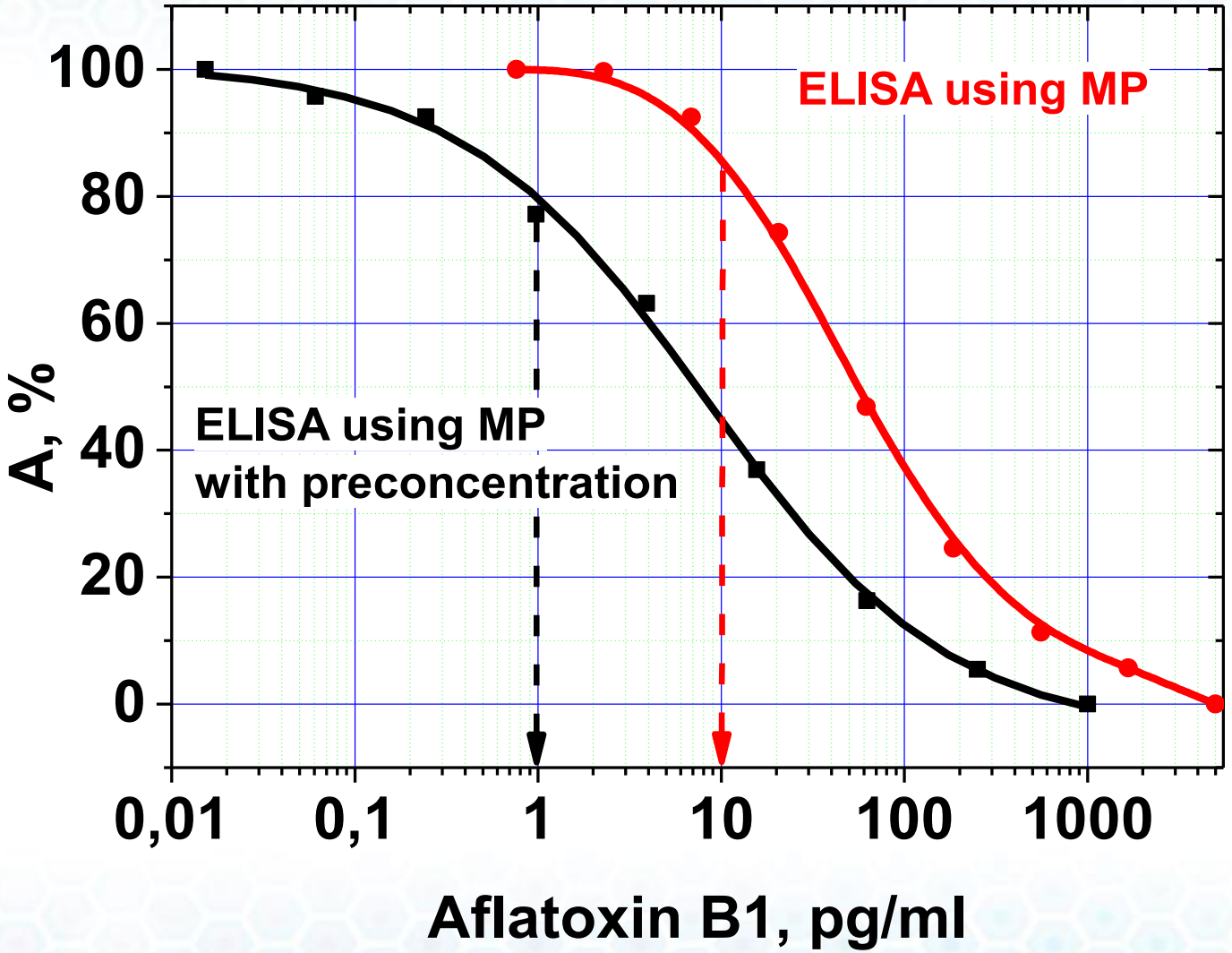
Detection



ELISA SCHEME WITH PRECONCENTRATION



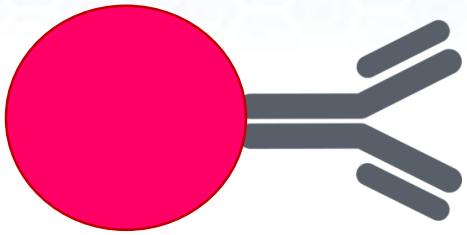
EFFECT OF PRECONCENTRATION



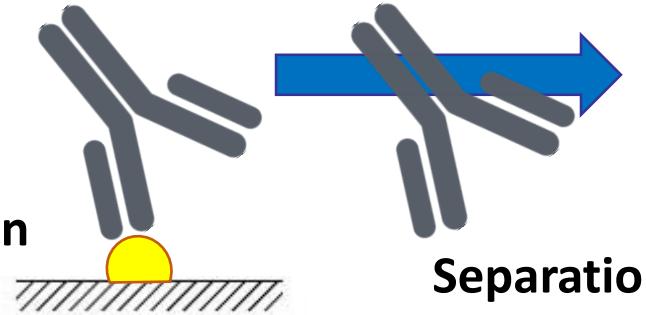
Reducing the detection limit of 10 times to 1 pg / ml

4. Efforts for high-sensitive LFIA

CONCEPT OF COMPETITIVE IMMUNOASSAY

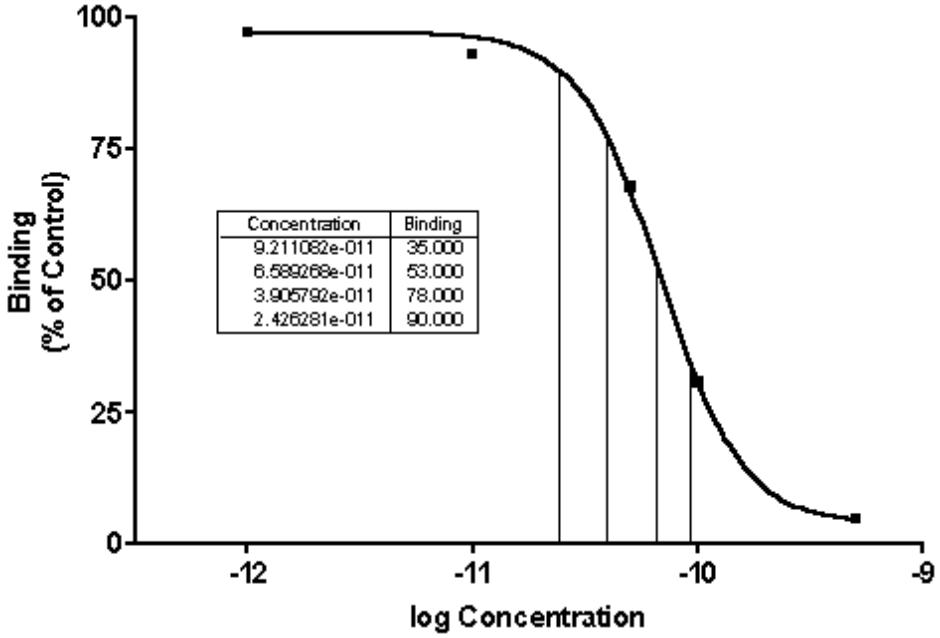


Labeling

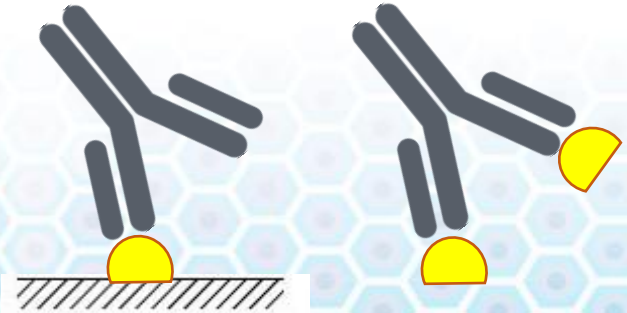


Immobilization

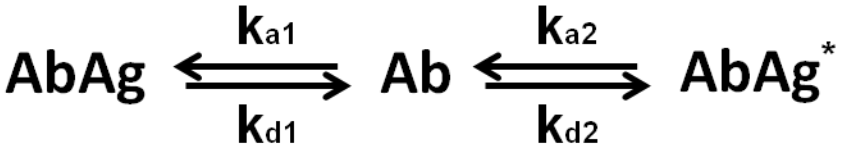
Separation



Competition



THEORETICAL DESCRIPTION OF COMPETITIVE IMMUNOASSAY



Solution

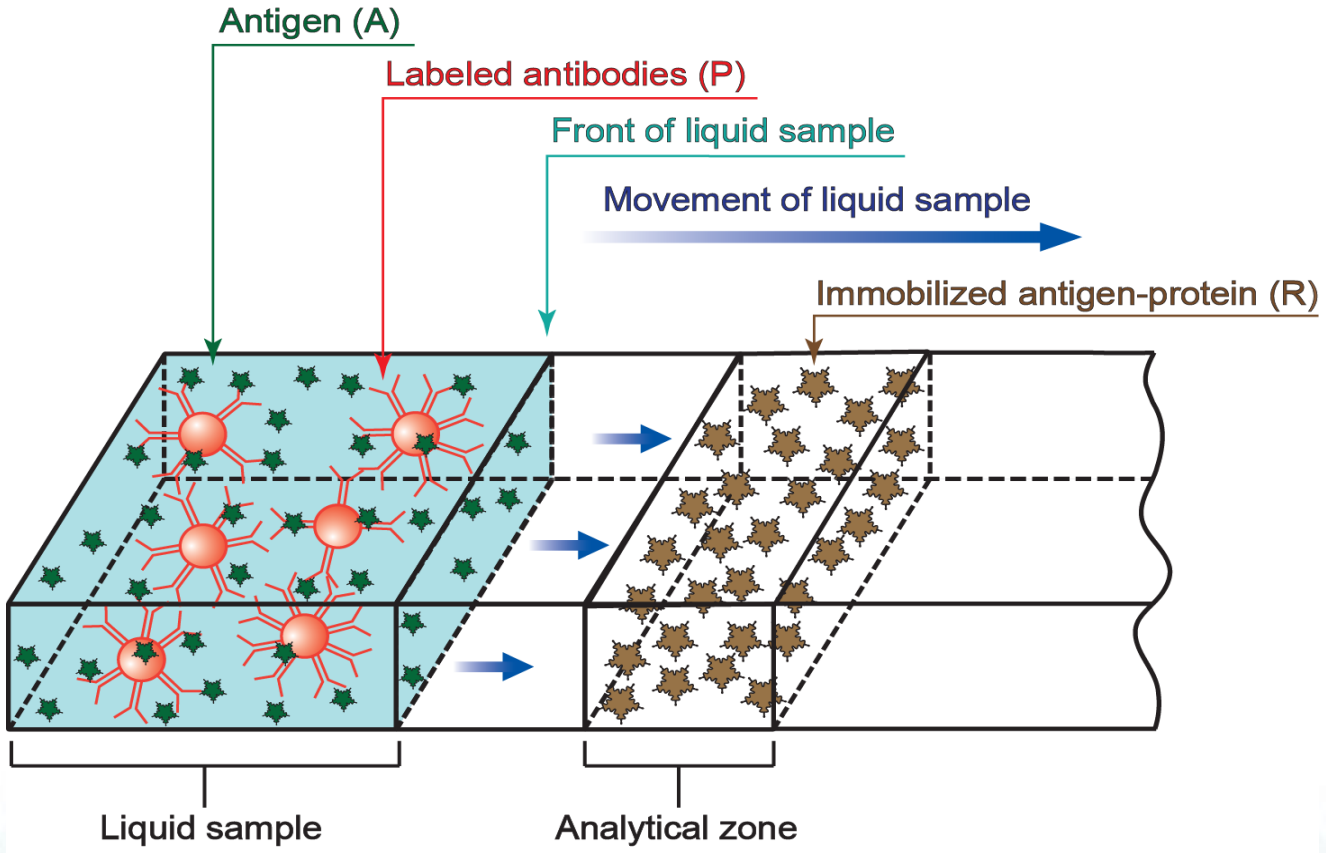
$$y = \frac{[Ab]_0+[Ag]_0+[Ag^*]_0+K_d+\sqrt{([Ab]_0+[Ag]_0+[Ag^*]_0+K_d)^2-4[Ab]_0([Ag]_0+[Ag^*]_0)}}{2([Ag]_0+[Ag^*]_0)}$$

Limitations:

- Non-known constants
- Immobilization
- Diffusion
- Non-equilibrium regime
- Flow of reactants

MATHEMATICAL MODELLING OF IMMUNOCHROMATOGRAPHY

Scheme for competitive immunoassay



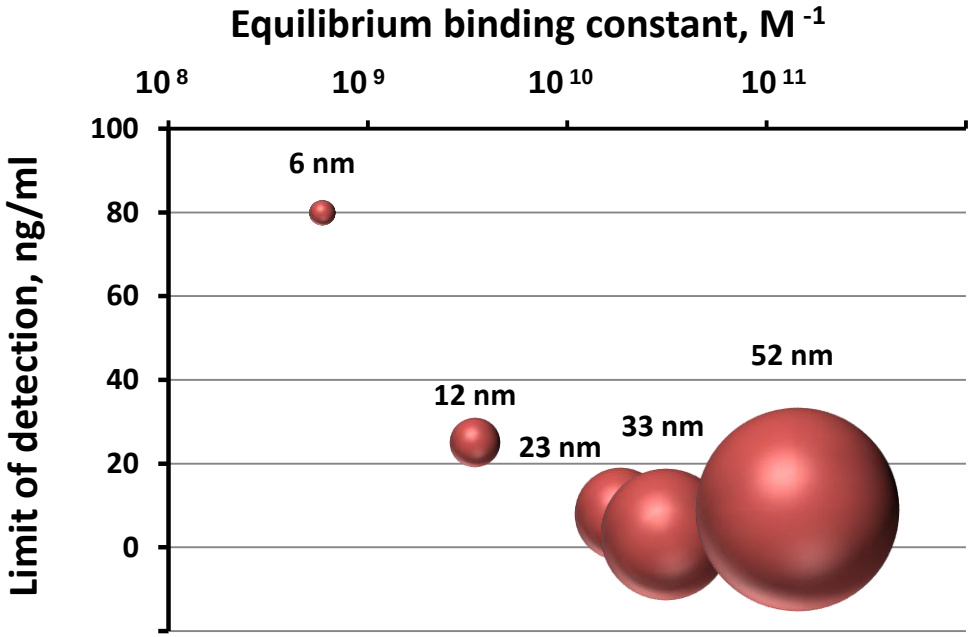
Reaction:

$$A + P \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AP$$

Reaction:

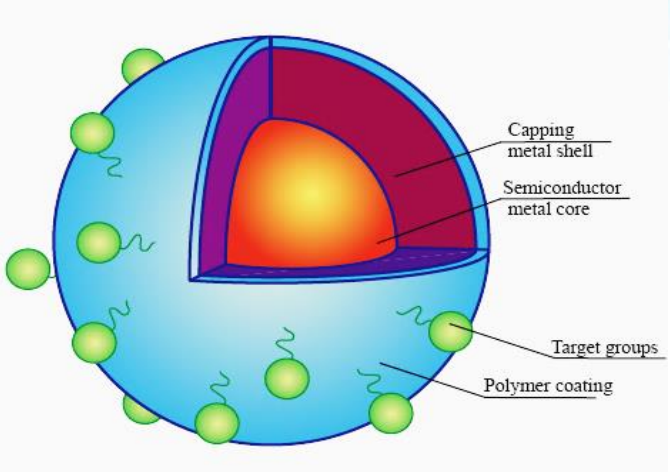
$$P + R \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} PR$$

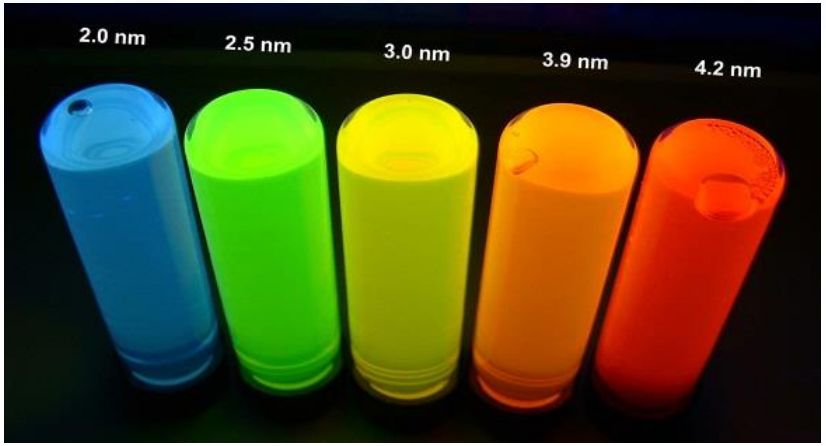
INFLUENCE OF GOLD CONJUGATE SIZE ON THE ASSAY CHARACTERISTICS



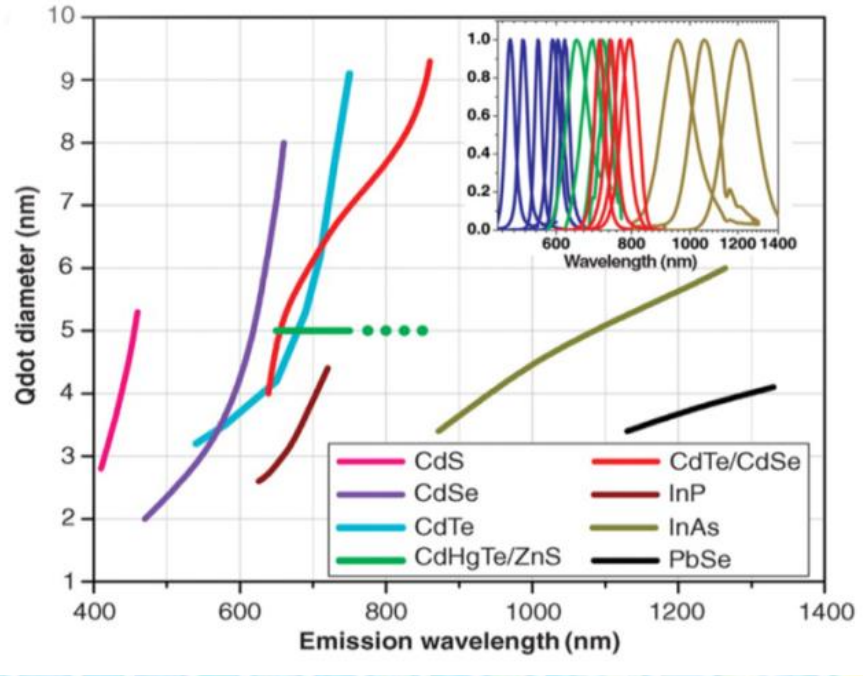
- **The variation of the conjugates composition leads to a change of the PVX detection limit from 80 to 2 ng/ml**
- **The lowest limit of detection is reached for the conjugates with 33 nm diameter**

FLUORECENT MARKERS: QUANTUM DOTS

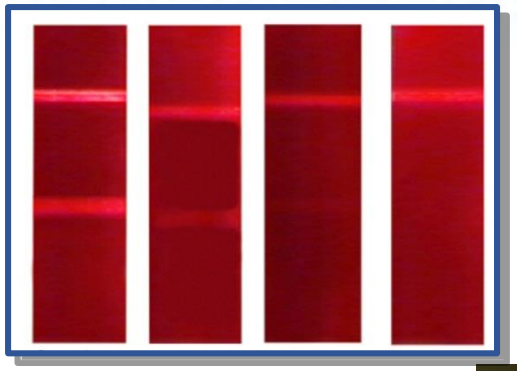




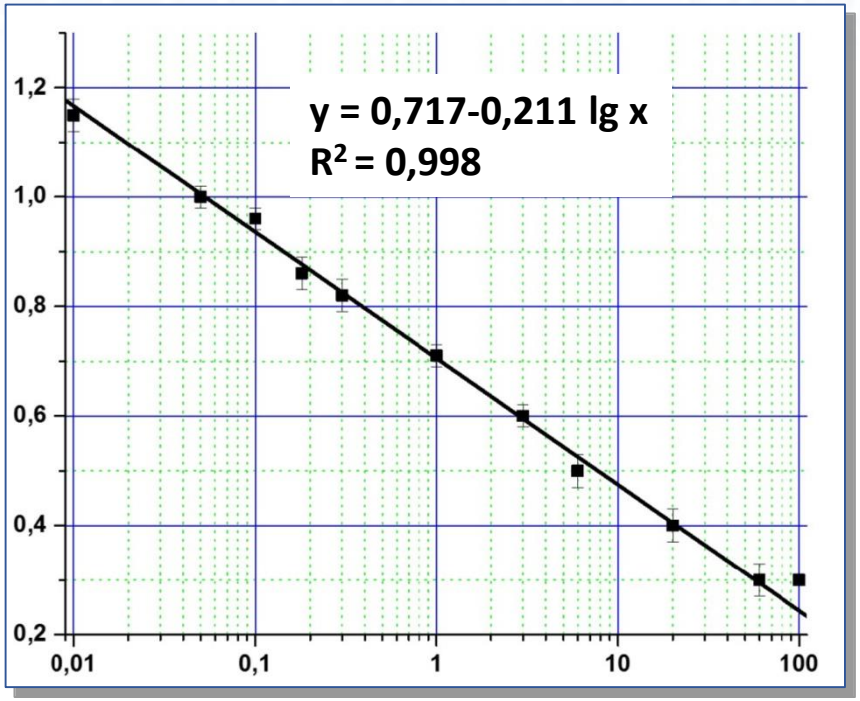
Property	Result
Cores from elements of II/VI and III/V groups of the Periodic Table	High storage stability High photostability
Different polymeric coatings	Biocompatibility Surface stabilization High quantum yield
Variable core size (1-10 nm)	Use of size-dependent energy transfers
Size-dependent fluorescence (emission peak at 500-1000 nm)	Simple identification in multiassays. Absence of biomatrix influence
Stable and reproductive emission	«On-off» regulation of the test-systems response



LFIA WITH QUANTUM DOTS

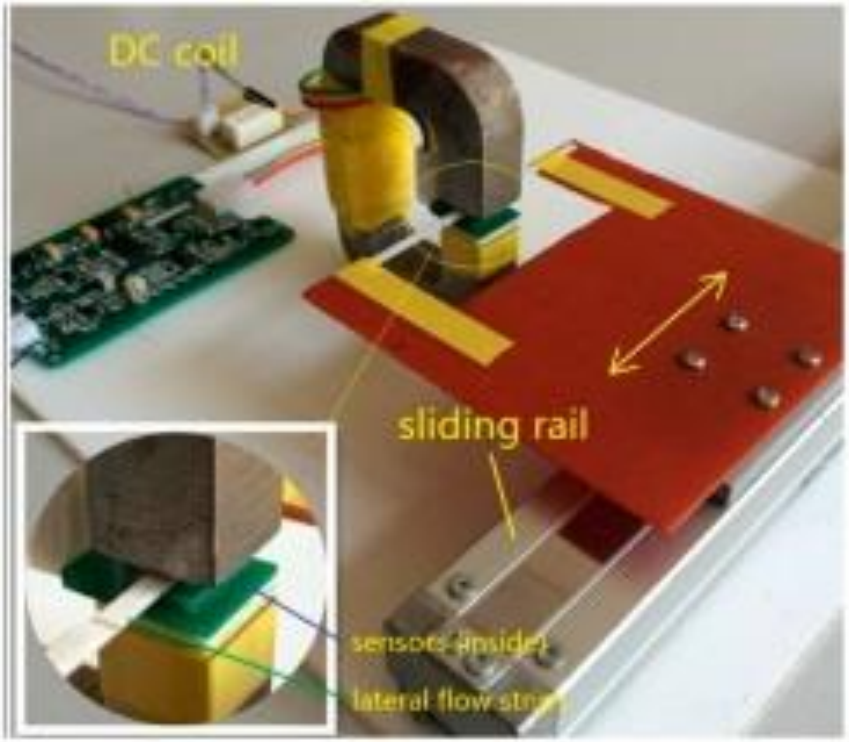


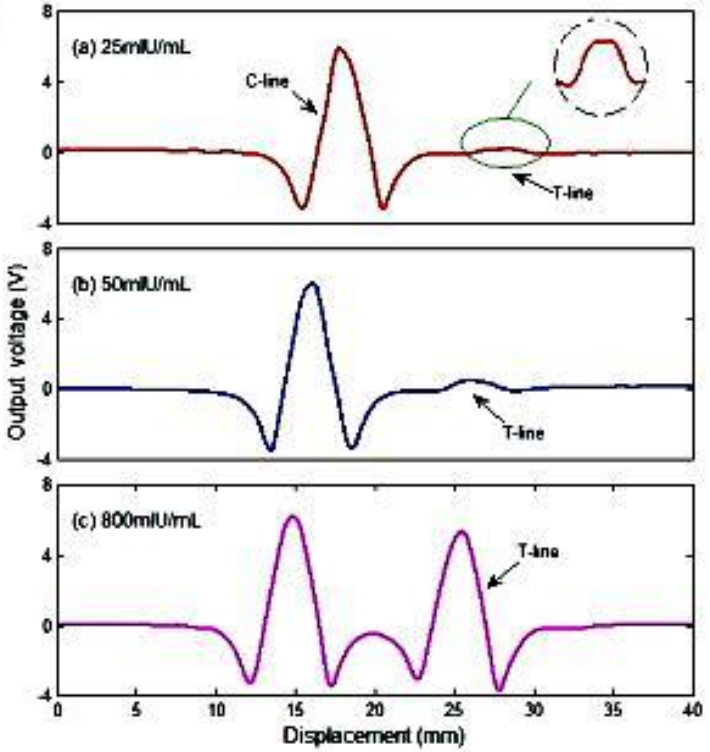
Portable fluorescent detector



	Quantum dots	Colloidal gold
Limit of detection, ng/мл	0.2	4.8
Working range, ng/ml	0.3–10	8.7–214

MGNETIC NANOPARTICLES AS LABELS

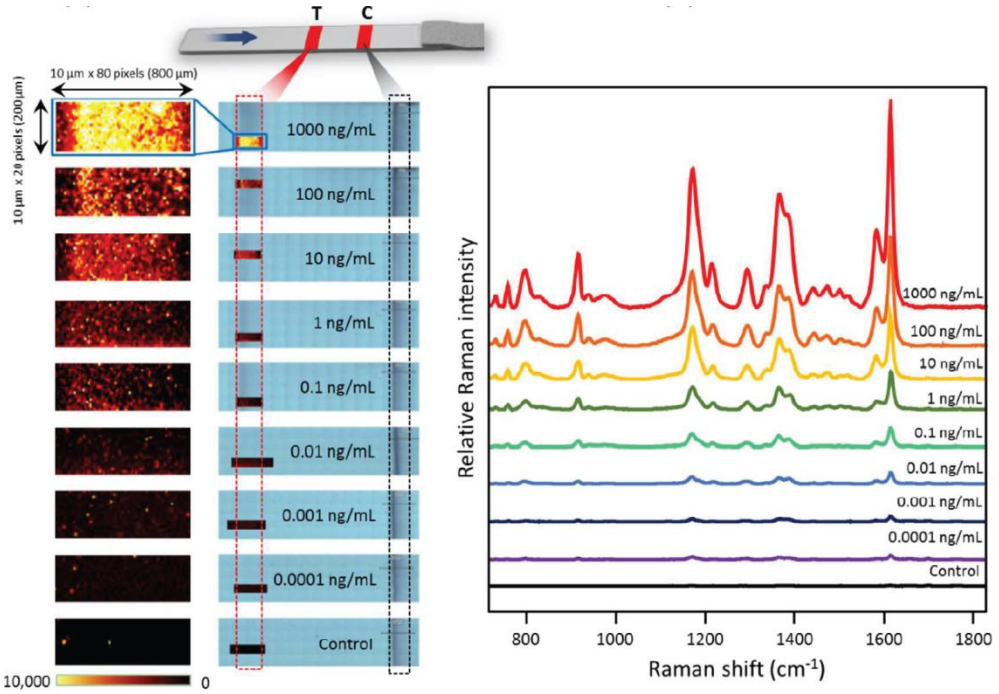




Lei H. et al. (2016) *Sensors*, **16**: 2130

SERS-IMMUNOCHROMATOGRAPHY

SERS-LFIA

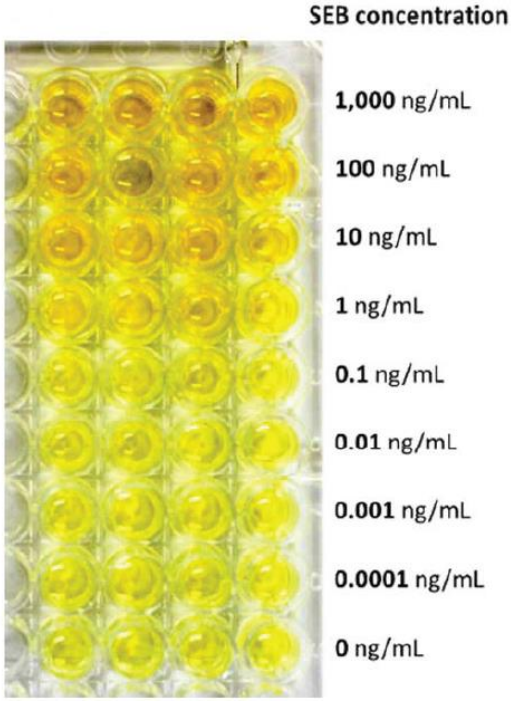


LOD 0.001 ng/ml

COMMON LFIA

LOD 10 ng/ml

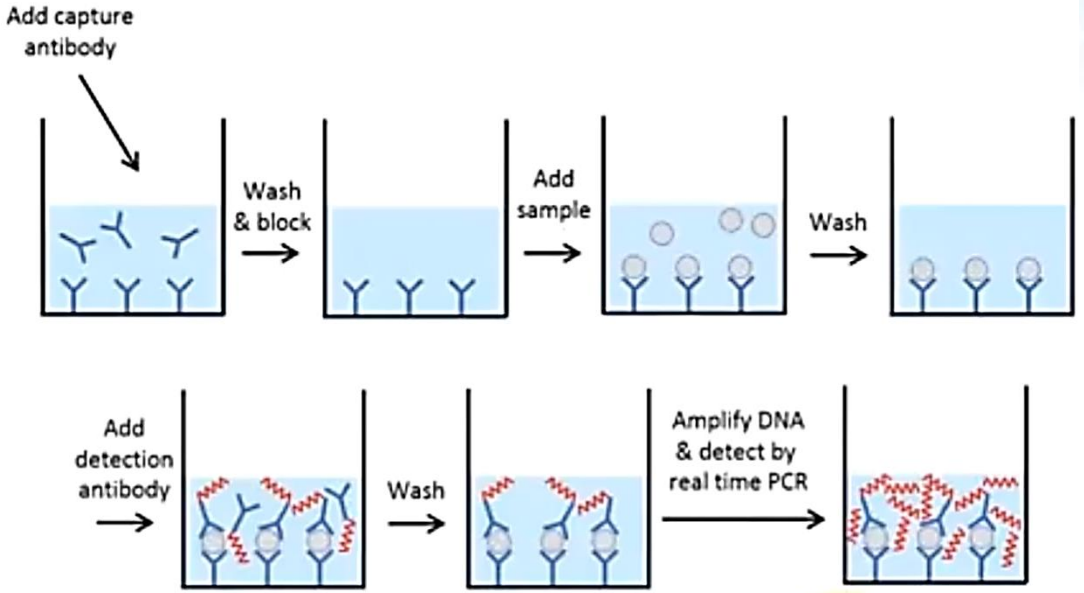
ELISA

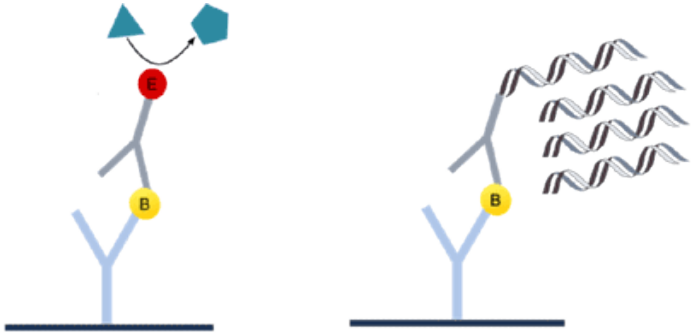


LOD 1 ng/ml

Hwang J. et al. (2016) *Nanoscale*, **8**: 11418-11425

IMMUNO-PCR

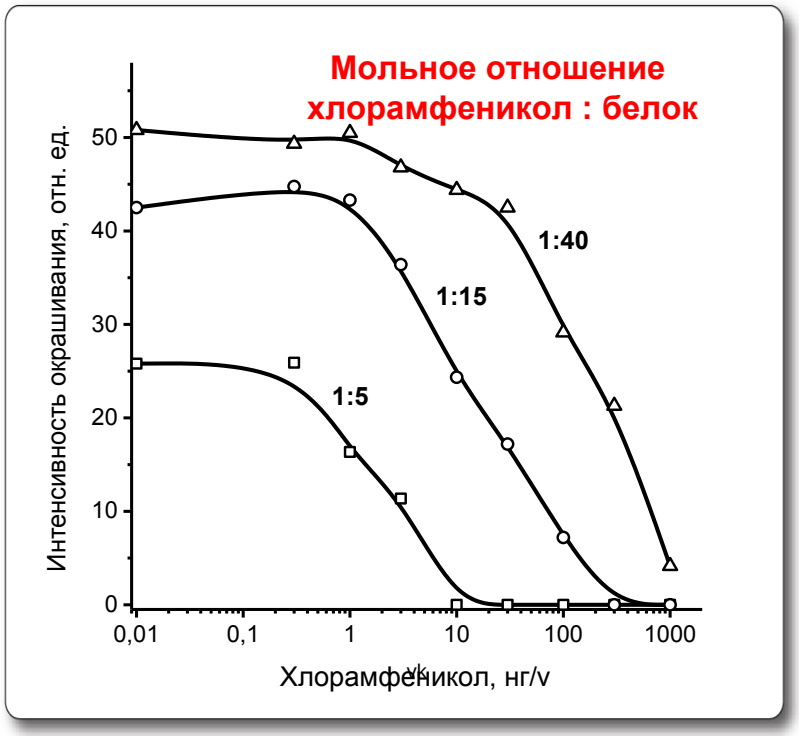




	ELISA	Immuno-PCR
Detection Limits	µg to pg	pg to fg
Amount of starting material required	µl	pl

Варьирование состава конъюгатов и его влияние на предел определения

Отношение гаптен : белок в иммобилизованном конъюгате

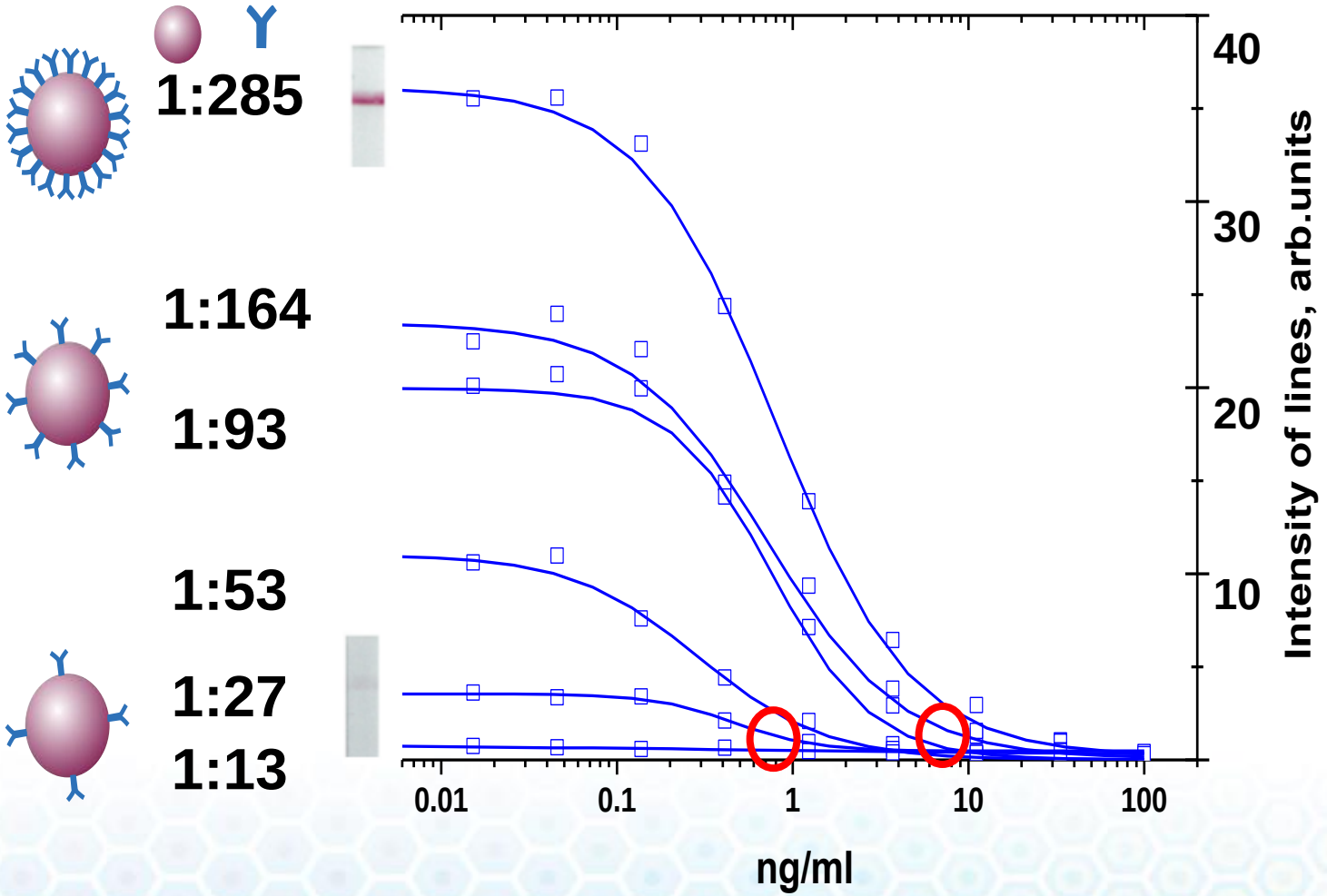


Содержание антител в конъюгате с коллоидным золотом

Данные факторы позволяют изменить пороговые уровни тестов на два порядка (с 1000 до 10 нг/мл в случае хлорамфеникола)

Zvereva E.A. et al. (2015) *Anal. Methods* **7**: 6378-6384

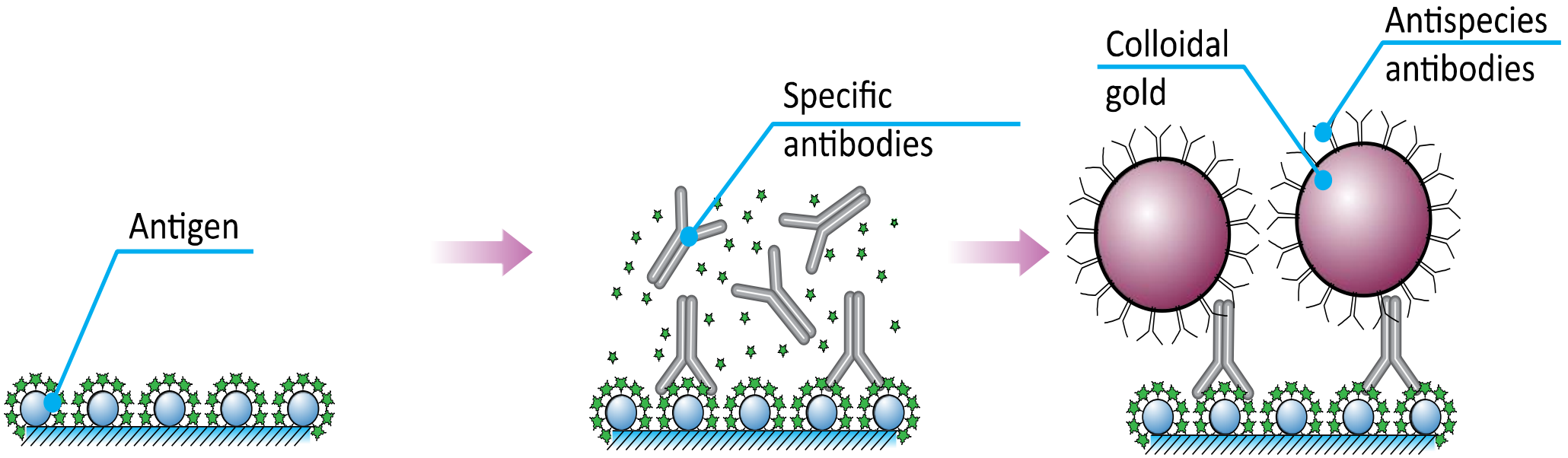
LFIA WITH CONJUGATES OF DIFFERENT COMPOSITION



Better LOD is strongly associated with fall of coloration

Urusov A.E. et al. *Analytical Biochemistry* (2015). *491*, 65-71.

INDIRECT LABELING IN LFIA

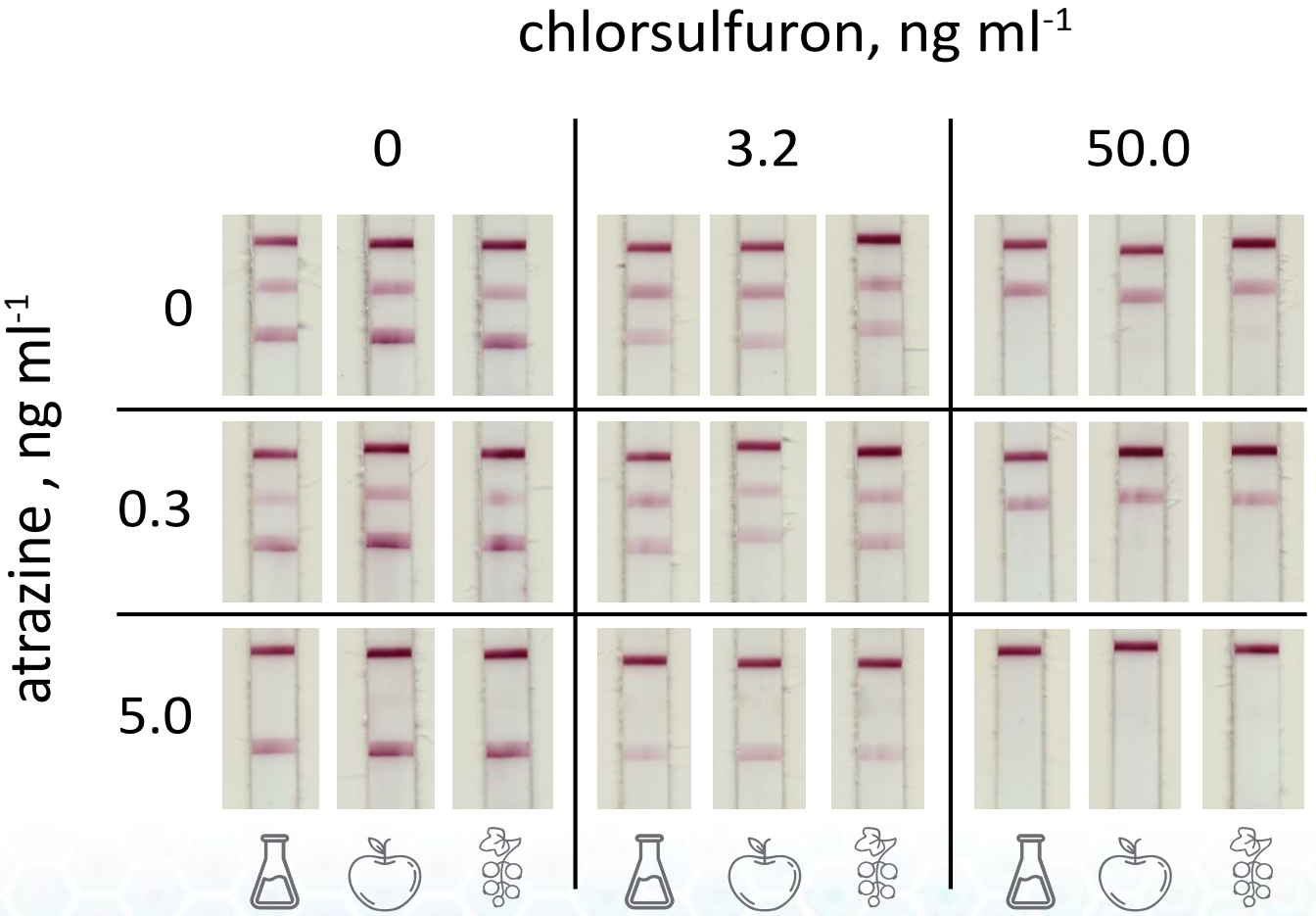


Competitive immune reaction

Labeling of immune complexes

The approach provides possibility to vary values of antibody and label independently and by this way to integrate sensitivity and high coloration

DETECTION OF TWO PESTICIDES USING INDIRECT LABELING



COMPARICON OF MONOTESTS AND DOUBLE TEST

	IC_{50}, ng/ml	Visual LOD, ng/ml	Instrumental LOD, ng/ml
Atrazine			
monotest	0.5 ± 0.01	5.0 ± 0.26	0.08 ± 0.01
Diuble test	0.43 ± 0.03	5.0 ± 0.01	0.1 ± 0.08
Chlorsulfuron			
monotest	5.3 ± 0.09	50.0 ± 0.87	0.7 ± 0.08
Diuble test	4.4 ± 0.15	50.0 ± 0.67	0.7 ± 0.1

1. Actual tasks of pesticides monitoring

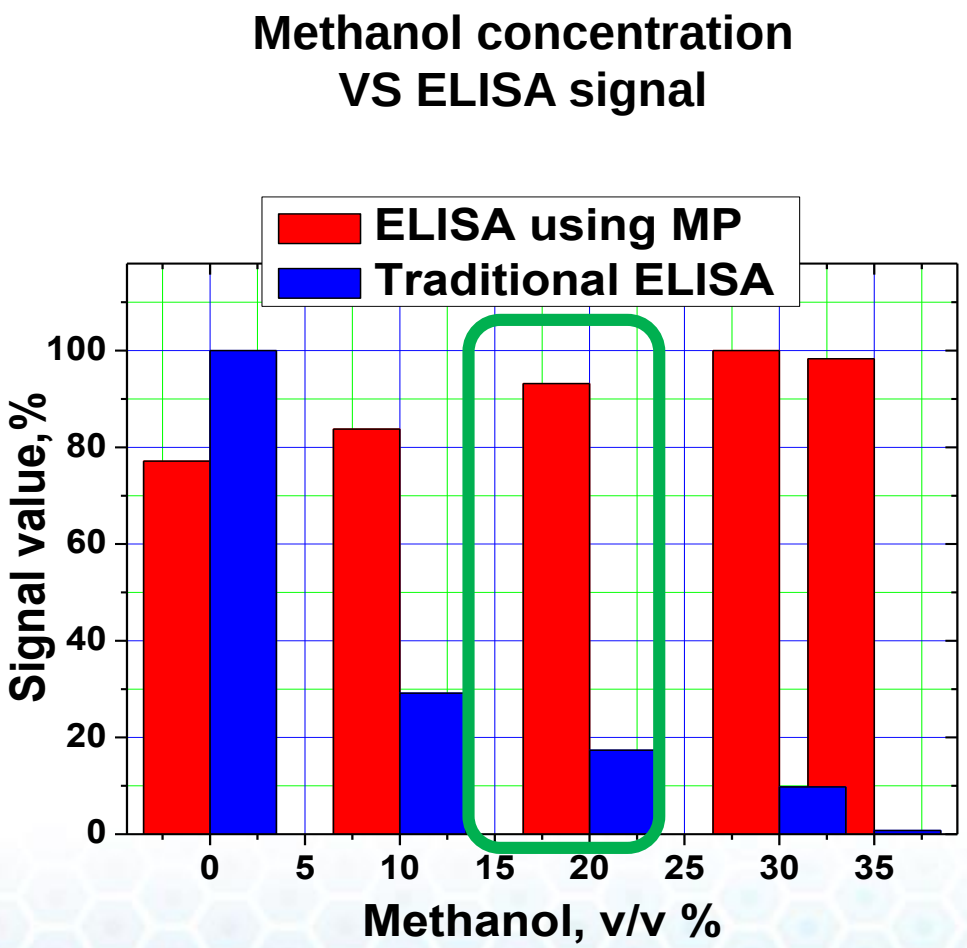
2. Range and variety of immunochemistry

5. Efforts for efficiency of whole assay procedures

IMPROVEMENTS FOR IMMUNOCHROMATOGRAPHIC TESTS

1. Proper sample
2. Proper receptor
3. Proper interaction
4. Proper responce
5. Proper output

INFLUENCE ON ORGANIC SOLVENTS TO NATIVE AND IMMOBILIZED ANTIBODIES

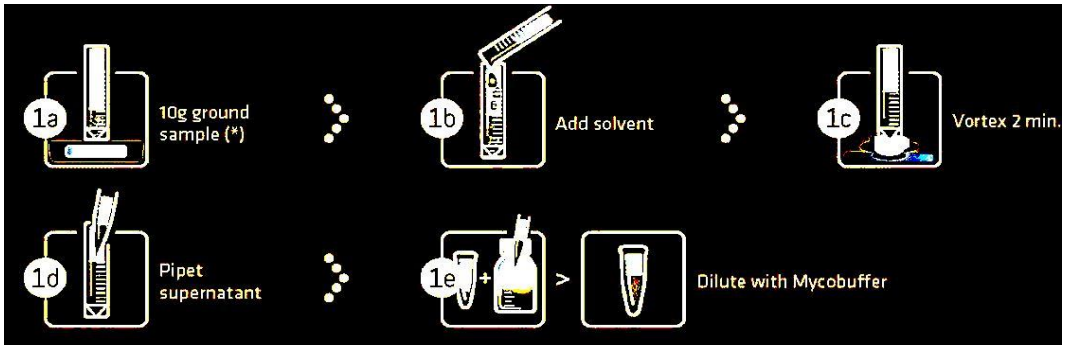


SAMPLING FOR COMPLEX MATRIXES

4MycoSensor (Unisensor, Belgium)

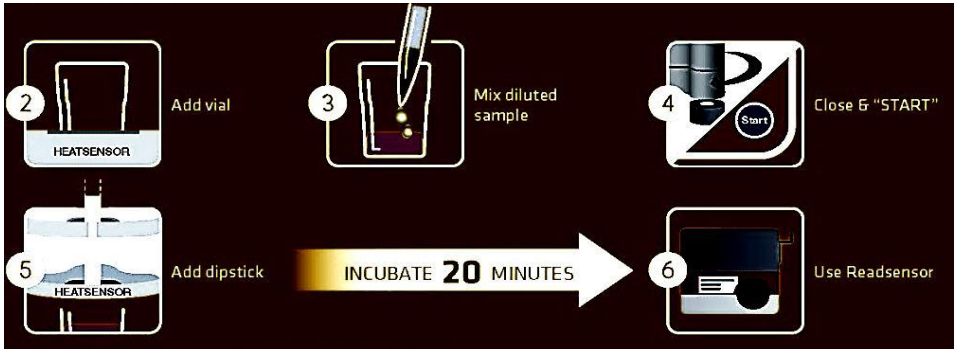
Mycotoxins control in cereals

Samples preparation

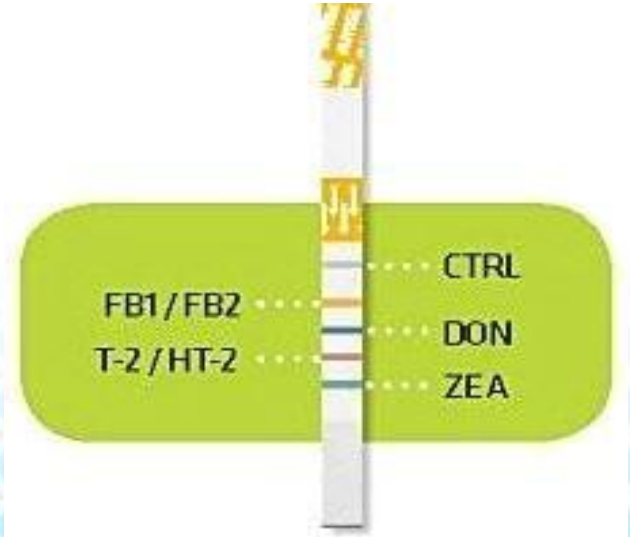


3 min at shaker (5 min for corn)

Assaying



20 min in thermostrat

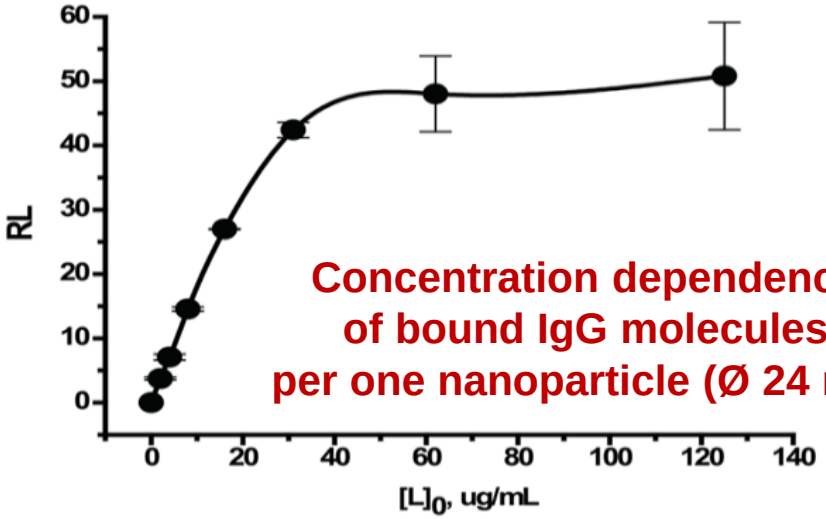


Minimal controlled levels:

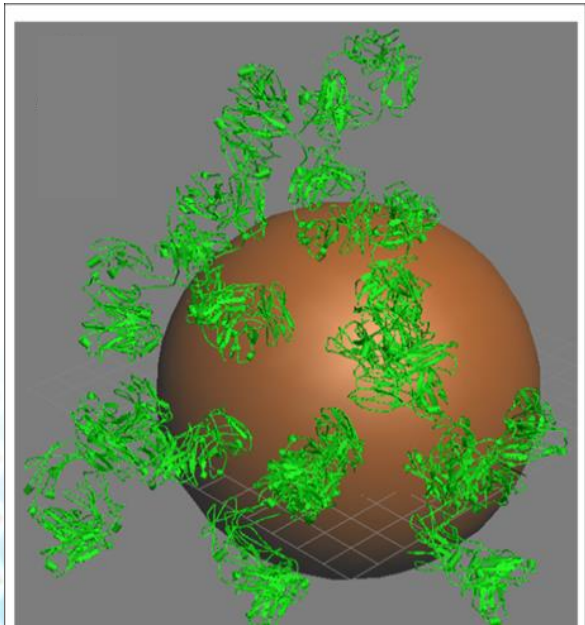
- zearalenone – 280 ug/kg
- T-2/HT-2 toxins – 200 ug/kg
- DON - 1400 ug/kg
- fumonisins B1/B2 – 3200 ug/kg

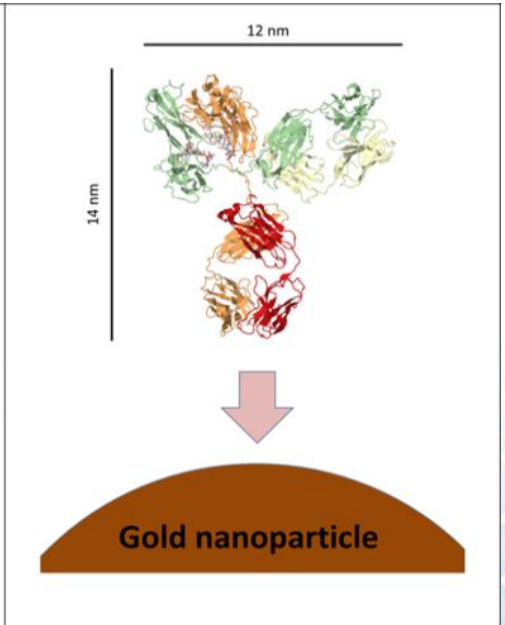
STUDIES OF IgG ADSORPTION ON GOLD NANOPARTICLES

Data of tryptophan fluorescence

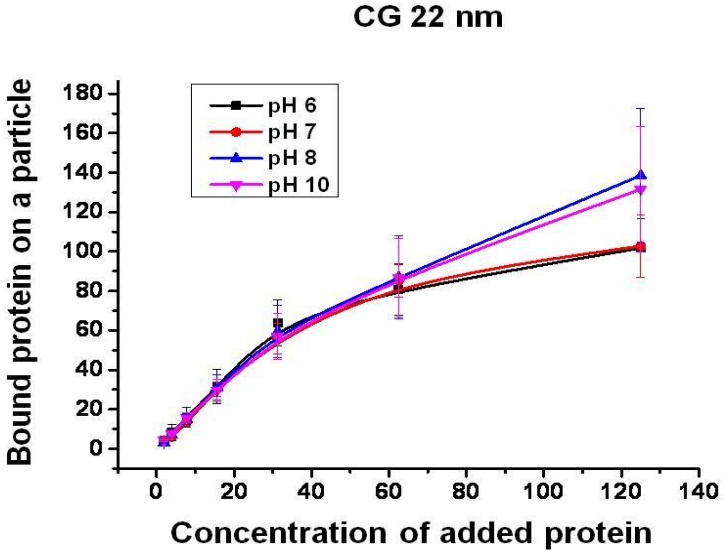


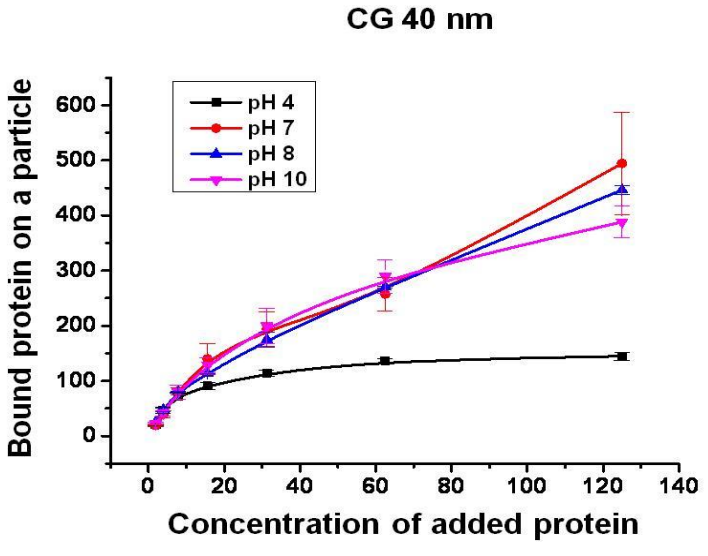
Concentration dependence of bound IgG molecules per one nanoparticle (Ø 24 nm)



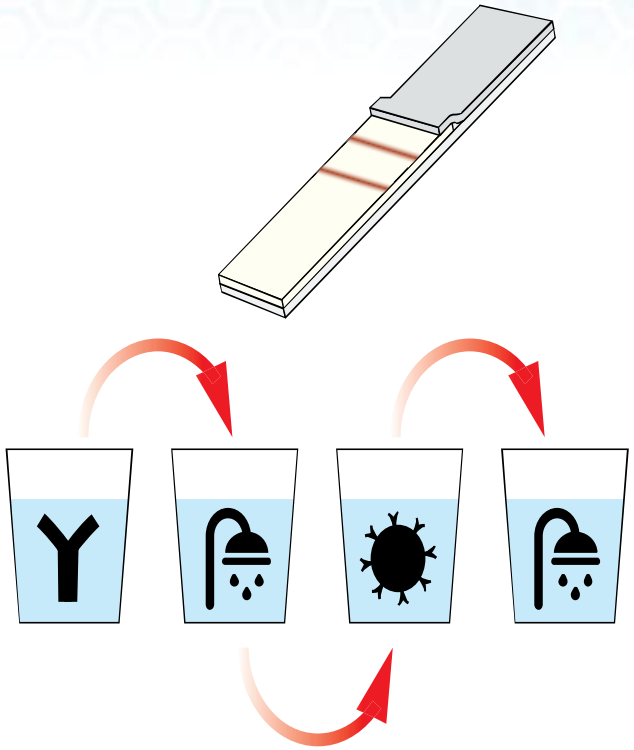


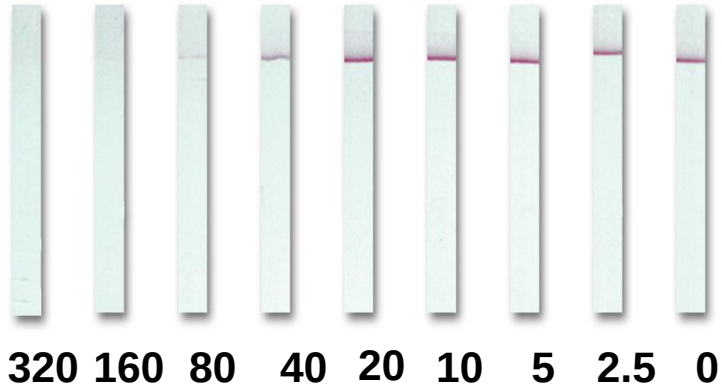
pH-dependent quantity of layers





Multistage LFIA with indirect labeling





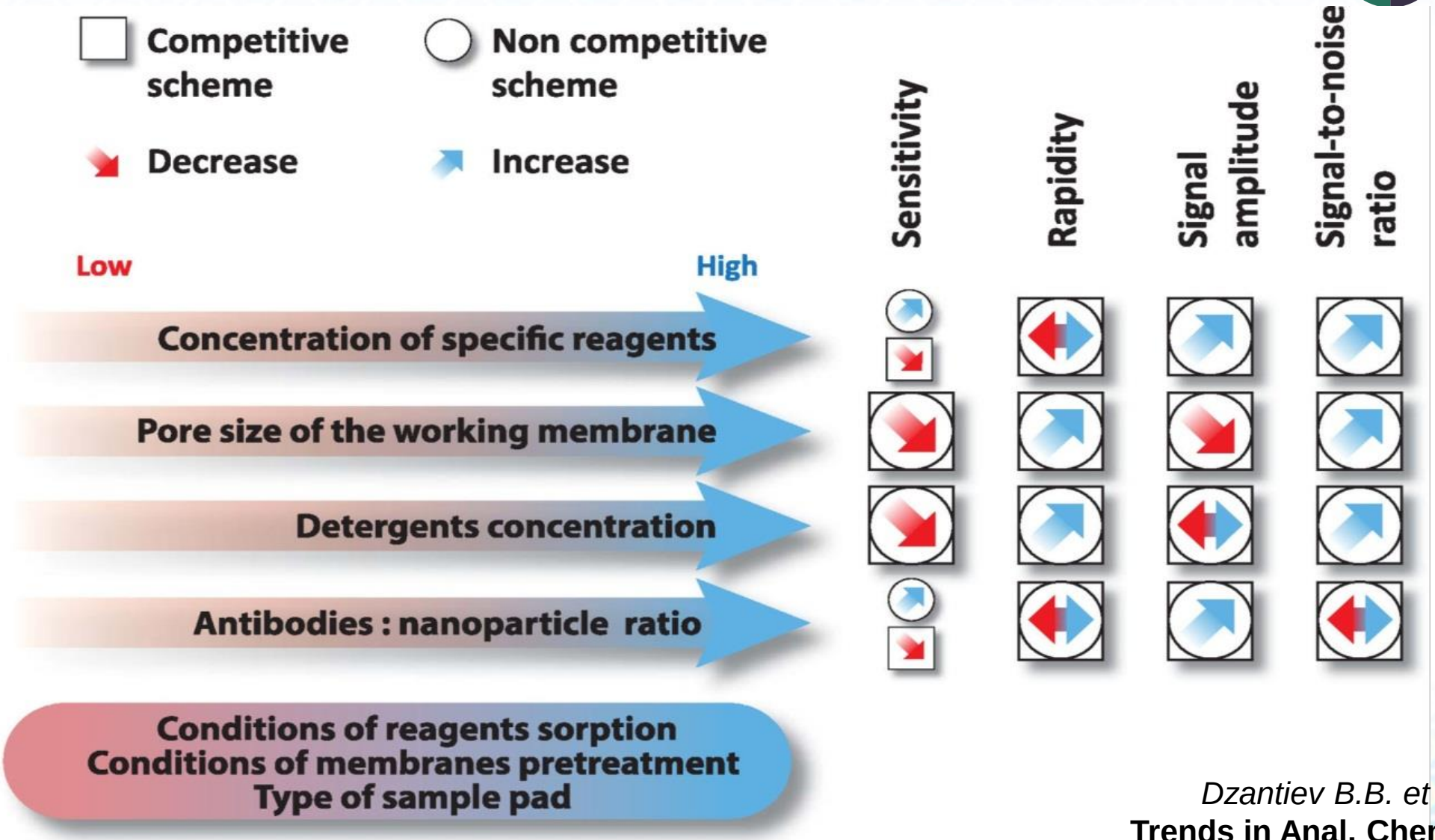
Aflatoxin B1, pg/ml

0.08 ng/ml **0.02 ng/ml** **30 min**

This approach exhibits more then 10-fold lower LOD for aflatoxin B1
As compared with traditional LFIA using the same reagents.
However, the stages are separated, require liquid reagents and additional manipulations.

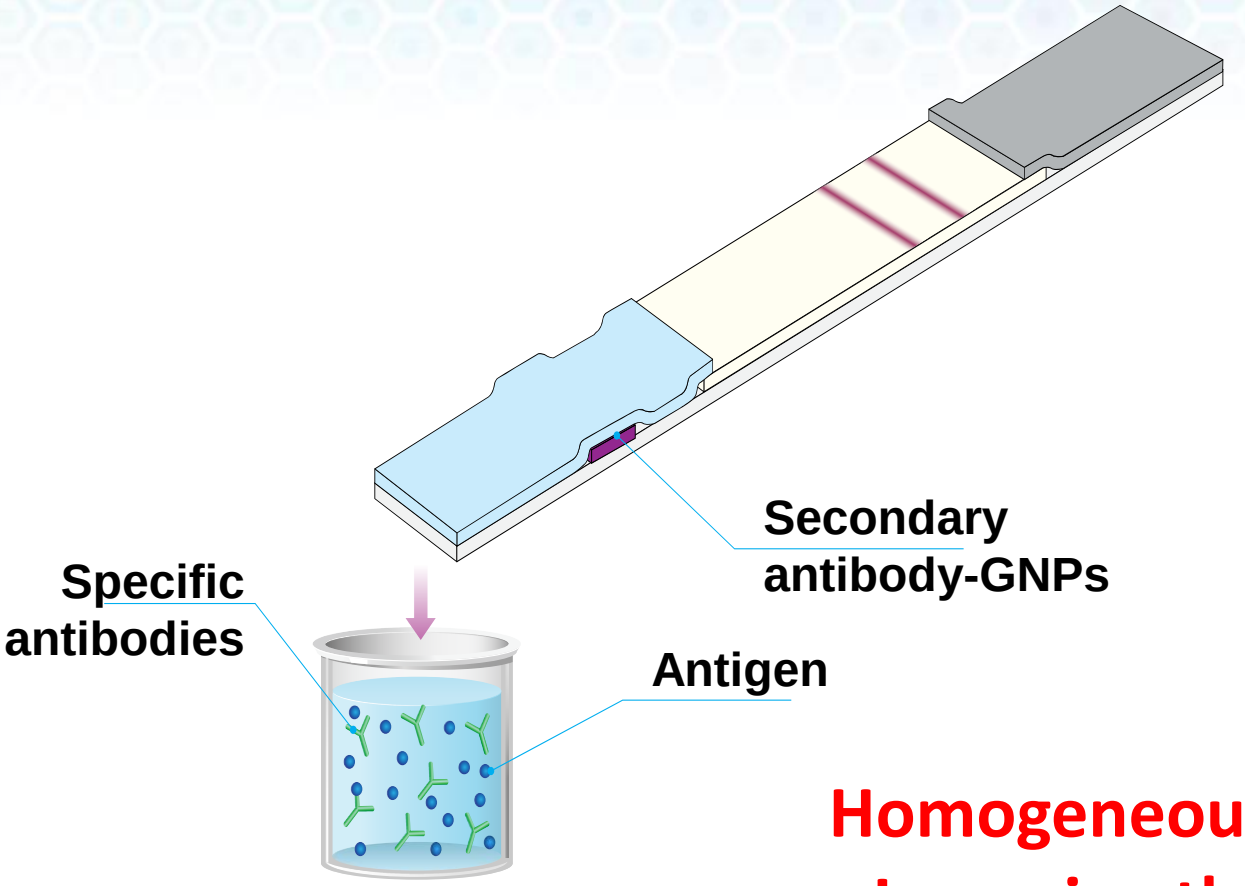
Urusov A.E. et al. *Microchimica Acta* (2014). *181*(15-16), 1939-1946.

MODULATION OF ASSAY PARAMETERS



Dzantiev B.B. et al.
Trends in Anal. Chem.,
2014, 55, 81-93.

LFIA with «external» specific antibodies



Homogeneous pre-incubation

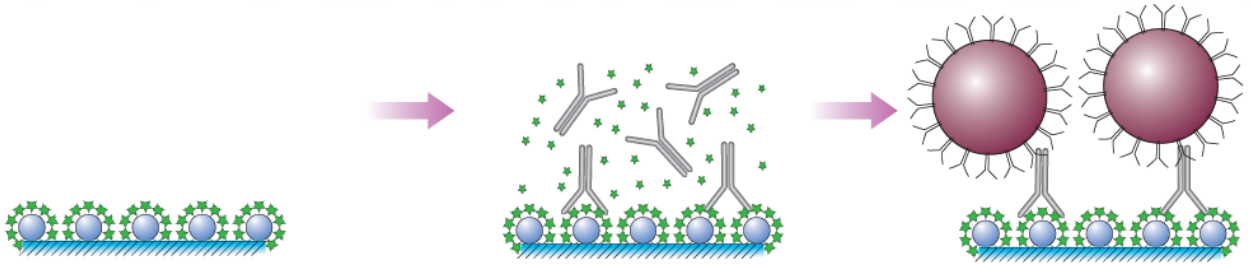
Lowering the detection limit

The strip includes all components, but specific free antibodies are moved to the diluting buffer.
Thus,

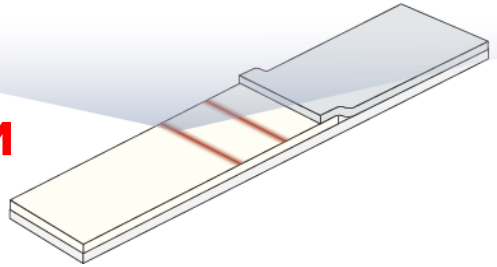
- the concentrations of the antibodies and markers can be chosen independently,
- and dilution ensures pre-incubation of the antibodies with the sample.

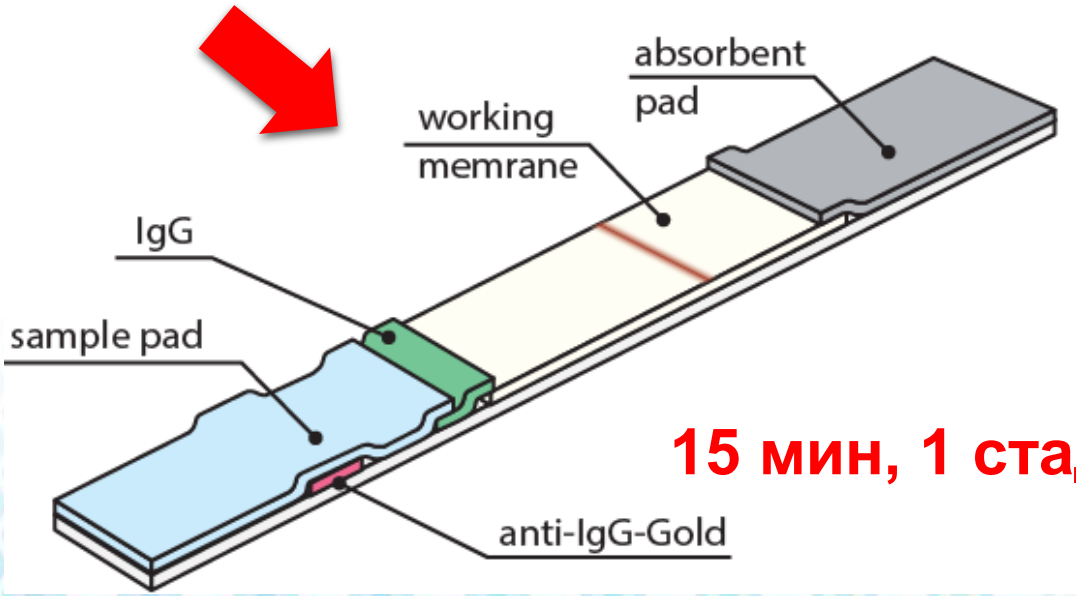
The both factors increase the sensitivity of the test.

Варианты тест-систем с непрямым мечением антител



30 мин, 4 стадии

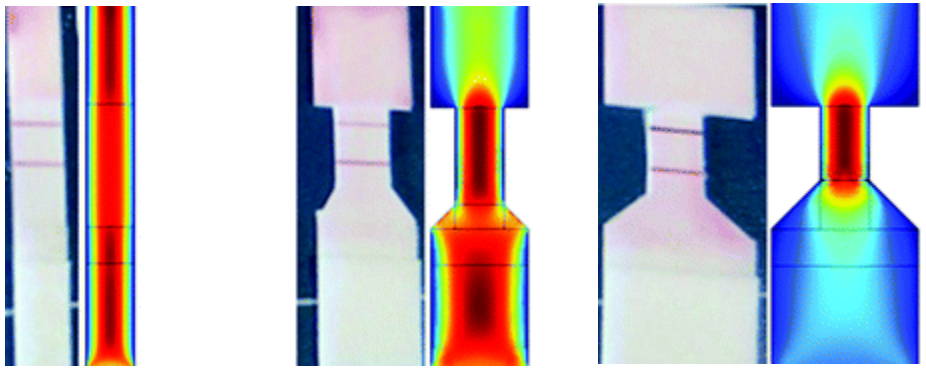


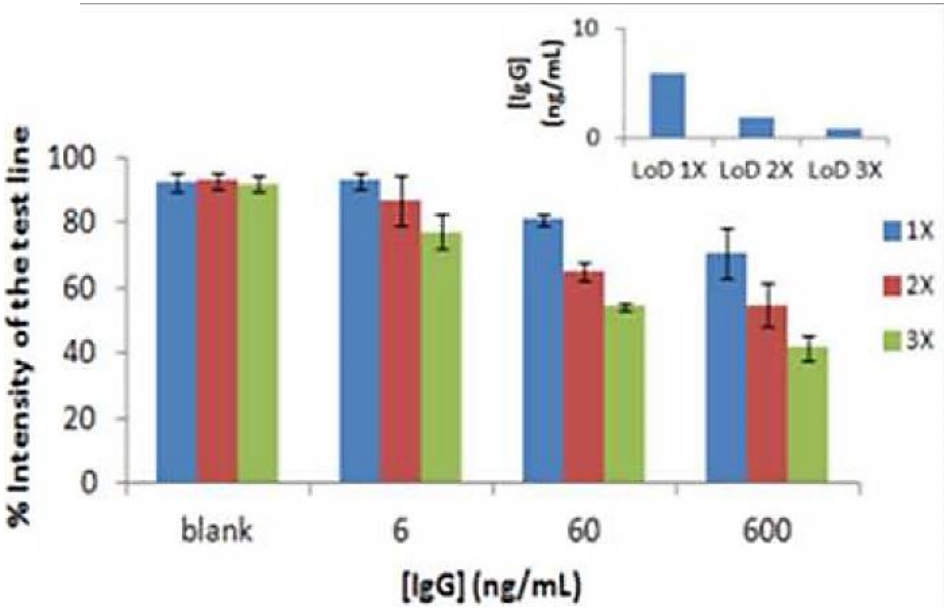


15 мин, 1 стадия

VARIATION OF STRIPS GEOMETRY

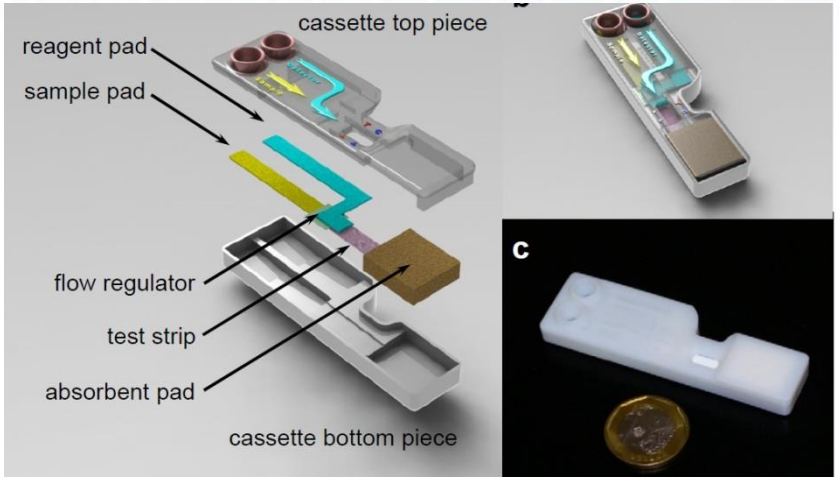
Common strip **Modified strips**

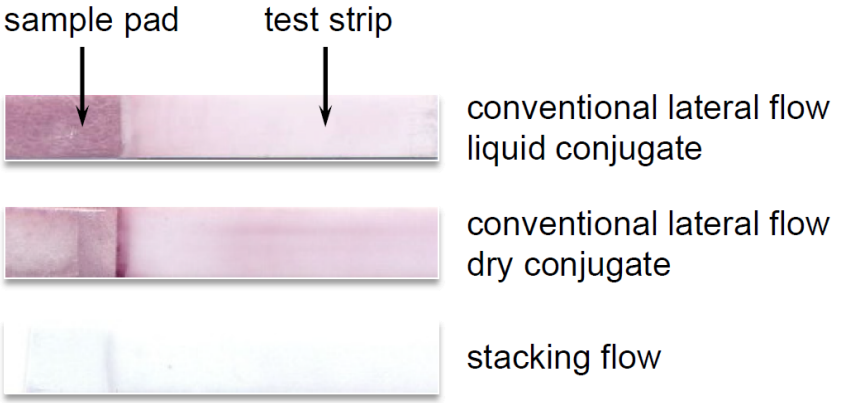


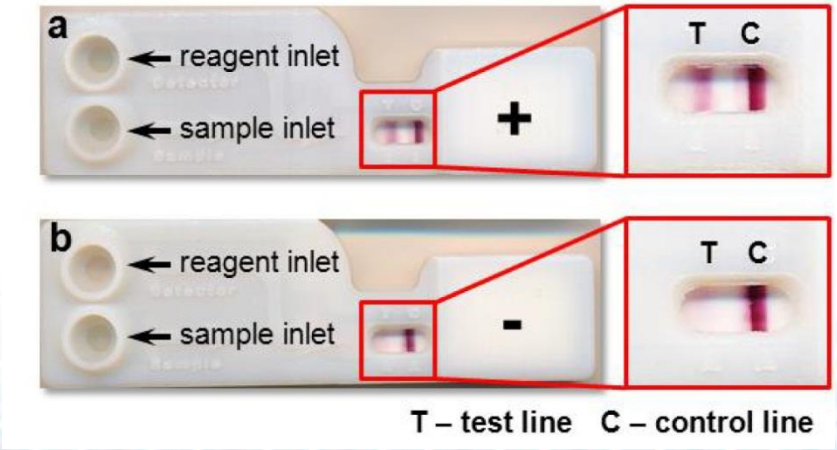


Parolo C. et al. (2013) *Lab Chip* **13**: 386-390

MICROFLUIDIC APPROACHES IN LFIA

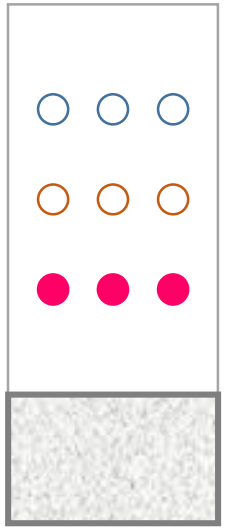






Zhang Y. et al. (2015) *Lab Chip*,15: 1465-1471

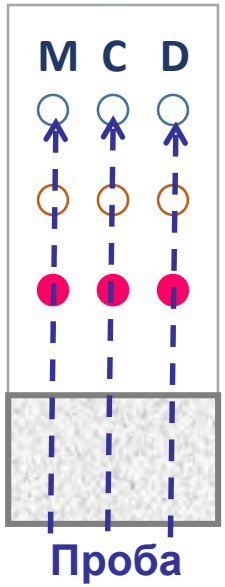
MULTI-TRACK CHROMATOGRAPHY



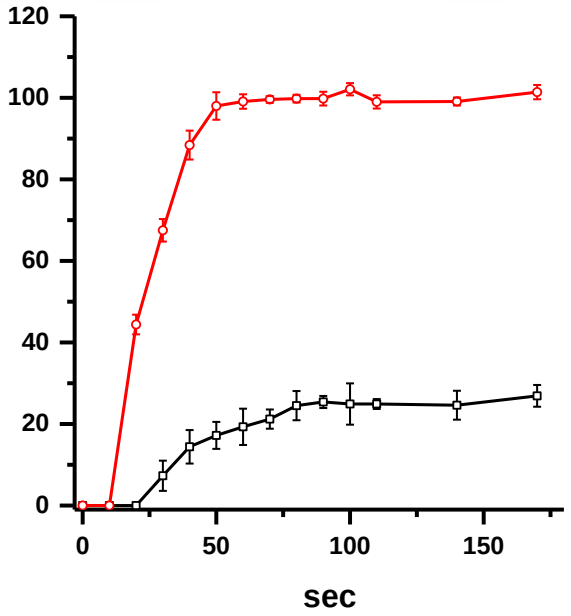
Control zone

Test zome

Gold conjugates



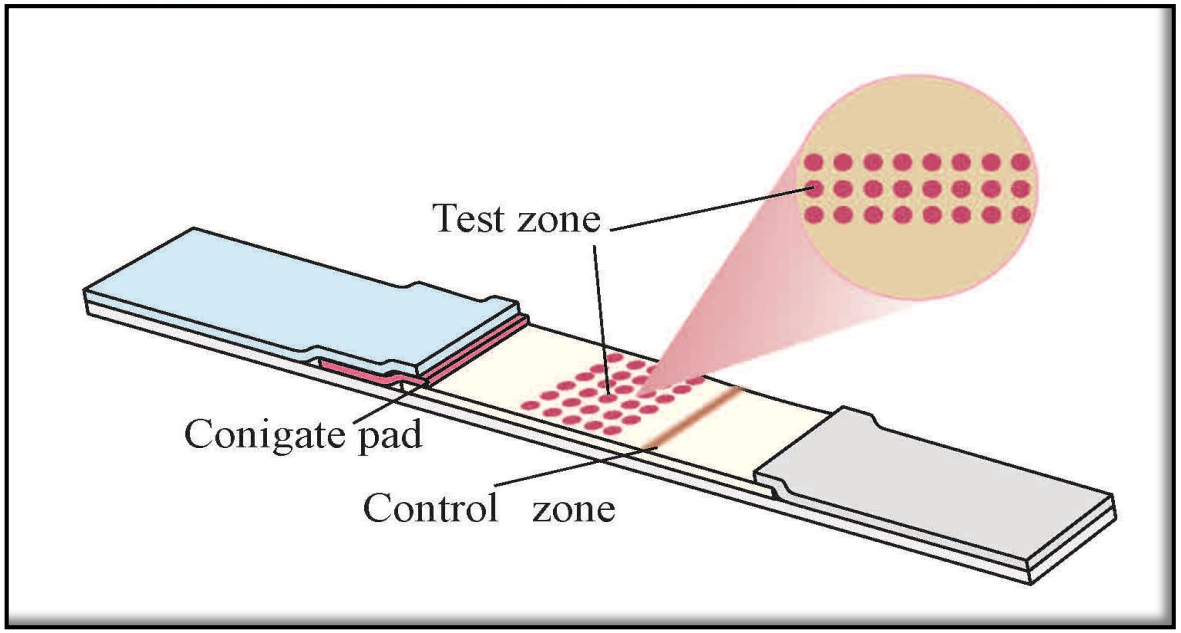
Dynamics of immune complexes formation



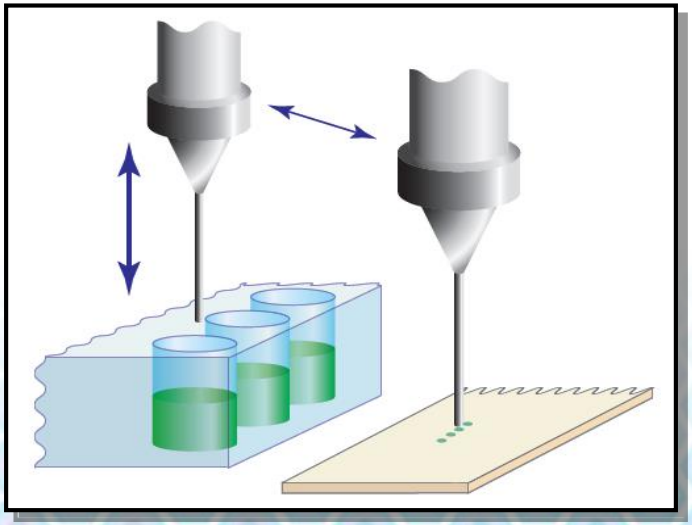
M C D	M C D	M C D	M C D	M C D	Analyte
0 + 0 + 30	0 + 30 + 0	3 + 0 + 0	3 + 0 + 30	3 + 30 + 30	Added, ng/ml
					← c.zone ← t.zone
– – +	– + –	+ – –	+ – +	+ + +	Result

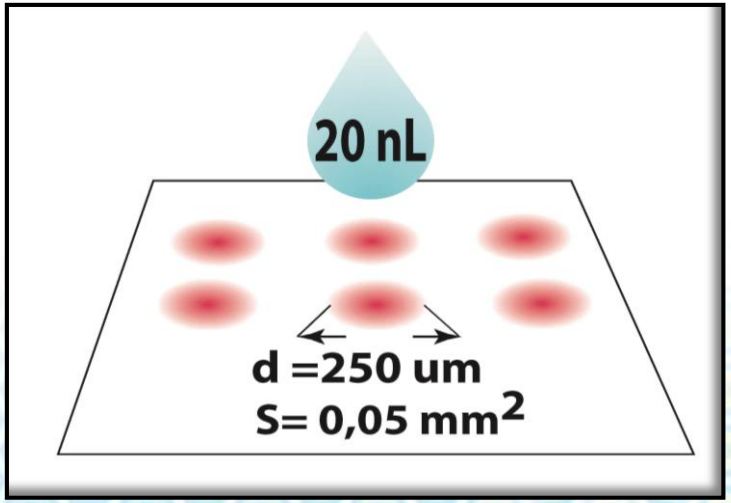
PRINCIPLE OF «2-D IMMUNOCHROMATOGRAPHY» FOR MULTIPLEX ASSAY

Construction of test strip



Application of reactants by pins





PORTABLE DEVICES TO REGISTER AND PROCESS THE ASSAY RESULTS

Camera-based solutions

- compact working place
- non-contact measurements

Scanner-based solutions

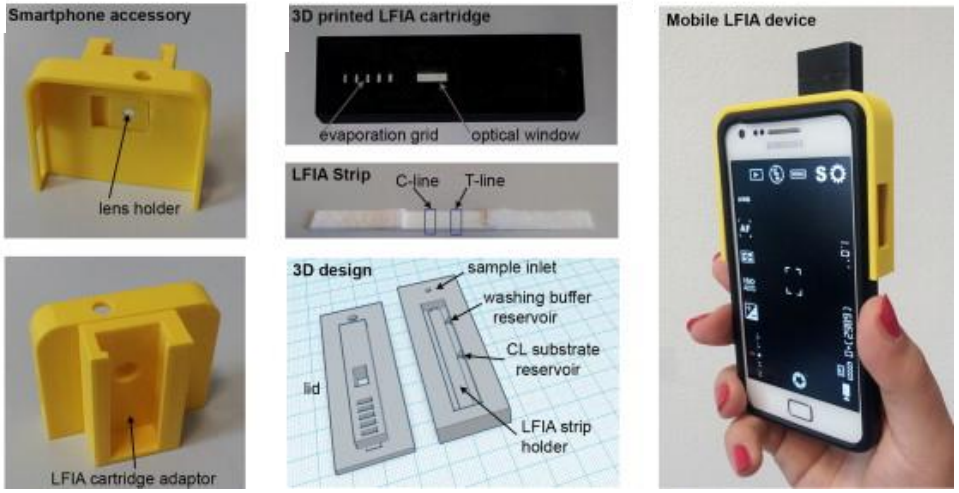
- high resolution
- only novel software for available equipment

Advantages of video-digital detection

- ✓ Clear identification of each binding zone
- ✓ Grounded solutions about zone's borders
- ✓ Qualitative and quantitative assays
- ✓ Compatibility with different test-strips
- ✓ Computer output and process control
- ✓ Storage of complete data about assays

APPLICATION OF COMMON OPTICAL TOOLS FOR IMMUNODETECTION

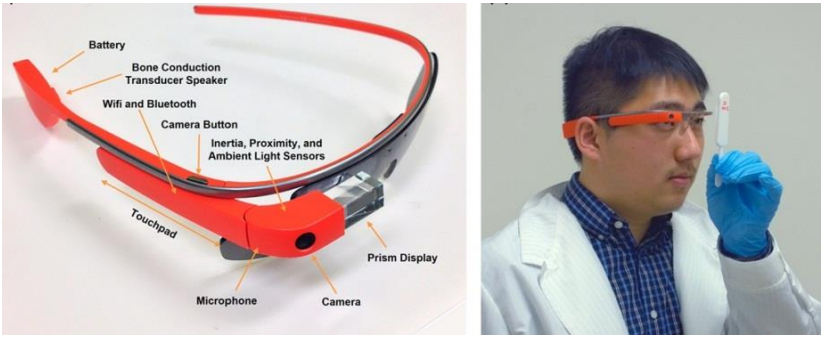
Mobile phone / Smartphone

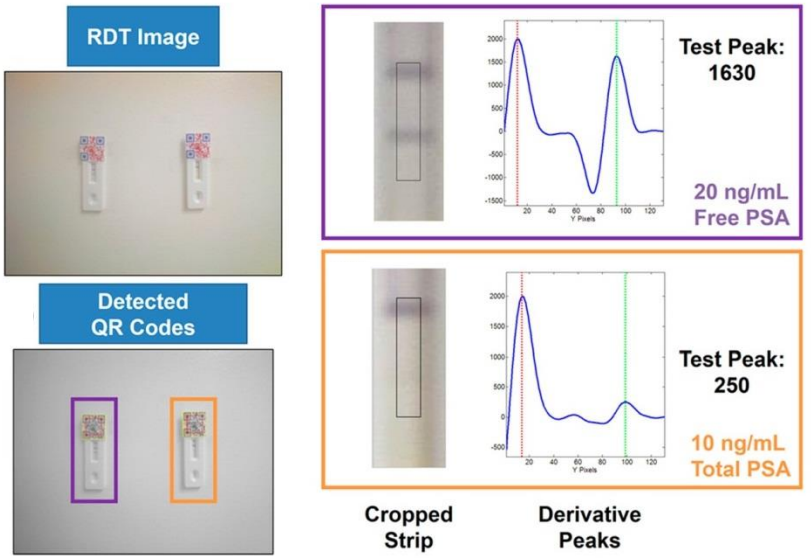


Zangheri M. et al. (2015) *Biosens. Bioelectron.* **15**: 63-68

Mudanyali O. et al. (2012) *Lab Chip* **12**: 2678-2686

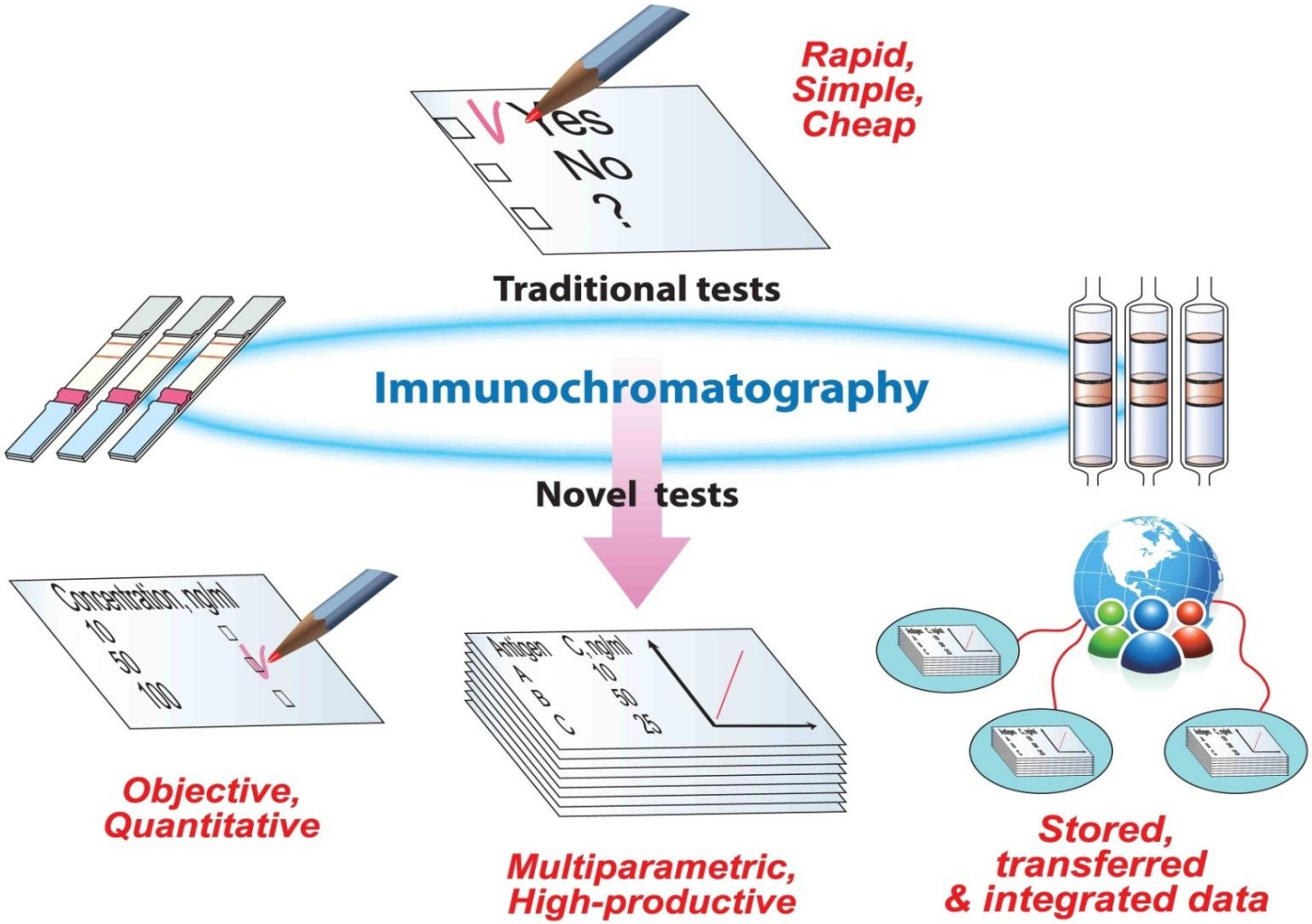
Google-glasses





Fend S. et al. (2014) *ACS Nano* **8**: 3069-3079

FROM FIRST TESTS TO INTEGRATED SYSTEMS OF DATA PROCESSING AND TRANSFER



Dzantiev B.B. et al. Trends in Anal. Chem., 2014, 55, 81-93.

Any questions?

Thank you for your attention!

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