About OMICS Group

OMICS Group is an amalgamation of Open Access Publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Nb4D
NANOBIOTECHNOLOGY
FOR DIAGNOSTICS

Rapid, accurate and simple microfluidic device based on fluorescent read-out immunoassay for the determination of oral anticoagulants in treated patients

J.-Pablo Salvador

CIBER-BBN/IQAC-CSIC

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

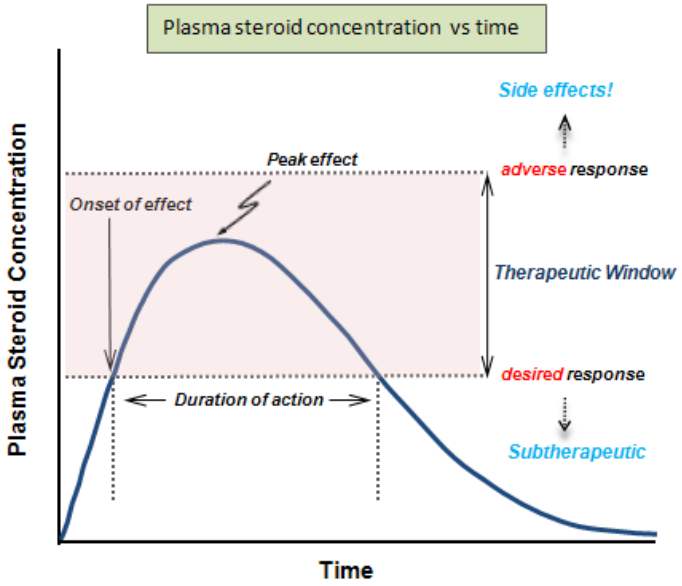
ciber-bbn

Outline

- **INTRODUCTION**
- **PART I:**
  - **PRODUCTION OF ANTIBODIES FOR OAC**
  - **ANALYTICAL CAPABILITIES FOR OAC DETECTION IN PLASMA**
- **PART II**
  - **MICROFLUIDIC DEVICE WITH FLUORESCENT READ-OUT**

Introduction to TDM

- **Therapeutic Drug Monitoring (TDM)** aims to accomplish a more personalized medicine by assisting on adjusting doses, according to each patient needs, reducing toxicity due to unnecessary overdosing, and follow-up of the treatments.



http://www.anabolicsteroidcalculator.com/pharmacology101.php

TDM responds to

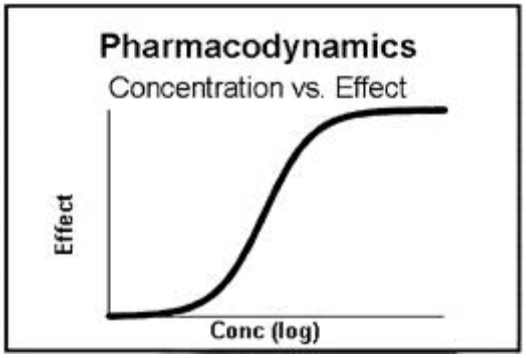
- Drugs that have a very narrow therapeutic range
- Concentrations above the upper limit of the therapeutic range of a drug can produce adverse or very toxic effects
- Concentrations below the lower limit of the therapeutic range of a particular drug may be completely ineffective
- Not all patients have the same response to similar doses, means the therapeutic range can be different.

Introduction to TDM

- **Therapeutic Drug Monitoring (TDM)** aims to accomplish a more personalized medicine by assisting on adjusting doses, according to each patient needs, reducing toxicity due to unnecessary overdosing, and follow-up of the treatments.

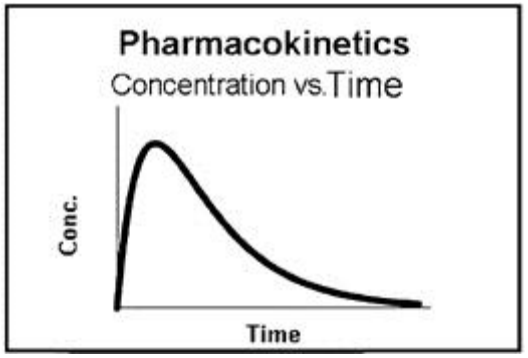
PHARMACODYNAMIC

End-point markers of effect



PHARMACOKINETIC

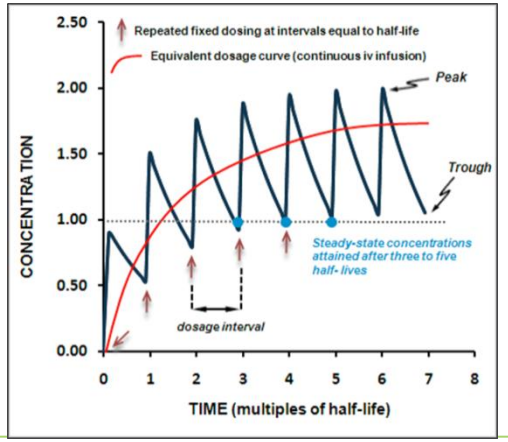
Measurement of blood concentrations of drugs



Commonly Monitored Drugs

Family	Congener EXAMPLES
Antiepileptics	Carbamazepine, Ethosuximide, Pirimidone, Phenobarbital
Antiarrythmics	Digoxin, Digitoxin, Lidocaine, Procainamide, Disopyramide
Antibiotics	Gentamicin, Vancomycin, Tobramycin, Amikacin,
Antineoplastics	Methotrexate, 5-fluorouracil
Psycopharmcs	Lithium, Phenobarbital, Phenytoin, Thioridazine, e
Broncodilators	Caffeine, Theophylline
Immunosupressives	Cyclosporine, Everolimus, Mycophenolic Acid, Tacrolimus
Anticoagulants	Warfarin, Acenocoumarol

TDM usually implies the measurement of specific drugs at intervals in order to maintain a relatively constant concentration of the medication in the bloodstream.



Current Analytical Methods for TDM

Traditionally **Chromatographic Methods** were employed, which had good specificity and sensitivity.

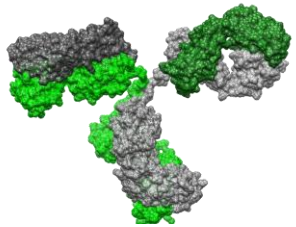
- **HPLC-UV**
- **GC-MS**
- **HPLC-MS (or MS/MS)**

- Labor-intensive
- Specially trained operators required
- Expensive
- Turnaround time slow
- Difficult to implement for routine clinical monitoring

In contrast, **Immunochemical assays**

- **RIA**
- **PETINIA**
- **EIA**
- **EMIT**
- **FPIA**
- **CLIA**
- **etc**

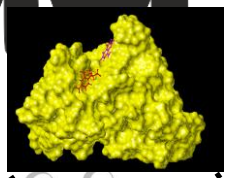
- Rapid tests for laboratory or point-of-care applications
- High detectability
- Selectivity
- Qualitative and/or accurate and precise quantitative tests
- Minimum sample pretreatment
- Low cost

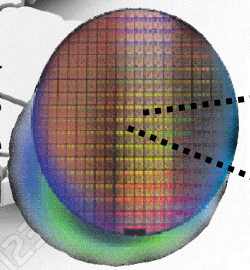


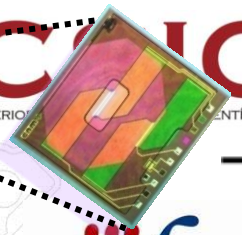
Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

Nb4D Research Objectives

- Development of bioreceptors with tailored properties, such as *affinity* and *selectivity*, versus different target biomarkers.
- Development of functional (bio)hybrid materials, by combining bioreceptors with inorganic micro(nano)structures with particular optical, magnetic or electrical properties.
- Investigation on novel micro & nano(bio)technological approaches to develop a new generation of more efficient *in vitro* diagnostic tools and devices









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Nb4D: Immunoreagent Collection Against Small Organic Molecules and Peptides

Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS

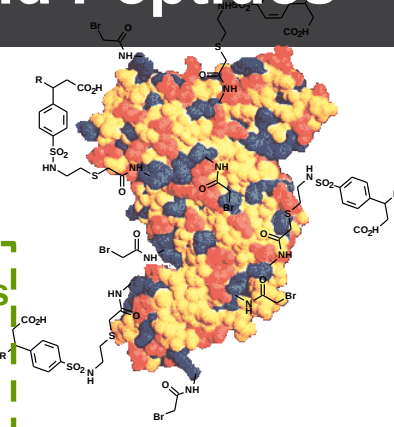
Antibiotics†
- Sulfonamides
- Fluoroquinolones
- Tetracyclines
- Chloramphenicol
- Tylosine A
- Tylosine A+B

Antineoplastics*
- *5-Fluorouracil*
- *Cyclophosamide*
- Tamoxifen

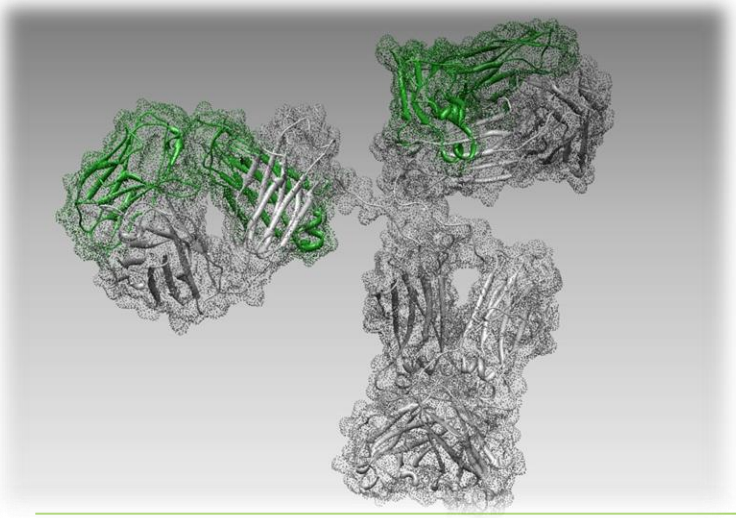
Peptides
- PBAN (Pheromone Biosynthesis Activating Neuropeptide)
- BNP (Brain Natriuretic Peptide)
- NT-pro-BNP (N-terminal proBNP)

Anabolic Androgenic Steroids (AAS)
- Stanozolol
- Boldenone
- Methylboldenone
- Tetrahydrogestrinone

Oral Anticoagulats
- Warfarin
- Acenocumarol



Industrial Residues
- Trichlorophenol(s) and Tribromophenol(s)
- LAS (linear alkylbenzenesulfonates) and SPCs
- Nonylphenol
- Trichloroanisol (TCA) and Tribromoanisol(s)
- Metolachlor (only As)
- PCBs (only As)



Pesticides
- Nitrophenol(s) (parathion, fenitrothiol, etc)
- Triazines: Atrazine, Simazine, Tertbutryne, etc
- Irgarol
- Dealkylated Triazines
- Paraquat
- Diquat

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**in process;* †, aminoglycoside, lincosamides, are also available for research purposes

Nb4D TDM Example: Oral Anticoagulants

Warfarin or Acenocoumarol: are Vitamin K antagonist inhibiting coagulation. These drugs are used on several cardiovascular diseases to stop or prevent thrombosis, embolism, stroke, myocardial infarction and dead.

- *In the US,* approximately 1% of the population or two million people are treated under OAs
- *In Spain, about 400000 patients are under oral anticoagulant treatment (OAT).*
- *In Spain expenses are estimated to be around 60 million € per year.*

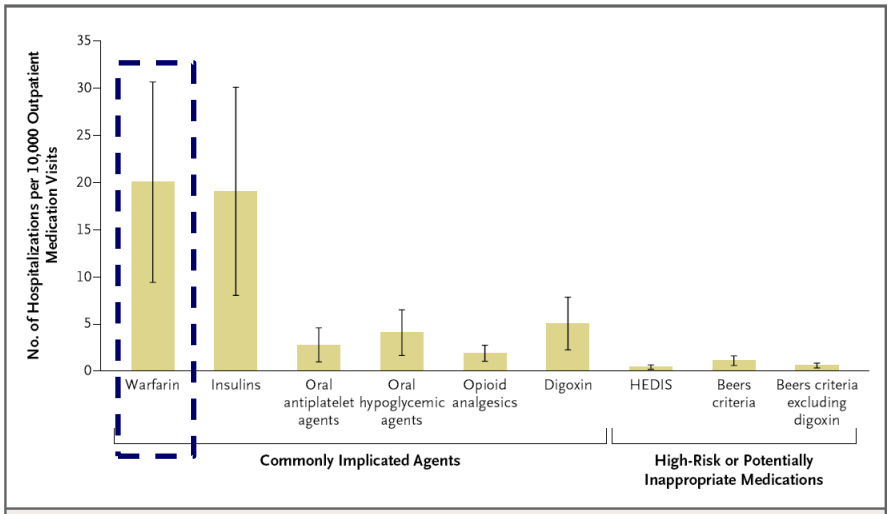


Figure 1. Estimated Rates of Emergency Hospitalizations for Adverse Drug Events in Older U.S. Adults, 2007–2009.

◀Estimated Rates of Emergency Hospitalizations for Adverse Drug Events in Older U.S. Adults 2007–2009.

Side effects: *hemorrhages* (inappropriate dosage)

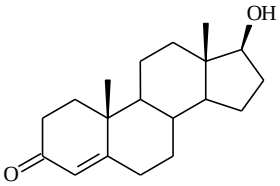
Budnitz, D. S.; Lovegrove, M. C.; Shehab, N.; Richards, C. L., *New England Journal of Medicine* ***2011, 365 (21), 2002-2012.***

Aim of the project

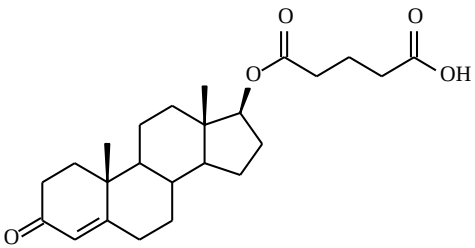
Establishment of an analytical methodology based on immunochemical techniques for the determination of oral anticoagulant drugs

Antibodies against low MW molecules

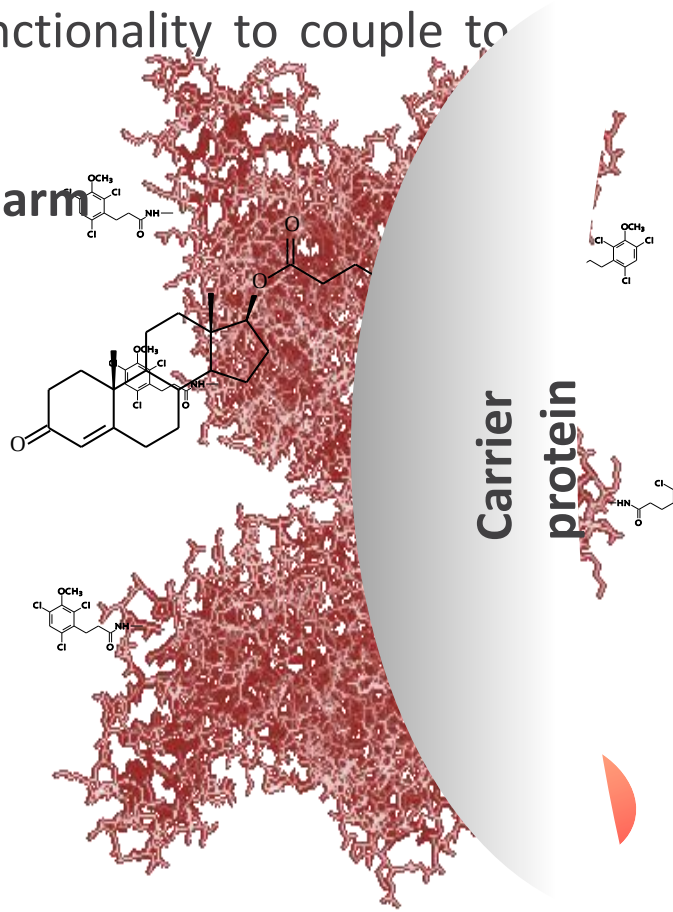
An **immunizing hapten** is a mimic of target analyte that has a similar physico-chemical properties with a additional functionality to couple to carrier protein.



Analyte



Hapten



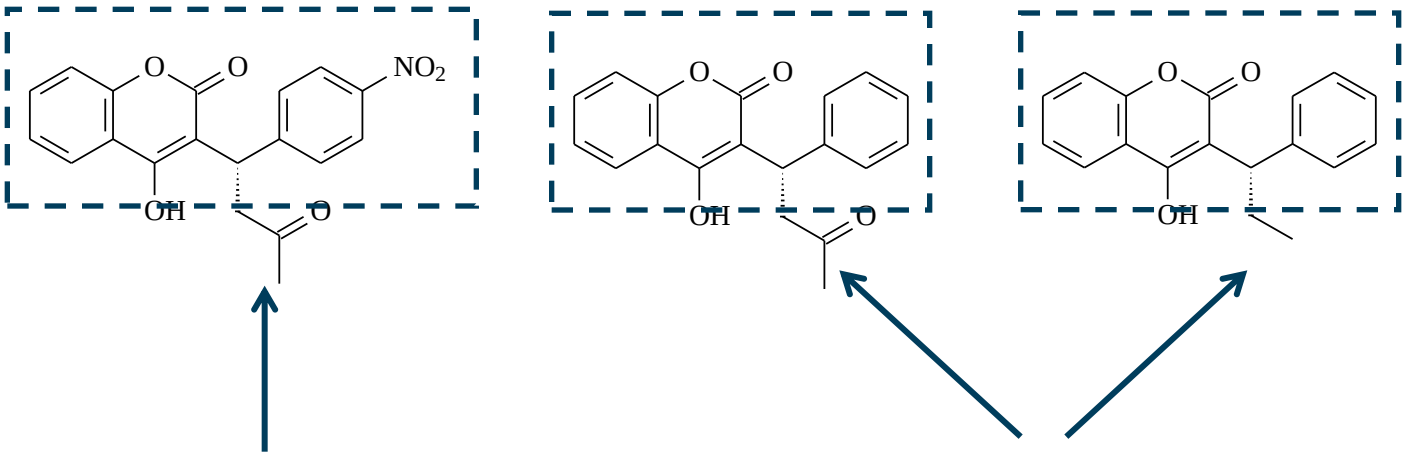
Immunogen

Immunizing haptens

Acenocoumarol
= Sintrom®

Warfarin

Phenprocoumon



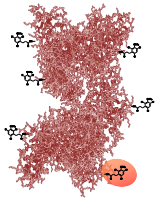
Hapten for Acenocoumarol
h_{ACL}
(specific for ACL)

Hapten for Warfarin
h_W
(recognize W and PPC)

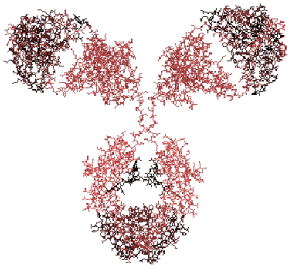
P201231836 (ES 2472290 A1)
PCT/ES2013/070816 (WO2014083226 A1)

Antibody production

Immunogens



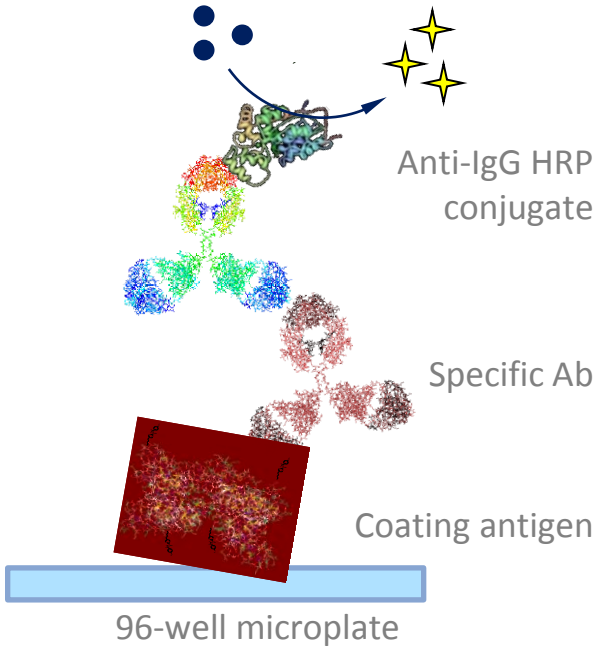
h_{ACL}- HCH h_W- HCH



Polyclonal Antibodies in Rabbit

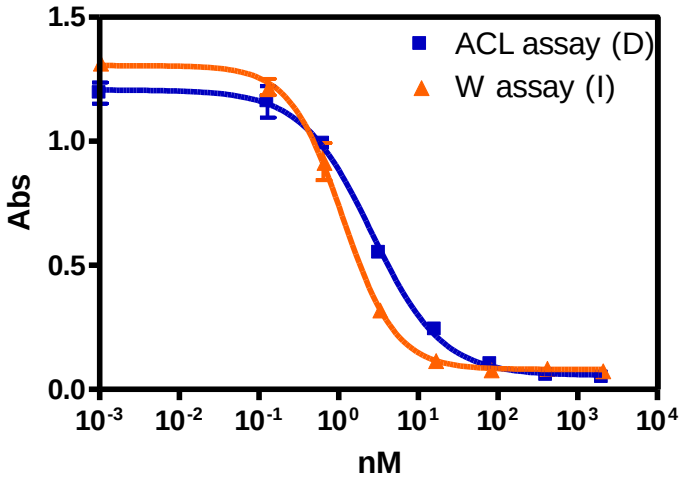
3 Rabbits/ immunogen
100 ug/immunization
6 immunizations

ELISA: Enzyme linked Immunosorbent Assay



ELISAs for ACL/W

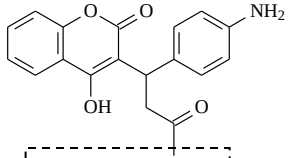
Calibration curves



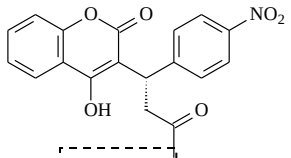
	ACL assay	W assay
Amin	0.056	0.080
Amax	1.207	1.305
Slope	-0.988	-1.305
IC_{50}	2.594	1.126
R^2	0.995	0.996

Selectivity assay

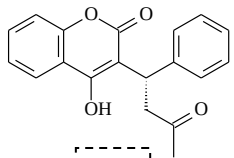
	W assay		ACL assay	
	IC_{50}	%CR	IC_{50}	%CR
ACL	4.3	**84**	2.7	**100**
W	3.7	**100**	0.8	**326**
4'-NH_2-ACL	46.9	8	5.0	**53**
4-OH-COU	>2000	<0.2	285	1
Testosterone	>2000	<0.2	>2000	<0.13
CAP	>2000	<0.2	>2000	<0.13



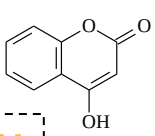
ACL-NH_2



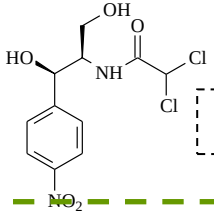
ACL



W

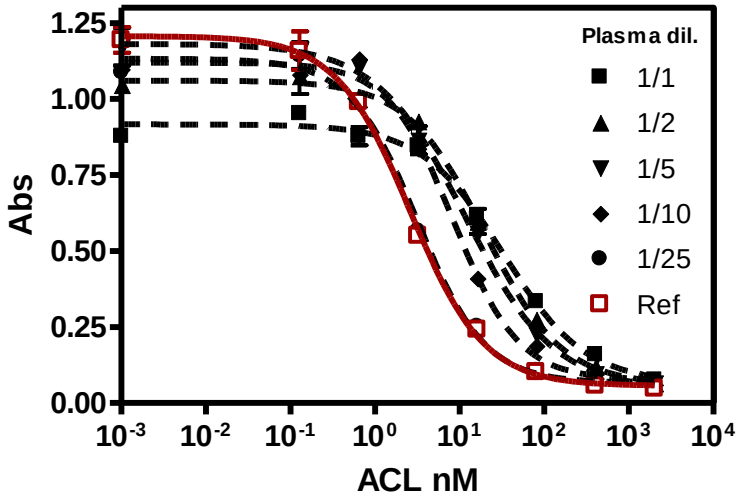


4OH-COU



CAP

Plasma Evaluation: As236/hACL-HRP



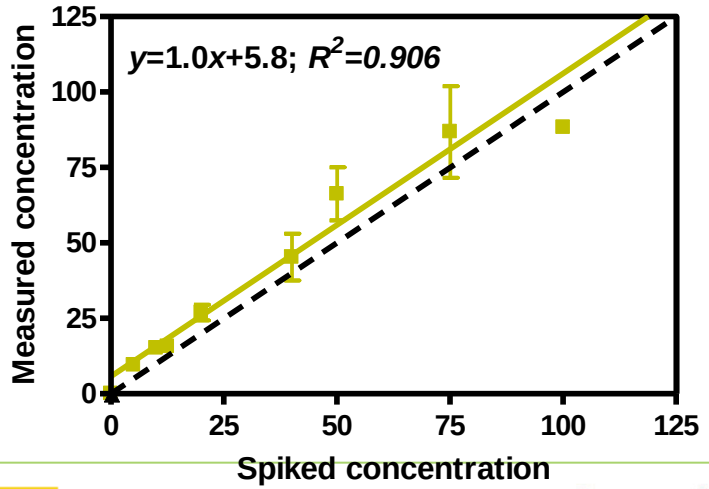
Matrix effect

◄**Plasma** produces only negligible effects on the **ACL assay**. **ACL** can be directly measured in plasma.

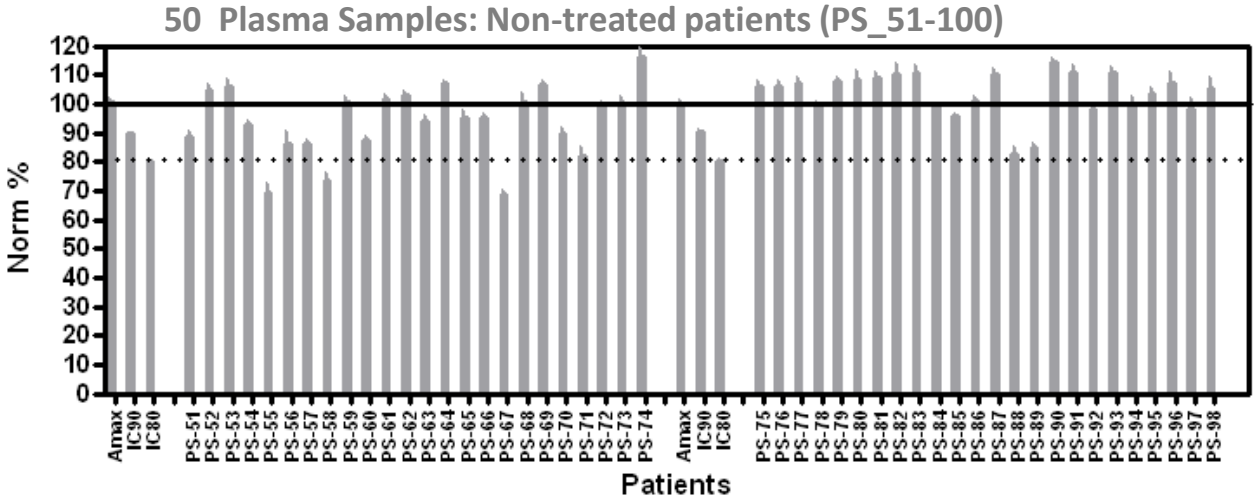
LOD = 1.23 nM (0.43 μg/L)

Accuracy

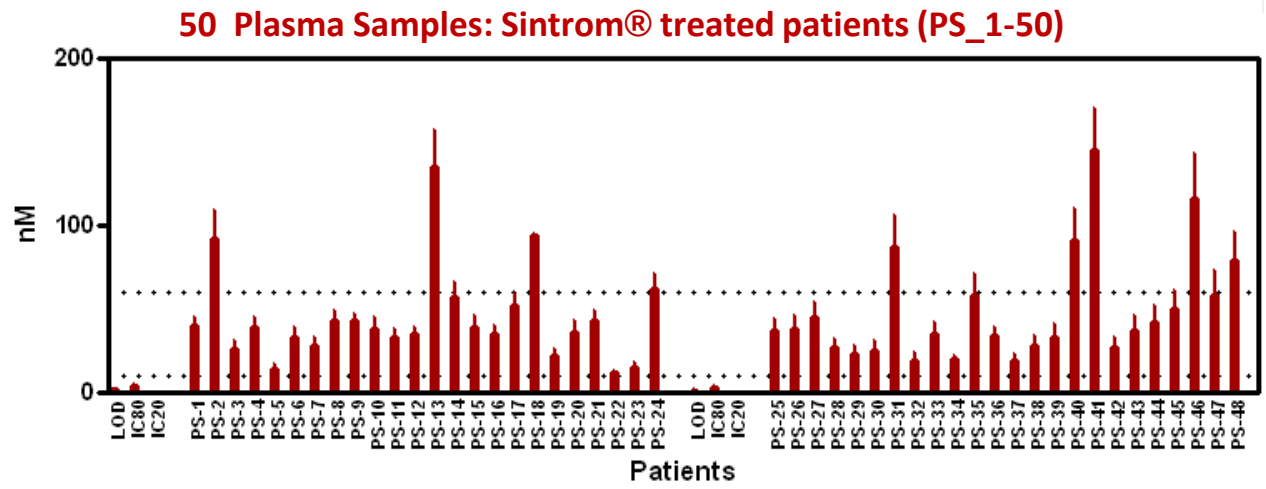
Assay accuracy was tested by measuring blind spiked samples prepared in plasma. Accuracy was found to be very good ►



Nb4D TDM ACN Example: Levels in Treated and Not Treated Patients



Zero-level



IDIBAPS Institut D'Investigacions Biomèdiques August Pi i Sunyer

CLÍNIC BARCELONA Hospital Universitari

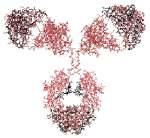
Dra. Tassies
Dr. Reverter

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Summary I



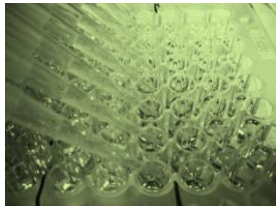
ACL
W



ORAL ANTICOAGULANT TREATMENT

Therapeutic Drug Monitoring

ELISA FOR ORAL ANTICOAGULANT DETECTION



In 1 hour, 96 samples
Directly in plasma
ACL range: 10-60 nM
LOD 0.43 μg/L

Portability

Requirements

- Fluorescent detection
- Desired format: Microarray
- Disposible



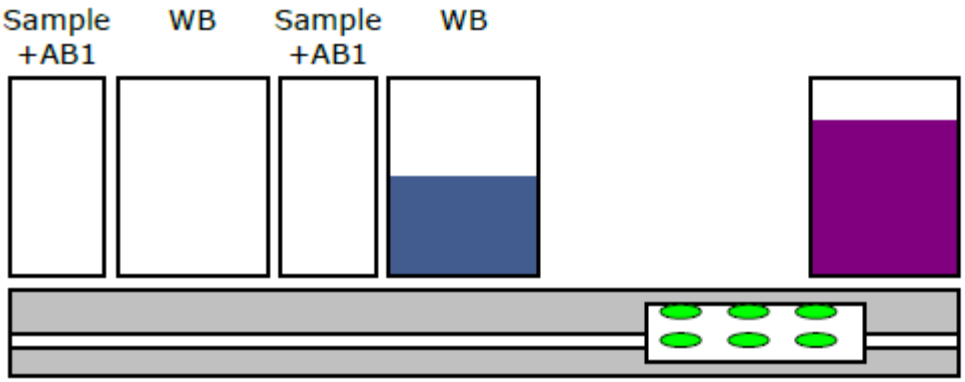
Point of care device for ORAL ANTICOAGULANT DETECTION

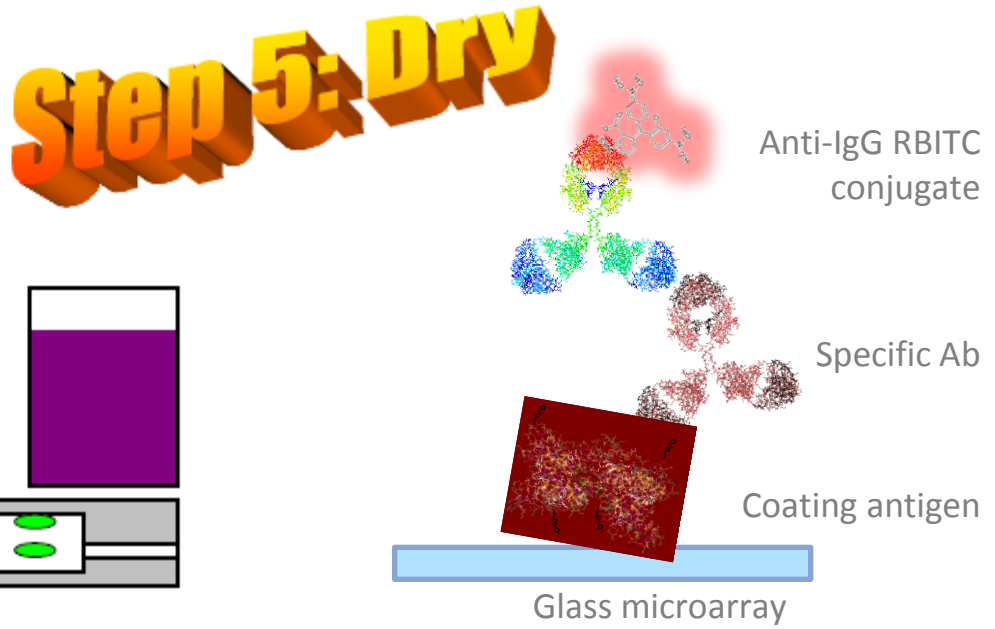
Outline

- INTRODUCTION
- PART I
  - » PRODUCTION OF ANTIBODIES FOR OAC
  - » ANALYTICAL CAPABILITIES FOR OAC DETECTION IN PLASMA
- **PART II**
  - » **MICROFLUIDIC DEVICE WITH FLUORESCENT READ-OUT**

Microfluidic system for microarray

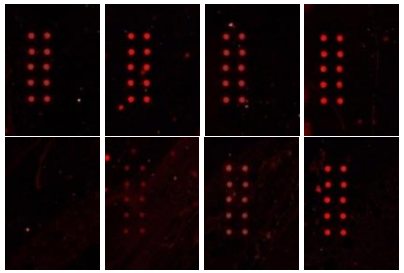
Step	Time[a]	Solution	Buffer diluent	Volume[c]	Temp
1	0’	Sample (or standard): Antibody[c] 1:1	PBST	100 µL	RT
2	30’	Washing[b]	PBST[f]	3x100 µL	RT
3	31’	Anti-IgG-FTIC[e]	PBST	100 uL	RT
4	41’	Washing[b]	PBST	3x100 µL	RT
5	42’	Drying	Air		RT
6	43’	Reading	dry		RT



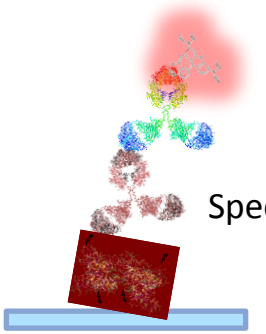


ACL assay in microarray format

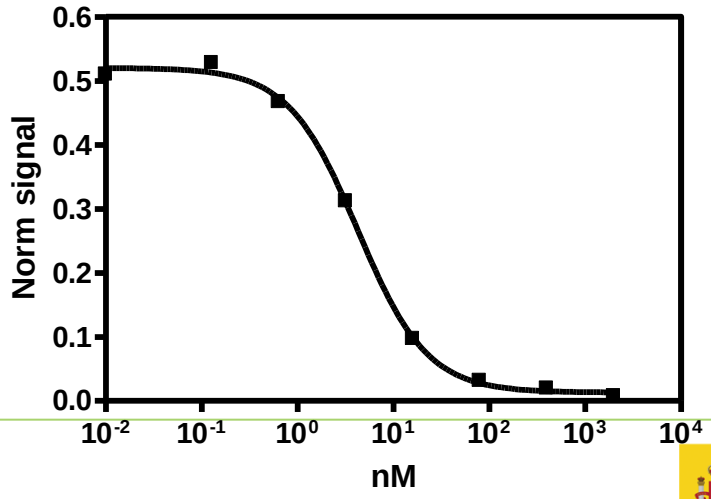




INDIRECT FORMAT: A2BSA/As233
+ Control (hA_BSA/As138)



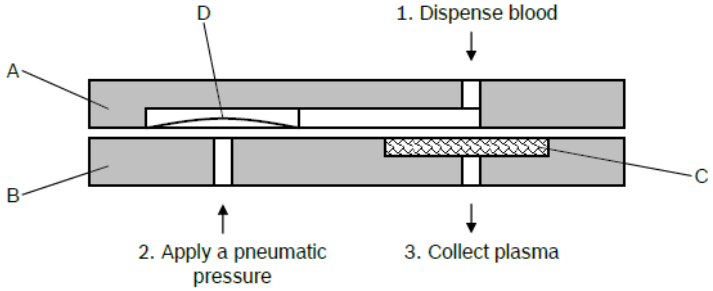
A2BSA/As233

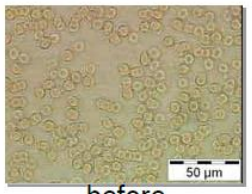


	ACL
$NORM_{min}$	0.01304
$NORM_{max}$	0.5203
Slope	-1.199
IC_{50}	4.249
N	4
R^2	0.998

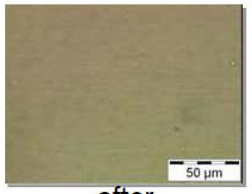
Sample prep unit

BOSCH
Invented for life

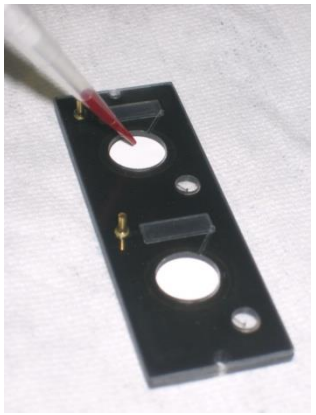


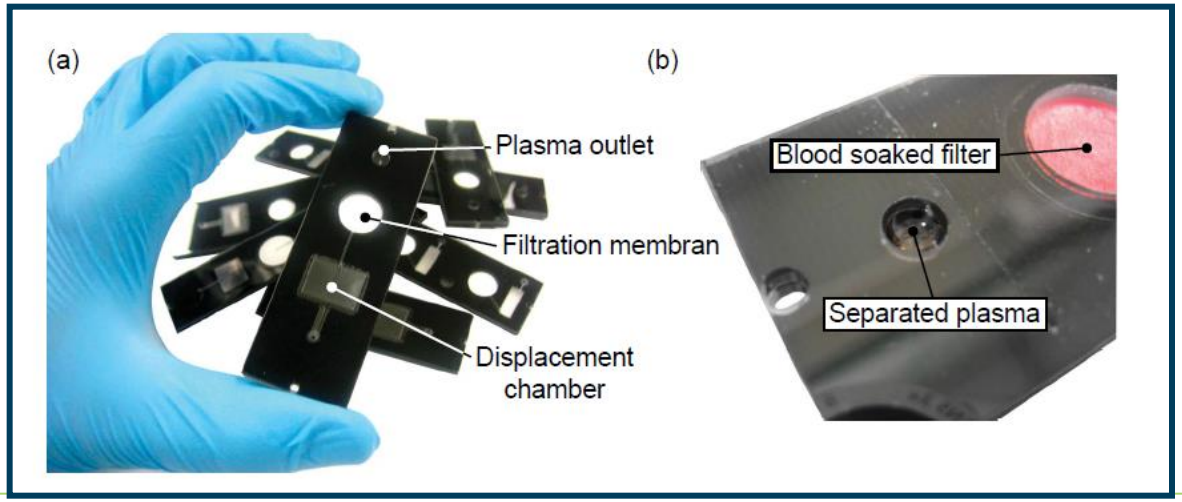


before



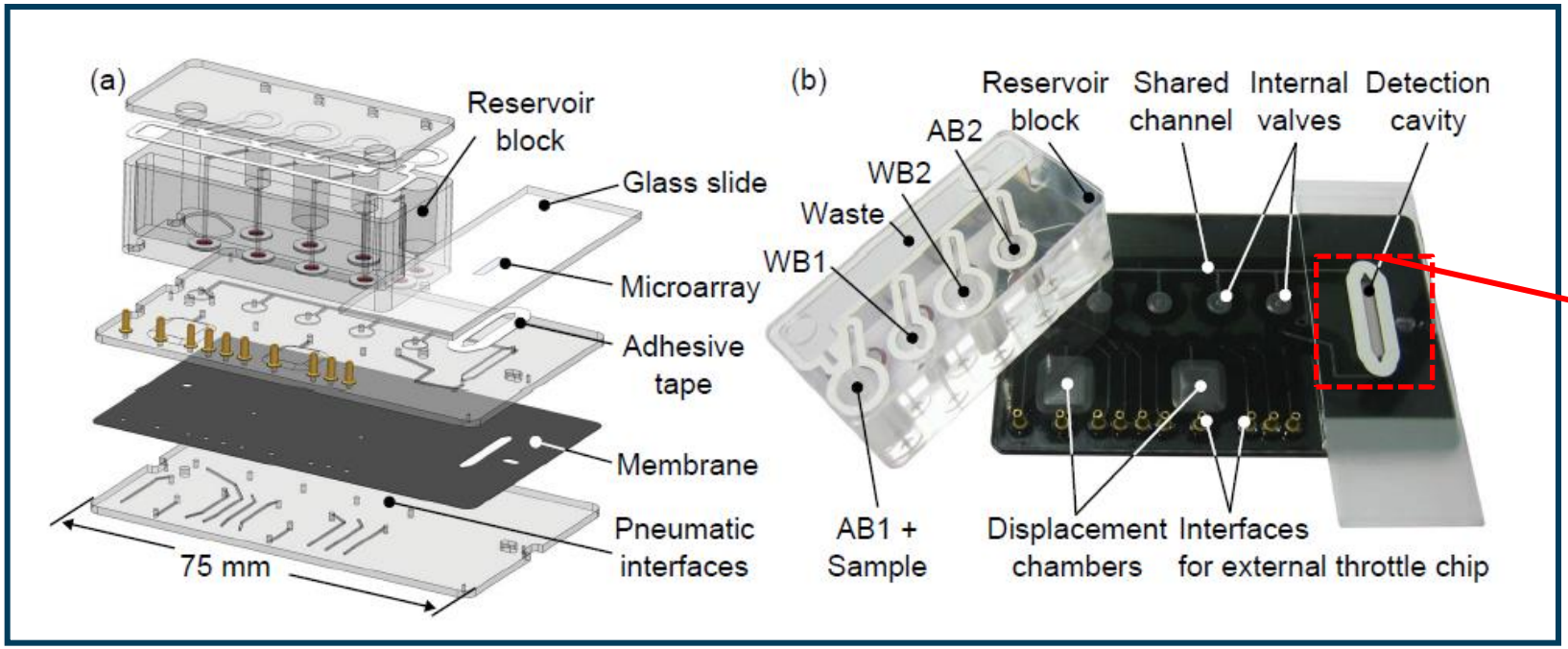
after





Design and prototyping

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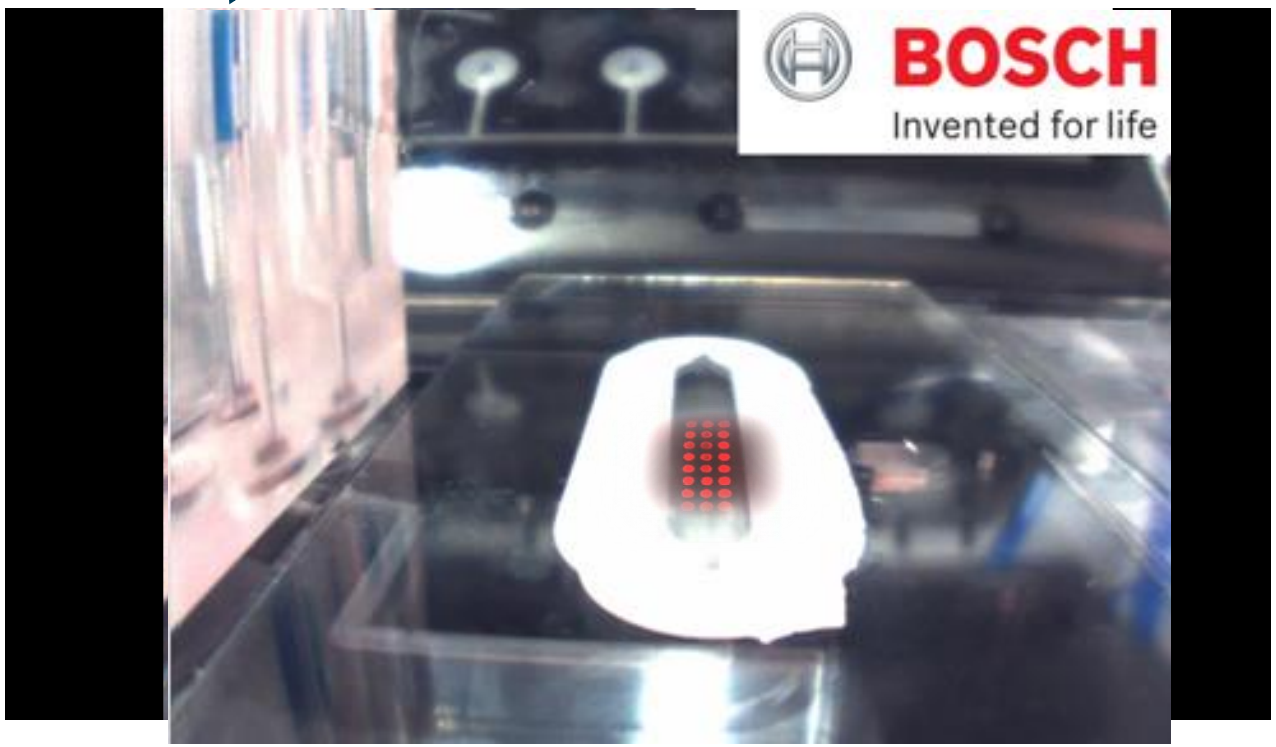
Assay on the microchip

Anti-IgG-TRITC

Sample + Ab + Control

Wash

Dry



Scanner
543 nm

Anti-IgG RBIT conjugat

Coating antigen

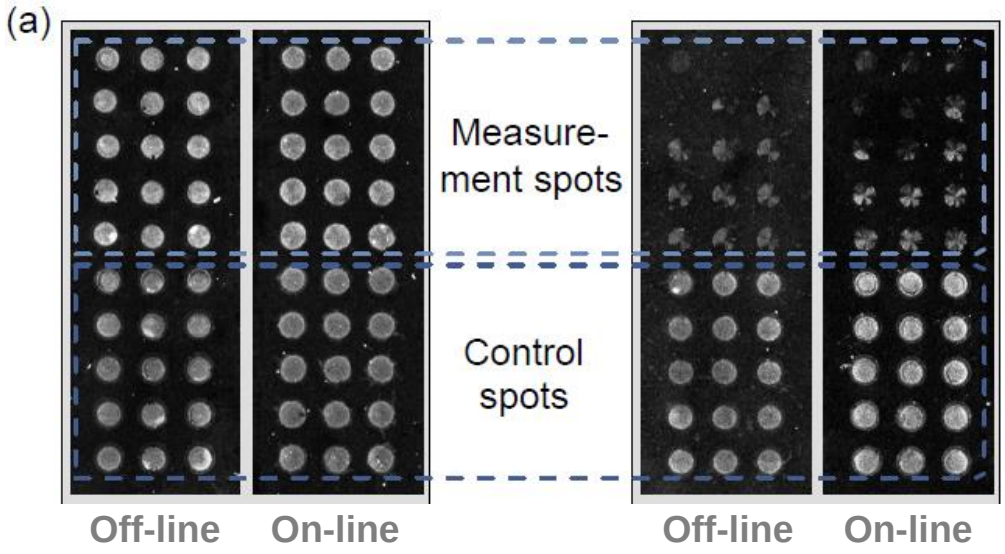
Glass microarray

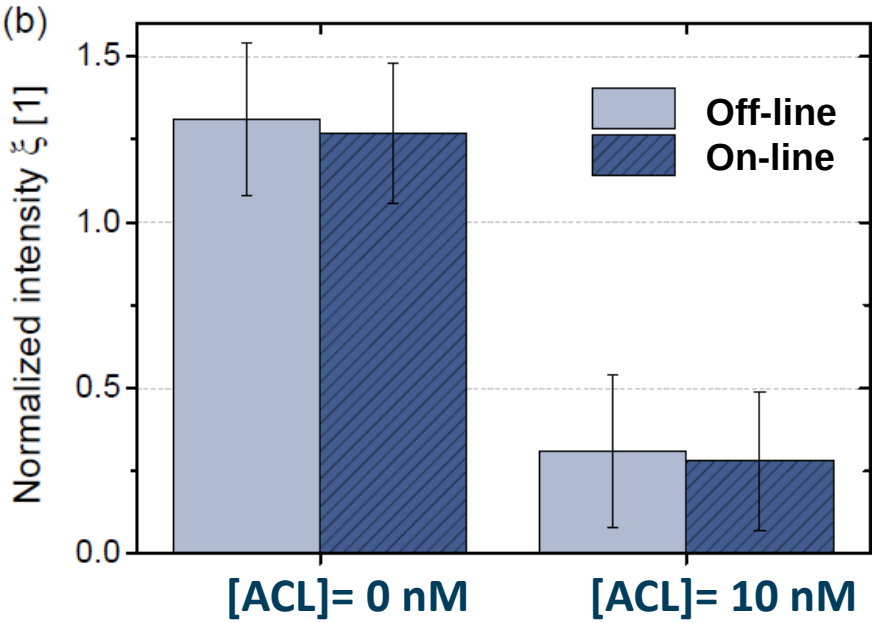
Waste

Total time assay: 2 min
Speed: 2x

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Nb4D NANOBIOTECHNOLOGY FOR DIAGNOSTICS





Conclusions II

- A microfluidic device has been developed for the monitoring of ACL.

- The system is able to distinguish the zero signal and the minim concentration of ACL found in patients (10 nM)

Acknowlegments

Foundings

ciber-bbn

CAJAL4EU

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Samples

IDIBAPS

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BOSCH Invented for life

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Dr. Christian Dorrer

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