Leiden University Medical Center

Point-of-Care Tests for Infectious Diseases

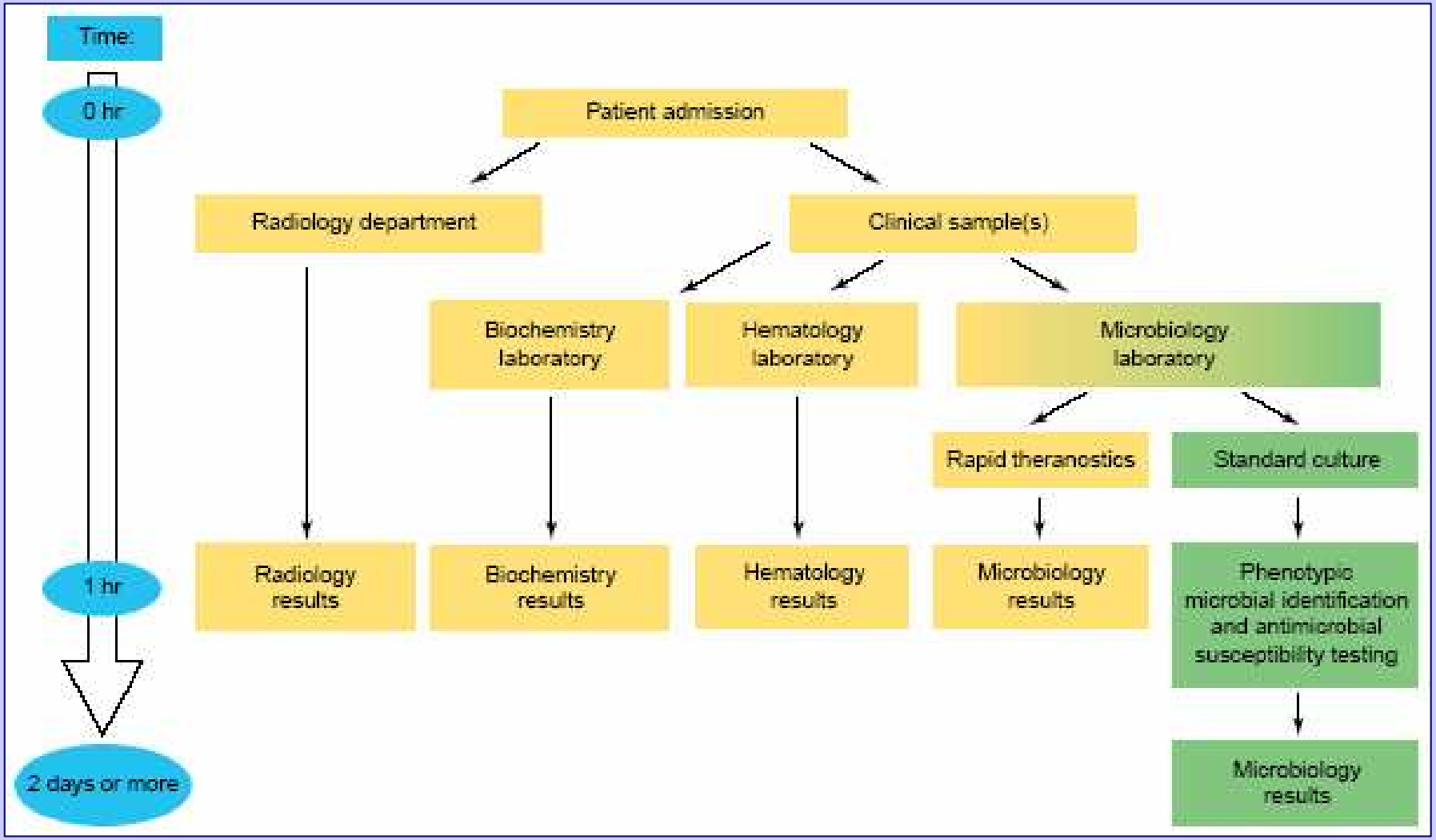
Trivandrum, India; January 21, 2010

Hans J. Tanke

LUMC

Contents:

- *Point of Care Testing - Multiplexing*
- *Up-Converting Phosphor Technology - UCP*
- *The Lateral Flow assay platform – LF*
- *Applications in infectious diseases*
- *Microfluidics - Chips and Actuators*
- *Handheld devices*


Time:
0 hr
1 hr
2 days or more
Patient admission
Radiology department
Clinical sample(s)
Biochemistry laboratory
Hematology laboratory
Microbiology laboratory
Rapid theranostics
Standard culture
Radiology results
Biochemistry results
Hematology results
Microbiology results
Phenotypic microbial identification and antimicrobial susceptibility testing
Microbiology results

Point of care testing

Sensitive/specific/reproducible

Rapid

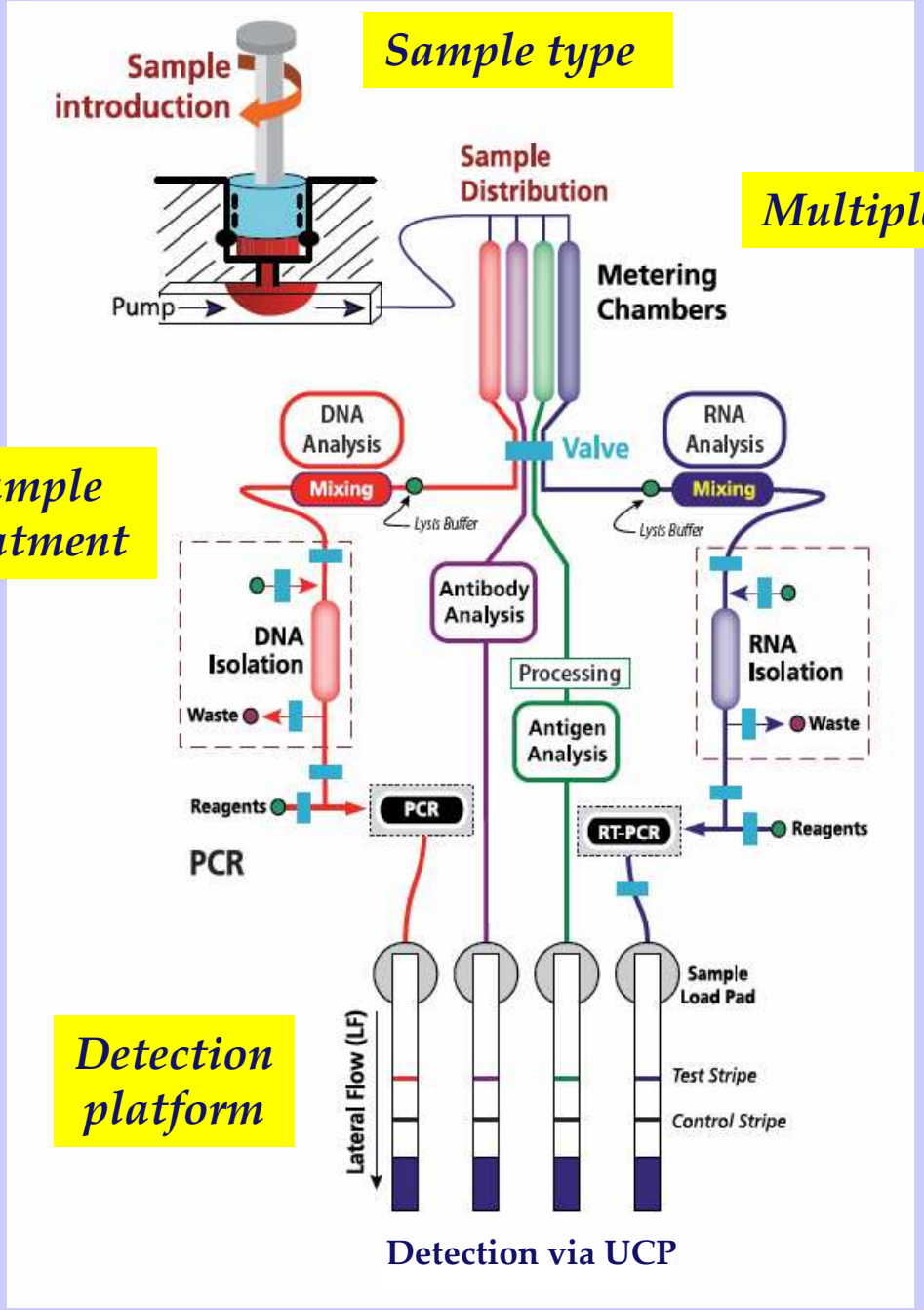
Robust

Low costs

Easy sampling (blood, urine, saliva)

Biomarkers: DNA, RNA, antigens, antibodies

A generic platform for biomarker detection



New York University College of Dentistry

University of Pennsylvania
School of Engineering and Applied Science

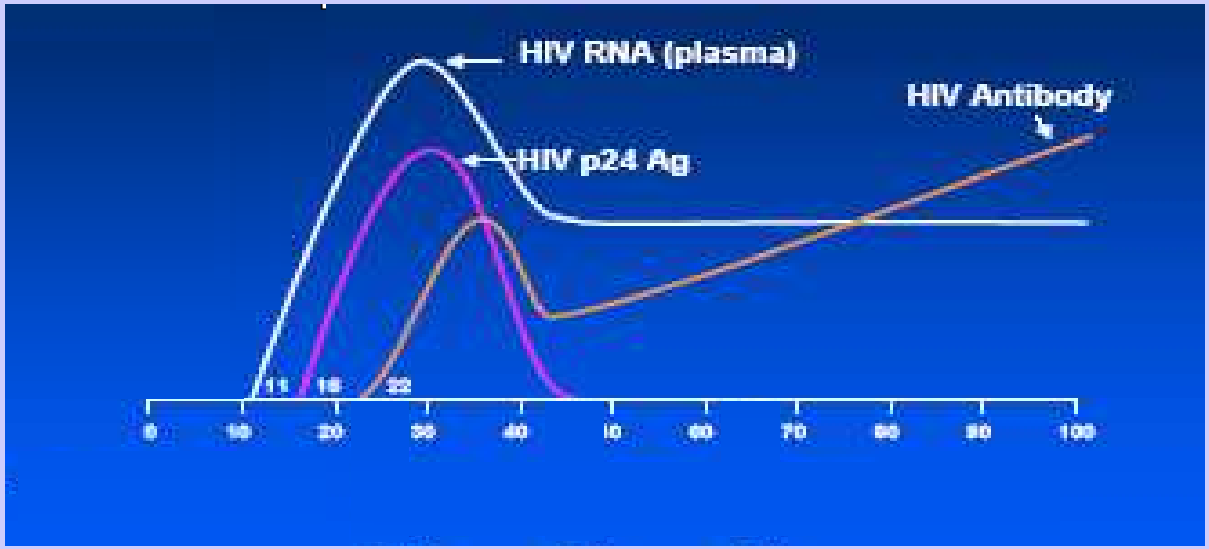
LEHIGH UNIVERSITY

A multidisciplinary collaboration to develop a point-of-care device for detection of infectious diseases in saliva

Funded by NIH
grant U01-DE017855

Why multiplex NA, Ab and Ag?

Rapid Point-of-Care Testing & Verification



Immediate viral load determination helps to establish earlier detection and may reduce the rate of HIV infection propagation.

Instantaneous start of counseling and appropriate anti-viral therapy will reduce the number of HIV-positive patients that remain untreated because they never return to the clinic for their verification results.

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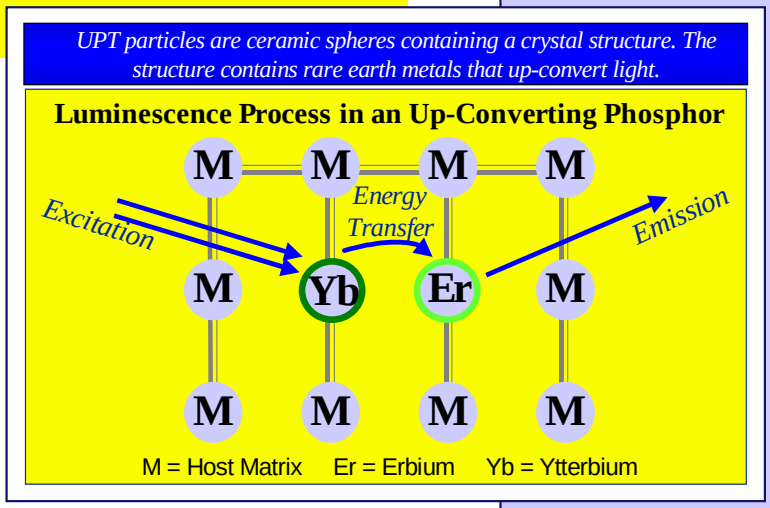
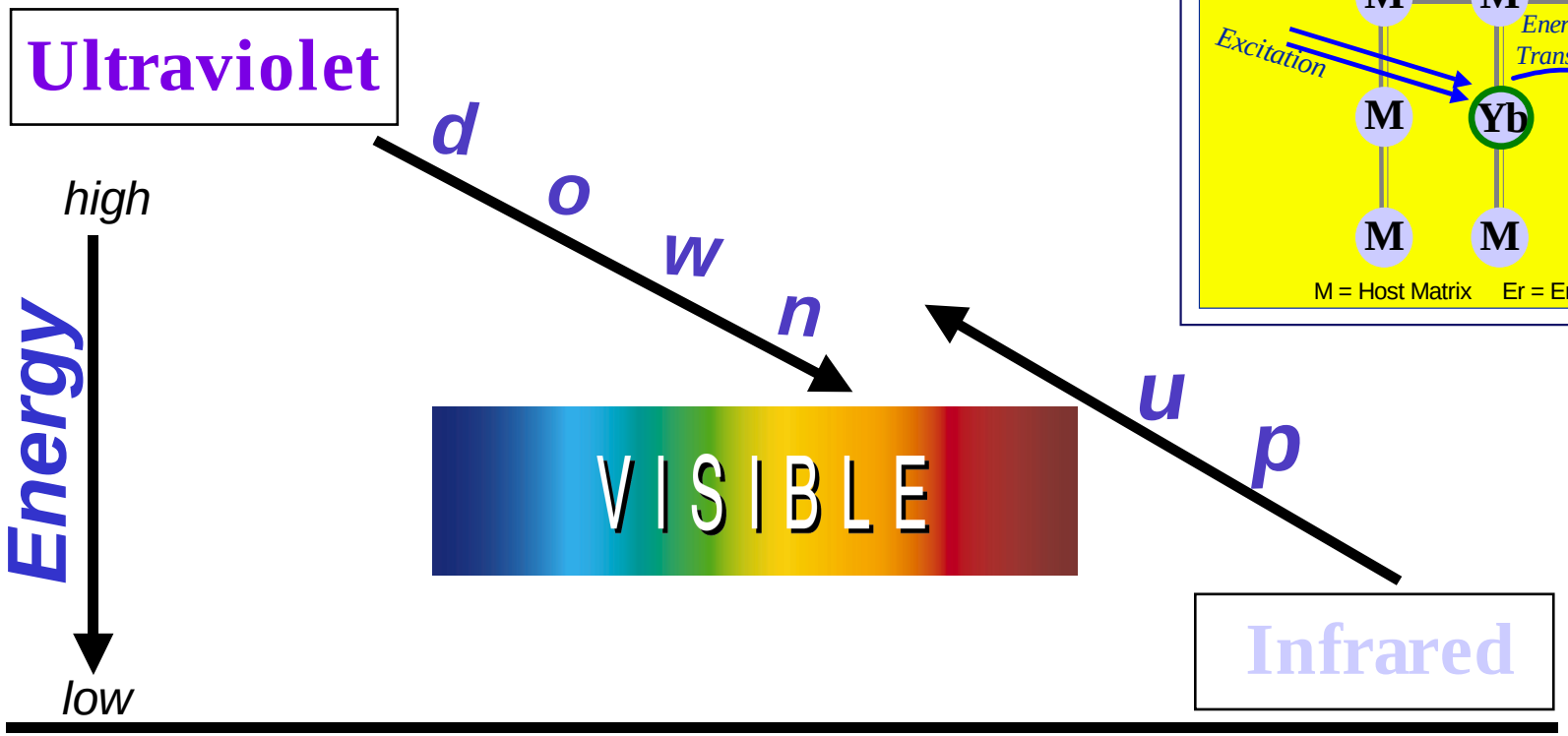
UCP: Up-Converting Phosphor

Up-conversion is a process in which light of lower energy is changed to light of higher energy.

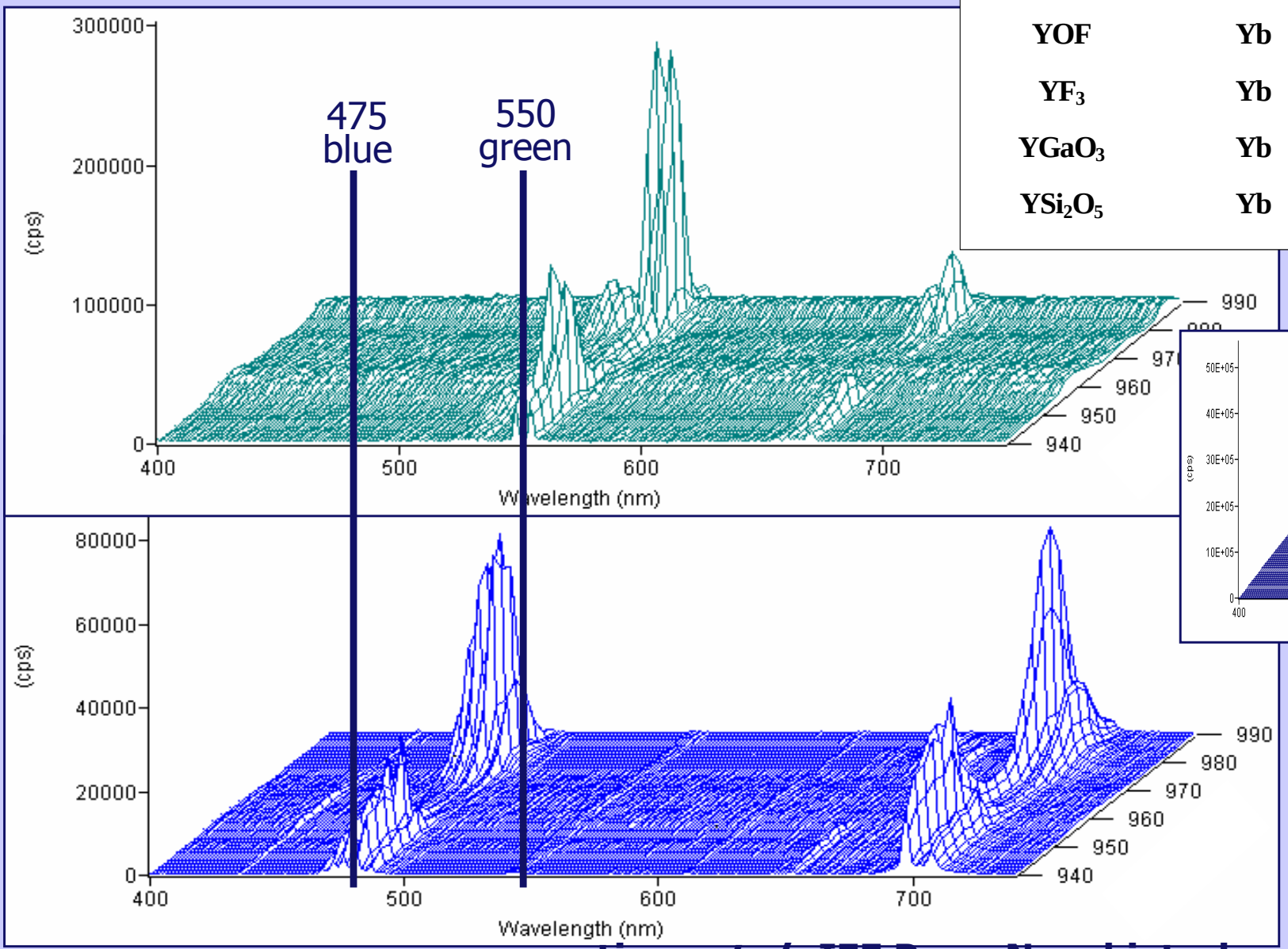


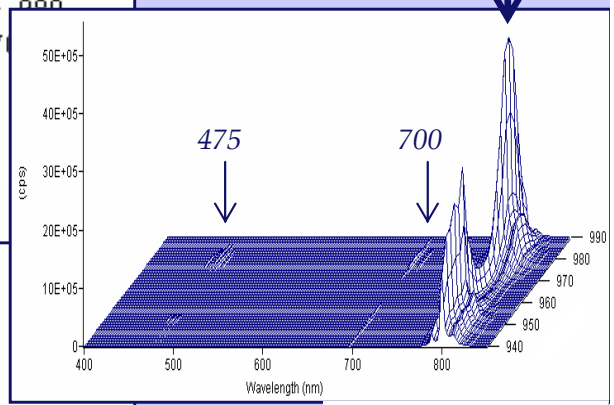


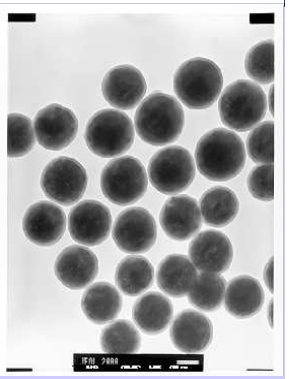
UCP Features

Emission/Excitation Spectra

Host Material	Absorber	Emitter	Color
Y_2O_2S	Yb	Er	Green
YOF	Yb	Tm	Blue
YF_3	Yb	Er	Red
$YGaO_3$	Yb	Er	Red
YSi_2O_5	Yb	Ho	Green

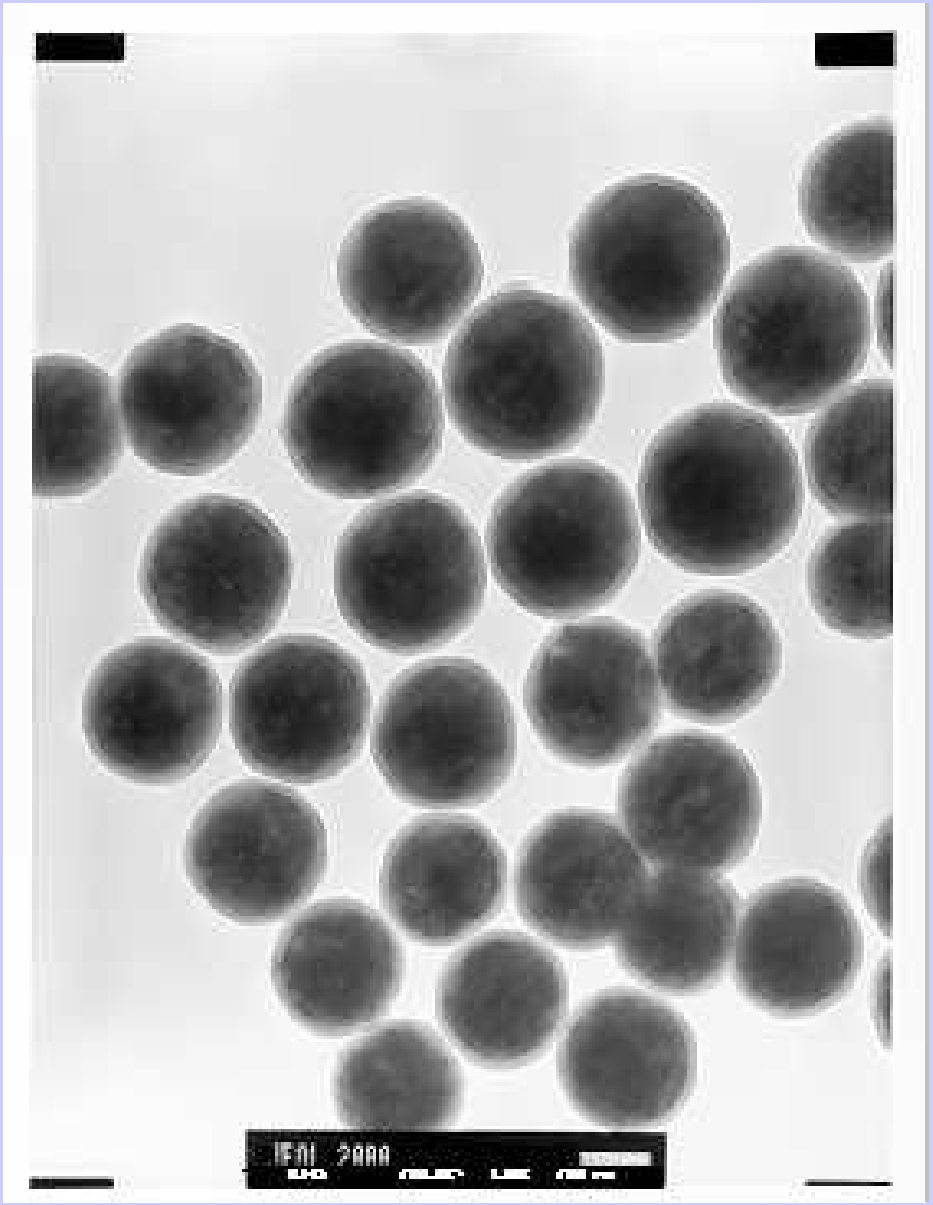






orstjens *et al.*, IEE Proc.-Nanobiotechnol. 2005, 152:64-72

UCP Features



TEM
silica coated UCP particles

400 nm particles
20 nm silica coating

Corstjens *et al.*, IEE Proc.-Nanobiotechnol. 2005, 152:64-72

UCP Features

Key Features

[1] Interference free

Process of up-conversion takes places within the lattice

[2] Multiple colors

Allows multiplexing; narrow well separated band widths

[3] Quantitative signal

Signal determined as Relative Fluorescent Units

[4] Unlimited signal integration time

Large anti-Stokes shift and label that does not fade; high sensitivity

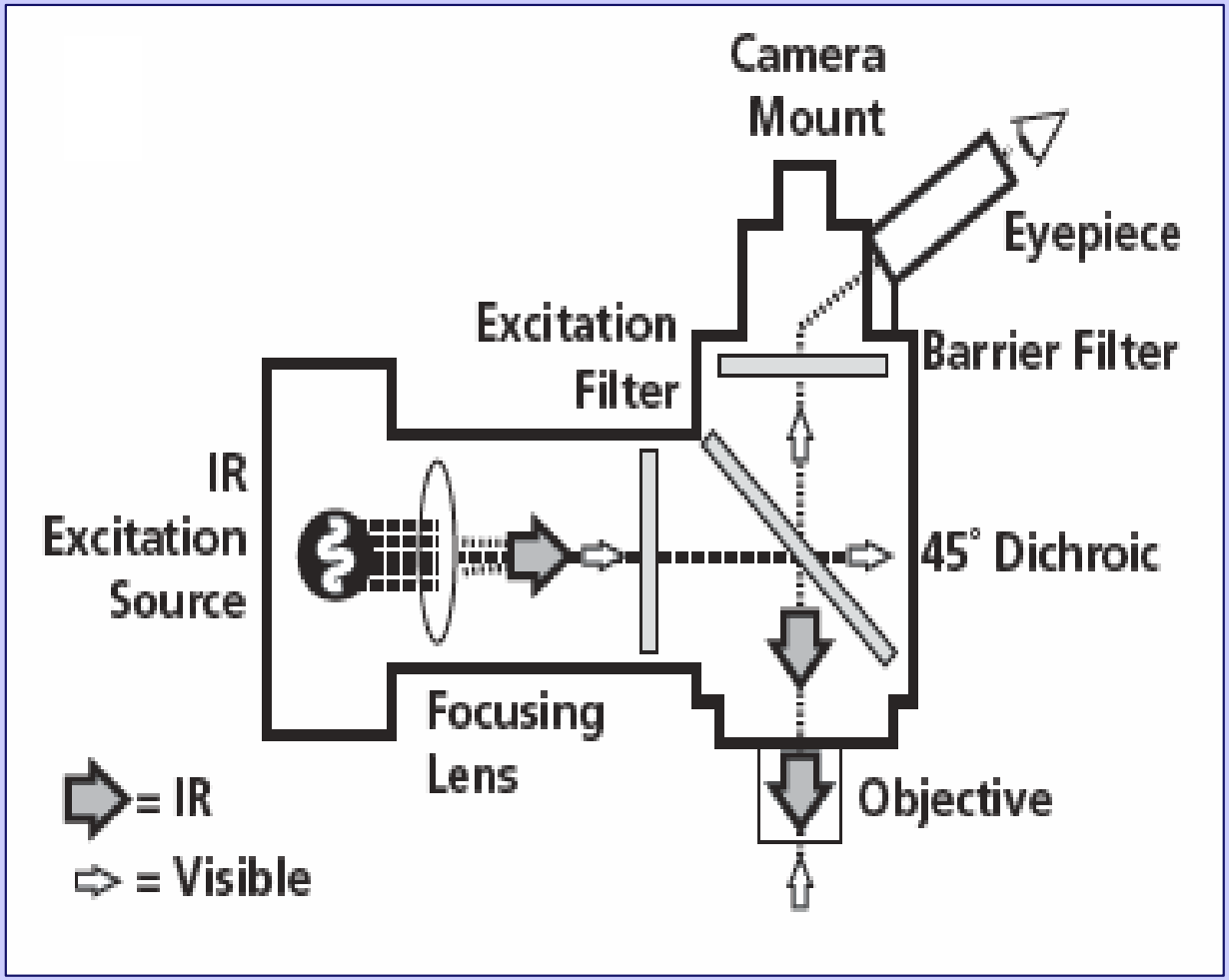
[5] Unmatched contrast

No autofluorescence of other assay components upon IR excitation

Some comments

- ***The UCP particles in this study used are large (400 nm); they can be made much smaller though (10-20 nm): NaYF4***
- ***Upconversion is a multi-photon process, but is 2-3 orders more efficient than "conventional multi-photon processes":***
- ***Thus: use of simple penlight lasers that produce 1 W of 980 nm light for $100-200***

Adapted Epifluorescence Microscope

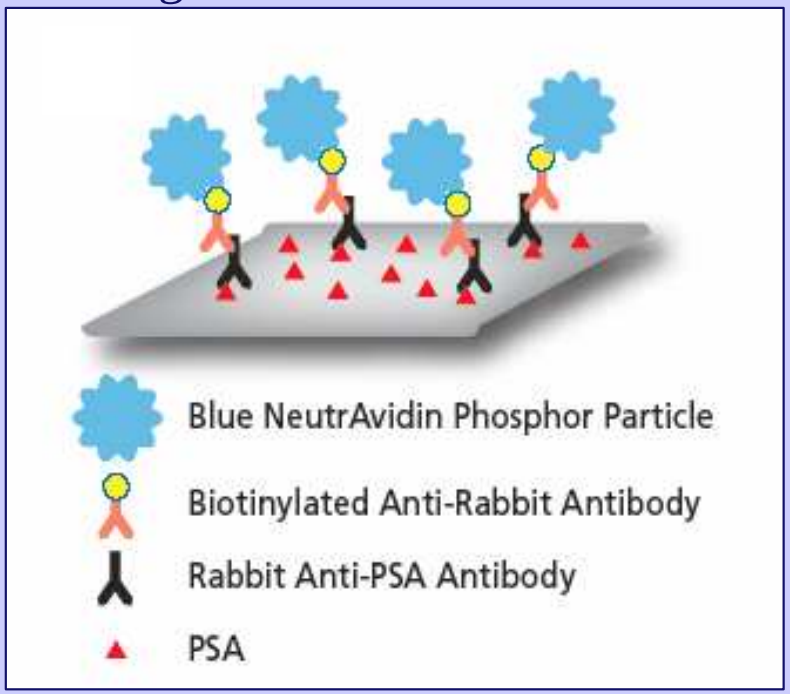


Microscope platform. Epifluorescence microscope modified (900-1000 nm BP filter [excitation]; 800 nm SP dichroic; 750 SP filter [emission]) to excite with infrared light from a common xenon lamp and to detect in the visible.

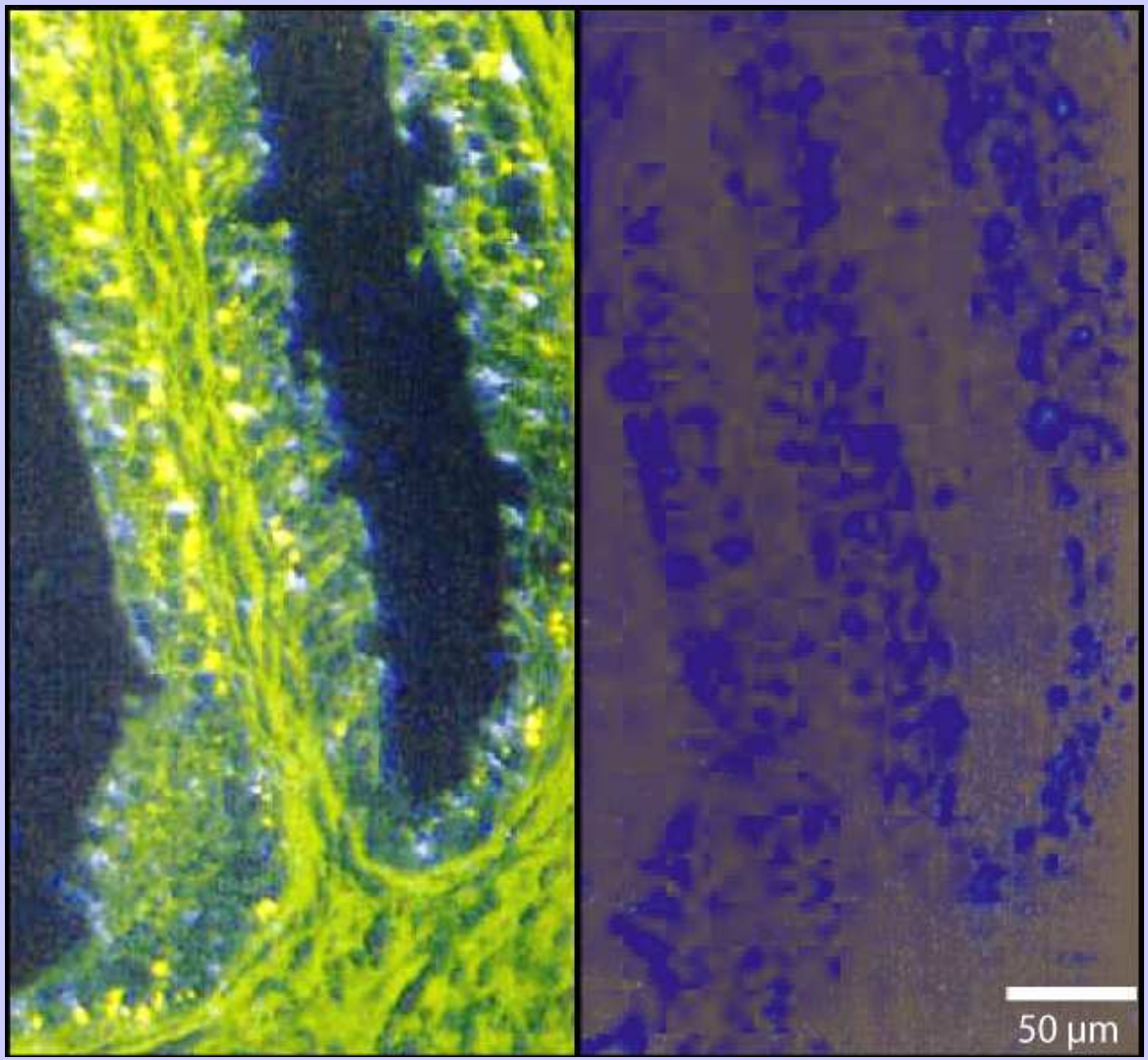
Zijlmans *et al.*, Anal. Biochem., 1999, 267:30-36

Immunohistochemistry

Detection of Prostate Specific Antigen in human tissue section



Unmatched contrast in comparison to many other fluorescent labels due to absence of autofluorescence



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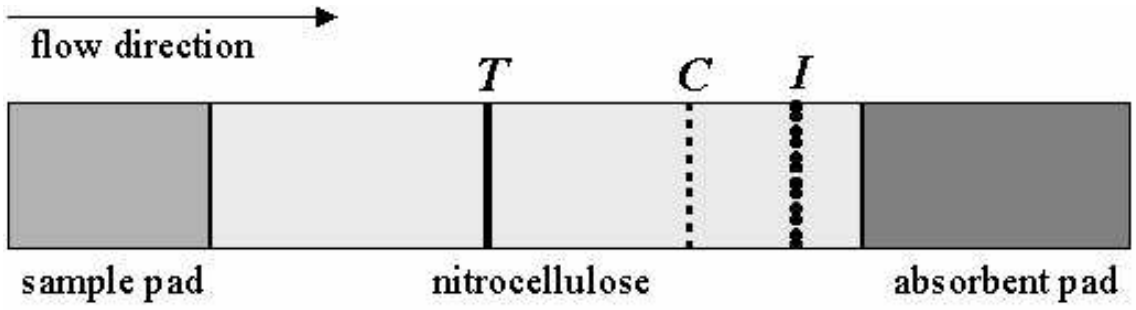
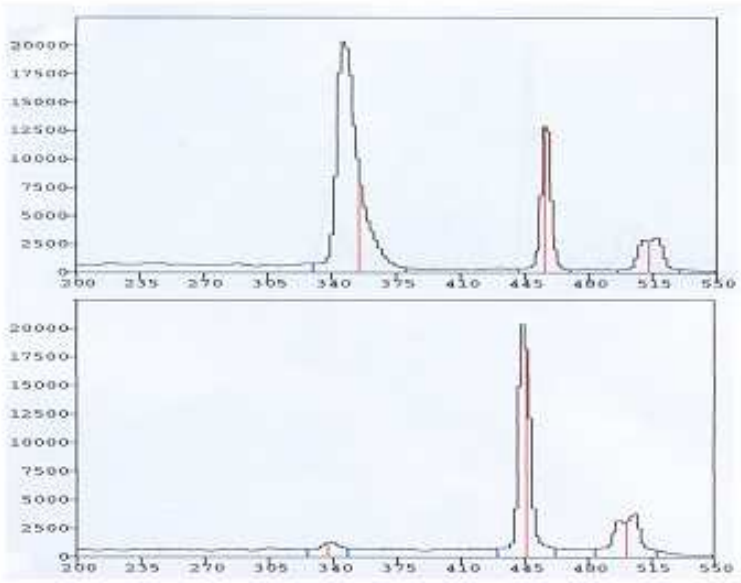
The Lateral Flow principle

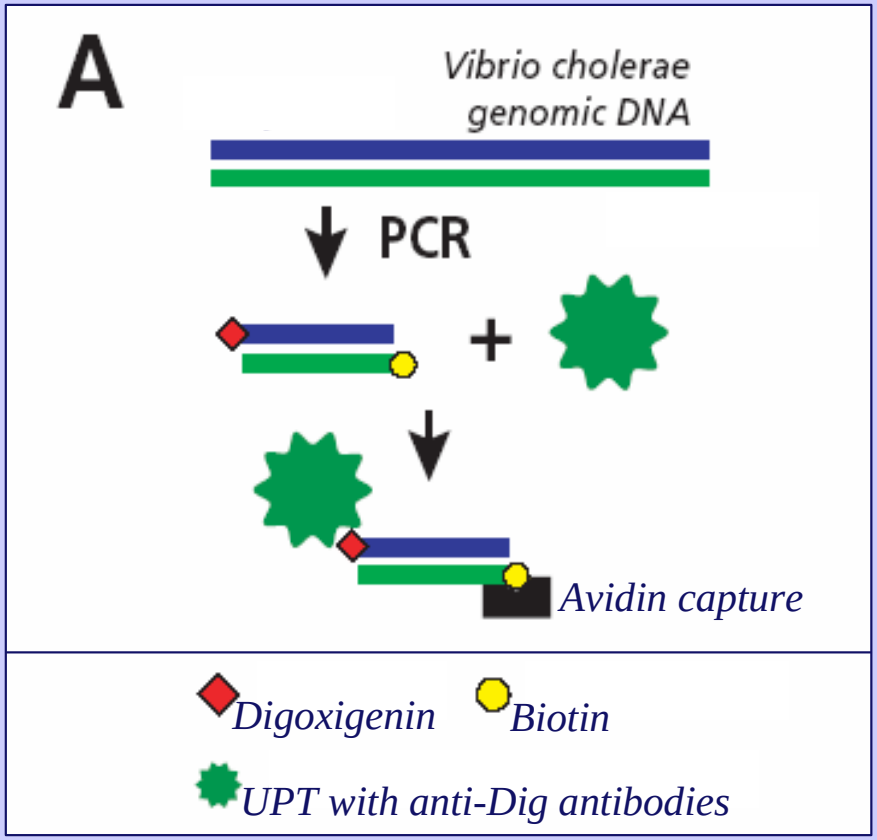


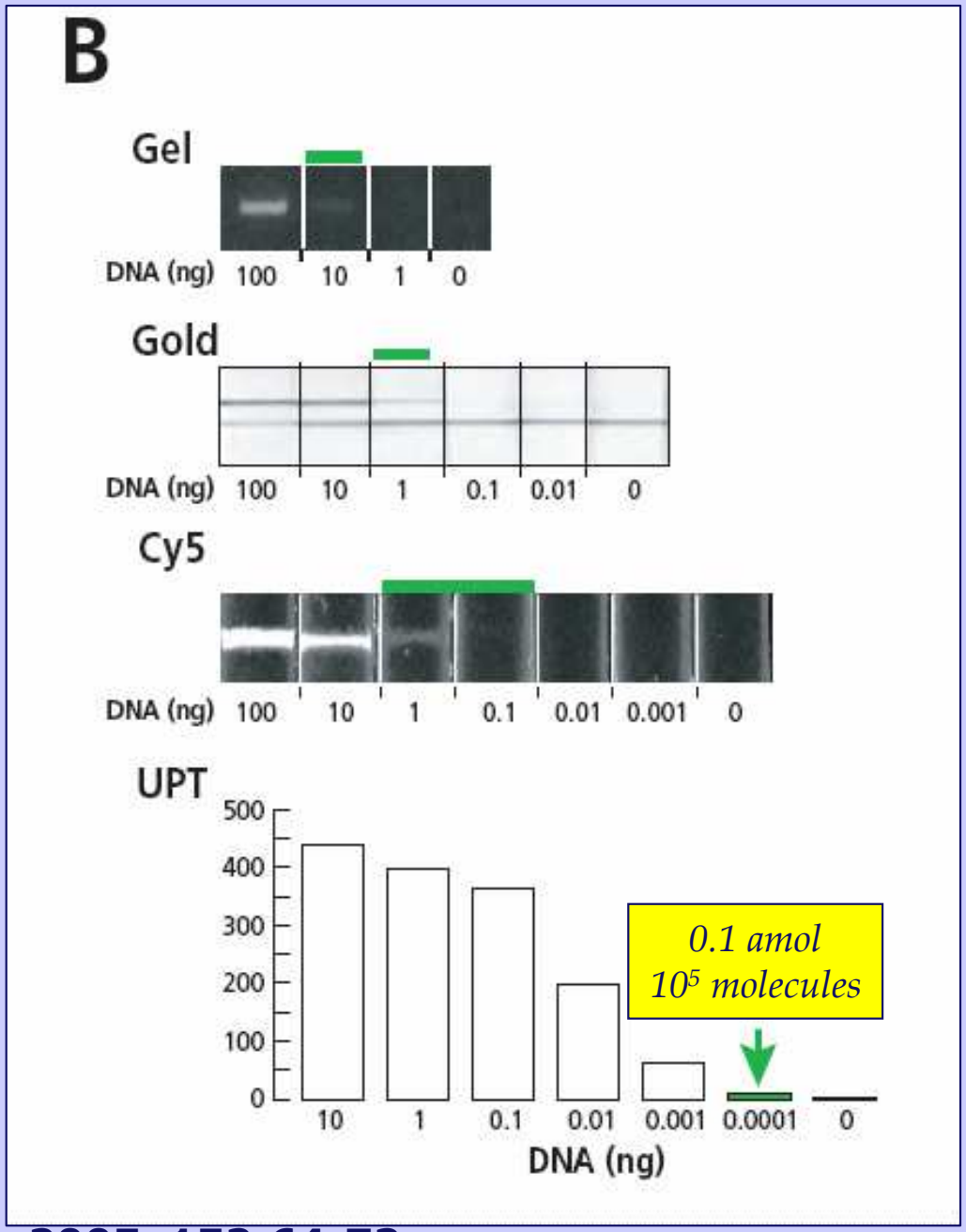


Lateral flow assays using UCP

Sensitivity
Limit of detection

Cholerae
V. cholerae



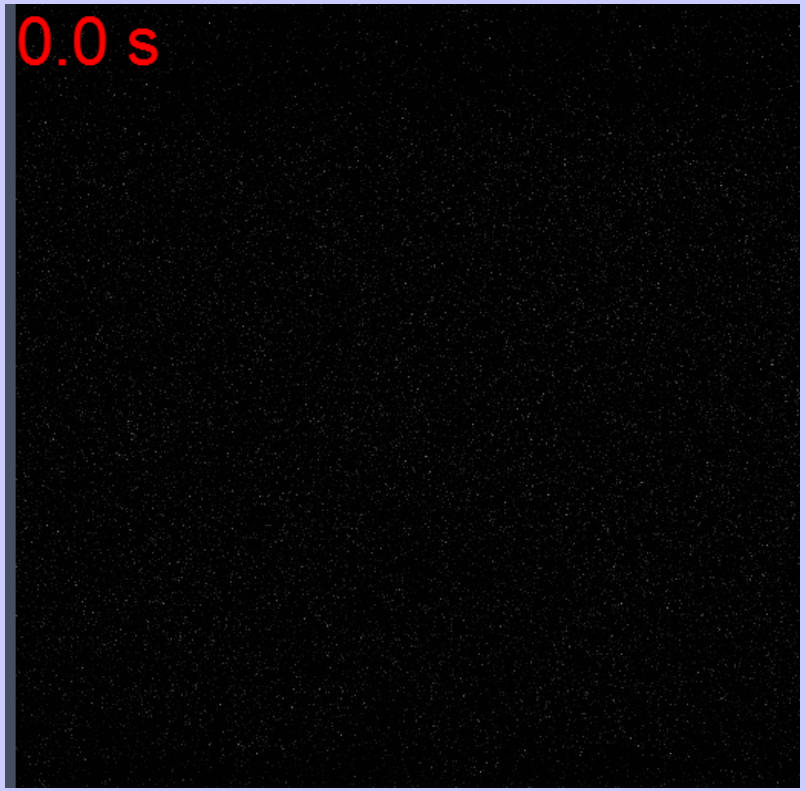


Corstjens *et al.*, IEE Proc.-Nanobiotechnol. 2005, 152:64-72

Imaging UCP in lateral flow strips: optimising conditions

Zeiss LSM710-NLO with IR laser

KPT particles
100 ng



Lateral flow assays using gold

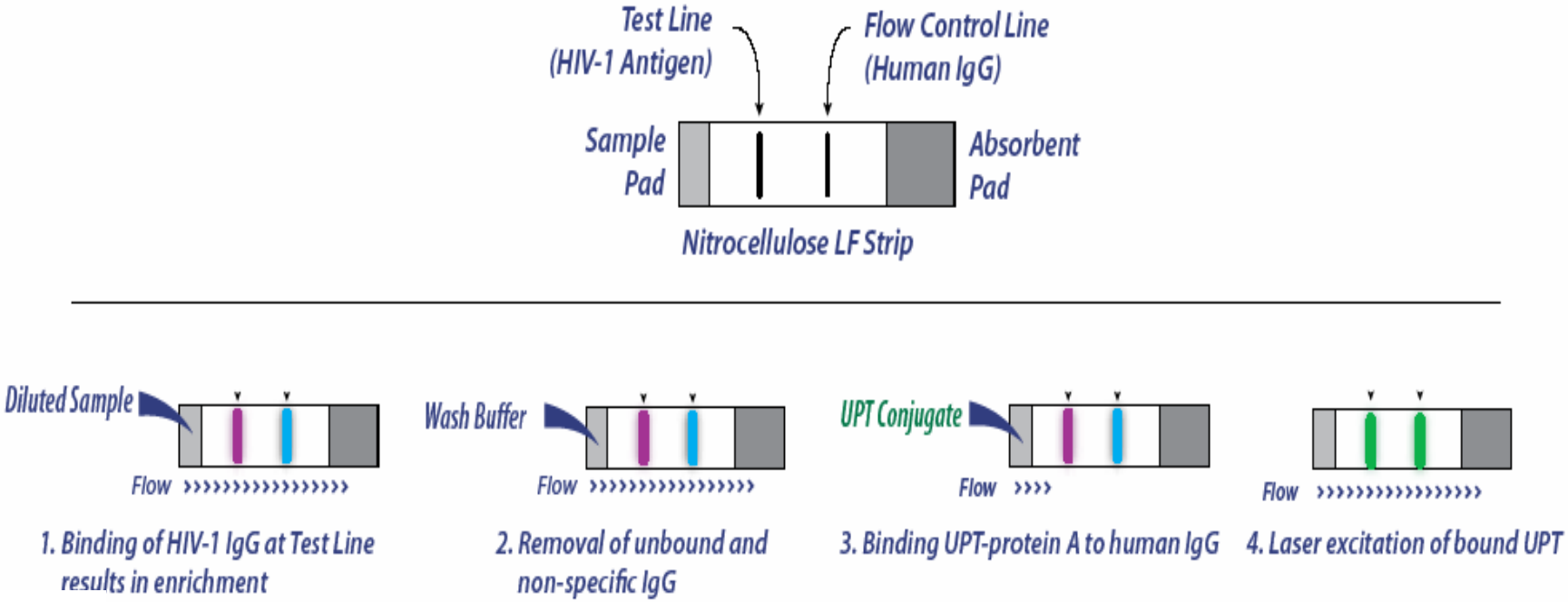
OraQuick

***OraQuick* ADVANCE**

- *detects HIV-1 & HIV-2*
- *applicable to oral fluid*
- *applicable to fingerstick whole blood*
- *applicable to blood serum and plasma*
- *results within 20 min*

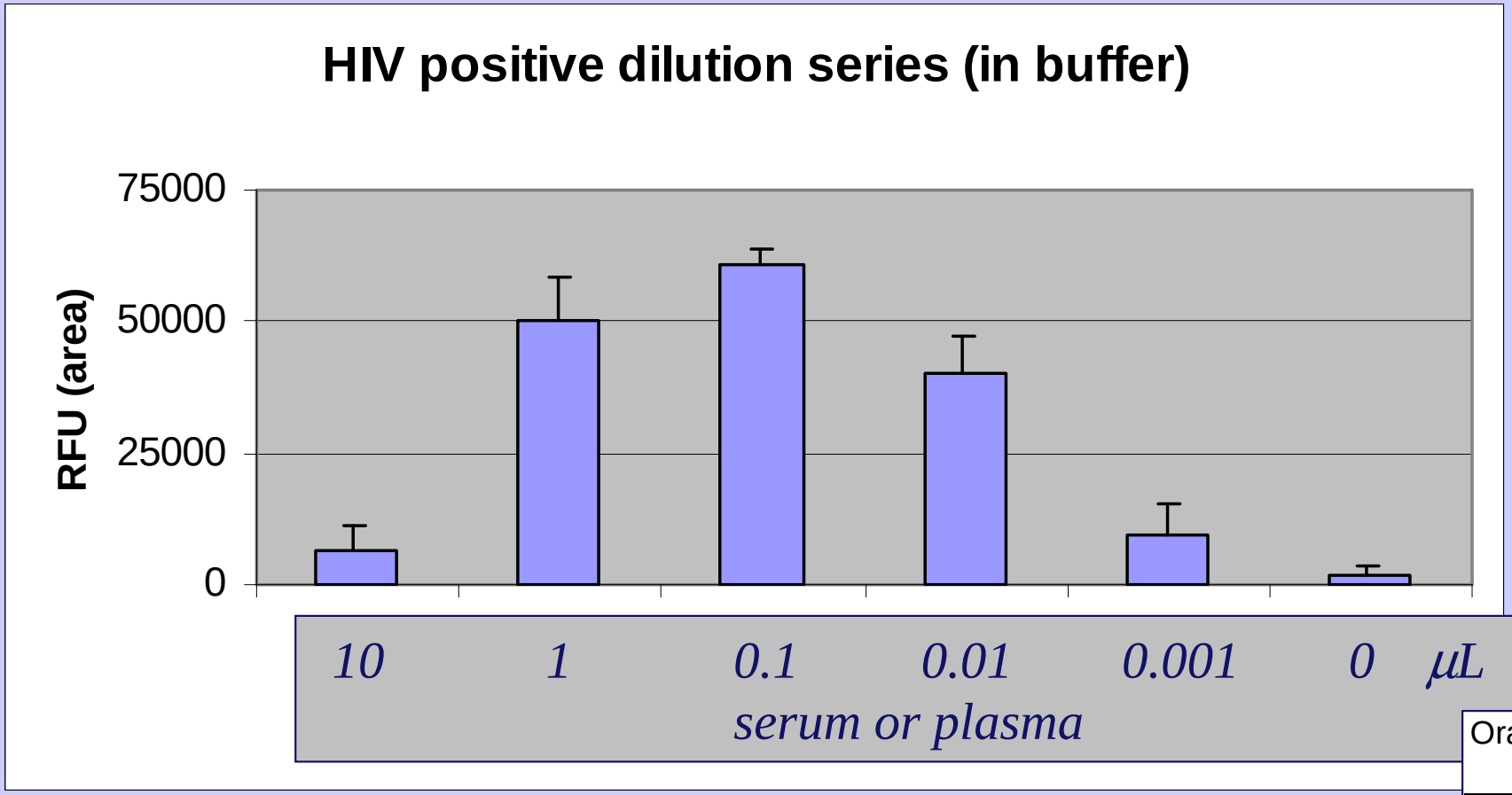
Lateral flow assays using UCP

Generic UPT-protA Consecutive Flow Antibody Detection Format



Schematic of LF strip configuration and consecutive lateral flow (cLF) analysis for detection of HIV

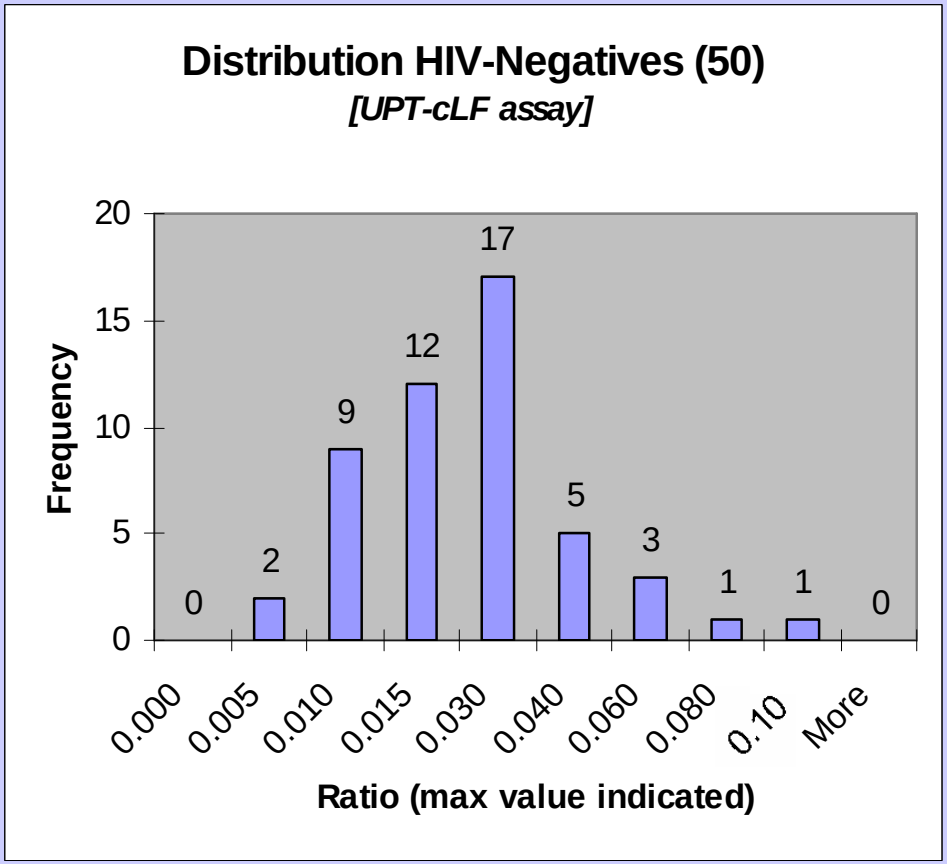
UPT-cLF: 10 uL sample in 30 µL buffer
OraQuick: 5 uL sample in 500 µL buffer

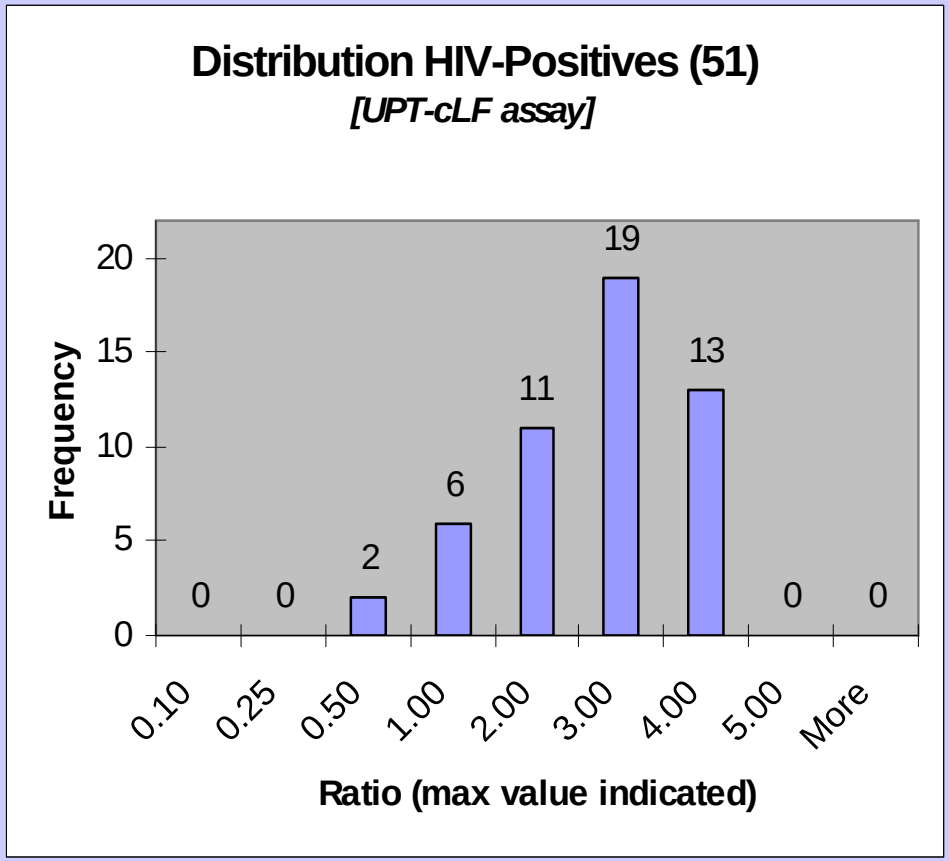


OraQuick gold	*dilution factor*
3	0
2,5	10
1	100
Tr	1000
0	10000
0	blanc

Lateral flow assays using UCP

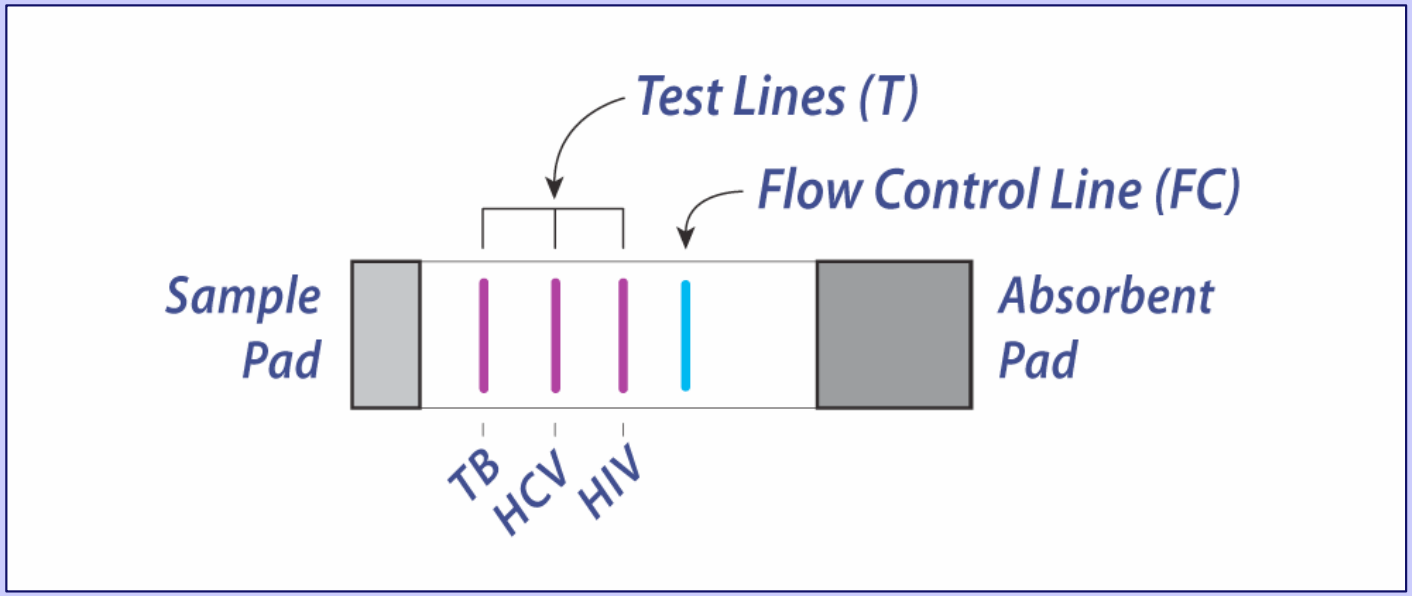
'Clinical'





Control assay is OraQuick HIV-1; agreement (qualitative) 100%

Lateral flow assays: spatial multiplexing



Corstjens *et al.*, Ann. N.Y. Acad. Sci. 2007, 1098:437-445

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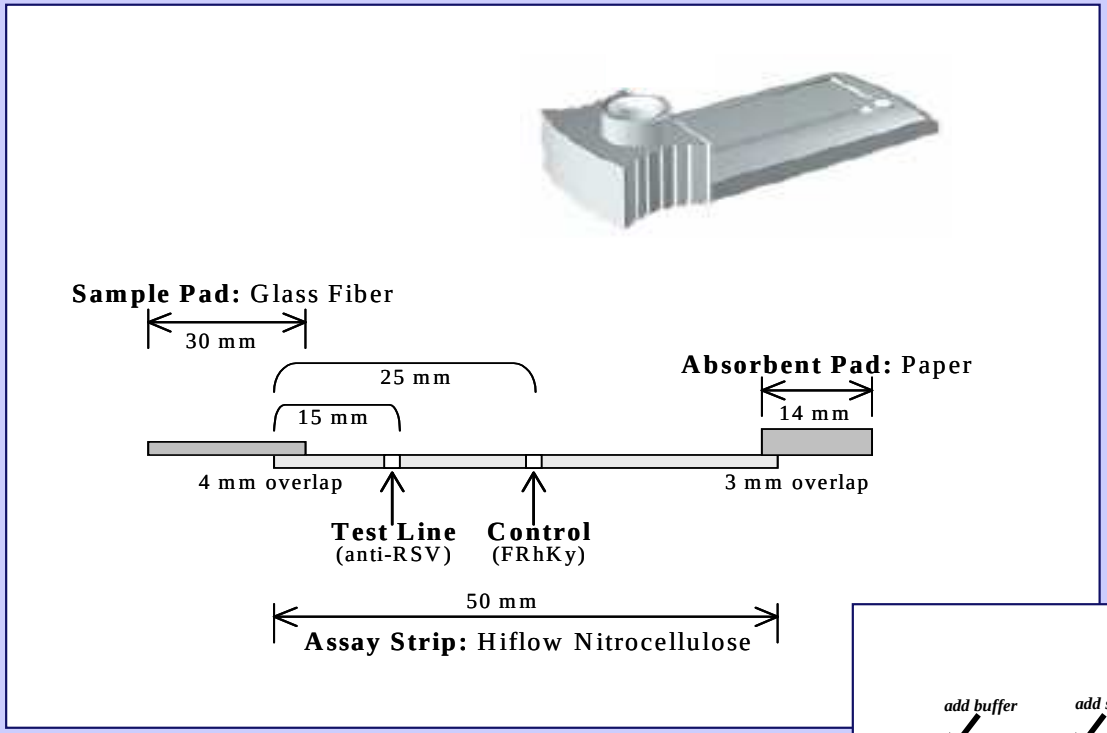
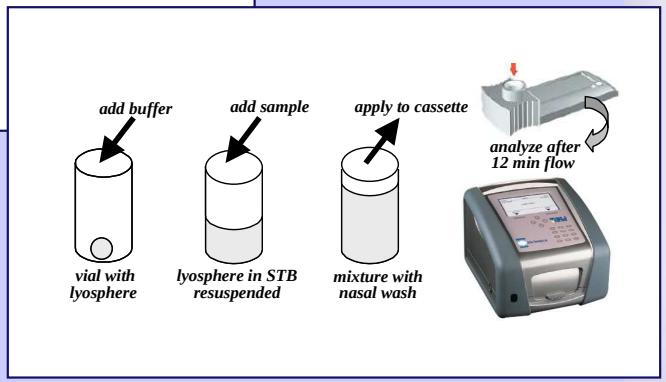
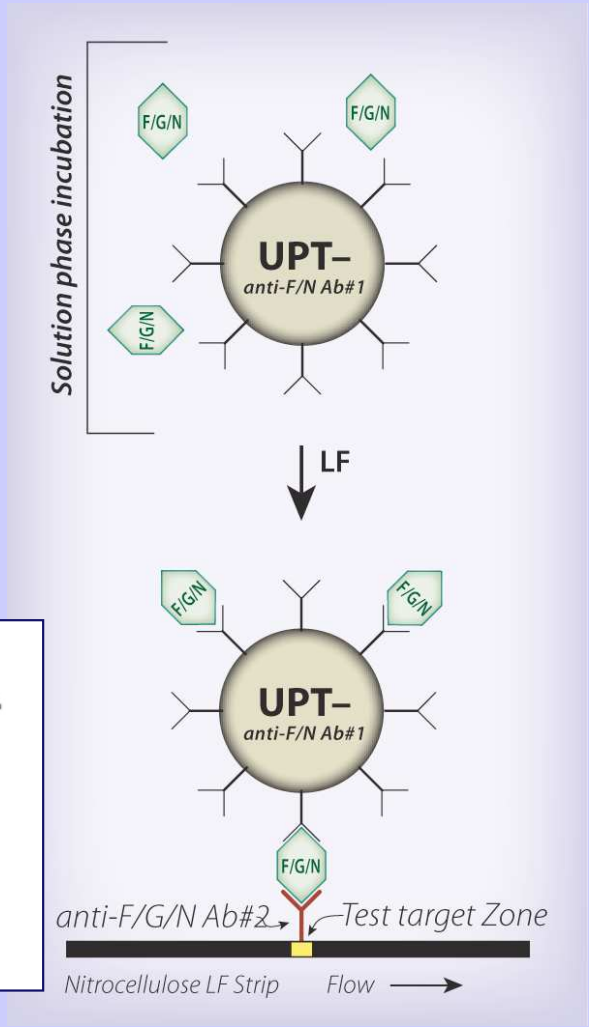
Quantitative analysis of results: the UPLink reader

Detection of RSV antigen in nasopharyngeal samples (immuno-sandwich)

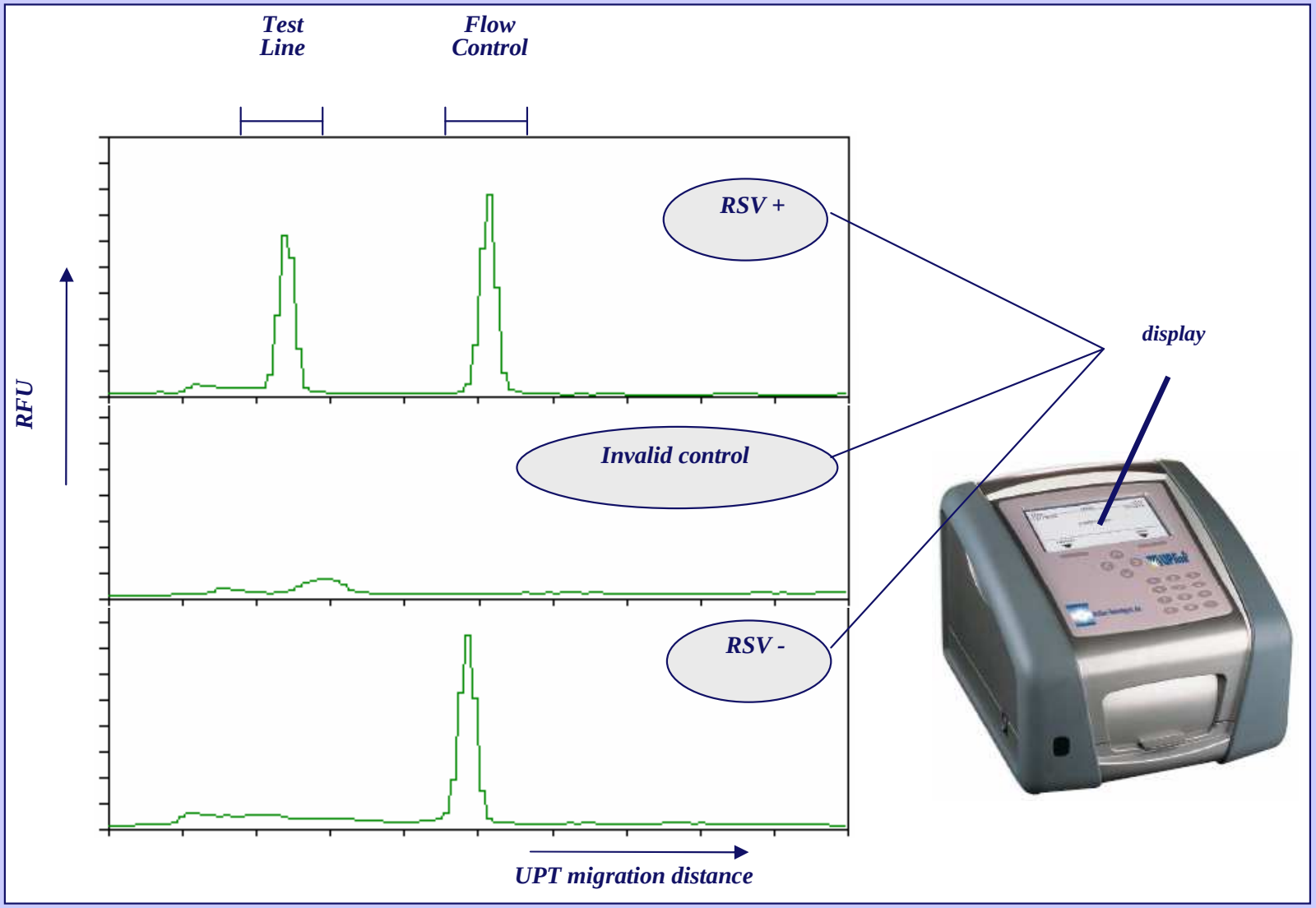






Mokkapti *et al.*, Ann. N.Y. Acad. Sci. 2007, 1098:476-485

RSV


Test Line
Flow Control
RSV +
Invalid control
RSV -
RFU
UPT migration distance
display

Pre-clinical

Two external test sites: Childeren's Hospital Medical Center in Cincinnati, Ohio (CHMCC) and Providence Health System in Portland, Oregon (PHS)

- ***Collected and tested their own samples***
- ***Golden standard was viral culture***
- ***Rapid antigen assays: DIR &UPR***
- ***Discrepancies resolved by RT-PCR***

Statistics: definitions

Diagnostic test:

a procedure which gives a rapid, convenient or inexpensive indication of whether a patient has a certain disease
an assay conducted for diagnostic purposes

Clinical sensitivity: **{Sn=TP/(TP+FN)}**

probability that test is positive when given to a group of patients with the disease

Clinical specificity: **{Sp=TN/(TN+FP)}**

probability that test will be negative among patients who do not have the disease

Positive predictive value: **{PPV=TP/(TP+FP)}**

probability that the patient has the disease when restricted to those patients who test positive

Negative predictive value: **{NPV=TN/(TN+FN)}**

probability that patient won't have the disease when restricted to all patients who test negative

TP *(true positive) = # who test* ***positive*** *and who have the* ***disease***
FN *(false negative) = # who test* ***negative*** *and who have the* ***disease***
FP *(false positive) = # who test* ***positive*** *and who are* ***healthy***
TN *(true negative) = # who test* ***negative*** *and who are* ***healthy***

Correlation:

percentage of agreement between two assays

RSV

Site I CHMCC (n=87)	Culture		
UPR	Positive	Negative	Invalid
Positive	17	9	3
Negative	4	54	0
Invalid	0	0	0

Site I CHMCC (n=87)	Culture		
DIR	Positive	Negative	Invalid
Positive	7	10	2
Negative	13 ←	50	1
Invalid	1	3	0

Site II PHS (n=78)	Culture		
UPR	Positive	Negative	Invalid
Positive	17	2	0
Negative	2	57	0
Invalid	0	0	0

Site II PHS (n=78)	Culture		
DIR	Positive	Negative	Invalid
Positive	15	1	0
Negative	4	58	0
Invalid	0	0	0

Both Sites (n=165)	Culture		
UPR	Positive	Negative	Invalid
Positive	34	11	3
Negative	6	111	0
Invalid	0	0	0

Both Sites (n=165)	Culture		
DIR	Positive	Negative	Invalid
Positive	22	11	2
Negative	17 ←	108	1
Invalid	1	3	0

UPR versus culture: Both sites (n=165-3) [a]		
UPR	Calculation	Percentage
Sensitivity	34/(34+6)	85,0
Specificity	111/(111+11)	91,0
Pos.Pred.Val.	34/(34+11)	75,6
Neg.Pred.Val.	111/(111+6)	94,9
Correlation	(34+111)/(165-3)	89,5

[a] Without 3 (culture) invalid assays

DIR versus culture: Both sites (n=165-7) [b]		
DIR	Calculation	Percentage
Sensitivity	22/(22+17)	56,4 ←
Specificity	108/(108+11)	90,8
Pos.Pred.Val.	22/(22+11)	66,7 ←
Neg.Pred.Val.	108/(108+17)	86,4 ←
Correlation	(22+108)/(165-7)	82,3 ←

[b] Wihout 7 (4 DIR, 3 culture) invalid assays

DIR scores more false negatives

RSV

Both Sites (n=165)	Culture		
UPR	Positive	Negative	Invalid
Positive	34	11	3
Negative	6	111	0
Invalid	0	0	0

Both Sites (n=165)	Culture		
DIR	Positive	Negative	Invalid
Positive	22	11	2
Negative	17 ←	108	1
Invalid	1	3	0

Culture only

UPR versus culture: Both sites (n=165-3) [a]		
UPR	Calculation	Percentage
Sensitivity	34/(34+6)	85,0
Specificity	111/(111+11)	91,0
Pos.Pred.Val.	34/(34+11)	75,6
Neg.Pred.Val.	111/(111+6)	94,9
Correlation	(34+111)/(165-3)	89,5

[a] Without 3 (culture) invalid assays

DIR versus culture: Both sites (n=165-7) [b]		
DIR	Calculation	Percentage
Sensitivity	22/(22+17)	56,4
Specificity	108/(108+11)	90,8
Pos.Pred.Val.	22/(22+11)	66,7
Neg.Pred.Val.	108/(108+17)	86,4
Correlation	(22+108)/(165-7)	82,3

[b] Wihout 7 (4 DIR, 3 culture) invalid assays

Both Sites (n=165)	Culture/RT-PCR		
UPR	Positive	Negative	Invalid
Positive	40	6	0
Negative	5	114	0
Invalid	0	0	0

Both Sites (n=165)	Culture/RT-PCR		
DIR	Positive	Negative	Invalid
Positive	27	7	0
Negative	9 ←	118	0
Invalid	1	3	0

Culture & RT-PCR

UPR, RT-PCR resolved: Both sites (n=165)		
UPR	Calculation	Percentage
Sensitivity	40/(40+5)	88,9
Specificity	114/(114+6)	95,0
Pos.Pred.Val.	40/(40+6)	87,0
Neg.Pred.Val.	114/(114+5)	95,8
Correlation	(40+114)/165	93,3

DIR, RT-PCR resolved: Both sites (n=165-4) [a]		
DIR	Calculation	Percentage
Sensitivity	27/(27+9)	75,0 ←
Specificity	118/(118+7)	94,4
Pos.Pred.Val.	27/(27+7)	79,4 ←
Neg.Pred.Val.	118/(118+9)	92,9
Correlation	(27+118)/(165-4)	90,0

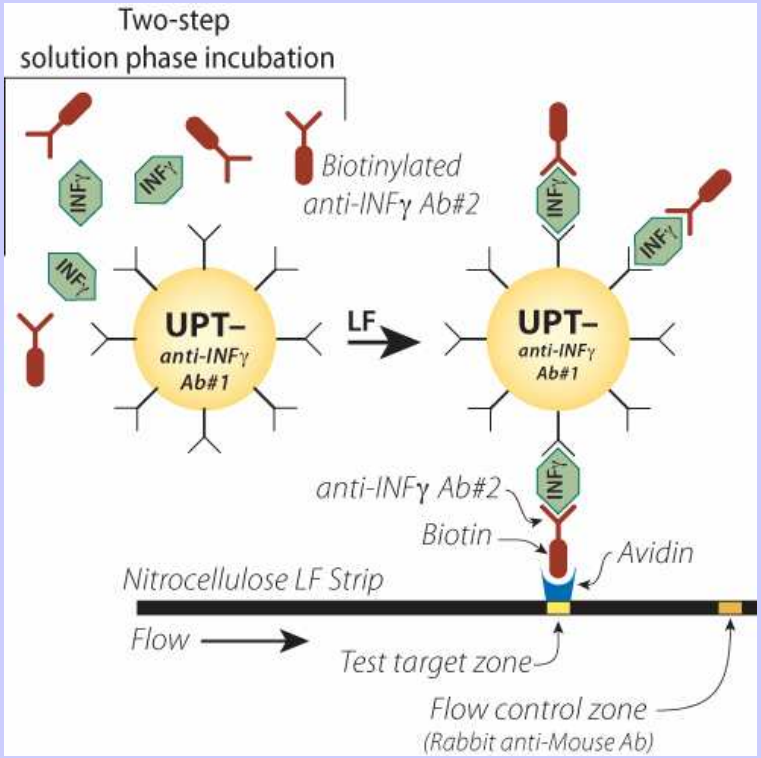
[a] Wihout 4 (DIR) invalid assays

Blind test (n=60)
Comparison of ELISA and UCP-LF

ELISA		ULIGA Positive	ULIGA Negative	ULIGA Discrepant
≥100	36	35	1	1
>50-100	5	4	1	1
>0-50	13	0	13	0
0	6	0	6	0
Total	60	39	21	2

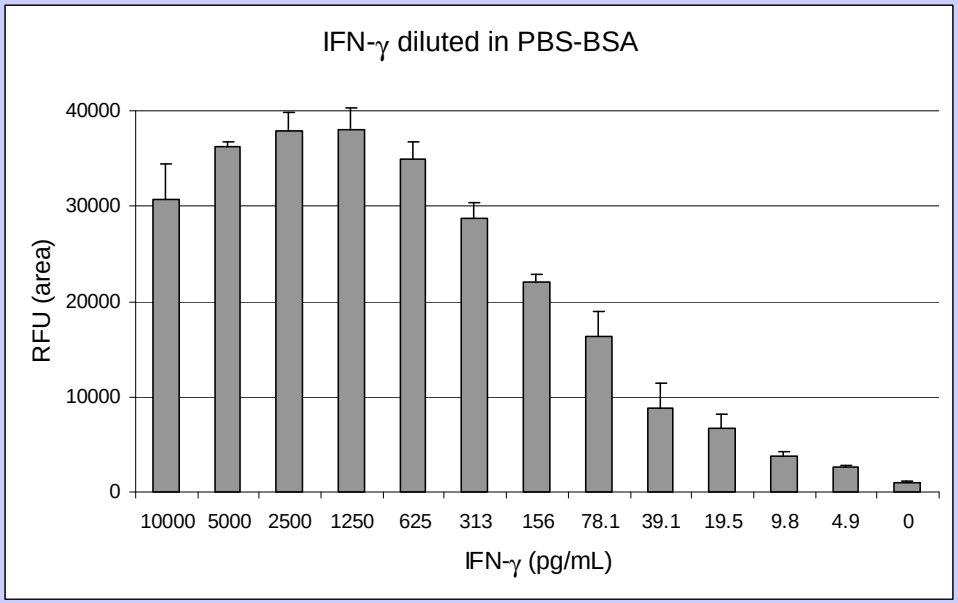
Mycobacteria – Lepra

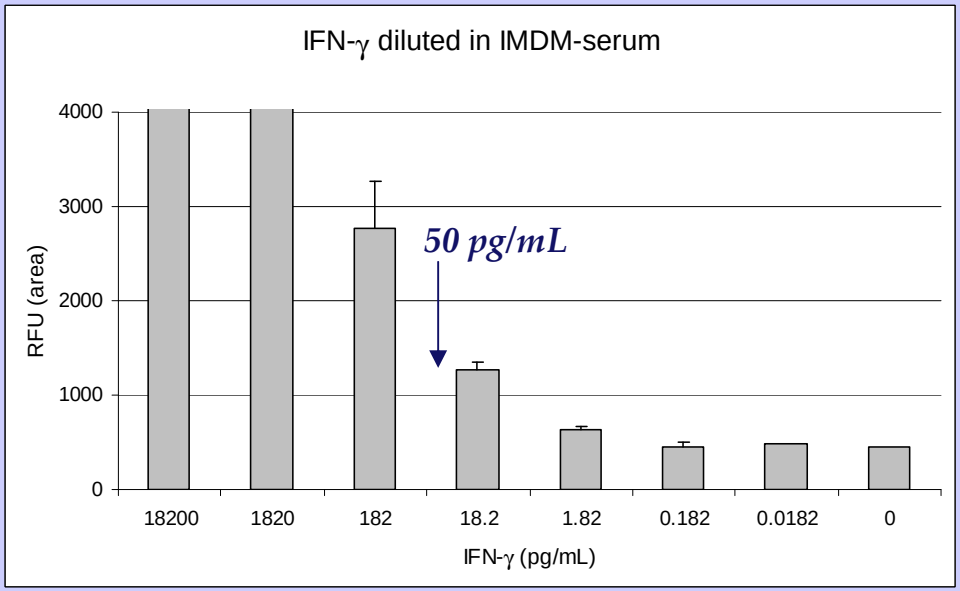
Cellular response – cytokine production



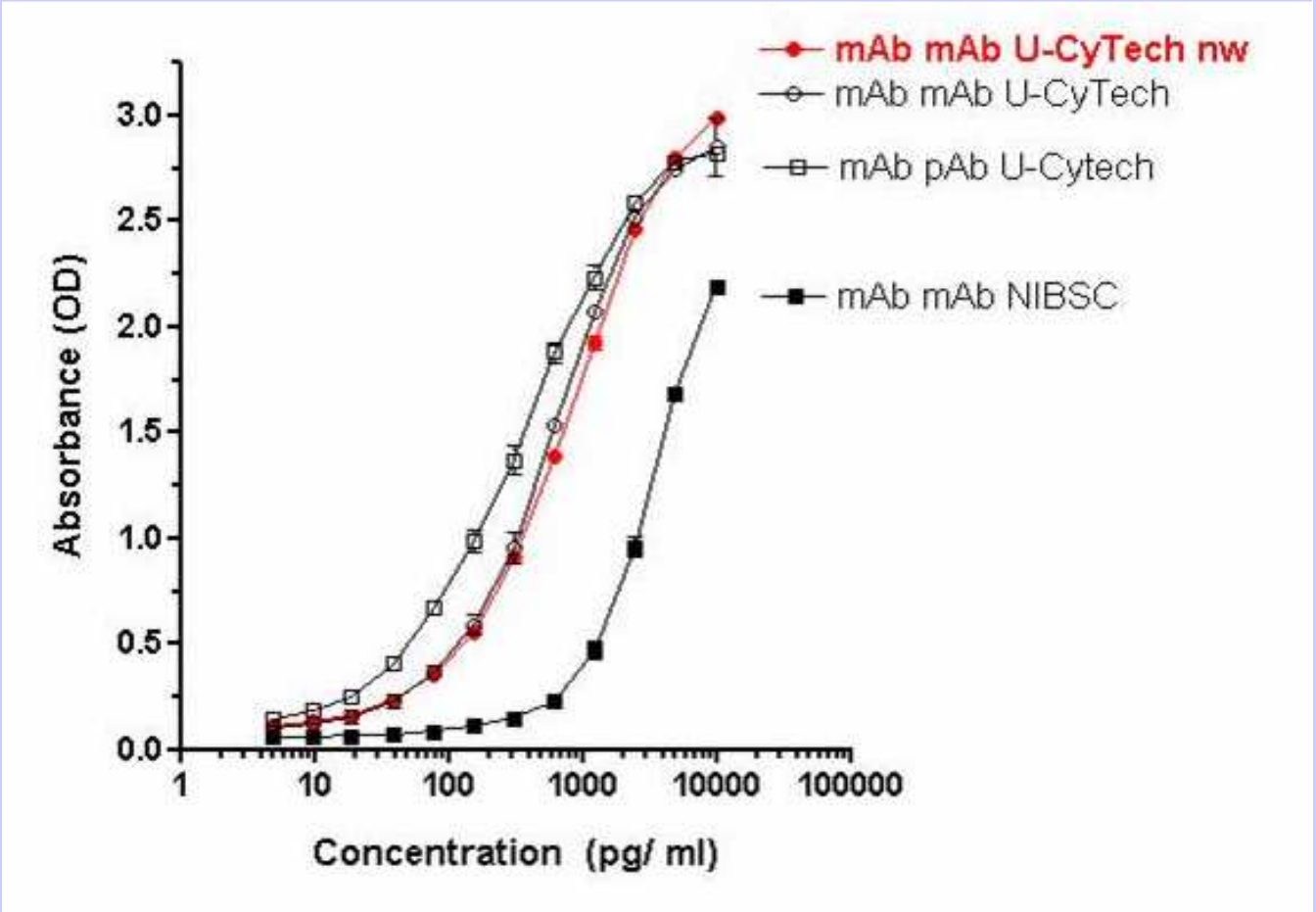
Format:
- Stepwise sandwich
- Monoclonal 1 bound to UCP
- Monoclonal 2 biotinylated in solution
- Avidin capture on strip
- Incubation 2x1 h, 37 ℃, 1200 rpm

Assay format allowing 40 µL sample

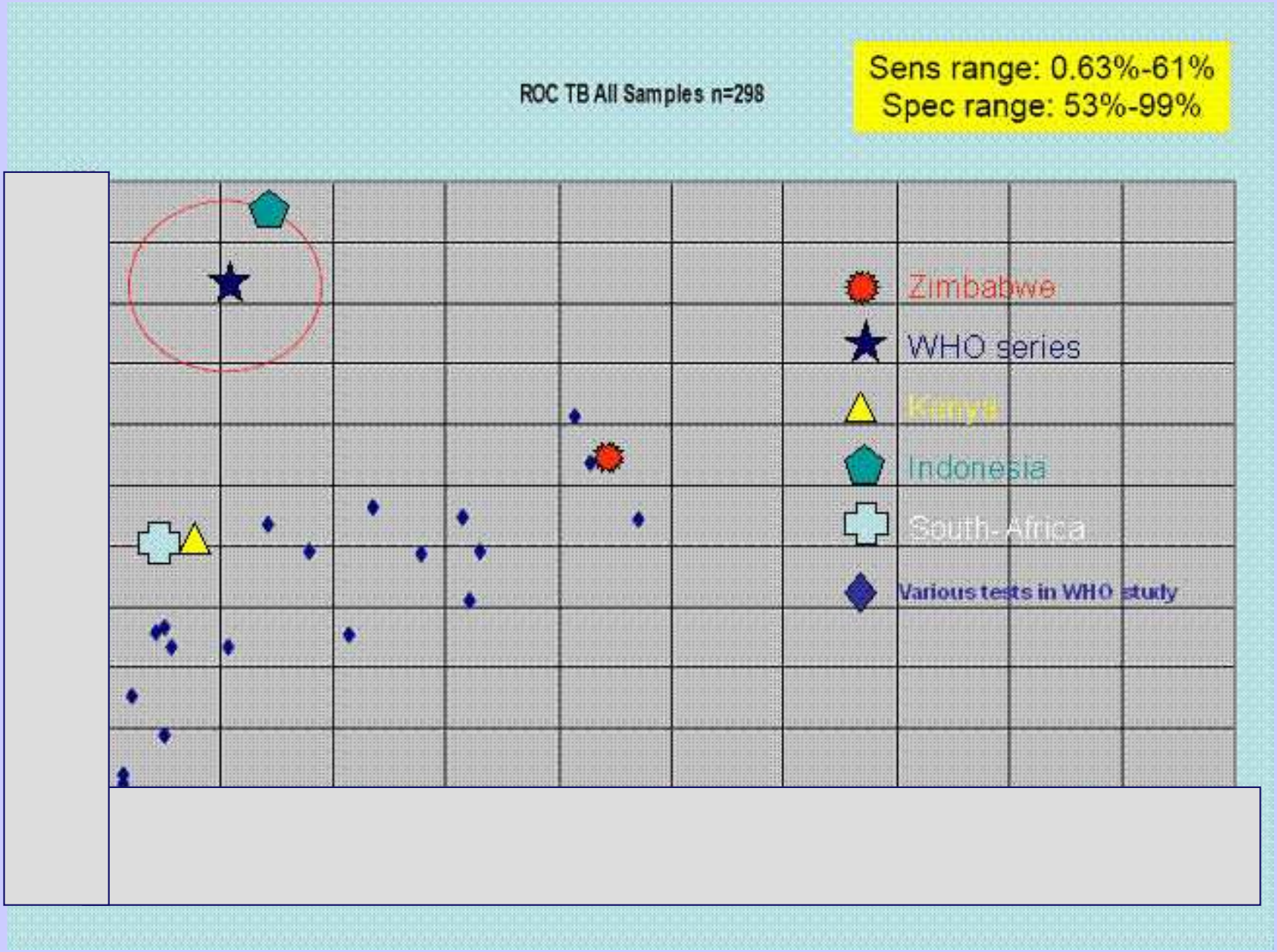




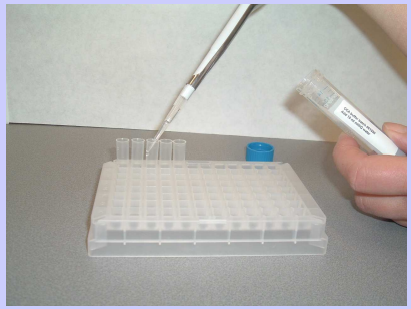
Antibody selection by ELISA

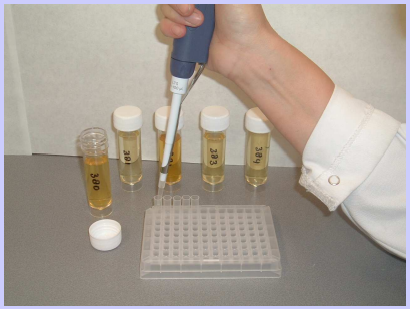


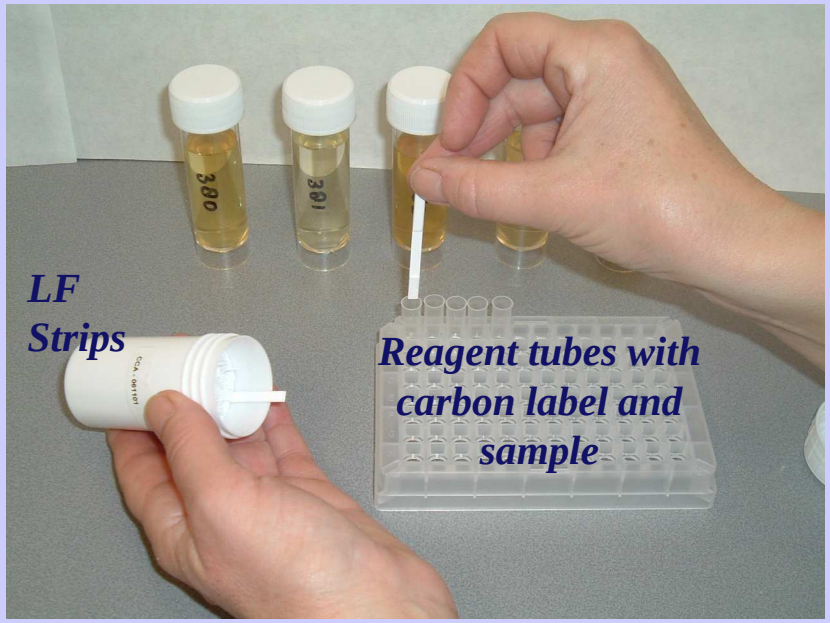
Good antibody-good assay: unexpected results (TB)



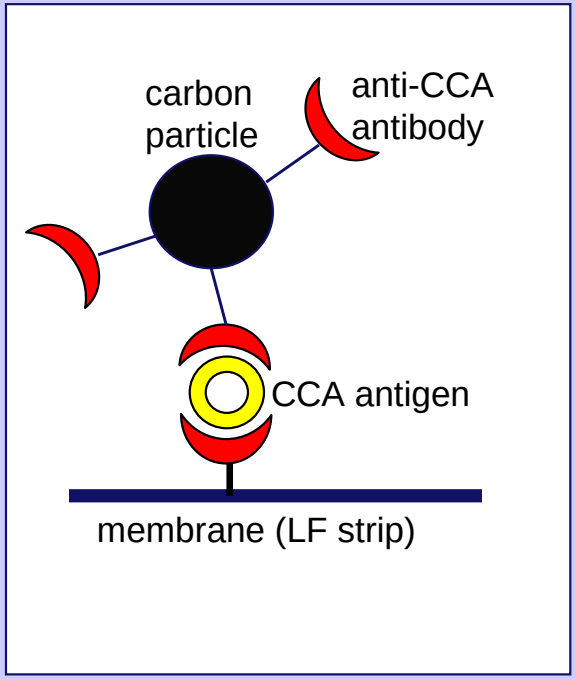
Mycobacteria – Lepra & TB



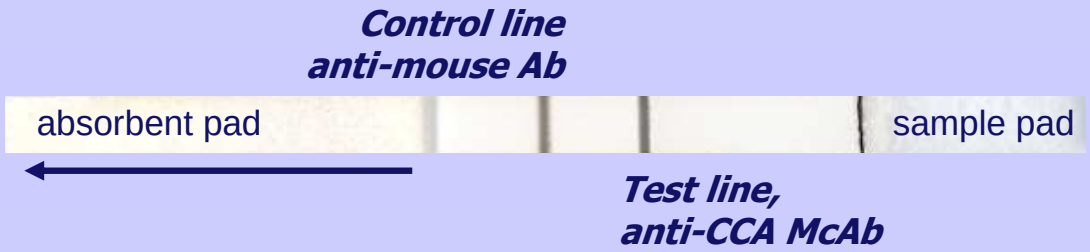




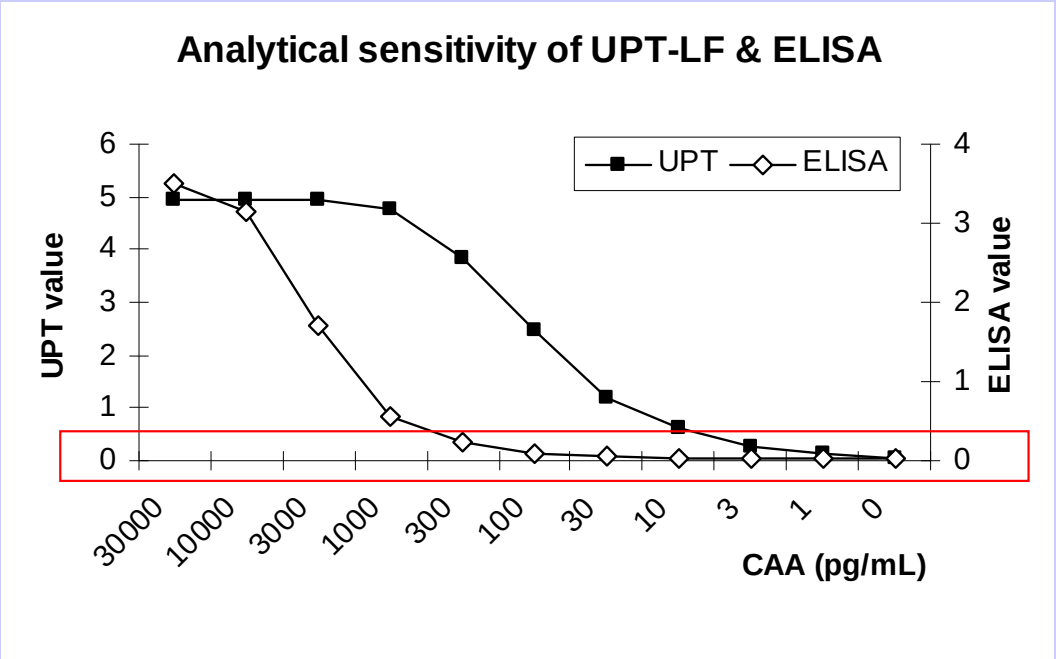
Eyeballing or quantitative results? Schistosomiasis

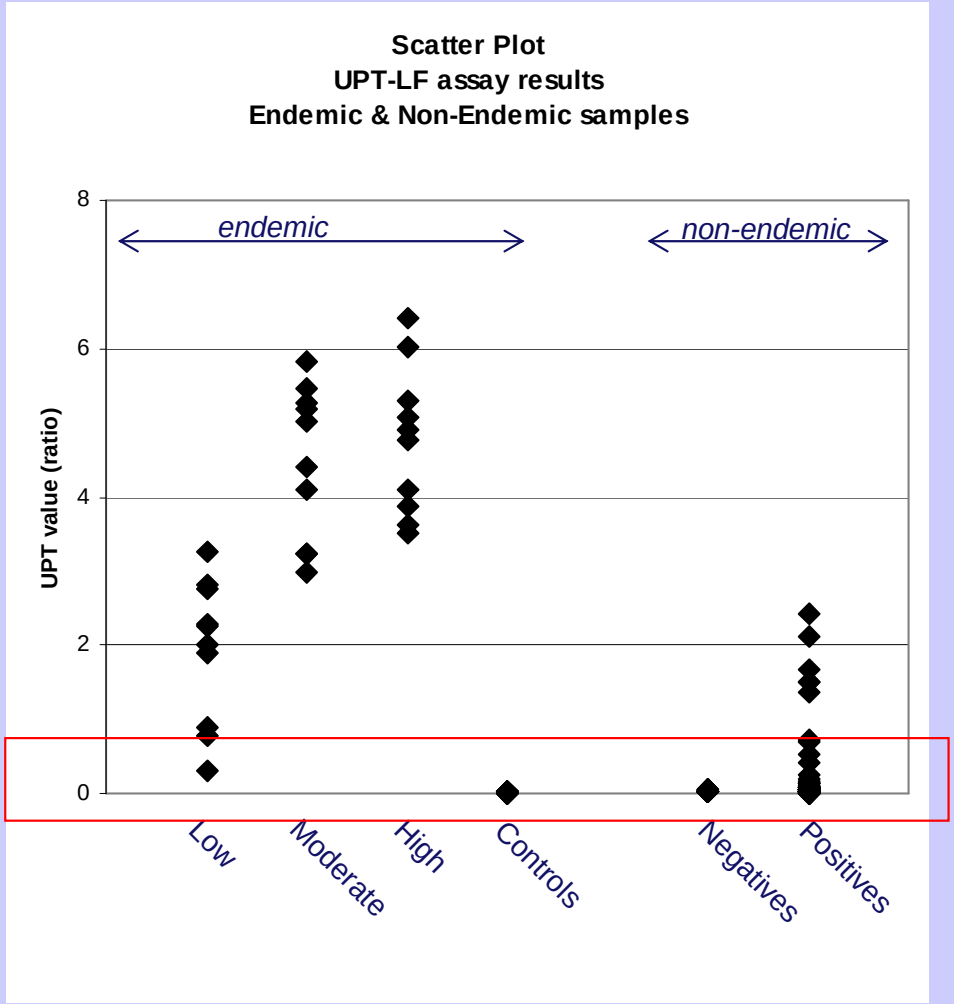


sample	GF#33 Schleicher&Schuell
10000 ng AT/ml ur 35	
1000 ng AT/ml ur 35	
500 ng AT/ml ur 35	
100 ng AT/ml ur 35	
50 ng AT/ml ur 35	
ur 35	
QC ur 2 (34 ng AT/ml)	
QC ur 3 (145 ng AT/ml)	
QC ur 14 (121 ng AT/ml)	
QC ur 16 (344 ng AT/ml)	



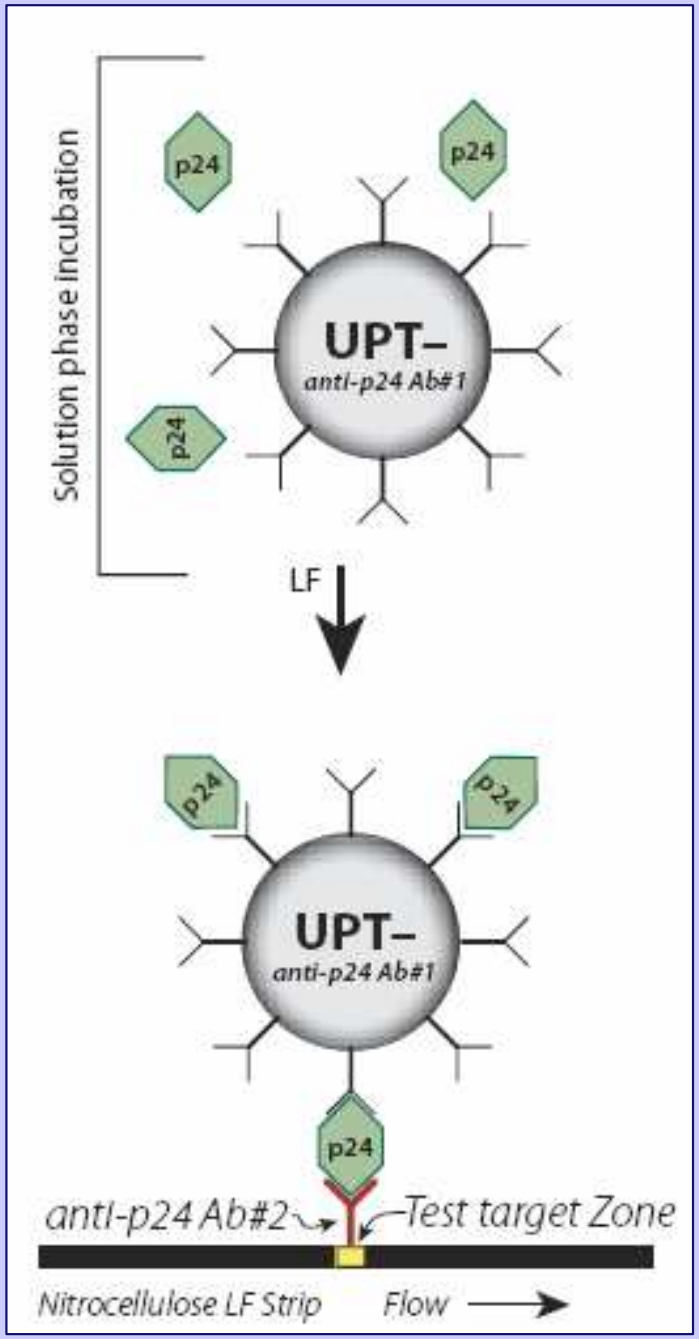
Schistosomiasis

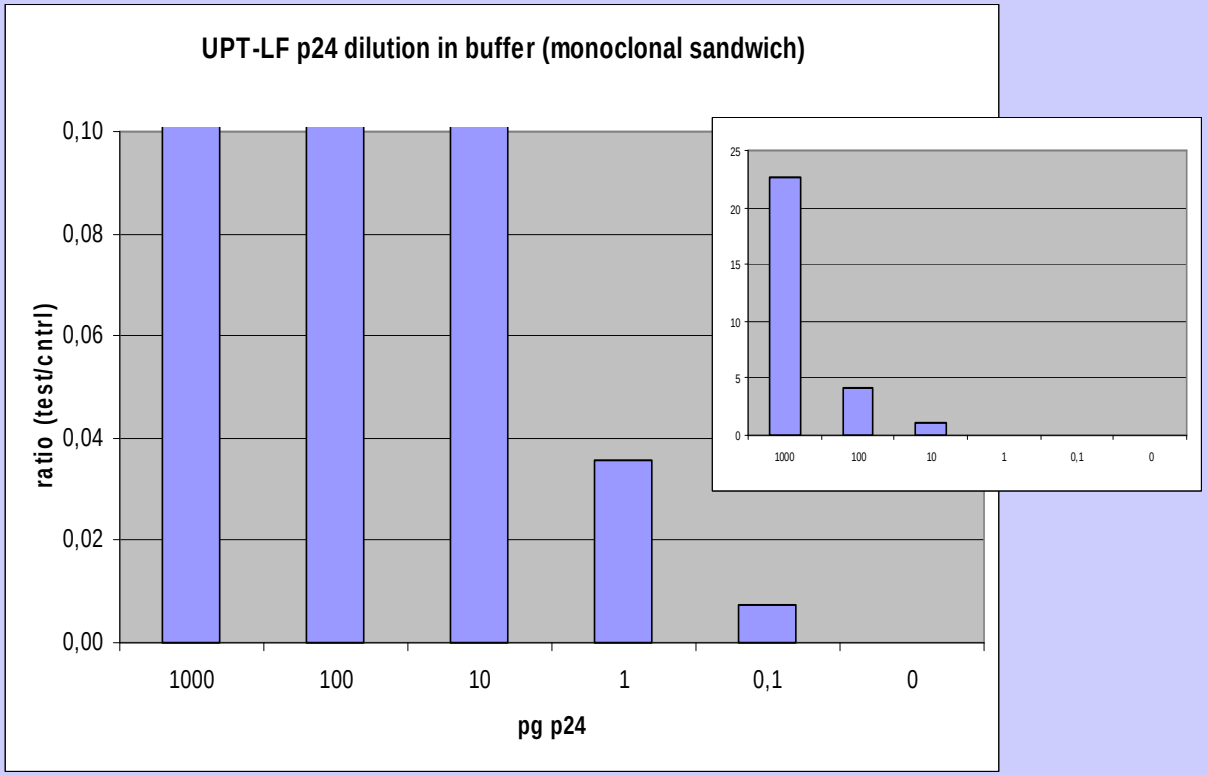




HIV – p24 antigen assay

Sandwich-assay

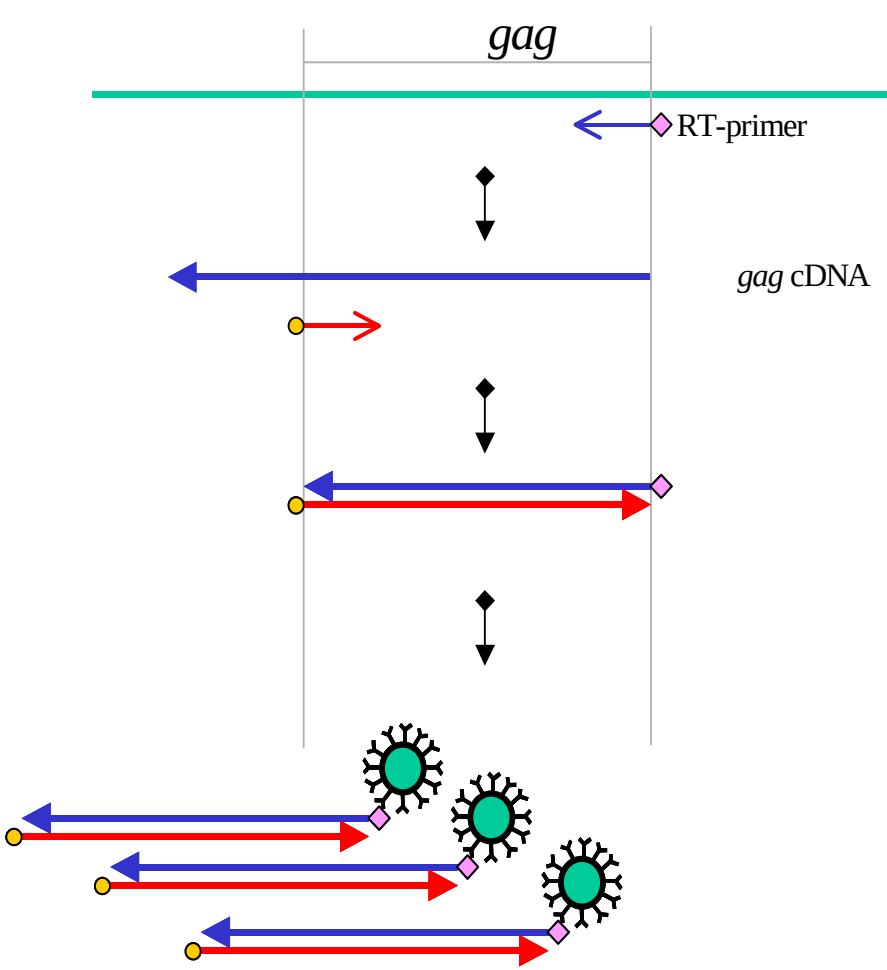




Detection sensitivity (LOD):
0.1 pg in 100 µL (1 pg/mL)

HIV RNA Assay - Amplification

System

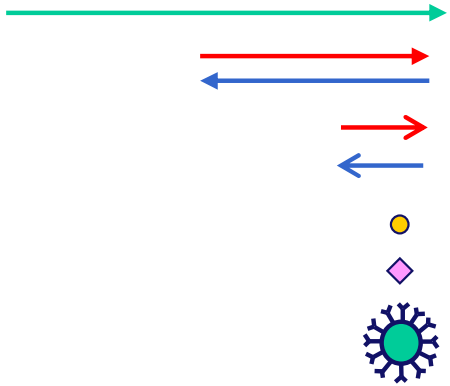


I RNA isolation

II cDNA synthesis

III PCR amplification

IV LF & UPT detection

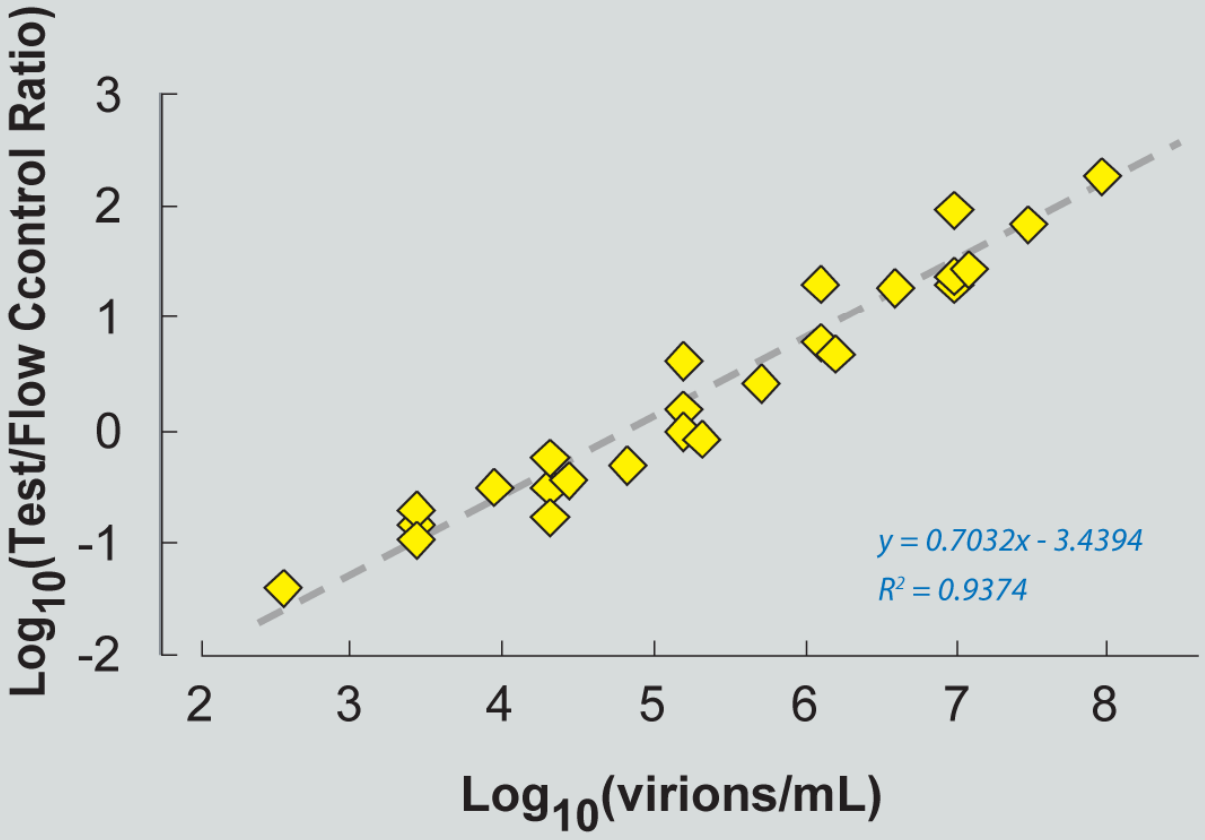


Note:
In the currently used system the dig and bio hapten are switched; RT is performed with a biotin labeled primer.

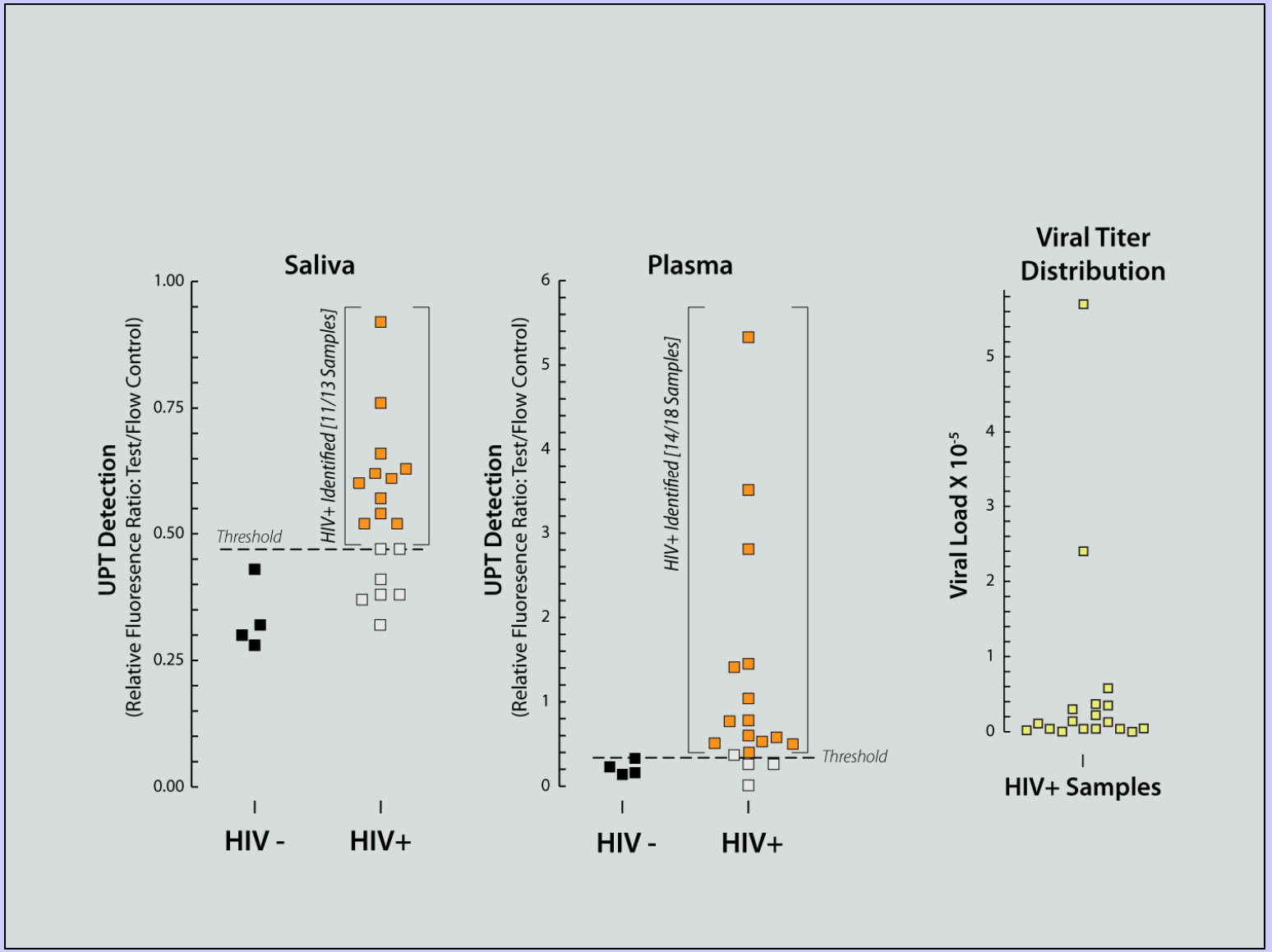
HIV RNA Assay - Amplification

(Quantitative) HIV Assay

– LOD 350 HIV virions mL –

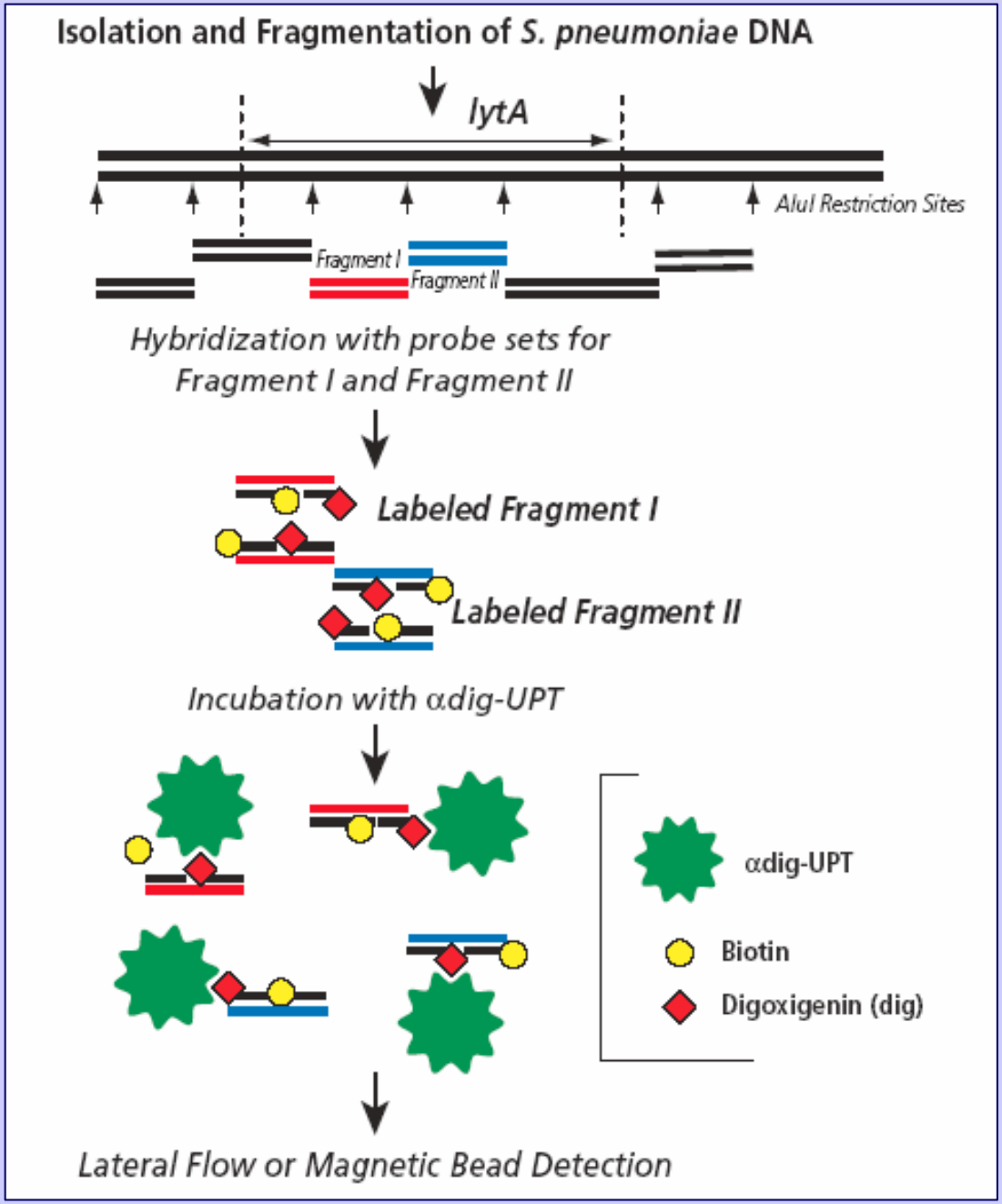


HIV RNA Assay - Amplification



Amplification free detection of Streptococcus pneumoniae

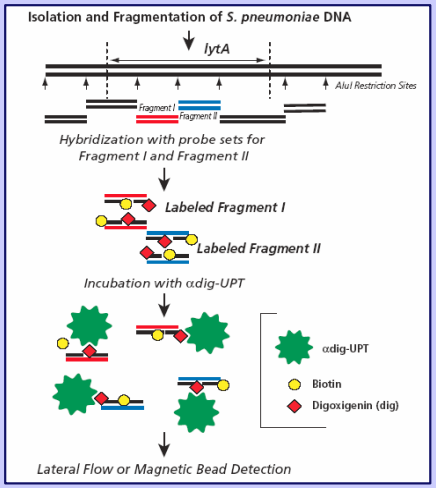
Meningitis
S. pneumoniae

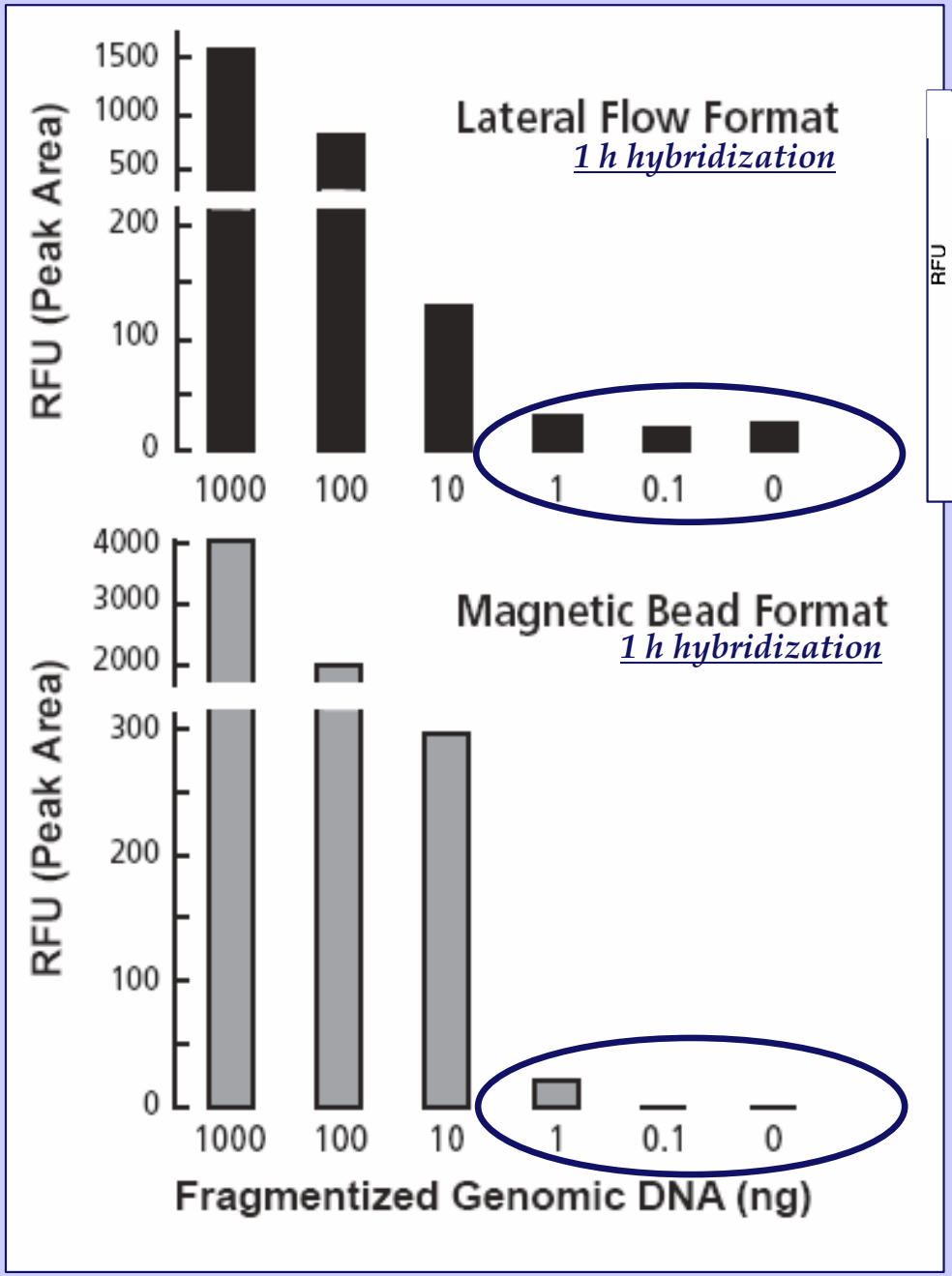


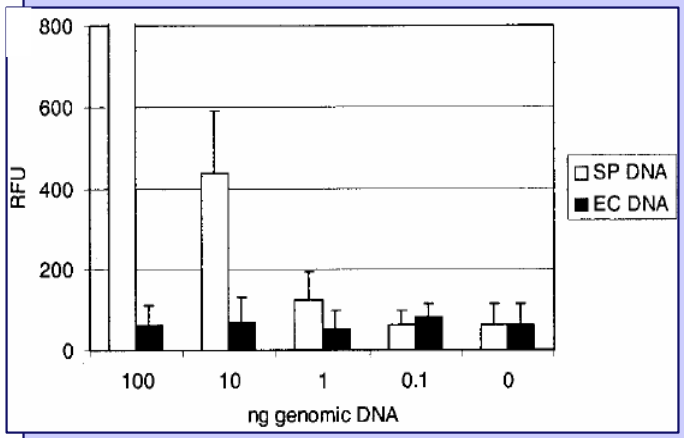
Amplification free
Hybridization based assay

Zuiderwijk *et al.*,Clin. Biochem., 2003, 36:401-403

Amplification free detection of Streptococcus pneumoniae







Amplification free Hybridization based assay

Magnetic bead format increases resolution

Single copy gene detection:

10^6 cells / genome equivalents

1 pg viral DNA
1 ng bacterial DNA
1 µg human DNA

Applications of lateral flow assays using UCP

Infectious diseases:

		Antigen	Nucleic acid	Host response
Viral	Hepatitius C Virus (HCV)			antibody
	Human Immunodeficiency Virus (HIV)	core protein	RNA	antibody
	Human Papilloma Virus (HPV)		DNA	
	Influenza A virus (H1N1)		RNA	
	Respiratory Syncyticial Virus (RSV)	antigen mix		
Bacterial	*Clostridium botulinum*	neurotoxin		
	Mycobacterium leprae			antibody & cytokines
	Mycobacterium tuberculosis			antibody
	Staphylococcus aureus		DNA	
	Streptococcus pneumoniae		DNA	
	Vibrio cholera		DNA	
Other parasites	*Plasmodium falciparum*		DNA	
	Schistosomisa mansoni	glycoprotein		

Systemic diseases:

	Disease marker	Disease Therapeutic
Heart failure	small protein	
Inflammatory bowl disease	protein	immunotherapeutic
Rheumatism		immunotherapeutic

Miscellaneous:

	Marker
Fruit ripening process	protein

LUMC

Contents:

- *Point of Care Testing - Multiplexing*
- *Up-Converting Phosphor Technology - UCP*
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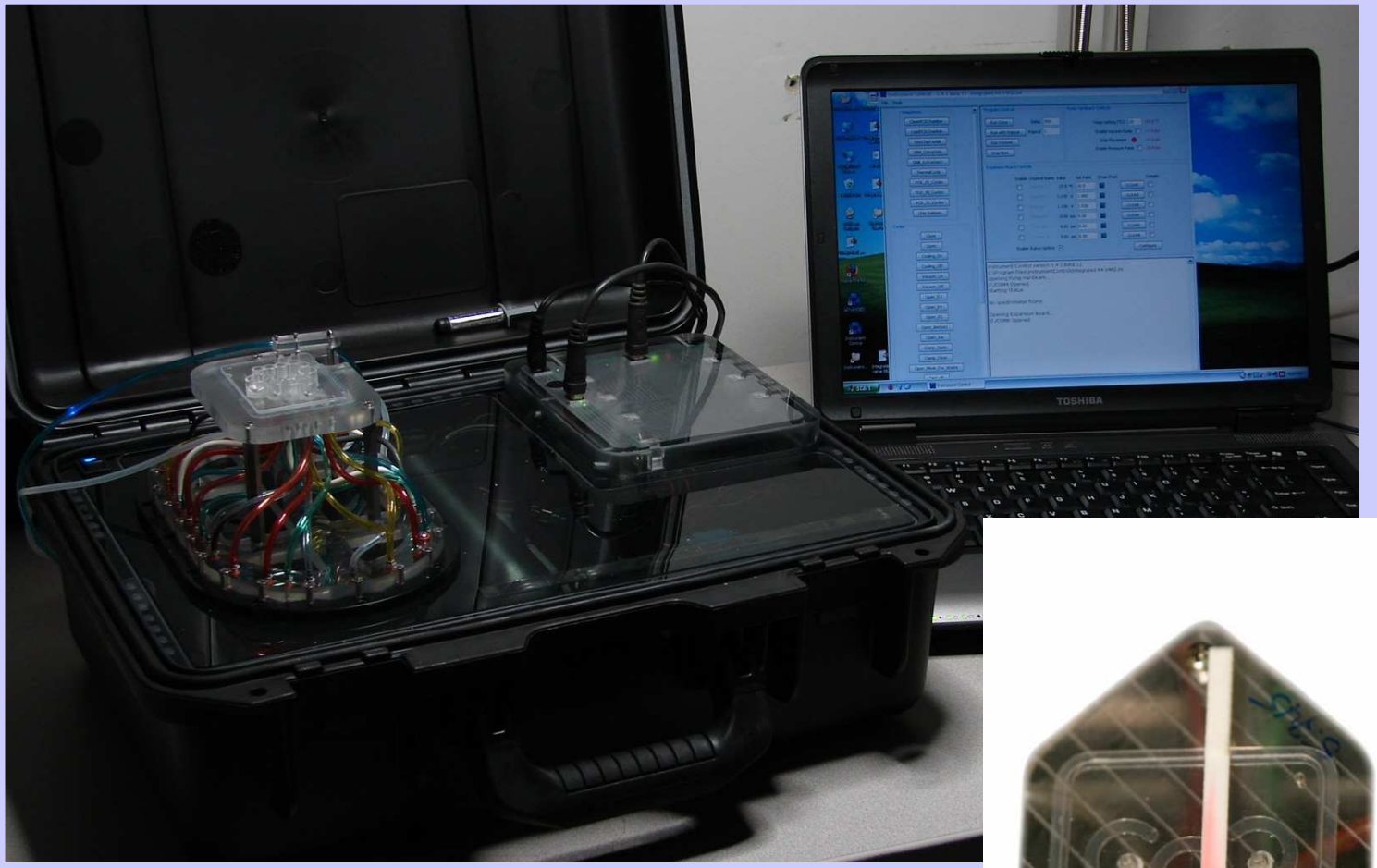
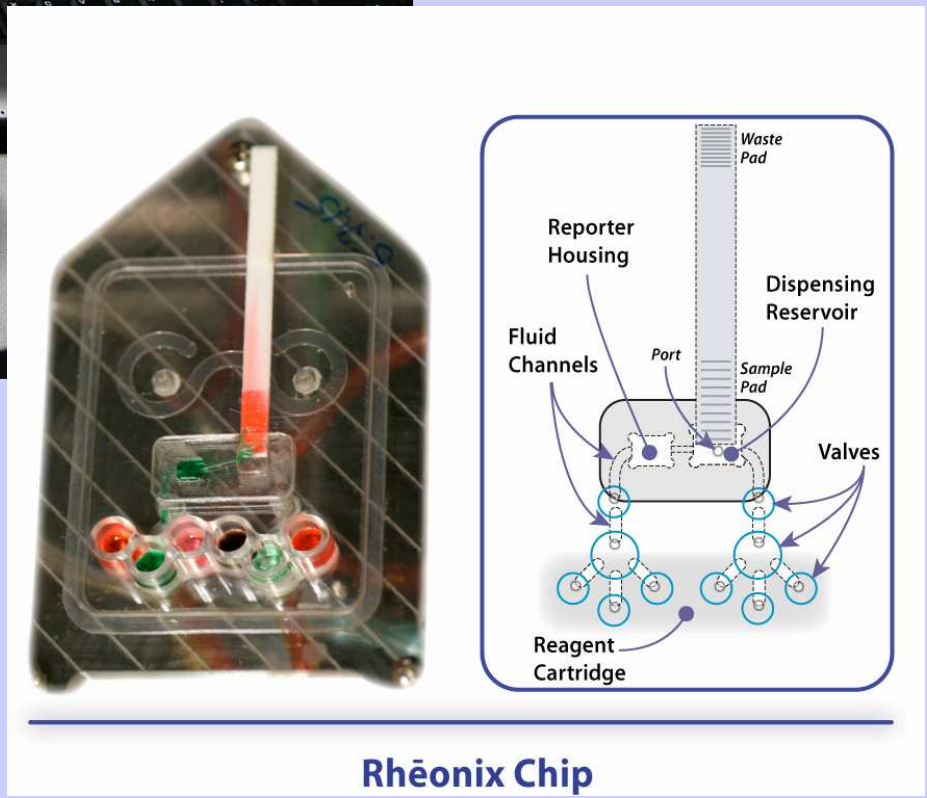
- *Applications in infectious diseases*
- *Microfluidics - Chips and Actuators*
- *Handheld devices*

High end microfluidic devices

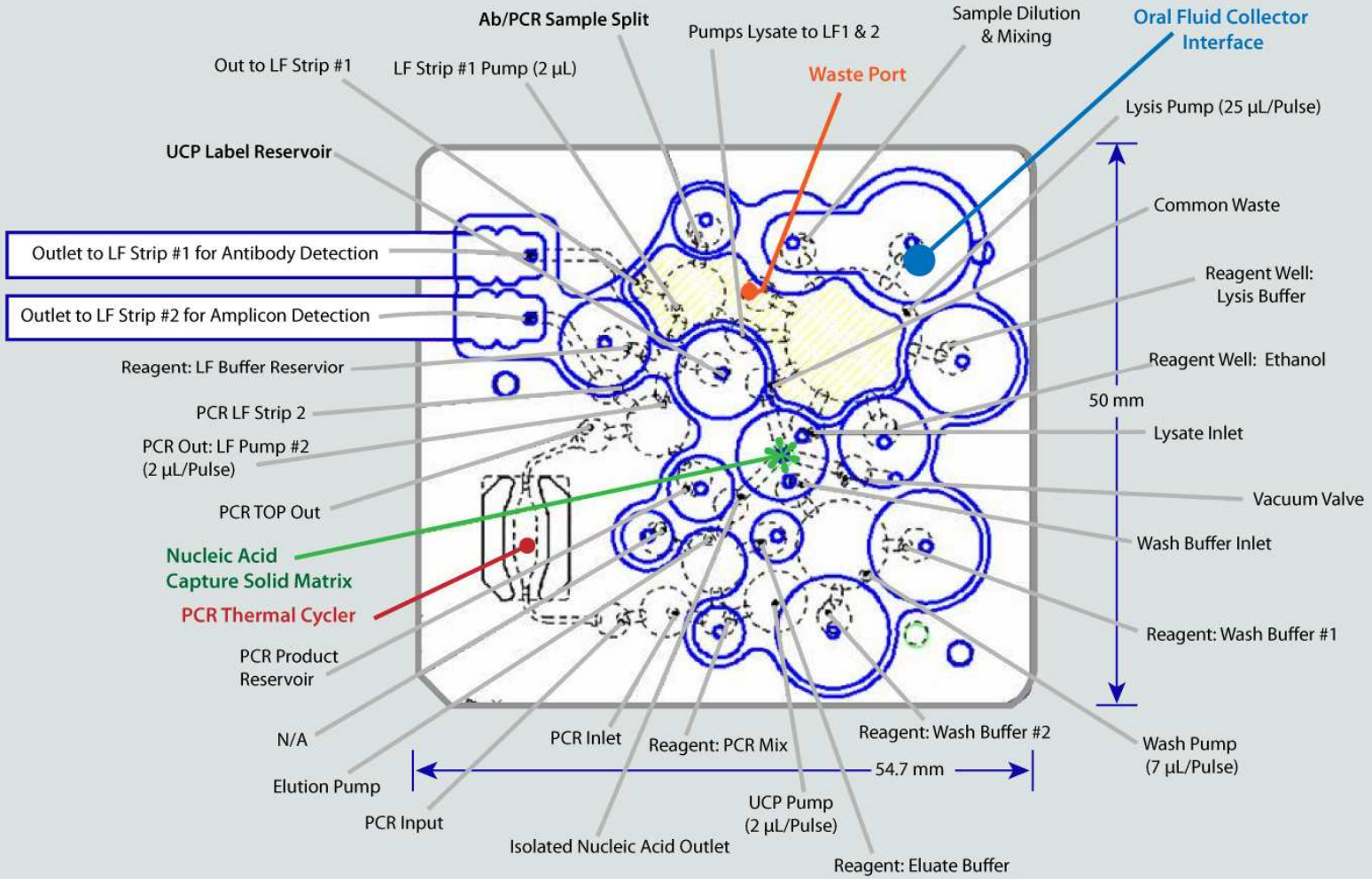


HIGH-TECH
Point-of-care
"Portable"



The development of fully integrated ***pneumatic chips*** for HIV testing is now performed in collaboration with Rheonix Inc. (Ithaca).

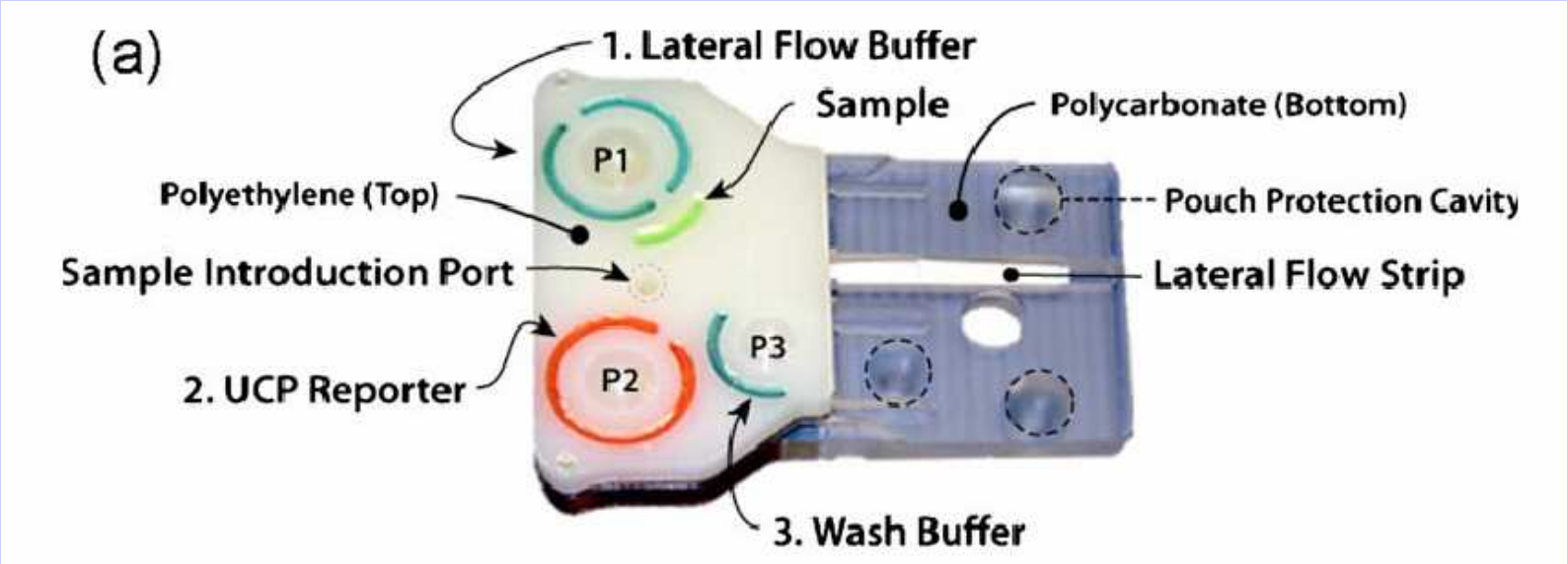
Top View


Ab/PCR Sample Split
Pumps Lysate to LF1 & 2
Sample Dilution & Mixing
Oral Fluid Collector Interface
Out to LF Strip #1
LF Strip #1 Pump (2 µL)
Waste Port
Lysis Pump (25 µL/Pulse)
UCP Label Reservoir
Common Waste
Outlet to LF Strip #1 for Antibody Detection
Outlet to LF Strip #2 for Amplicon Detection
Reagent Well: Lysis Buffer
Reagent: LF Buffer Reservior
Reagent Well: Ethanol
PCR LF Strip 2
50 mm
PCR Out: LF Pump #2 (2 µL/Pulse)
Lysate Inlet
PCR TOP Out
Vacuum Valve
Nucleic Acid Capture Solid Matrix
Wash Buffer Inlet
PCR Thermal Cycler
Reagent: Wash Buffer #1
PCR Product Reservoir
N/A
PCR Inlet
Reagent: PCR Mix
Reagent: Wash Buffer #2
Wash Pump (7 µL/Pulse)
Elution Pump
54.7 mm
PCR Input
UCP Pump (2 µL/Pulse)
Isolated Nucleic Acid Outlet
Reagent: Eluate Buffer

Chip with Oral Collector Inserted

(Lower!) Low end microfluidic devices

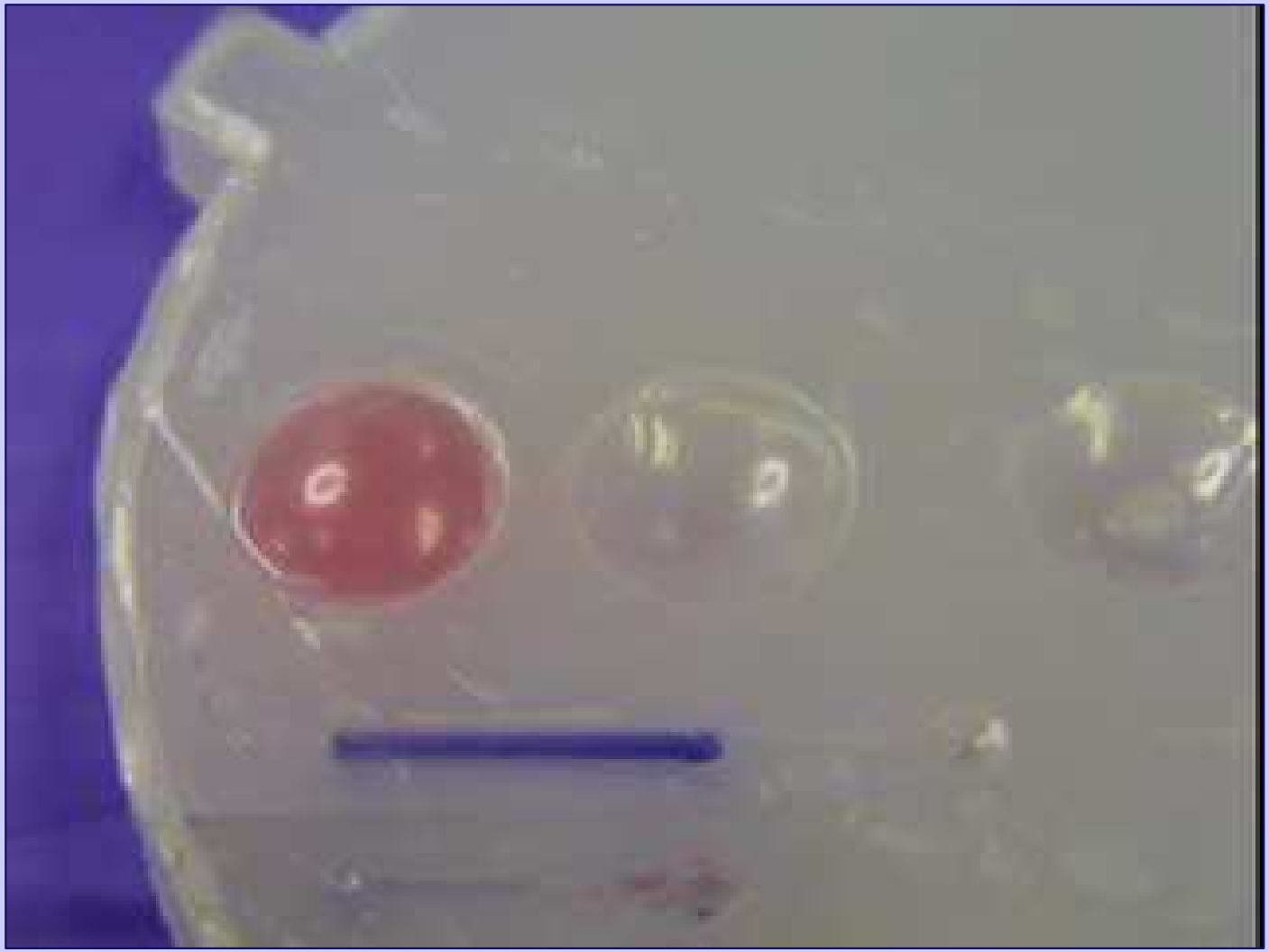
Finger actuated pouch chips



Qiu *et al.*, Biomed Microdevices 2009

(Lower!) Low end microfluidic devices

Pouch-based fluid propulsion

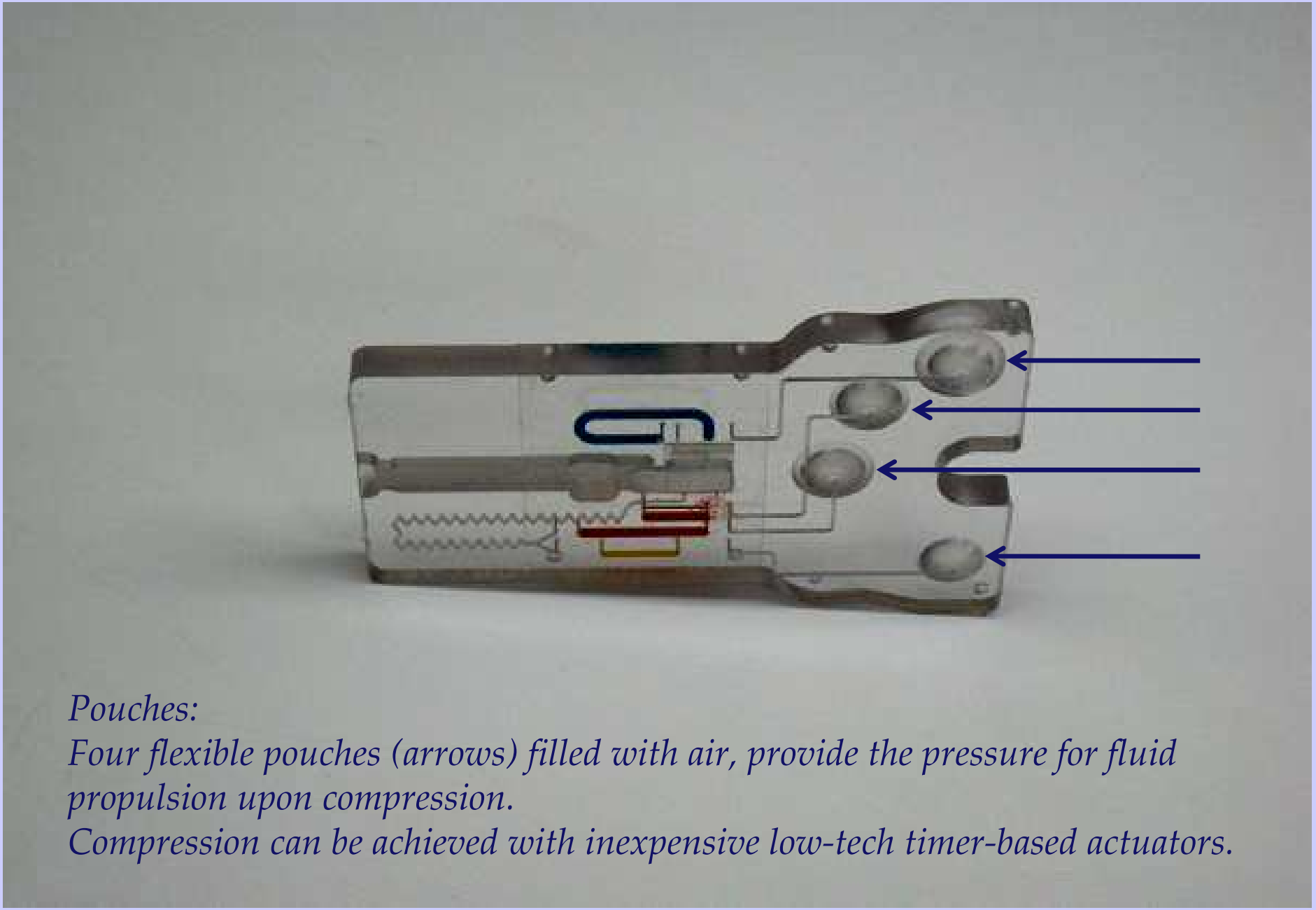


Applications of this lower-low end system?

Rapid detection of disease specific antibodies or antigens (<u>not DNA/RNA</u>) in:

- ***remote areas/developmental countries***
- ***home diagnostics***

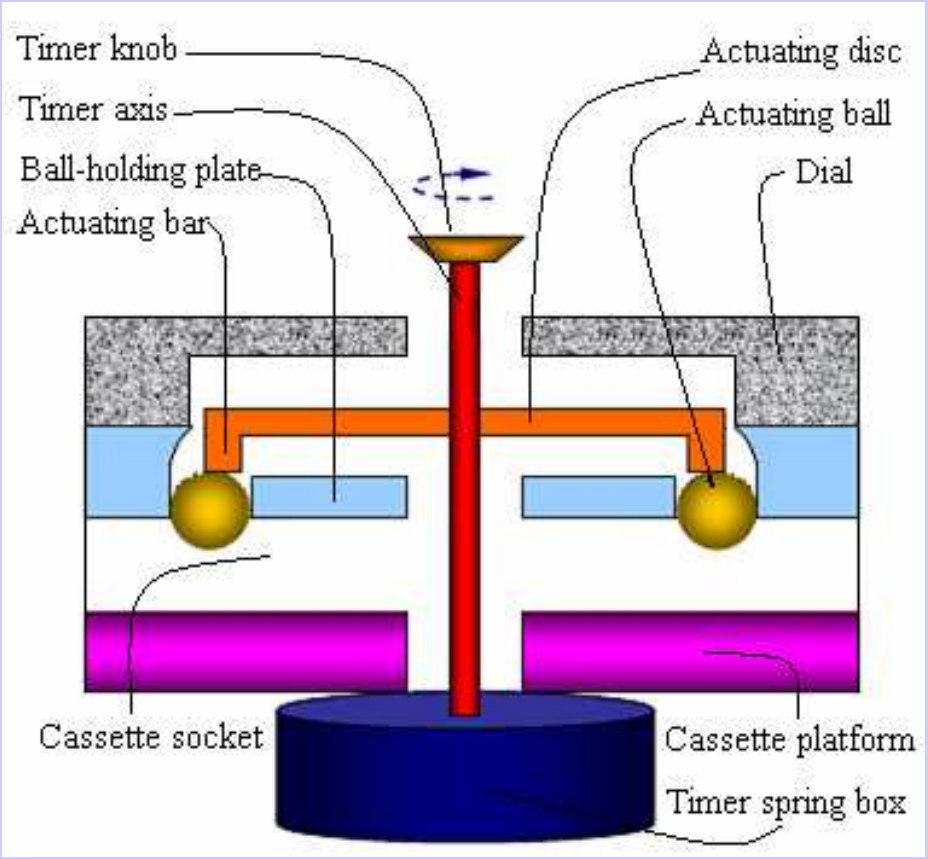
Low end microfluidic devices

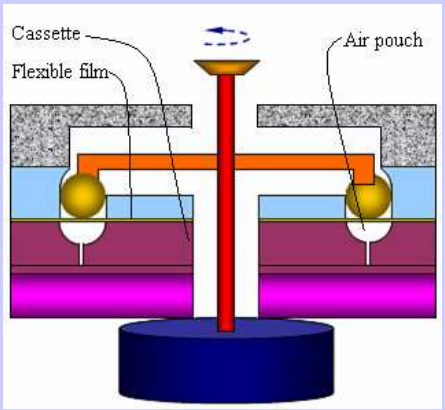


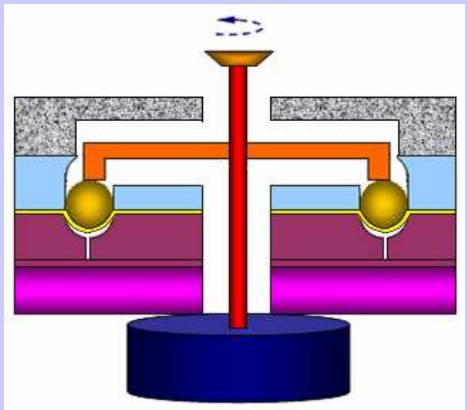
Pouches:
Four flexible pouches (arrows) filled with air, provide the pressure for fluid propulsion upon compression.
Compression can be achieved with inexpensive low-tech timer-based actuators.

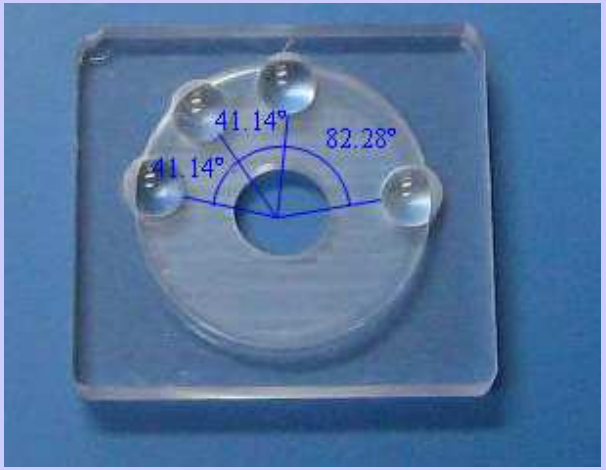
Liu *et al.*, Lab Chip 2009, 9:768-776

Low end microfluidic devices









Liu *et al.*, Lab Chip 2009, 9:768-776

Low end microfluidic devices

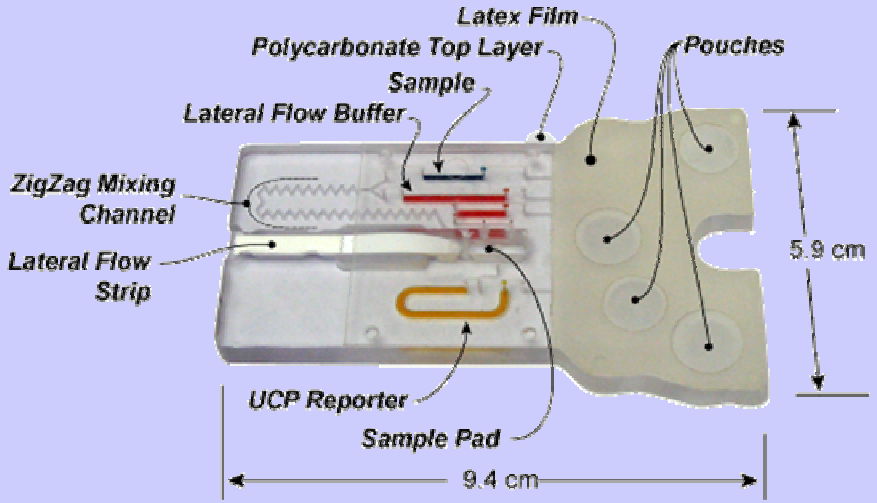
Changchun Liu

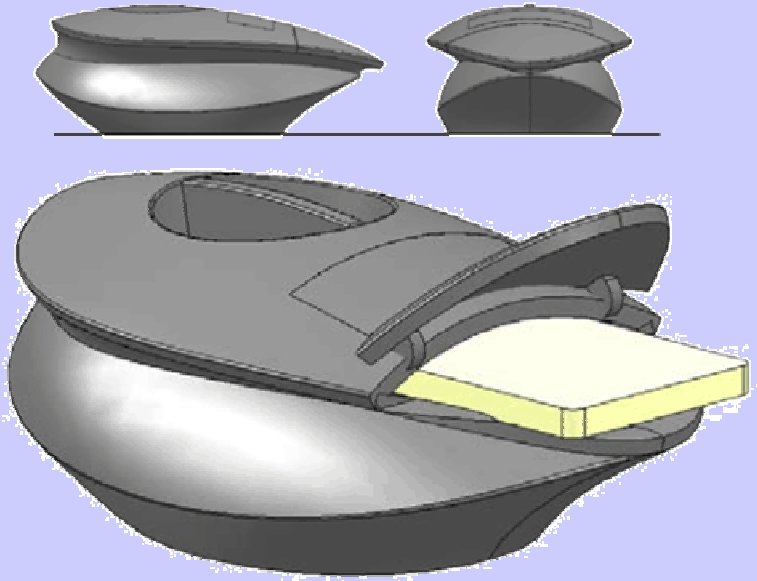
HIV Antibody Detection Chip

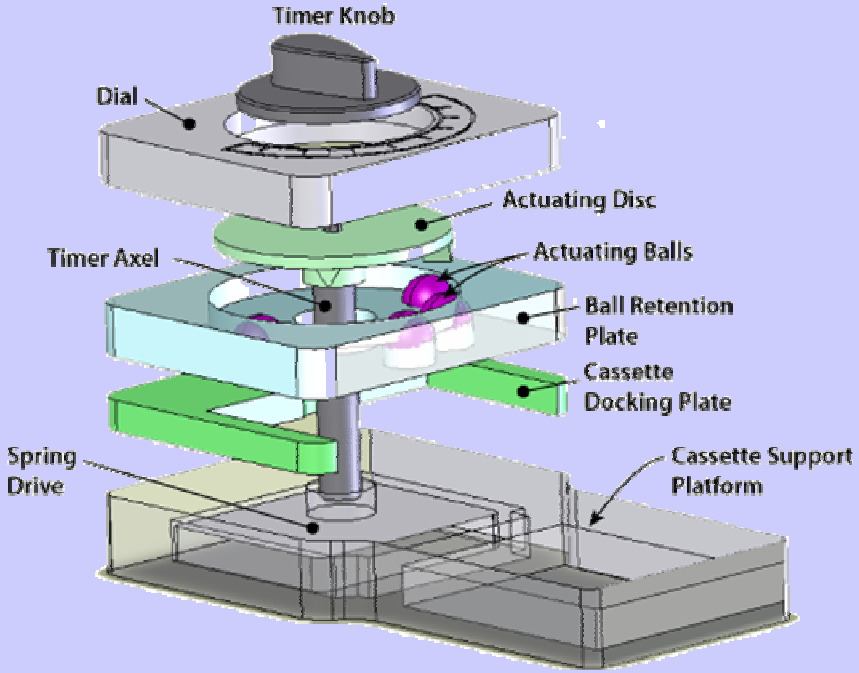
Hand-held Pouch Driven Chip with Timer Controlled Actuator

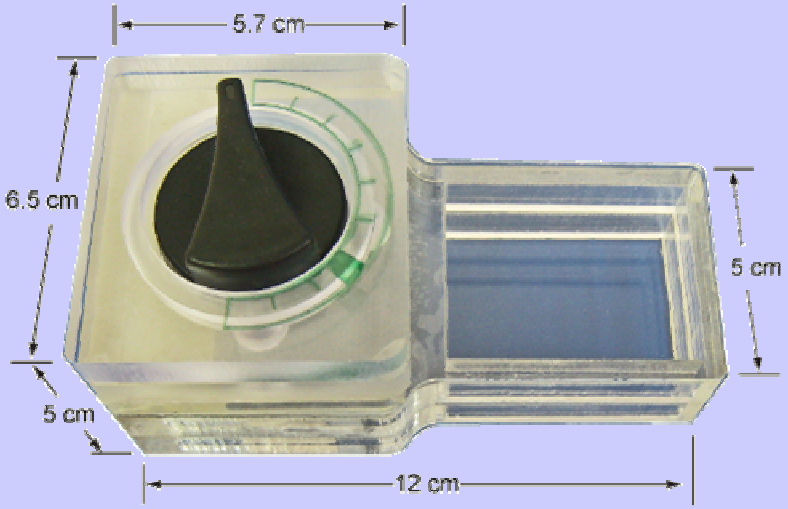
Liu *et al.*, Lab Chip 2009, 9:768-776

Pouch Cassette and Actuator









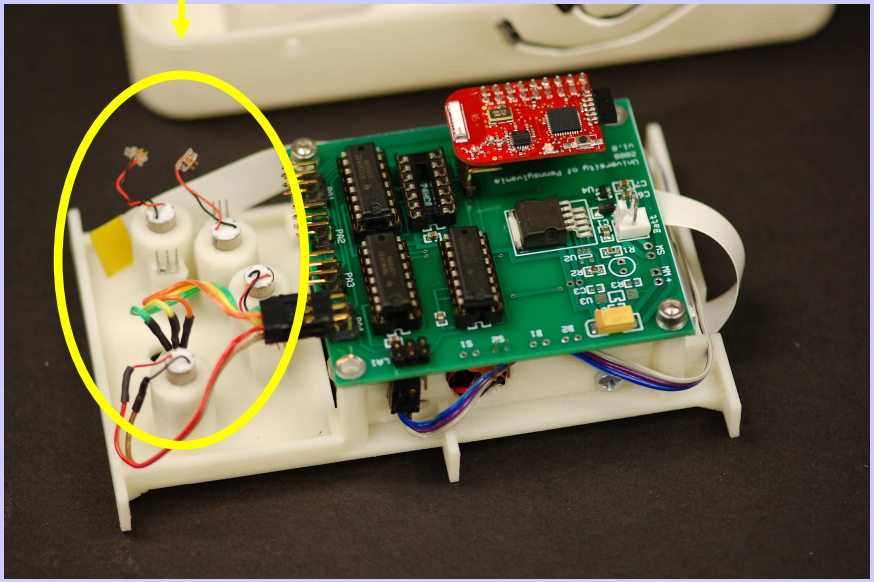
UCP readers

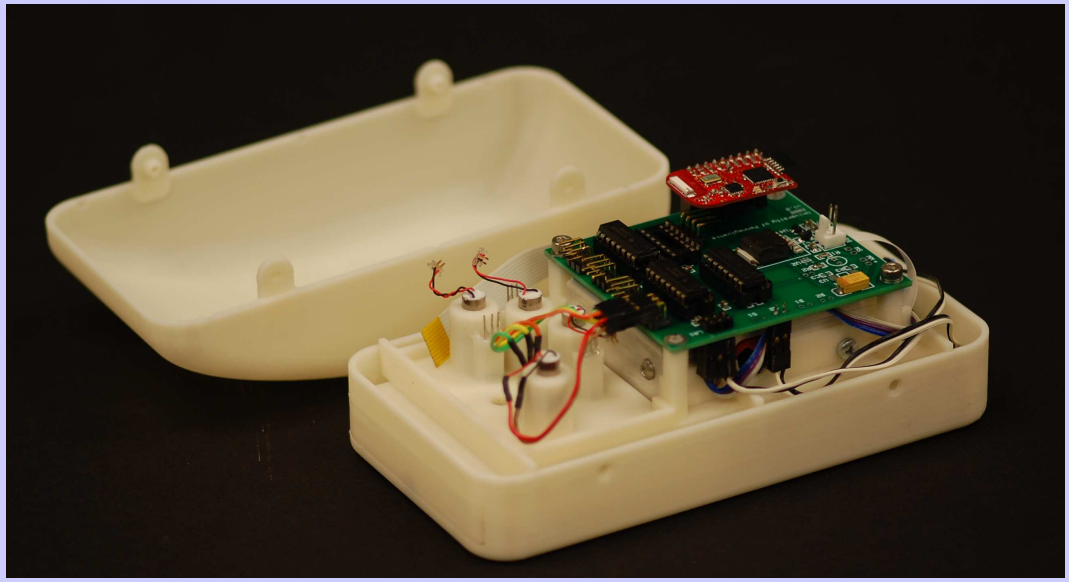
ESE
Handheld reader; size of mobile phone

90 gram confocal optoelectronic sensor.
Size comparison to a EURO coin

Electronic actuated pouch chips

Electronically controlled 'pins' to depress pouches





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UCP readers

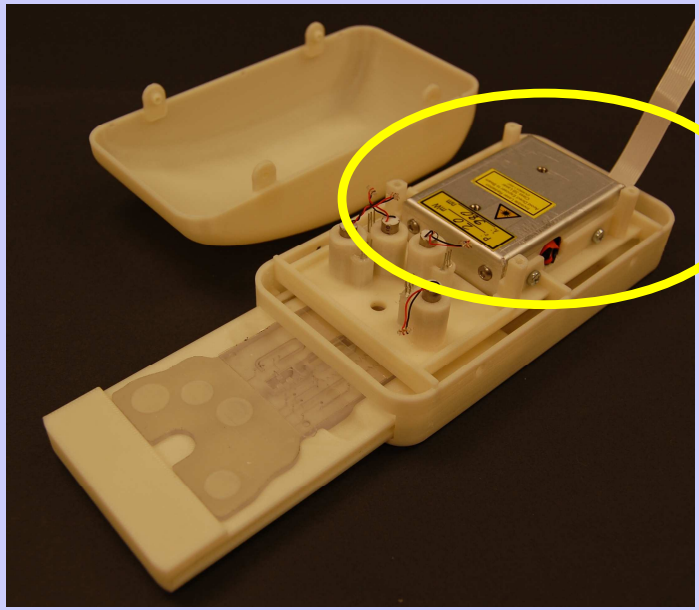
Modified Packard

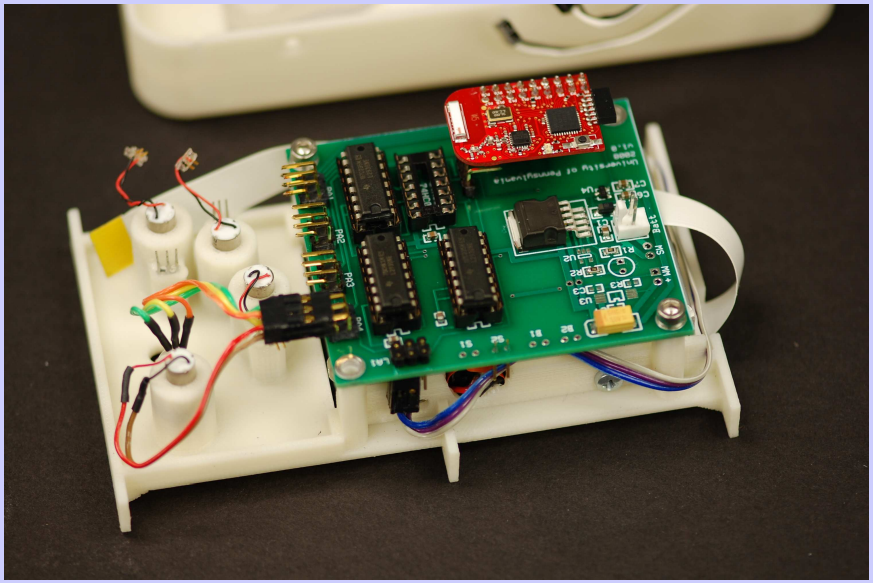
***UP*link**

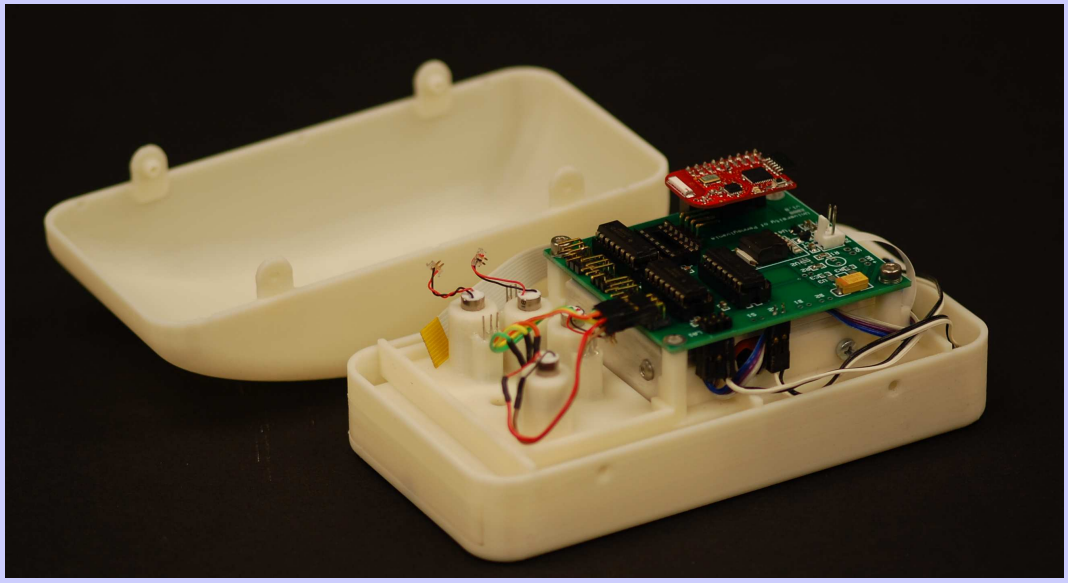
Quant

ESE Q-Scan
Handheld
580 gramms
Battery operated
18, 16, 4.5 cm (L,W,H)

Integrated system: chip and UCP reader







Bottlenecks and the future

Sampling: too complex

Lateral flow strips: OK, but old technology

UCP reporter, OK, but magnetic read out?

Microfluidics: OK, but nanofluidics…?

PCR in shoe boxes? Too big, other rapid amplification assays at ambient temperature: small disposable cartridges

Introduction for clinical use: within 3-5 years

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Haim Bau
Michael Mauk

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Pete Bourdelle

Paul Corstjens
Claudia de Dood
Karien Wiesmeijer
Christian Bertholle
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Hans Tanke

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Department of Infectious Diseases (Annemiek Geluk, Tom Ottenhof, Jaap van Dissel)