INSIDE INNOVATION

Corso di Biotecnologie applicate
A.A. 2024-2025

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Alifax Srl

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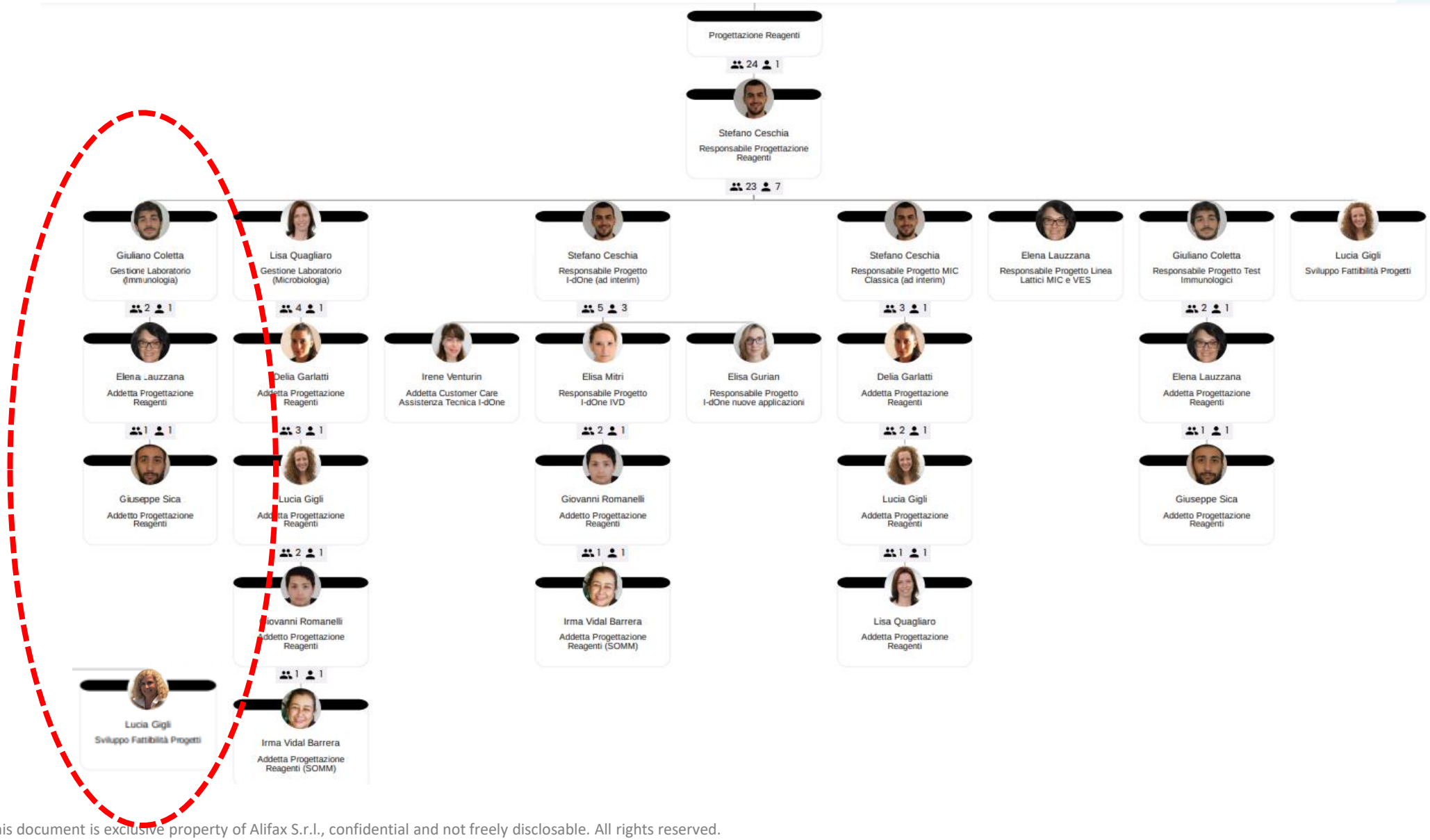
UFFICIO PROGETTAZIONE REAGENTI

INSIDE INNOVATION

1. Definizione di Immunoassay
2. Sviluppo di un Immunoassay
    a) ELISA
    b) CLIA
    c) LFA
    d) Cenni su Ottimizzazione
    e) Tecnologie emergenti
3. Case studies

UFFICIO PROGETTAZIONE REAGENTI

INSIDE INNOVATION



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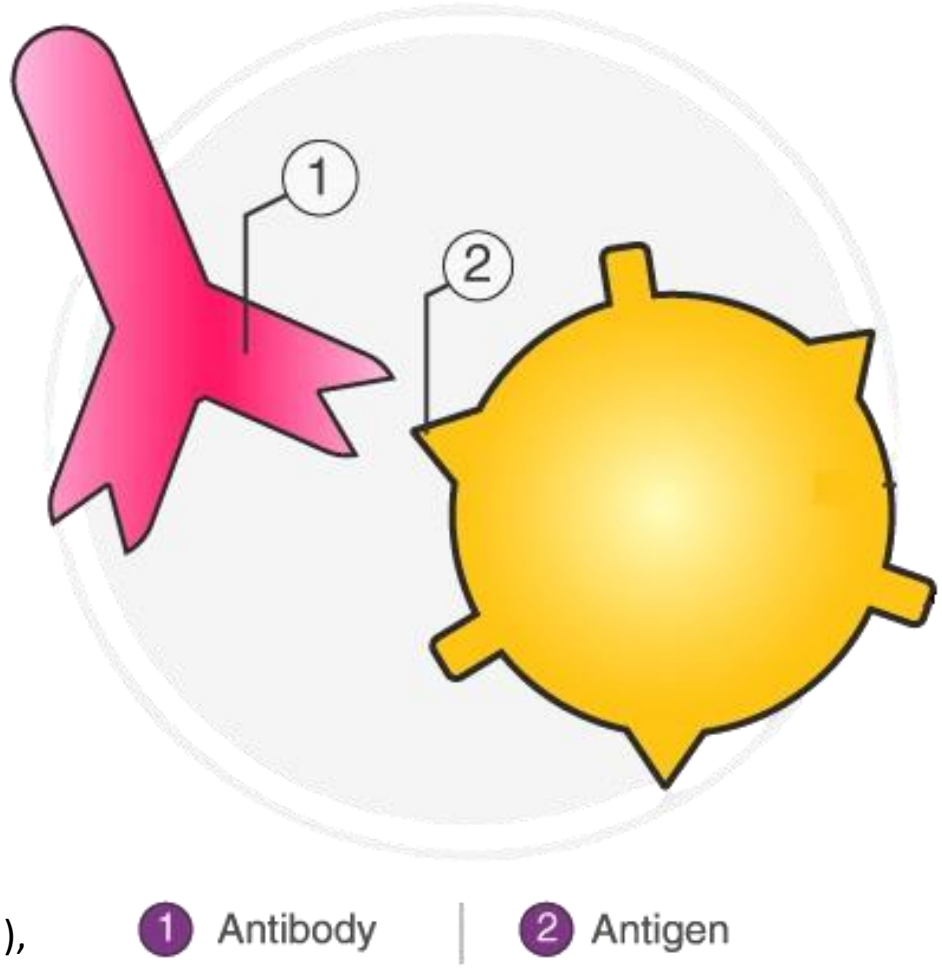
IMMUNOASSAY - DEFINIZIONE

Il saggio immunologico è un metodo bio analitico altamente selettivo che misura la presenza o la concentrazione di un analita in soluzione utilizzando un anticorpo o un antigene come parte sensibile.

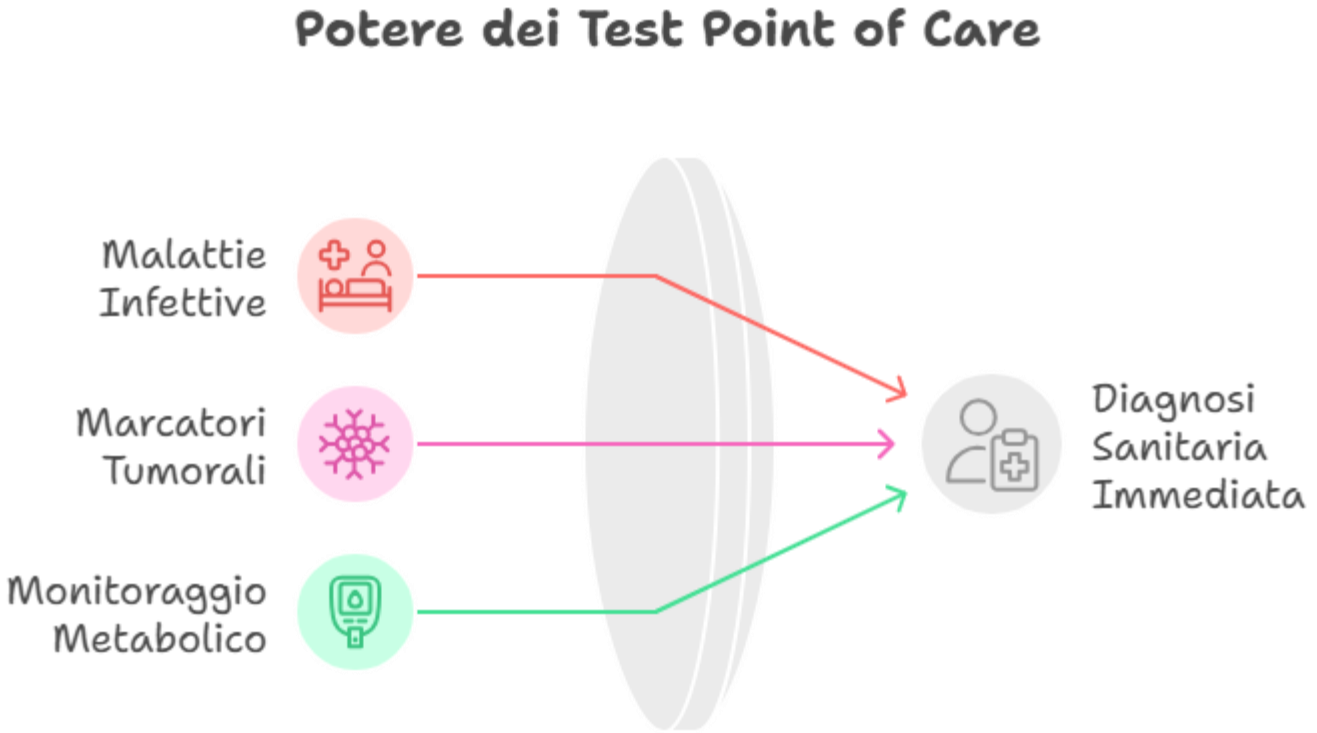
È basato sull'immunoreazione anticorpo-antigene e può raggiungere alta specificità e sensibilità attraverso l'amplificazione del segnale



R.Dorsey, G.Emmett, H.Salem, Chapter 27 - Ricin, Editor(s): Ramesh C. Gupta, Handbook of Toxicology of Chemical Warfare Agents (Second Edition), Academic Press,
2015, Pages 347-360, ISBN 9780128001592, https://doi.org/10.1016/B978-0-12-800159-2.00027-0.

POCT - DEFINIZIONE

I test point-of-care (POCT) sono test clinici di laboratorio condotti vicino al luogo di cura del paziente, dove vengono effettuate le cure o i trattamenti.
I POCT consentono di ottenere rapidamente i risultati dei test, in modo da poter attuare un trattamento appropriato, migliorando i risultati clinici o economici rispetto ai test di laboratorio.
Il POCT può essere eseguito da diversi operatori sanitari e, in alcuni casi, anche dai pazienti stessi.

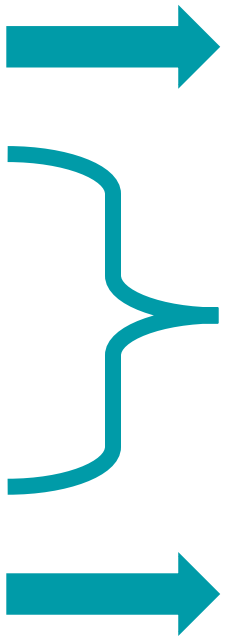


Larkins, M. C., & Thombare, A. (2023, May 29). *Point-of-Care testing*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK592387/

POCT - VANTAGGI

SVILUPPO DI UN IMMUNOASSAY

1. Identificazione del bisogno clinico
2. Selezione del biomarcatore
3. Sviluppo del test
4. Ottimizzazione e validazione
5. Studi clinici e analisi statistica
6. Regolamentazione e approvazione



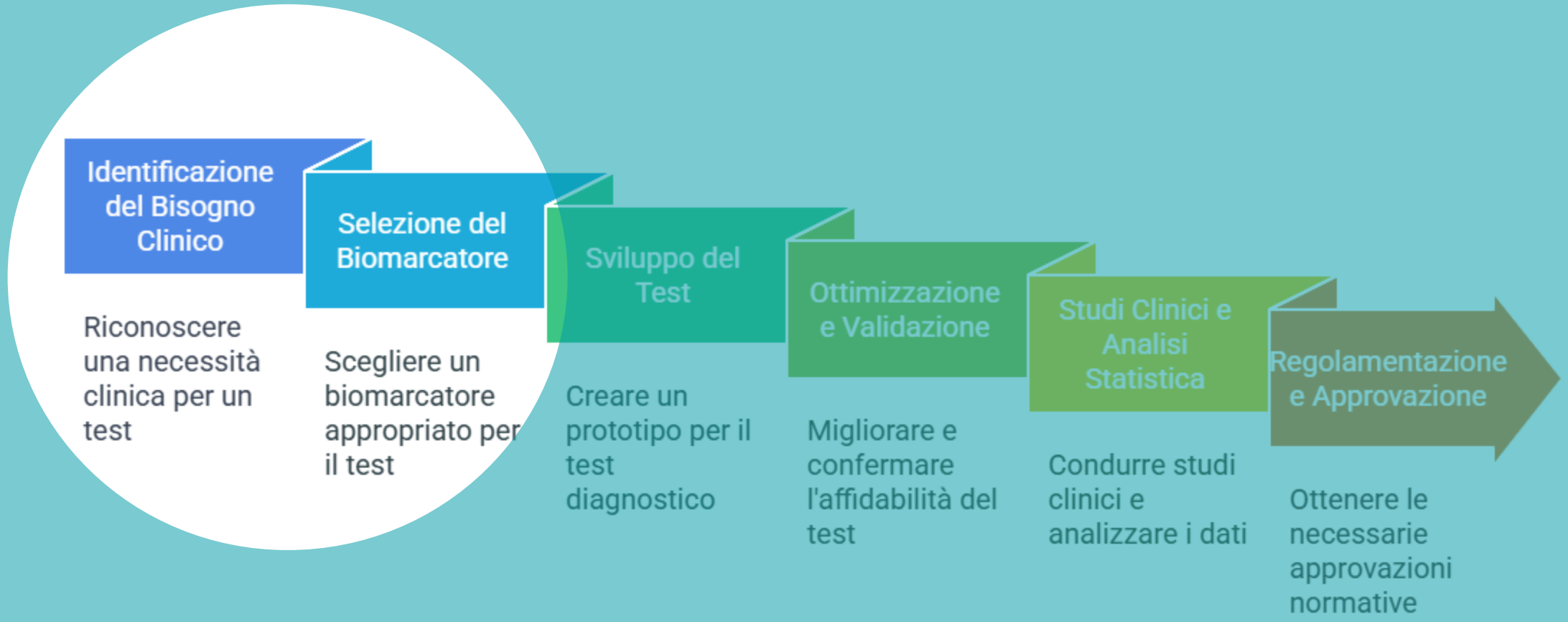
Stakeholdes
Direzione

Progettazione Reagenti

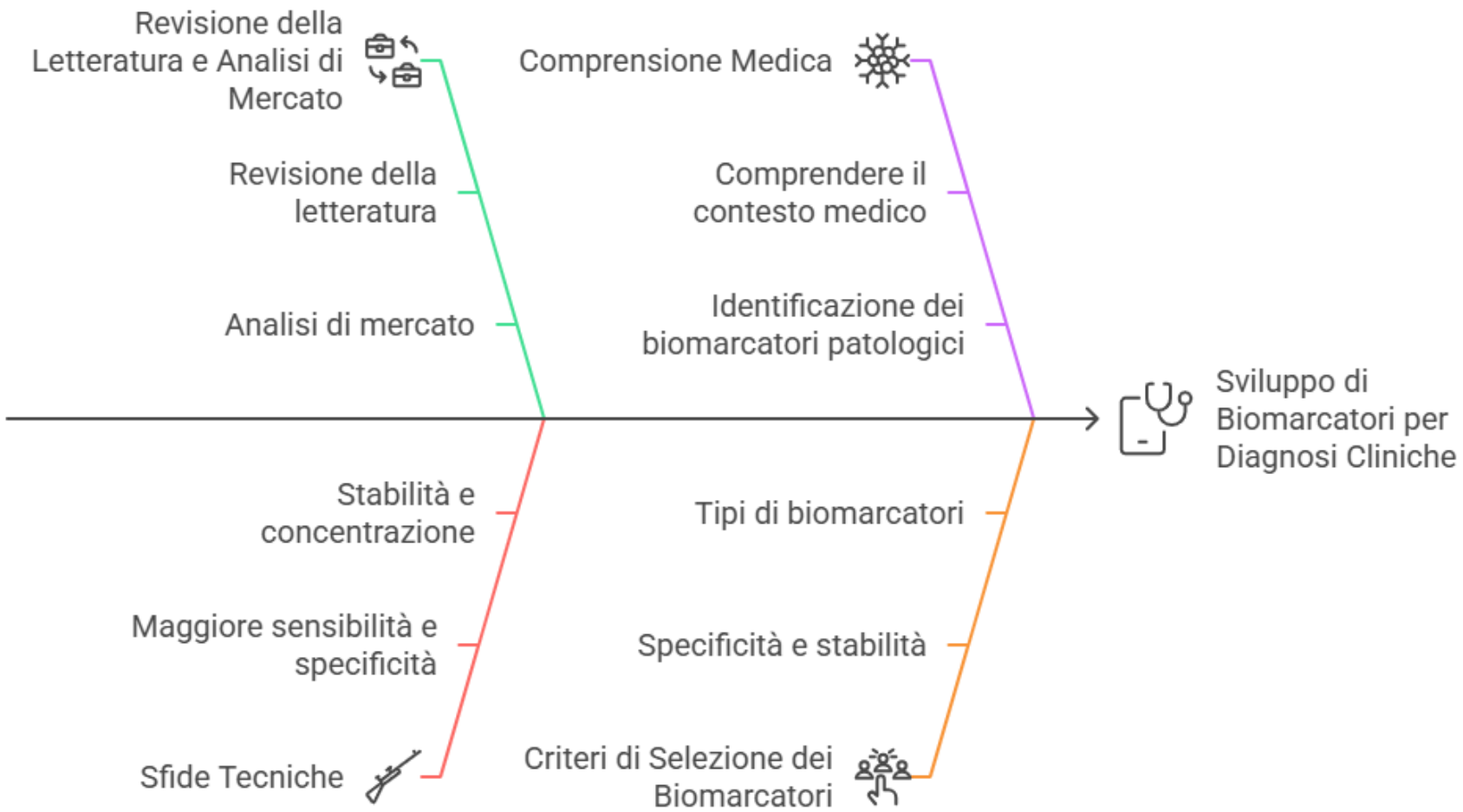
Progettazione Reagenti
Design Compliance
Regolatorio Tecnico

SVILUPPO DI UN IMMUNOASSAY

INSIDE INNOVATION



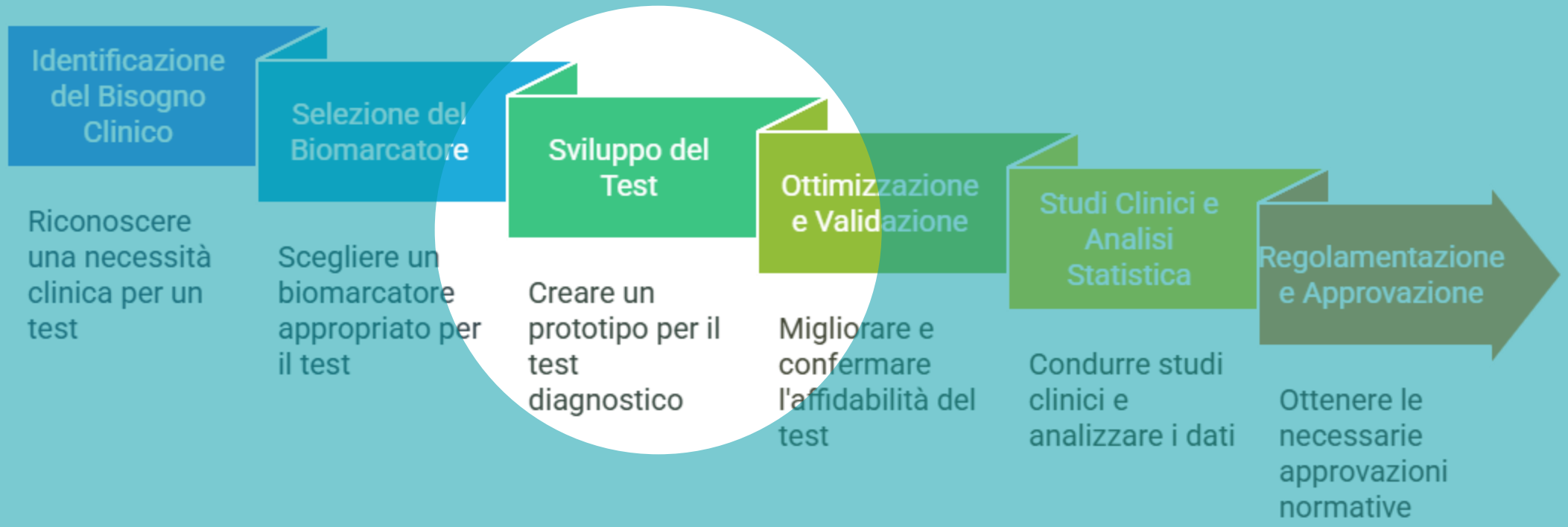
1. IDENTIFICAZIONE DEL BISOGNO CLINICO
2. SELEZIONE DEL BIOMARCATORE



Made with Napkin

SVILUPPO DI UN IMMUNOASSAY

INSIDE INNOVATION



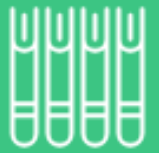
3. SVILUPPO DEL TEST

Selezione della Tecnologia

Scelta della tecnologia diagnostica più adeguata.

Design del Formato del Test

Progettazione del formato del test come quantitativo, qualitativo o semi-quantitativo.

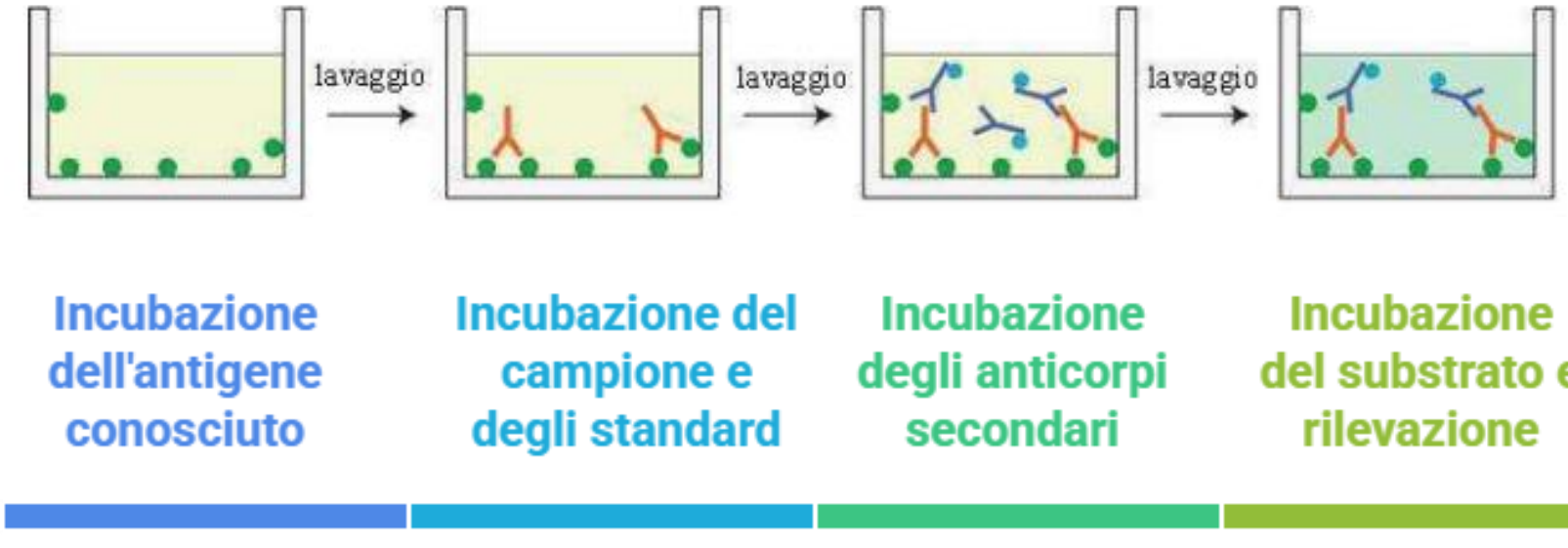


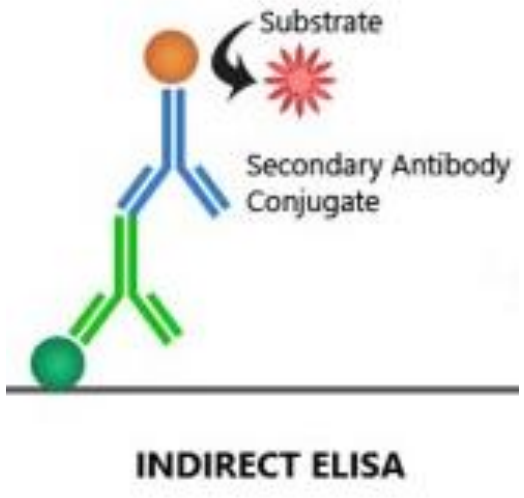
Selezione della Coppia di Anticorpi

Selezione della coppia di anticorpi e ottimizzazione dei reagenti.

TIPI DI IMMUNOASSAY - ELISA

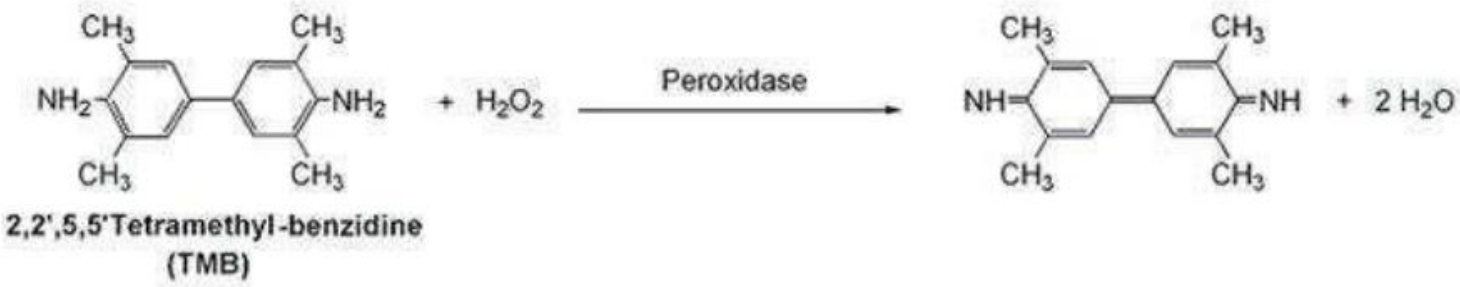
La tecnica di ELISA (Enzyme-Linked Immuno Assay) è principalmente utilizzato in immunologia al fine di rilevare e/o dosare la presenza di proteine, anticorpi o antigeni nel campione



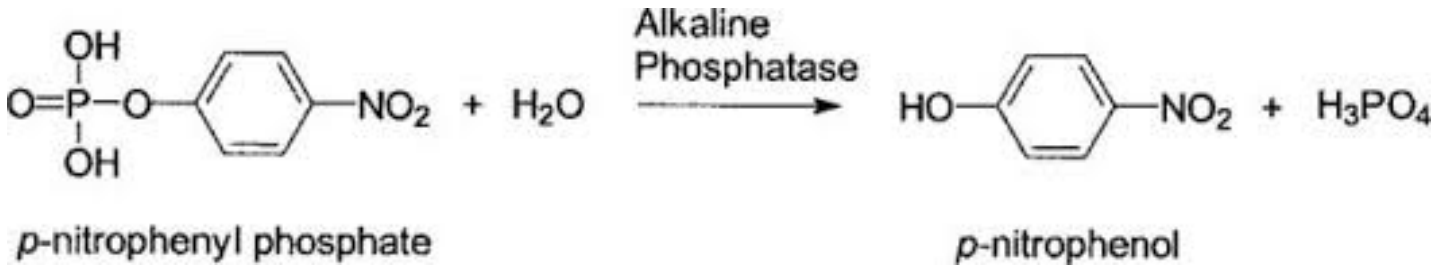


ELISA - SUBSTRATI

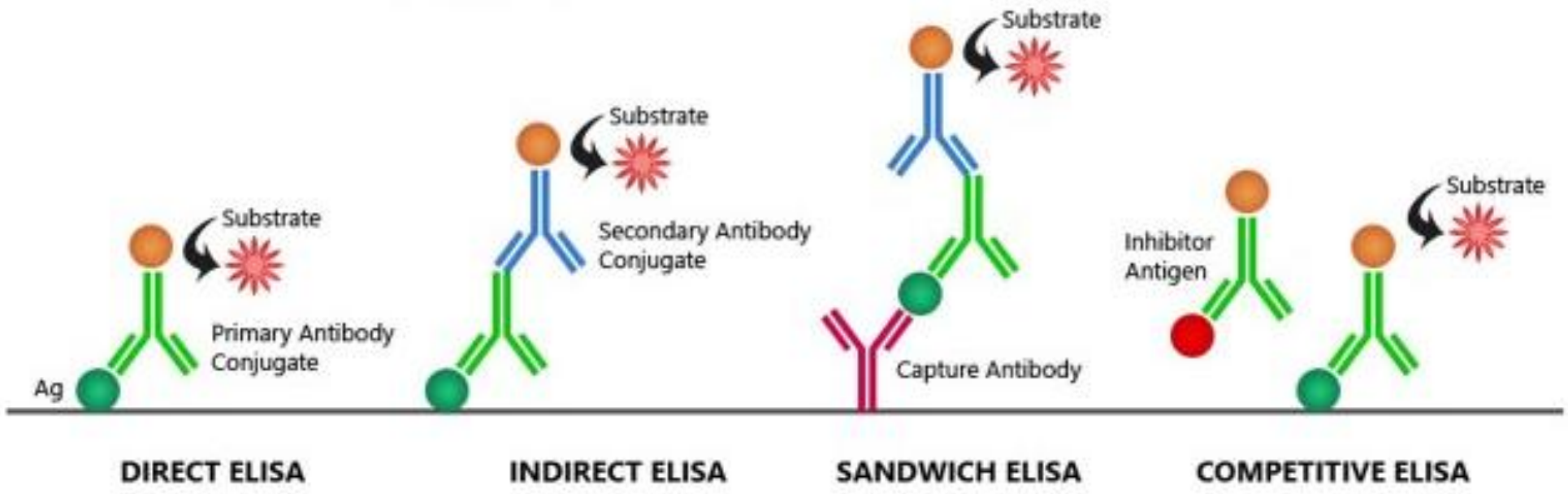
La perossidasi è un enzima di tipo ossidasi, che permette la degradazione dei perossidi. L'ossidazione di diversi substrati permette di ottenere un composto cromogenico come prodotto finale.



per anticorpi coniugati alla fosfatasi alcalina si usa in genere il substrato p-nitrophenylphosphate (pNPP), che sviluppa un intenso colore giallo misurabile a 410 nm.



ELISA - TIPOLOGIE

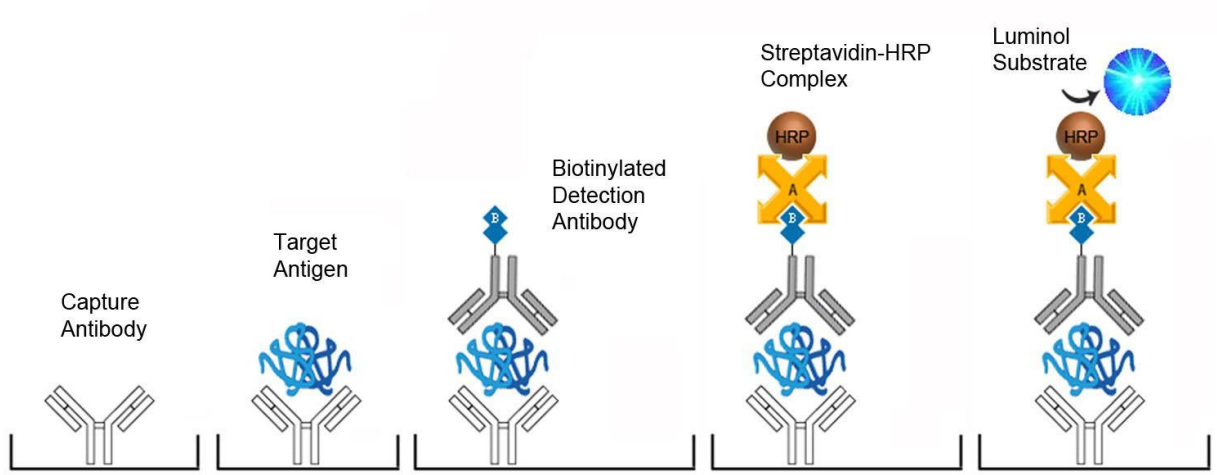


ELISA - TIPOLOGIE

	Direct ELISA	Indirect ELISA	Sandwich ELISA	Competitive ELISA
Advantages	Quick due to few protocol steps	Higher sensitivity than direct ELISA due to using a secondary antibody for detection More flexible than direct ELISA as it eliminates the need to source conjugated primary antibodies	High specificity due to using a matched antibody pair for capture and detection	Highly flexible as it can be based on direct, indirect or sandwich ELISA
Disadvantages	Requires a conjugated primary antibody Has limited sensitivity as there is no amplification from a secondary antibody May suffer from high background since antigen immobilization is not specific	More protocol steps than direct ELISA Risk of cross-reactivity between the secondary antibody and the target analyte	More protocol steps than direct and indirect ELISA Identifying a matched antibody pair can be costly and time-consuming	More difficult to develop and optimize than the other ELISA formats
Potential applications	Analyzing the immune response to an antigen (e.g., during allergy testing or in response to an infection)	Measuring the antibody concentration in a sample (e.g., in response to a vaccination)	Detecting an analyte in complex samples, such as cell or tissue lysates	Useful when only one antibody is available for the target antigen or when detecting small analytes (e.g., banned drug substances

TIPI DI IMMUNOASSAY - CLIA

La chemiluminescenza (CL) è l'emissione di luce dovuta a una reazione chimica, spesso biochimica, dove un prodotto della reazione torna allo stato fondamentale emettendo energia sotto forma di fotoni. L'immunodosaggio a chemiluminescenza (CLIA) è un test che combina la tecnica della chemiluminescenza con le reazioni immunochimiche. Analogamente ad altri immunodosaggi marcati (RIA, FIA, ELISA), il saggio CLIA utilizza probes chimici che possono generare un'emissione di luce attraverso una reazione chimica per marcare l'anticorpo.



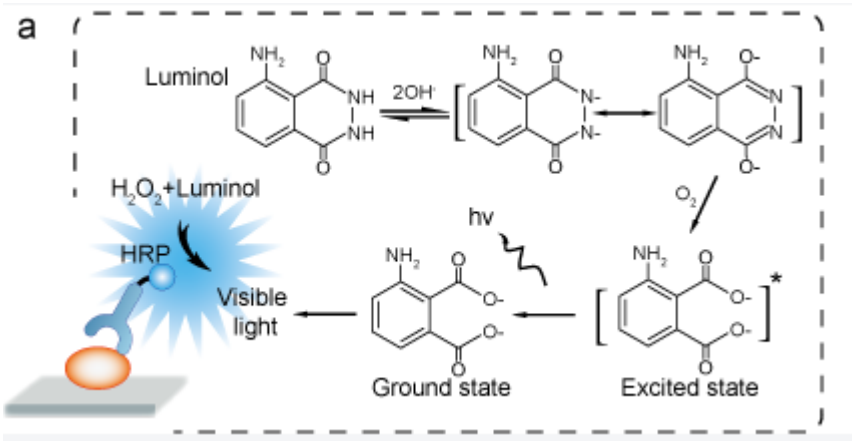
CLIA ENZIMATICA

Questo tipo di chemiluminescenza utilizza enzimi per marcare gli anticorpi.

Gli enzimi più utilizzati sono la perossidasi di rafano (HRP) e la fosfatasi alcalina (AP), ciascuno con i propri substrati luminescenti.

La RP catalizza la decomposizione del luminolo in presenza di perossido per produrre un intermedio allo stato eccitato. Al decadimento dell'intermedio singoletto viene emessa luce visibile (massimo a 425 nm).

L'AMPPD è un derivato di substrati di 1, 2-diossetano. Presenta un meccanismo simile di chemiluminescenza. Al momento della scissione enzimatica del gruppo fosfato, questo composto si destabilizza e si decompone attraverso un anione intermedio, AMPD, moderatamente stabile. La lunghezza d'onda della massima emissione luminosa è di 470 .



CLIA ACRIDINIO

Questo tipo di chemiluminescenza non utilizza enzimi per marcare gli anticorpi, ma l'eccitazione dell'estere di acridinio

L'esposizione dell'estere di acridinio a una soluzione alcalina di perossido di idrogeno provoca l'emissione di luce.

I composti marcati con acridinio hanno un'intensità di chemiluminescenza 100 volte superiore rispetto a quelli marcati con luminol, e gli esteri di acridinio hanno la caratteristica di non perdere l'efficienza della luminescenza anche dopo il legame con l'antigene o l'anticorpo.

Inoltre, la chimica è semplice e non richiede l'intervento di alcun enzima.

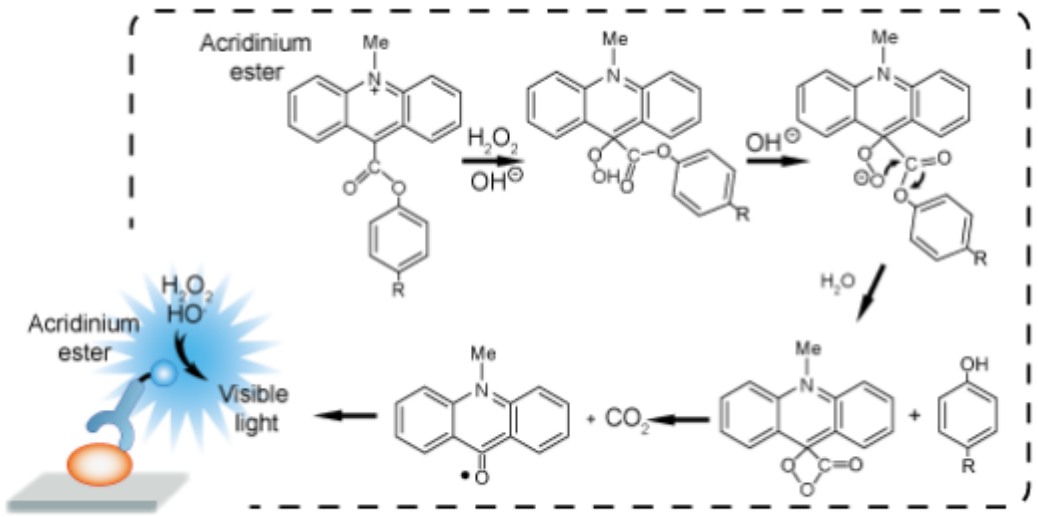


Figure 1. Mechanism of acridinium ester mediated chemiluminescence.

CLIA REDOX

Un altro sistema di chemiluminescenza degno di nota - perché il reagente è rigenerato e quindi può essere riciclato - è quello redox.

Questo sistema utilizza la tris-bipiridina di rutenio (BPY) come label, prevede la reazione di $Ru(bpy)_3$3+ e Ru(bpy)3+ per produrre uno stato eccitato di $Ru(bpy)_3$2+, una specie che decade allo stato fondamentale emettendo un'emissione arancione a 620 nm.

$Ru(bpy)_3$3+ e Ru(bpy)3+ possono essere elettrogenerati da $Ru(bpy)_3$2+ mediante riduzione a circa -1,3 V e ossidazione a circa + 1,3 V.

Questo sistema è dedicato all'elettrochimiluminescenza con altissima sensibilità e specificità.

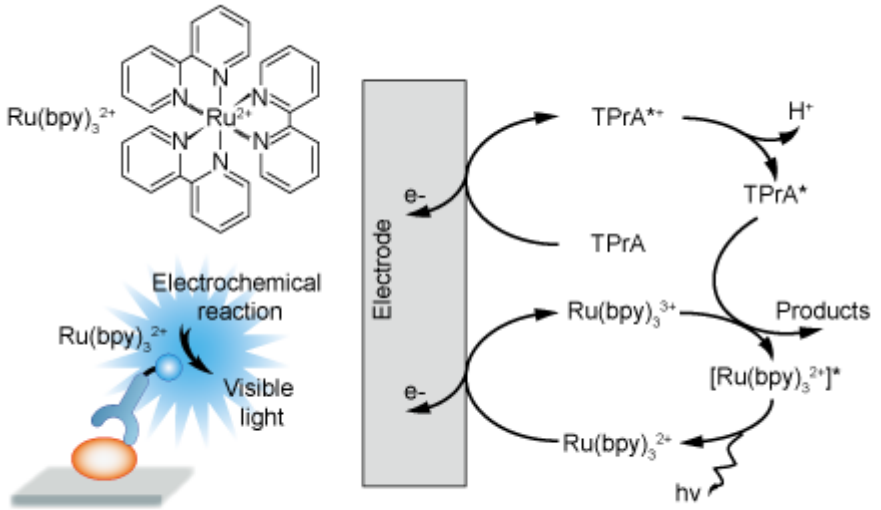
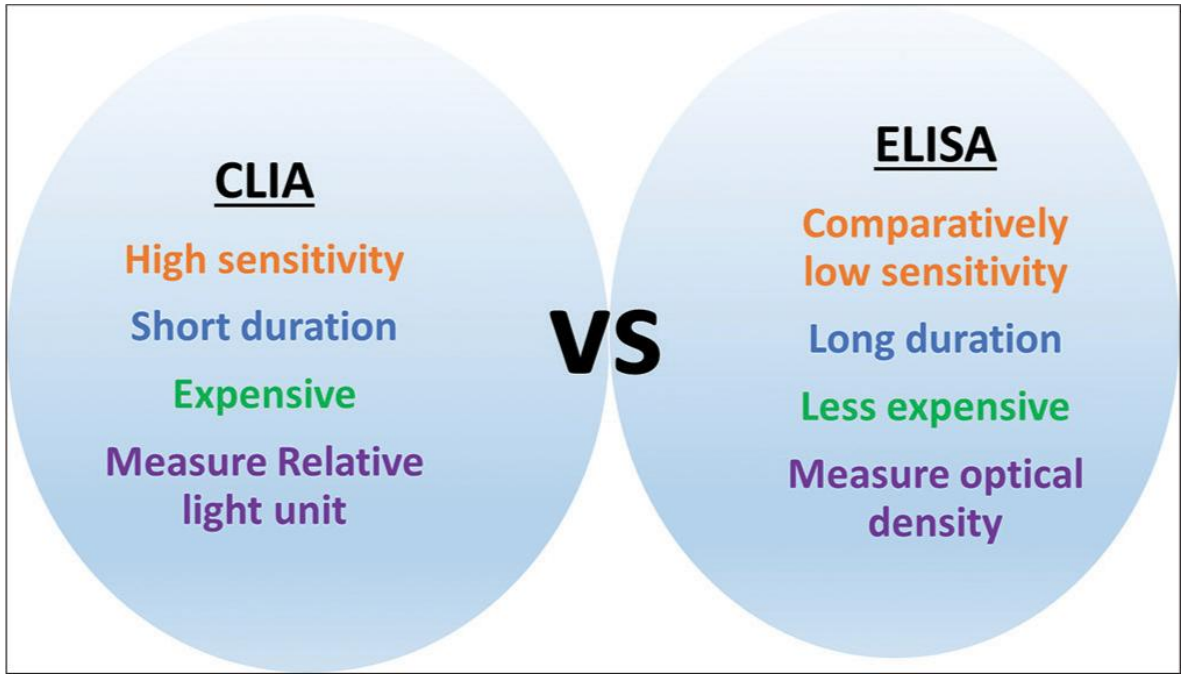


Figure 3. Mechanism of $Ru(bpy)_3$ electrochemiluminescence system.

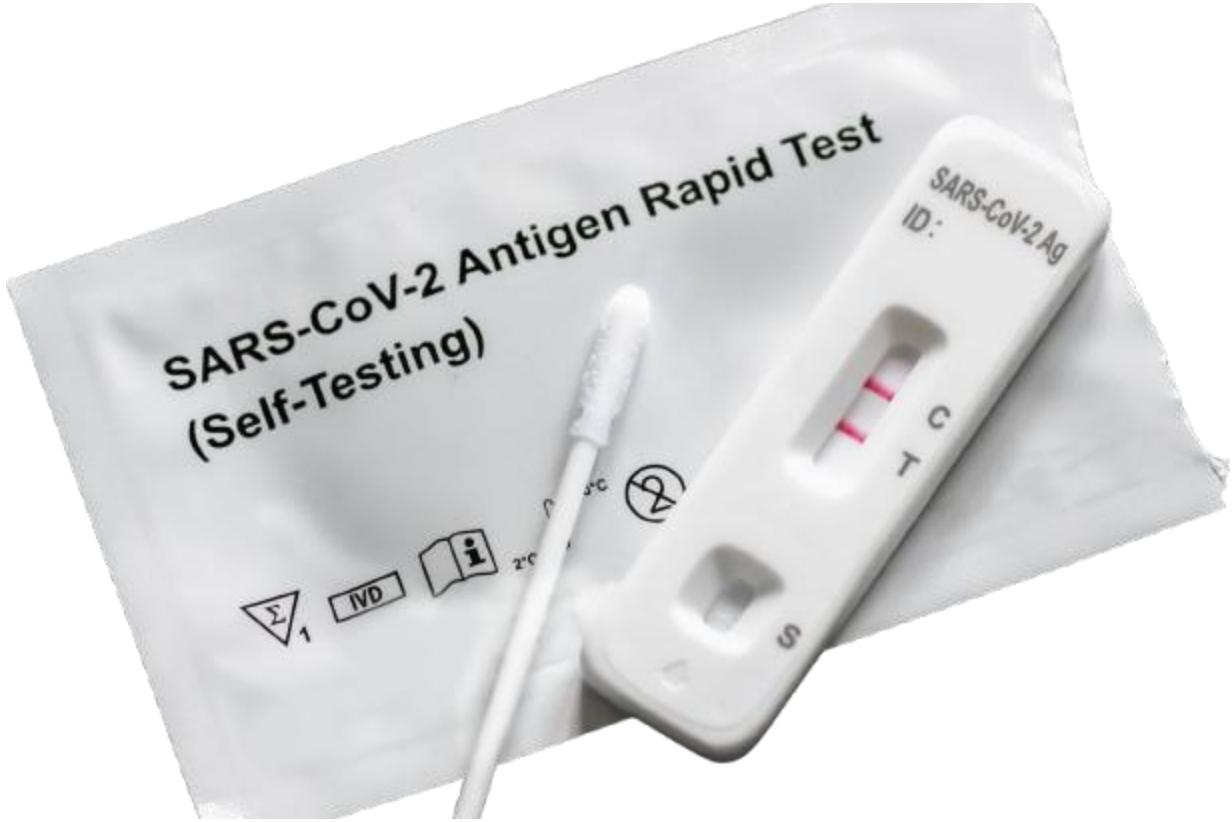
CLIA vs ELISA



TIPI DI IMMUNOASSAY - LFA

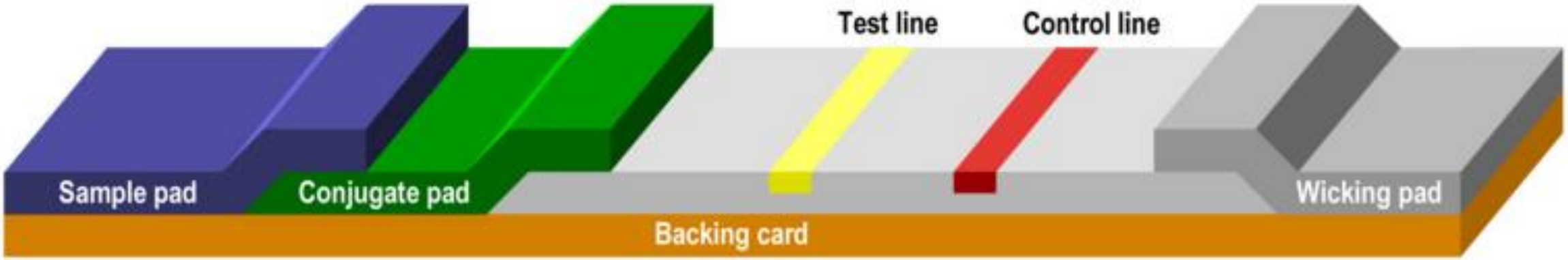
I test rapidi (LFA) sono dispositivi diagnostici rapidi ed economici che possono essere utilizzati per analizzare una sostanza target (analita) in un campione.

- Lifetime elevata
- Storage a RT
- Basso costo
- Applicazioni POCT



LFA - COMPONENTI

INSIDE INNOVATION



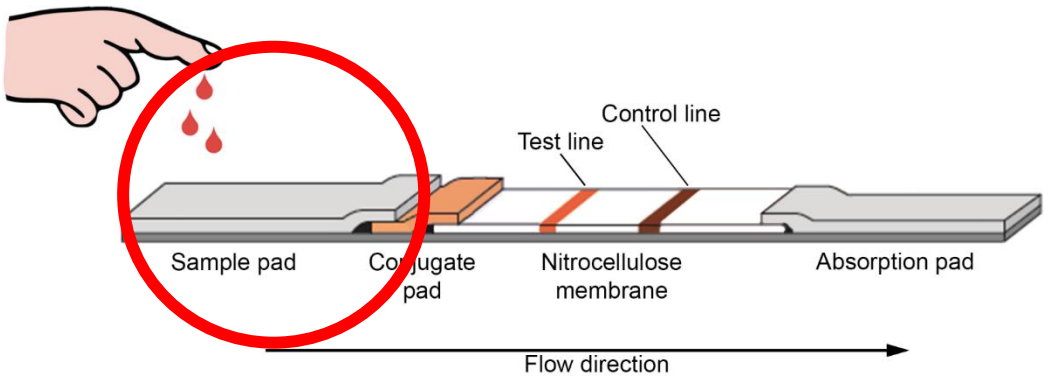
LFA – SAMPLE PAD

Key points:

- Cellulose fiber
- Low protein binding
- Pretreating sample
- Pretreated with proteins (BSA-aspecificity) detergents (Tween/Triton fluency) salt (viscosity)
- Suitable with different specimen

Utility:

- Preteating the sample
- Minimize aspecificity
- Reduce viscosity
- Uniform pH



LFA – CONJUGATE PAD

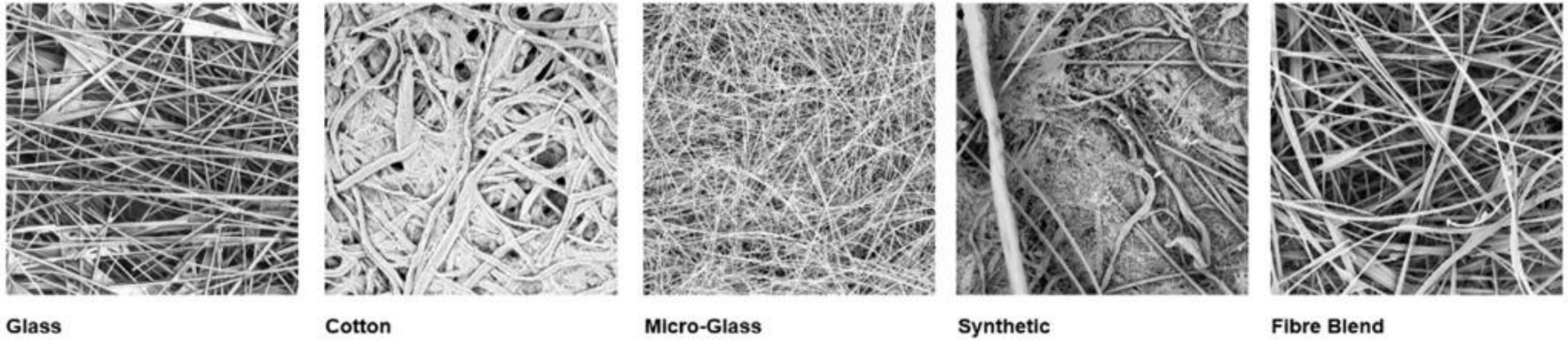
INSIDE INNOVATION

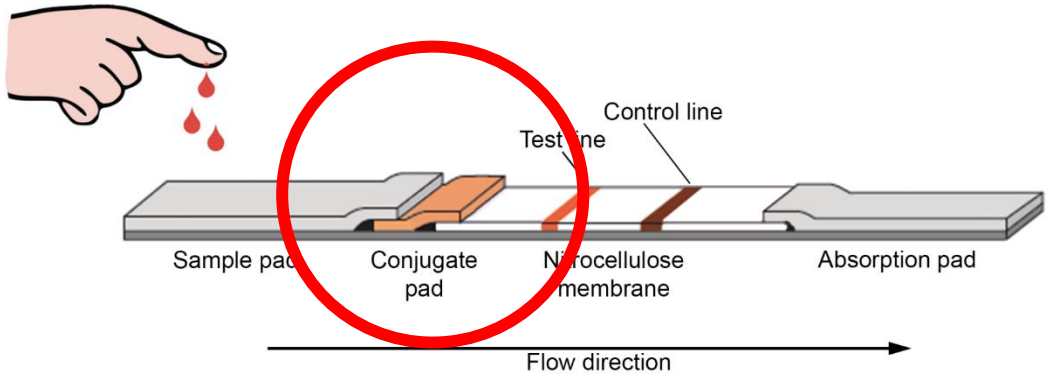
Key points:

- Glass fiber
- Low nonpecific binding
- Pretreated with protein, sugars and detergents

Utility:

- Drying and rewetting detection reagent

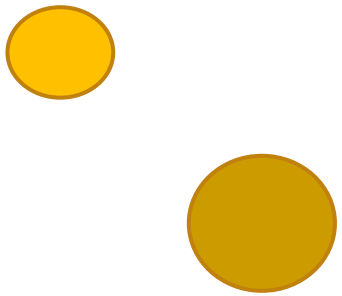




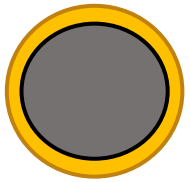
LFA - PROBES

Il reagente di rivelazione utilizzato in un immunodosaggio a flusso laterale consiste tipicamente in anticorpi coniugati a particelle colorate o fluorescenti.
L'efficacia complessiva dell'immunodosaggio a flusso laterale dipende, tra l'altro, dalla qualità degli anticorpi e della sostanza di rilevazione.

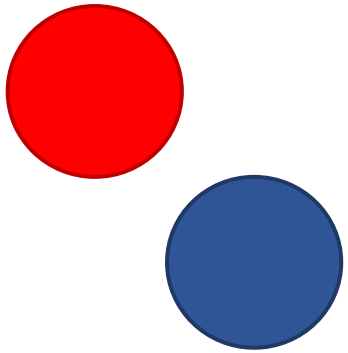
Gold Nanoparticles



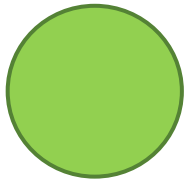
Gold Nanoshells



Latex Microspheres

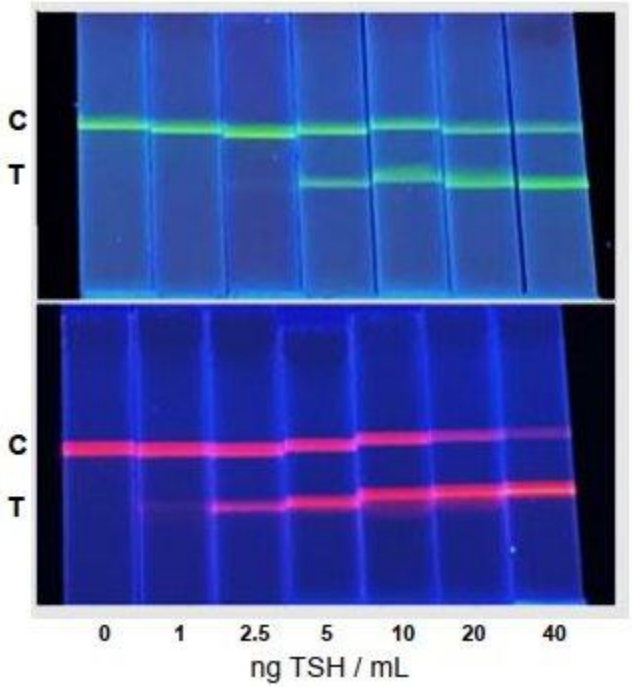


Fluorescent Microspheres

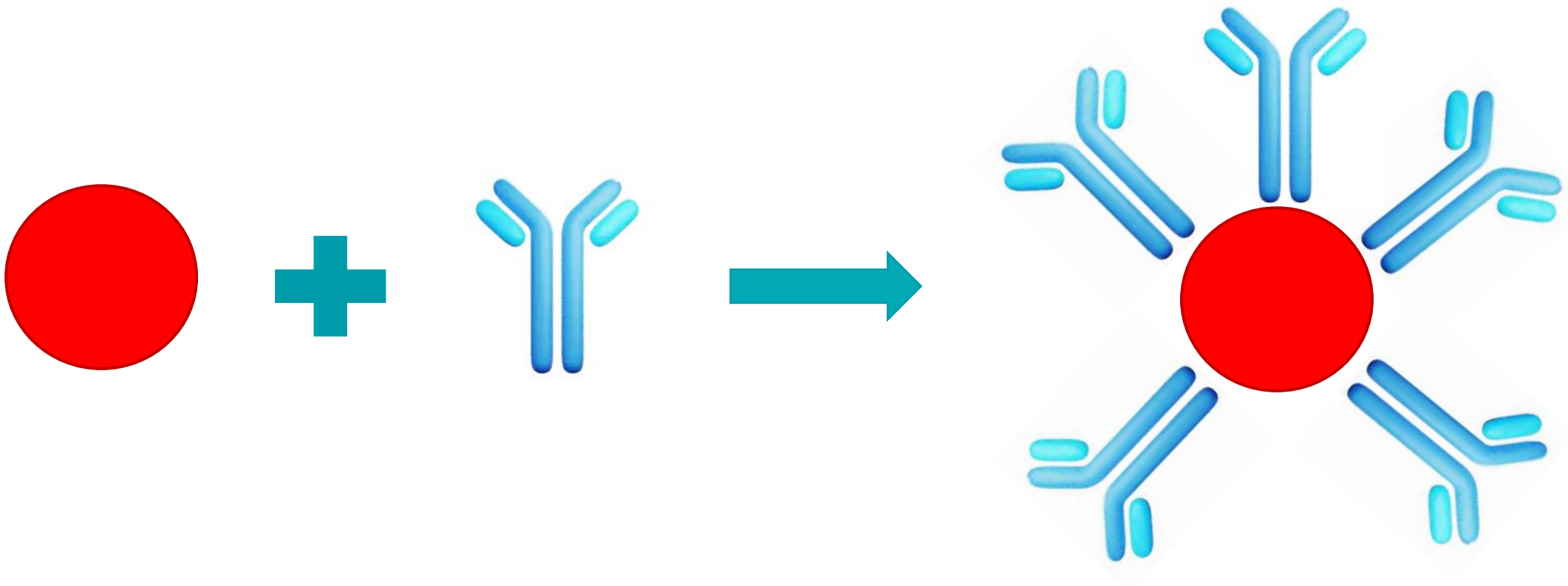


LFA - PROBES

Property	Colloidal Gold	Latex Microspheres
Colors	Deep red	Full color spectrum, black
Size Range	15 to 80 nm	0.1 to 0.7 μm
Typical Size	40 nm	0.4 μm
Antibody Binding	• Passive adsorption • Covalent attachment	• Covalent attachment
Membrane Compatibility	All flow rates	Faster flowing membranes
Aggregation	Detectable by color change – shift from deep red to blue	No visual evidence; must be checked under a microscope
Availability	• Commercial Suppliers • "Make your own"	Commercial Suppliers
Optimal Shape	Spherical	Spherical

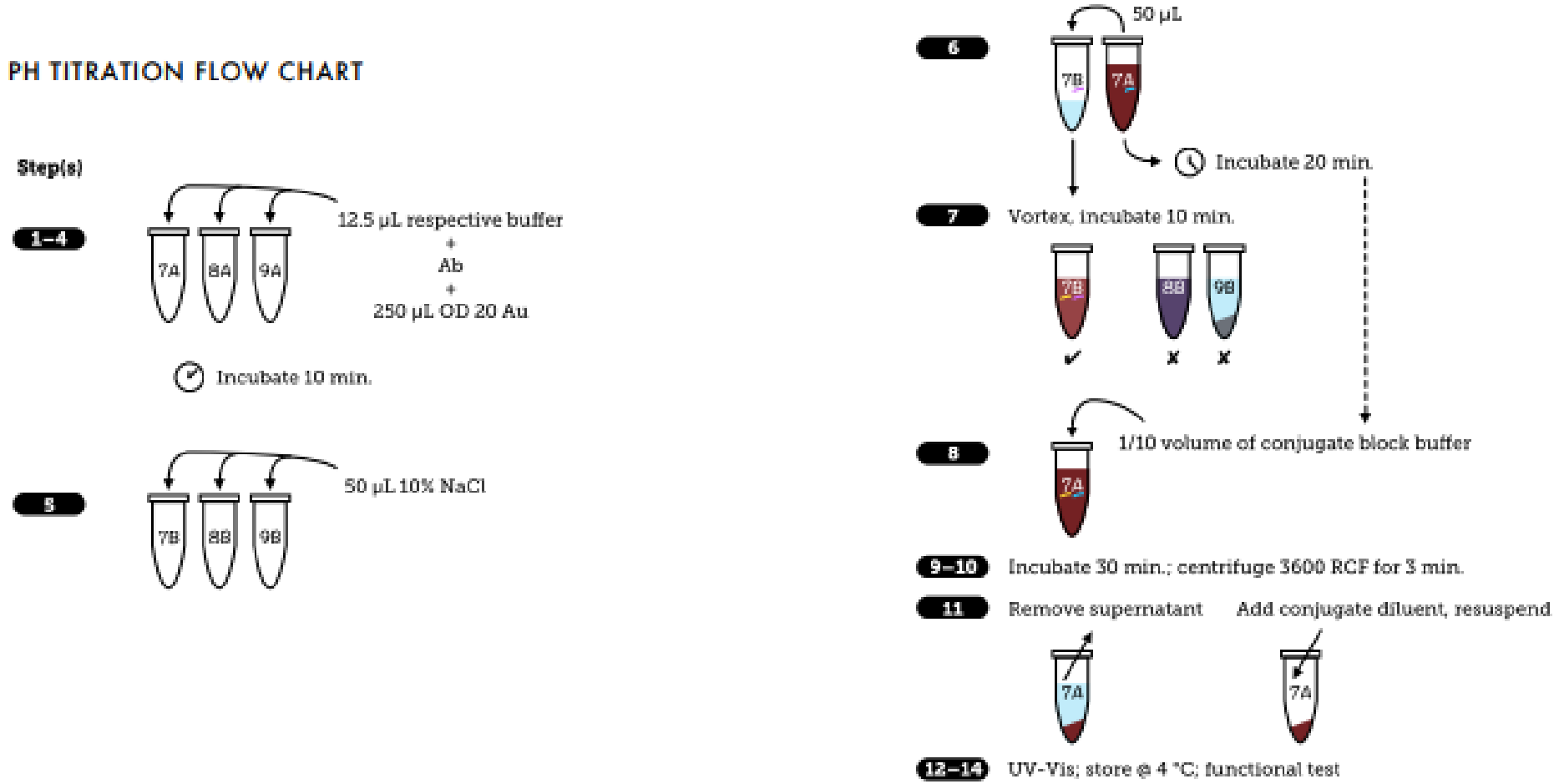


LFA - CONIUGATI

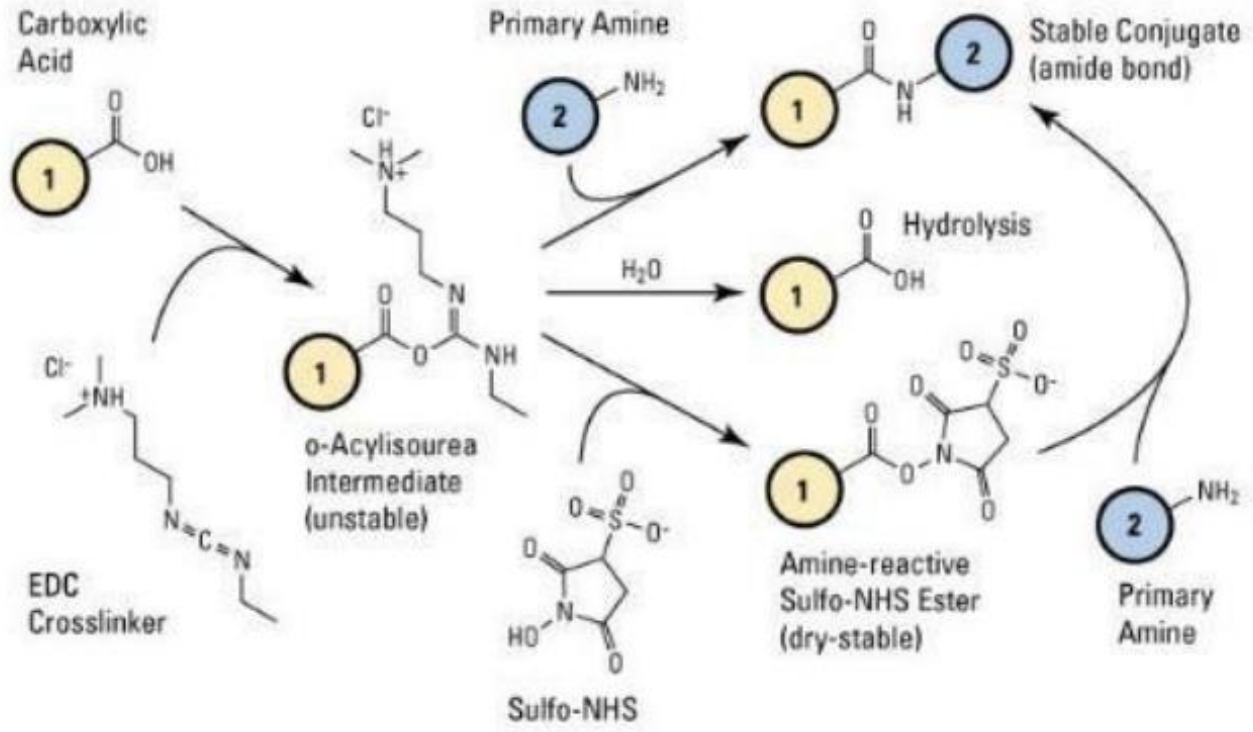


LFA – CONIUGAZIONE PASSIVA

INSIDE INNOVATION



LFA – CONIUGAZIONE COVALENTE

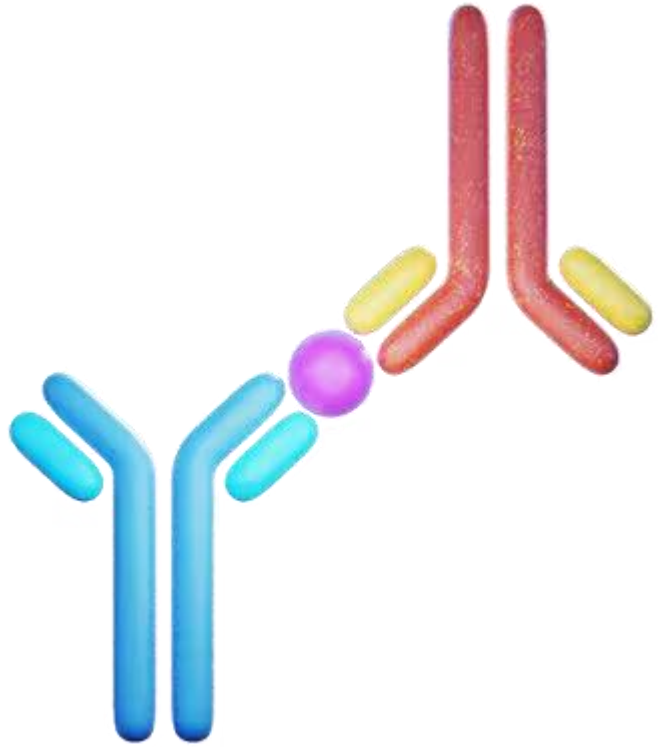


CONIUGAZIONE PASSIVA			CONIUGAZIONE COVALENTE	
VANTAGGI	SVANTAGGI		VANTAGGI	SVANTAGGI
Metodo tradizionale di preparazione dei coniugati	Il processo va ottimizzato (pH) per ogni anticorpo		Anticorpi interi, frammenti di anticorpi e piccole molecole possono essere legati in modo irreversibile	Richiede un passaggio aggiuntivo per attivare la superficie -COOH con la chimica EDC/Sulfo-NHS
Pochissima chimica coinvolta	Sono necessari anticorpi interi o ligandi tiolati		Generalmente è necessario meno anticorpo rispetto all'adsorbimento passivo	Meno anticorpi sulla superficie rispetto ai metodi di accoppiamento passivo per una dimensione di particella equivalente
Metodo di sintesi delle nanoparticelle altamente riproducibile con bassa varianza da lotto a lotto	Le proteine non sono legate in modo covalente alla superficie delle particelle e possono desorbire		Si forma un legame ammidico stabile e irreversibile	
Costo contenuto	Rischio di aggregazione se le condizioni non sono ottimizzate		Migliore controllo del rapporto anticorpo/particella	
	Il meccanismo di legame dipende dall'anticorpo		Stabile in un intervallo di pH più ampio e con un elevato carico di tensioattivi e detergenti	

LFA – SELEZIONE DI ANTICORPI

	Ab #1 on particle	Ab #2 on particle	Ab #3 on particle	Ab #4 on particle
Ab #1 on strip		X	X	X
Ab #2 on strip	X		X	X
Ab #3 on strip	X	X		X
Ab #4 on strip	X	X	X	

Table 1: Antibody (Ab) evaluation matrix.



LFA – SELEZIONE DI ANTICORPI

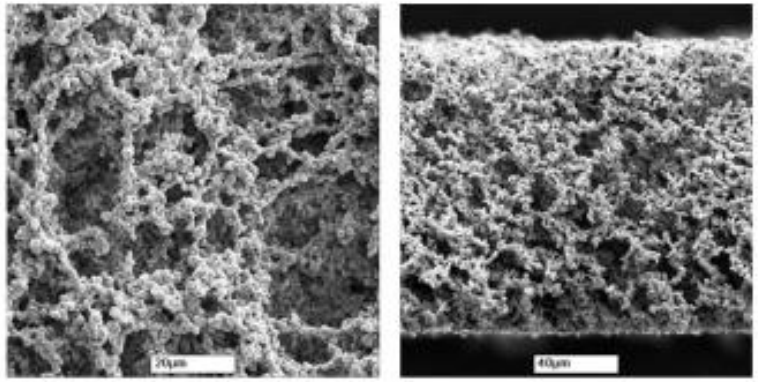
	POLYCLONAL	MONOCLONAL
ADVANTAGES	• Inexpensive to produce • High affinity • Will recognize multiple epitopes (generally provides more robust detection) • Polyclonal antibodies are often the preferred choice for detection of denatured proteins • Higher tolerance for differences in antigen (i.e. glycosylation of proteins)	• Constant and renewable source, all batches will be identical • Less background relative to polyclonal antibodies • Homogeneity is very high, ensuring reproducible results • Specificity of monoclonal antibodies make them extremely efficient for binding of antigen within a mixture of related molecules
DISADVANTAGES	• Prone to batch to batch variability • They produce large amounts of non-specific antibodies which can result in a background signal in some applications • Multiple epitopes make it important to check for cross reactivity	• Monoclonal antibodies may be too specific (e.g. less likely to detect across a range of species)

Table 2: Comparison of advantages and disadvantages of using polyclonal and monoclonal antibodies.

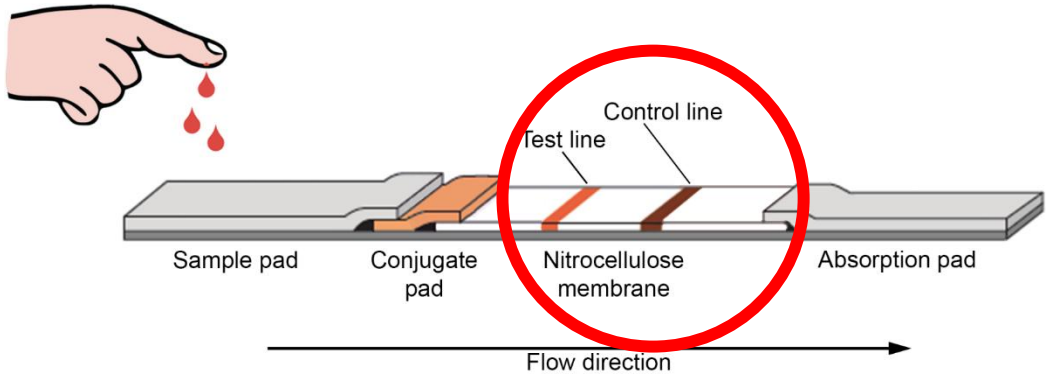
LFA – MEMBRANA IN NITROCELLULOSA

INSIDE INNOVATION

SEM

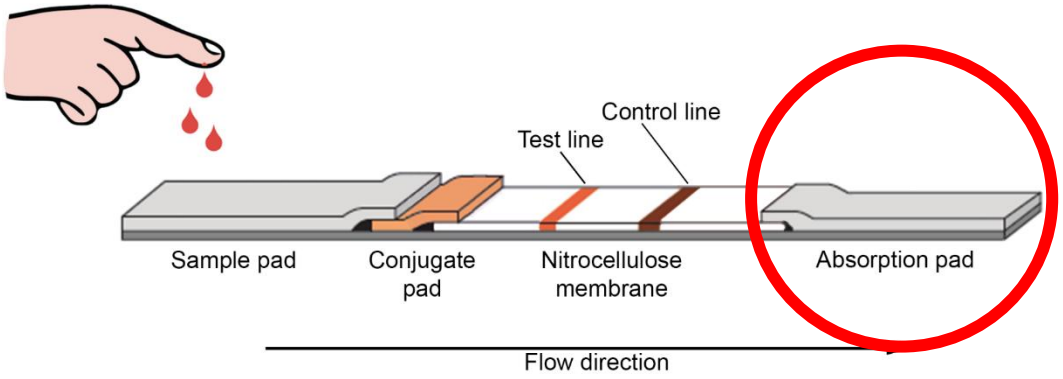


RELATIVE FLOW TIME	RELATIVE PORE SIZE	RELATIVE SENSITIVITY	EXAMPLES
FAST	LARGE	LOW	Millipore: HF 75, 90 Sartorius: CN 95 MDI: NC 15 µm Whatman/GE: AE 98, AE99
MEDIUM	MEDIUM	MEDIUM	Millipore: HF 120, 135 Satorius: CN 140, CN 150 MDI: NC 8 µm Whatman/GE: FF120 HP
SLOW	SMALL	HIGH	Millipore: HF 180 MDI: NC 5 µm Whatman/GE: FF170HP



LFA – WICKING PAD & CASSETTE

INSIDE INNOVATION



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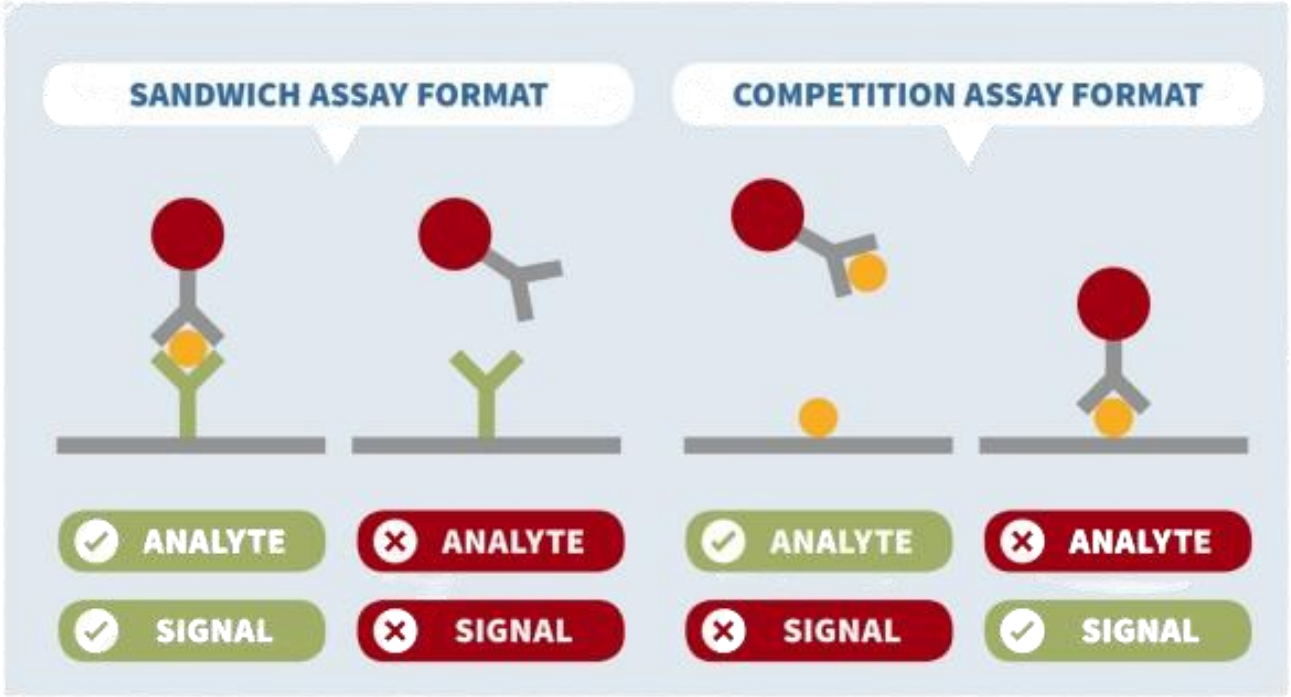
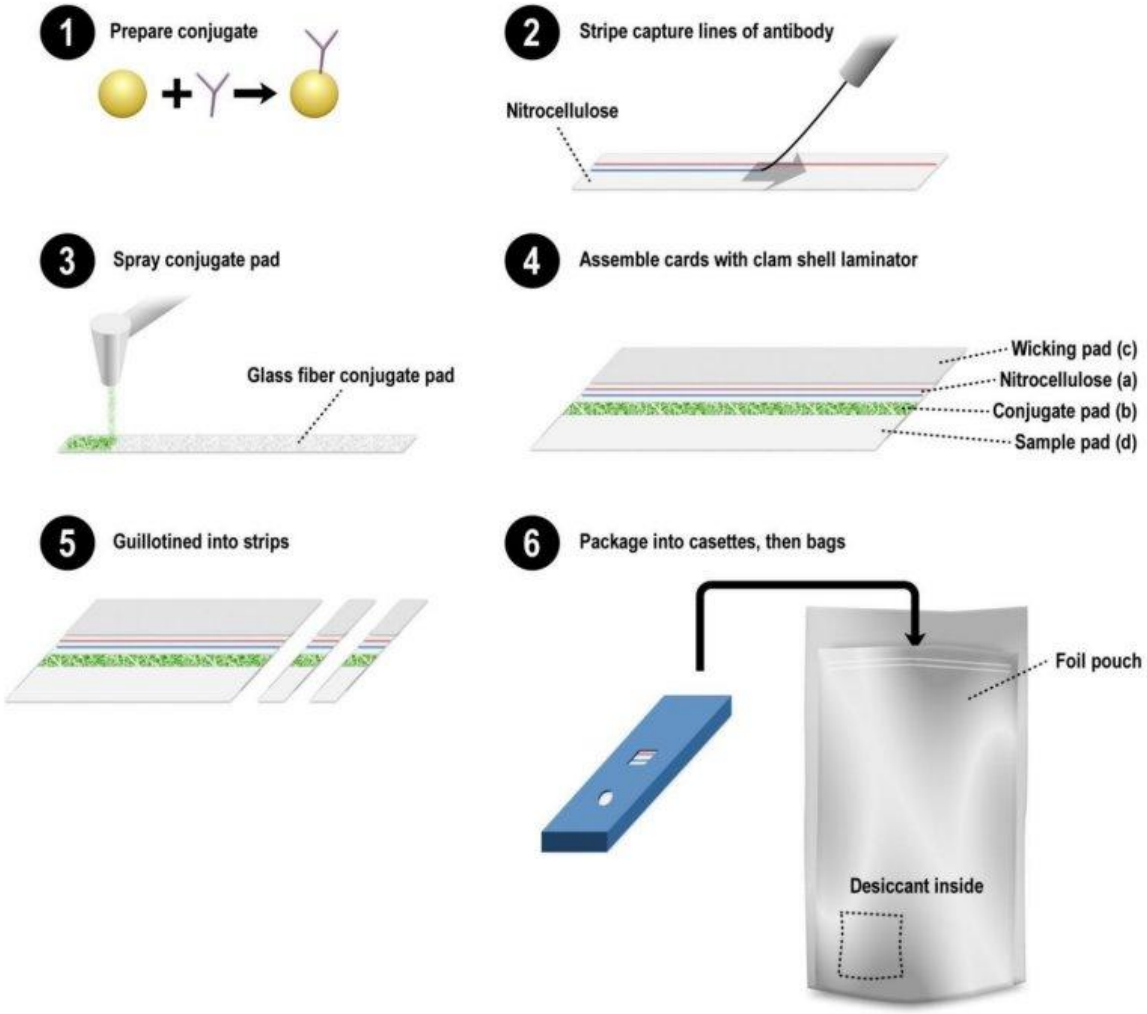
LFA - FORMATI



LFA - PRODUZIONE



CONFRONTO ELISA, LFA, CLIA

	ELISA	CLIA	LFA
Prestazioni (Se & Sp)			
Tempo di esecuzione			
Costi del test			
Operatività			
Possibilità di automazione			

SVILUPPO DI UN IMMUNOASSAY

INSIDE INNOVATION

OTTIMIZZAZIONE E VALIDAZIONE

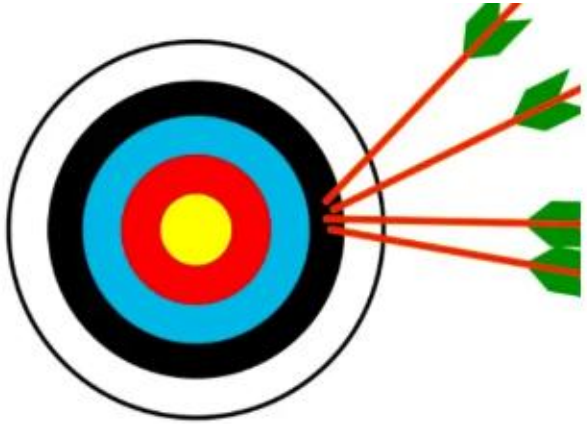
- Determinazione della sensibilità e specificità analitica
- Verifica della riproducibilità e robustezza
- Definizione dei parametri operativi (tempo di incubazione, temperatura, reagenti)
- Verifica della stabilità

OTTIMIZZAZIONE LFA

SENSIBILITÀ E SPECIFICITÀ ANALITICA

- **L'accuratezza** si riferisce alla capacità di uno strumento di indicare il vero valore di una misura.
- **La precisione** si riferisce alla capacità di indicare ripetutamente uno stesso valore di misura, indipendentemente dal fatto che corrisponda o meno al valore vero.

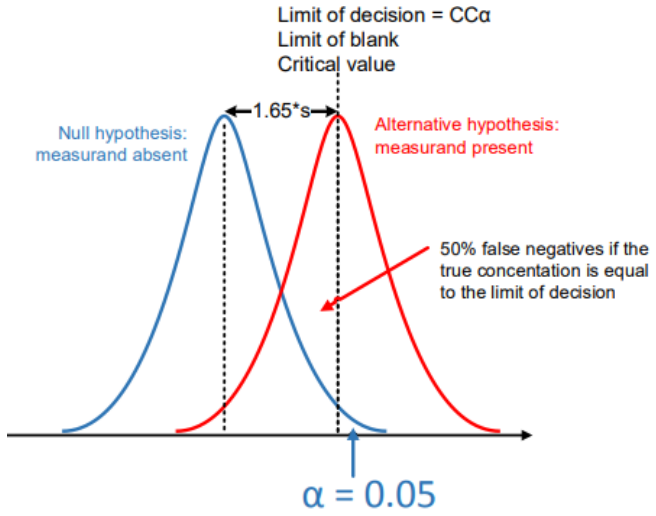
High Accuracy

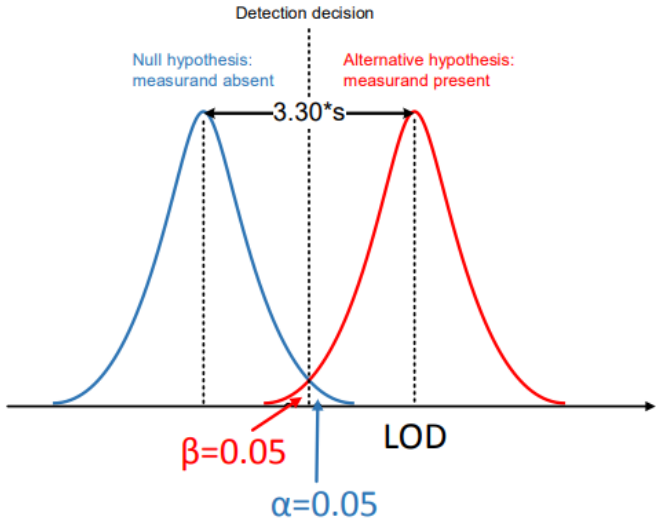


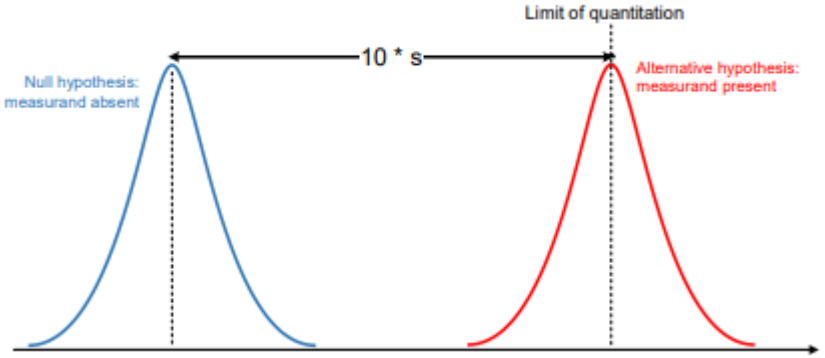
High Precision

LoB, LoD e LoQ

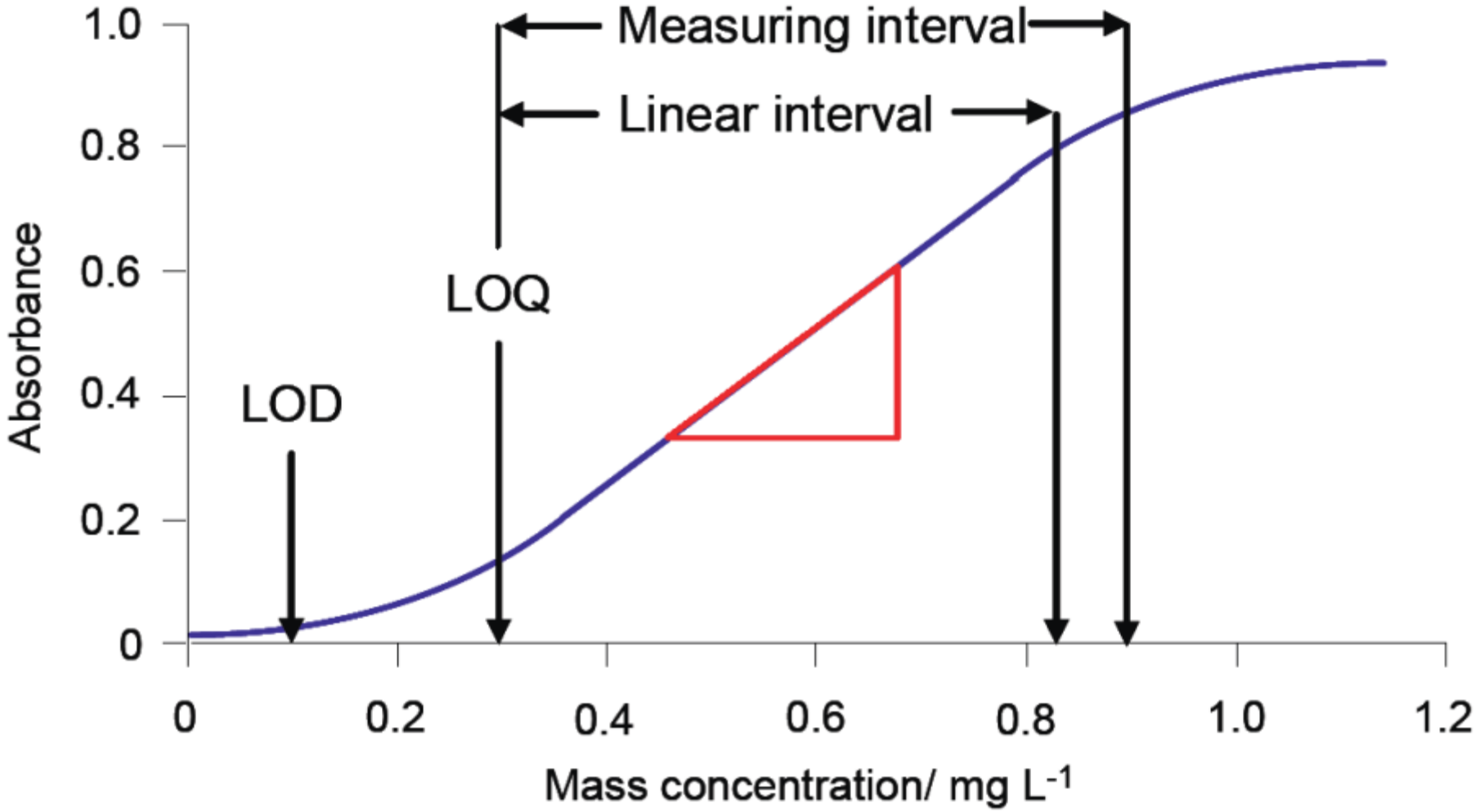
- Limit of Blank: è la concentrazione del misurando che è significativamente diversa da zero. Ovvero la più alta concentrazione apparente di un analita che si ha quando si fanno ripetizioni di una misura di bianco.
- Il Limit of Detection è la più bassa concentrazione di analita che può essere misurata ad uno specifico livello di confidenza.
- Il Limiti of Quantitation è la più bassa concentrazione di analita per cui sono accettabili le performance del metodo di misura



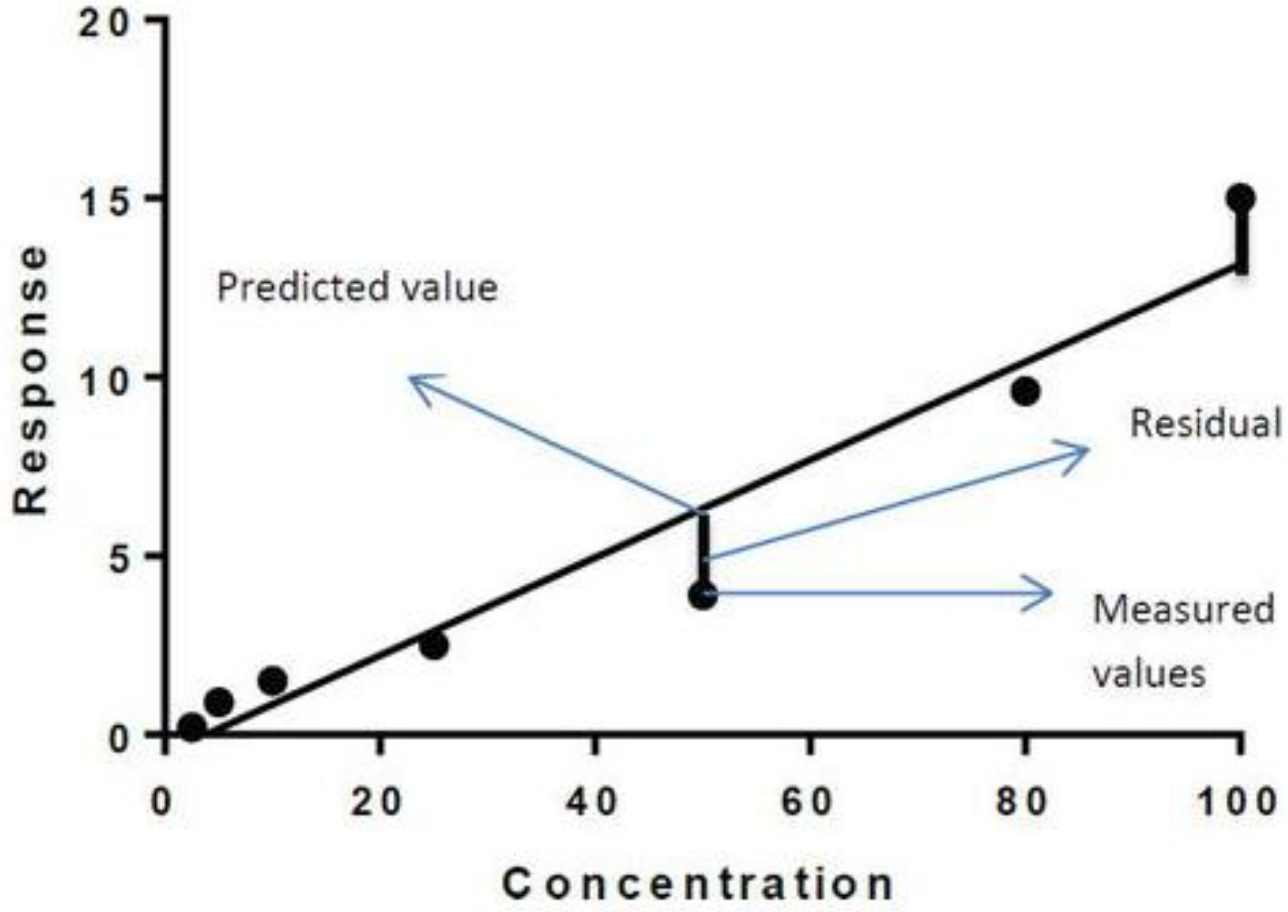




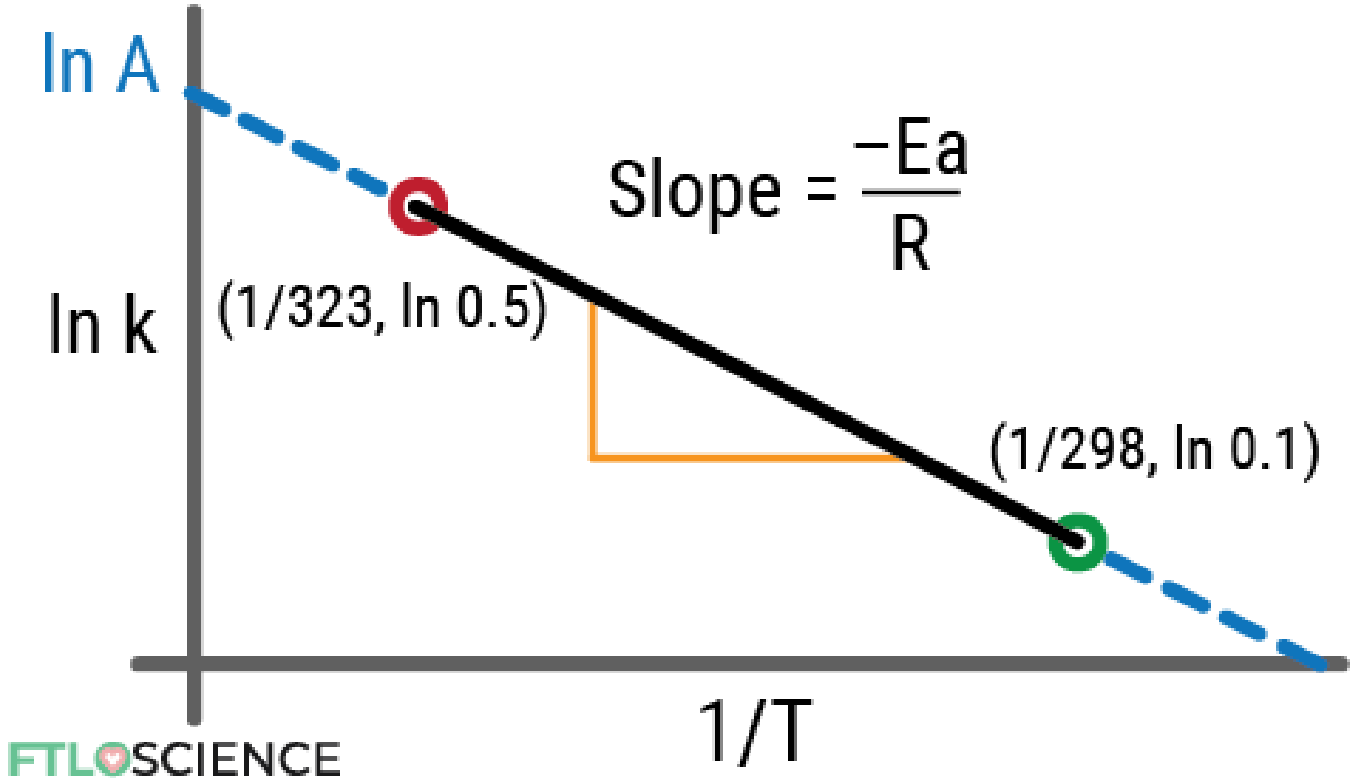
INTERVALLO DI MISURA



FITTING DEI DATI



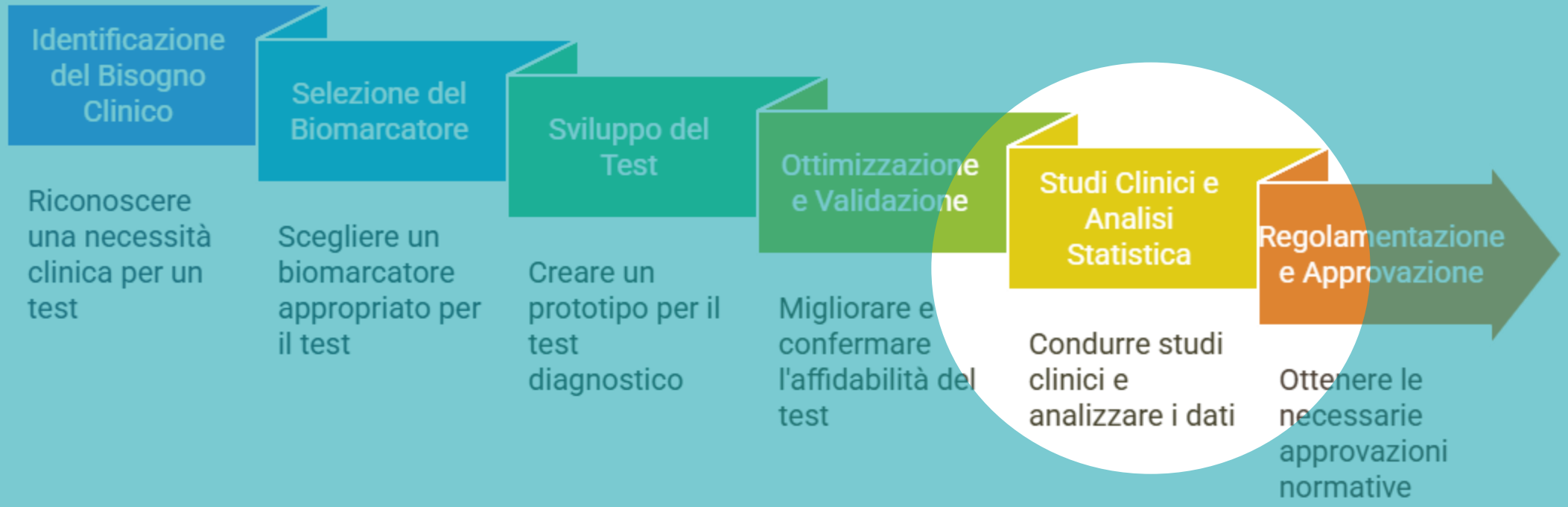
STABILITÀ



$$\ln(k) = -\frac{E_a}{RT} + lnA$$

SVILUPPO DI UN IMMUNOASSAY

INSIDE INNOVATION



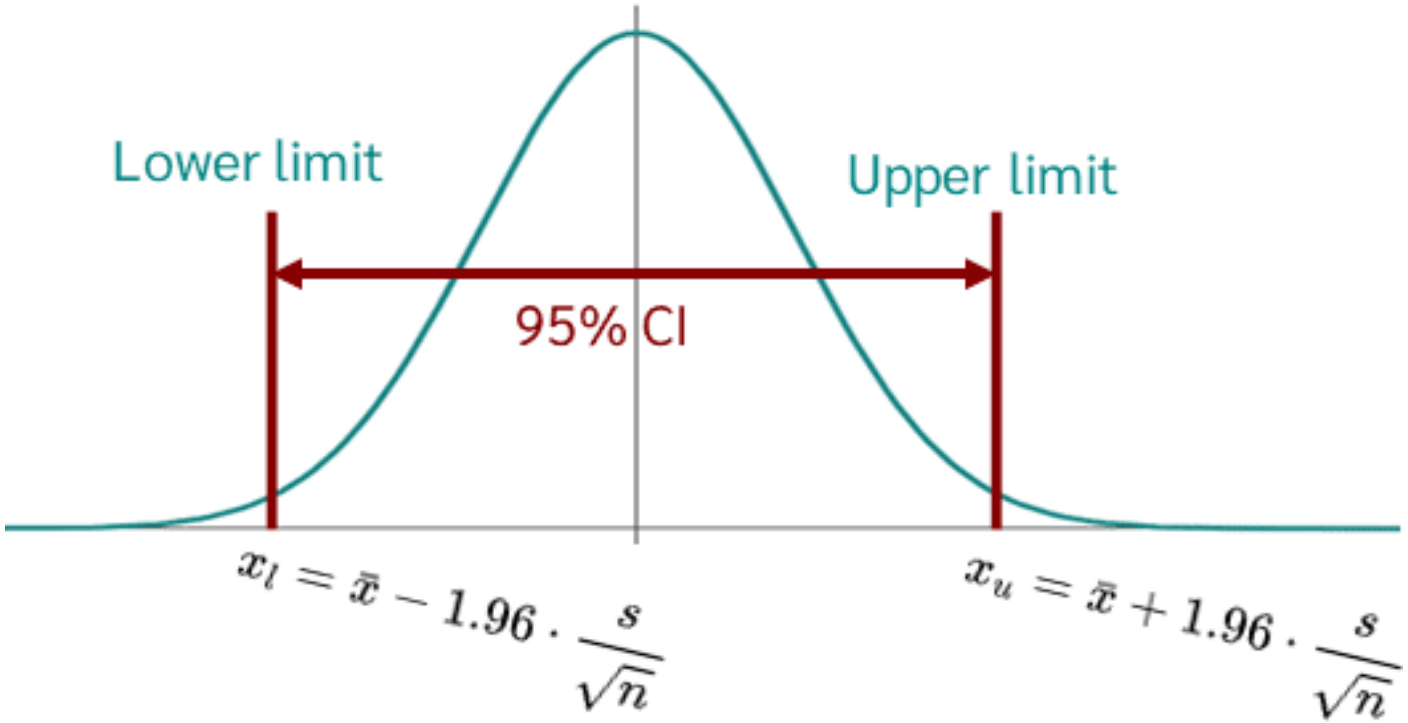
STUDI CLINICI E ANALISI STATISTICA

- Disegno dello studio clinico
- Raccolta di campioni reali per testare il dispositivo
- Analisi ROC per determinare cut-off e prestazioni cliniche

Se, Sp, PPV, NPV

		Disease		Predictive Value	
		⊕	⊖		
Test	⊕	**A** True Positive (TP)	**B** False Positive (FP)	Positive Predictive Value (PPV) $\frac{TP}{TP+FP} = \frac{A}{A+B}$	Total Positive Results (A + B)
	⊖	**C** False Negative (FN)	**D** True Negative (TN)	Negative Predictive Value (NPV) $\frac{TN}{FN+TN} = \frac{D}{C+D}$	Total Negative Results (C + D)
Sensitivity & Specificity		Sensitivity $\frac{TP}{TP+FN} = \frac{A}{A+C}$	Specificity $\frac{TN}{FP+TN} = \frac{B}{B+D}$		
		All diseased patients (A + C)	All non-diseased patients (B + D)		

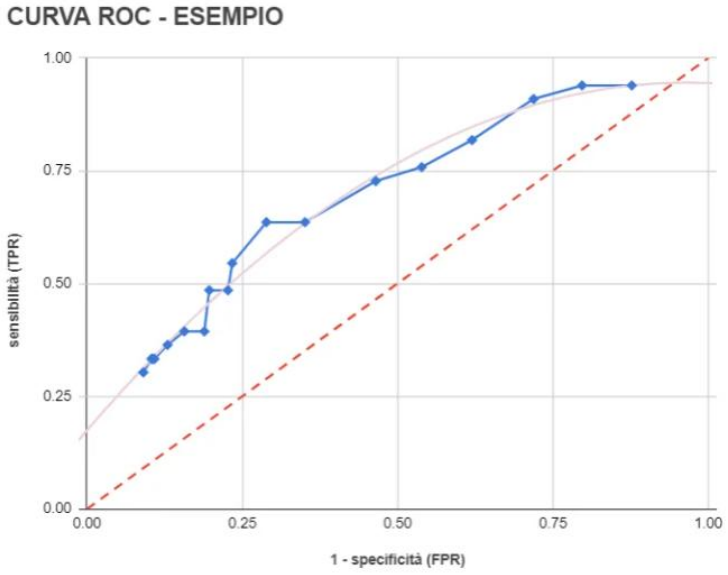
INTERVALLO DI CONFIDENZA

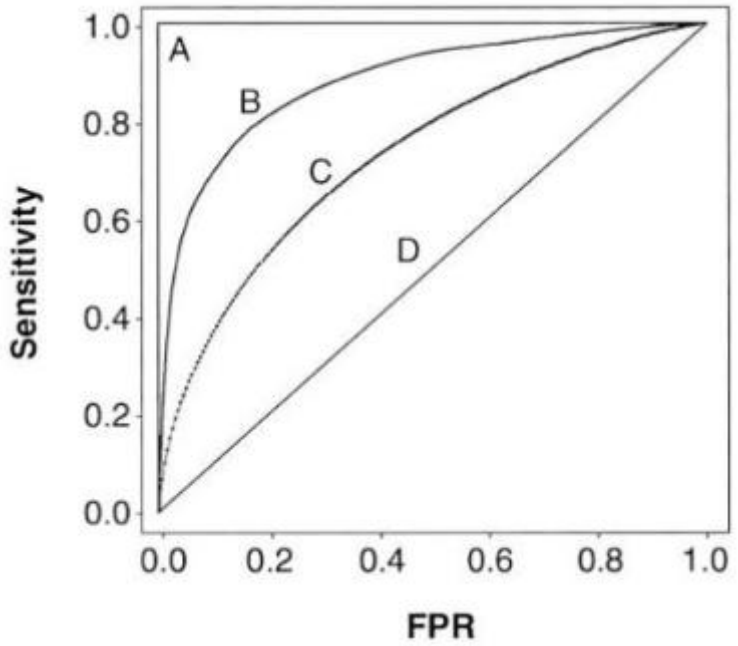


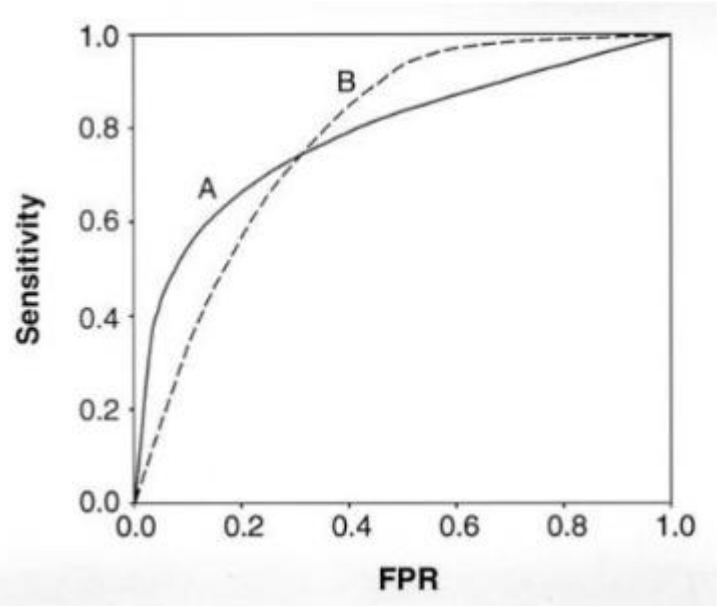
CURVA ROC

La curva ROC (Receiver Operating Characteristic) è un grafico che mette in relazione la sensibilità e la specificità di un test diagnostico al variare del valore di cut-off (valore soglia).

L'analisi della curva ROC di un test diagnostico permette di valutarne l'accuratezza, di determinare il valore di cut-off più appropriato e di confrontare le performance di due, o più, diversi test.

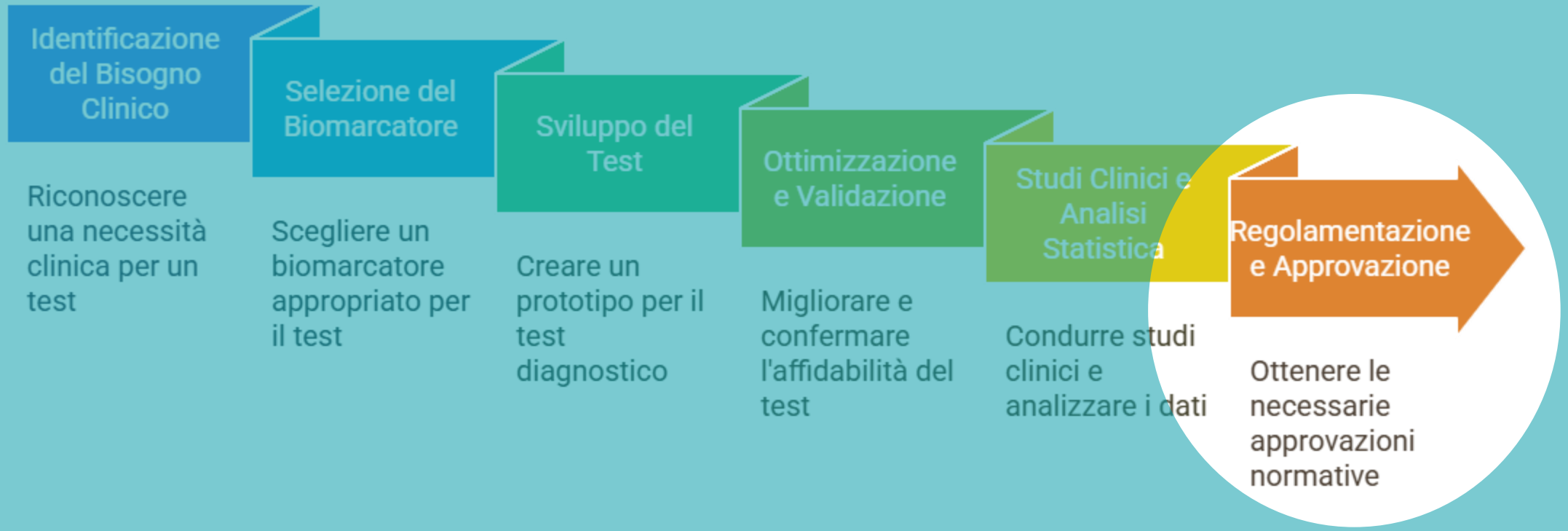






SVILUPPO DI UN IMMUNOASSAY

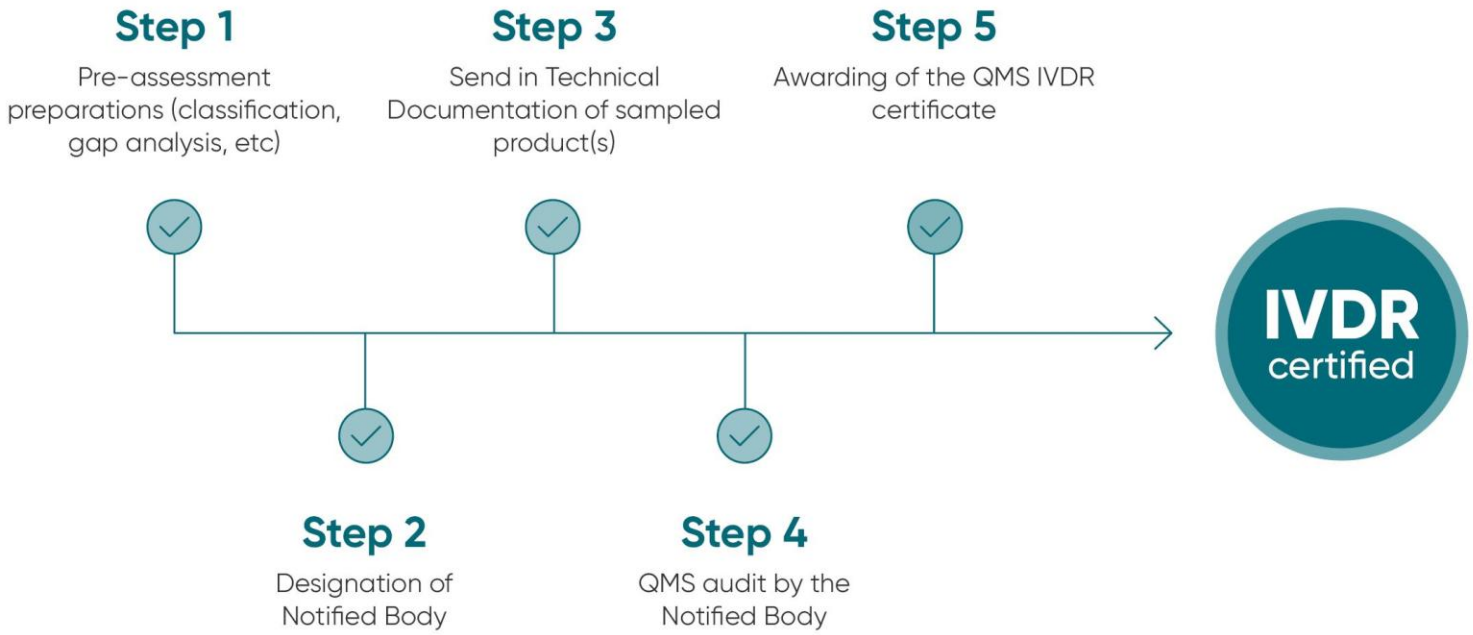
INSIDE INNOVATION



REGOLAMENTAZIONE E APPROVAZIONE

INSIDE INNOVATION

- Requisiti normativi per l'approvazione (FDA, IVDR, ISO 13485)
- Documentazione necessaria per la certificazione
- Test di conformità e produzione su larga scala

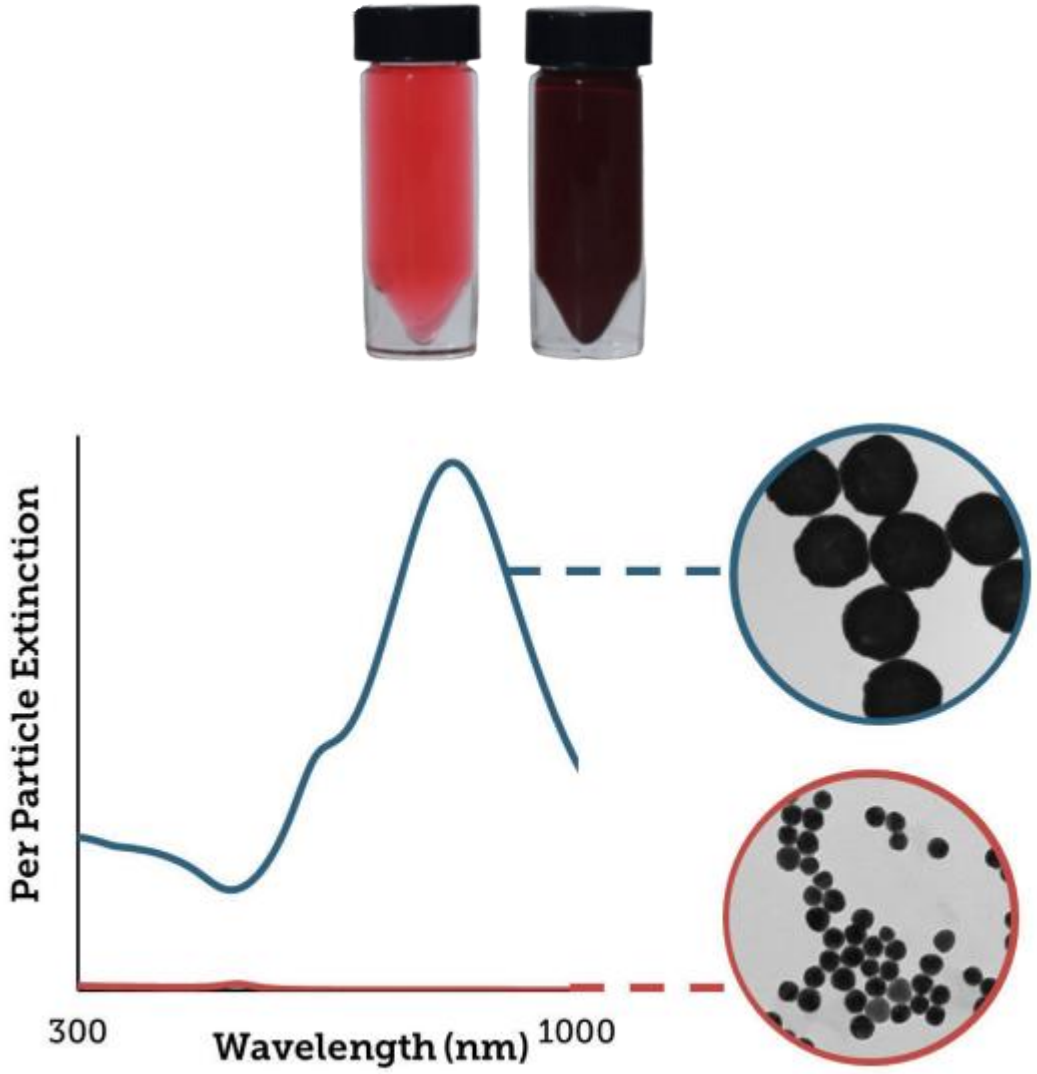


TECNOLOGIE EMERGENTI

- Test immunologici basati su nuove tipologie di nanoparticelle
- Screen Printed Electrode
- Microfluidica e biosensori avanzati
- Integrazione con intelligenza artificiale per analisi dei risultati

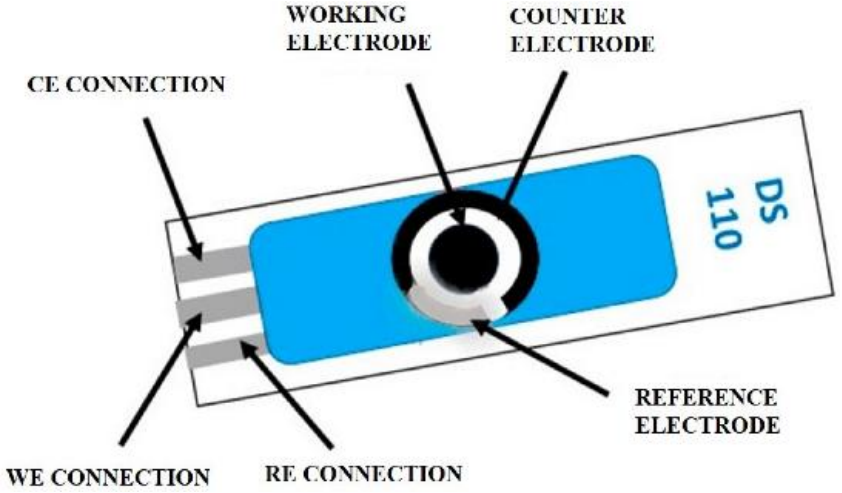
GOLD NANOSHELLS

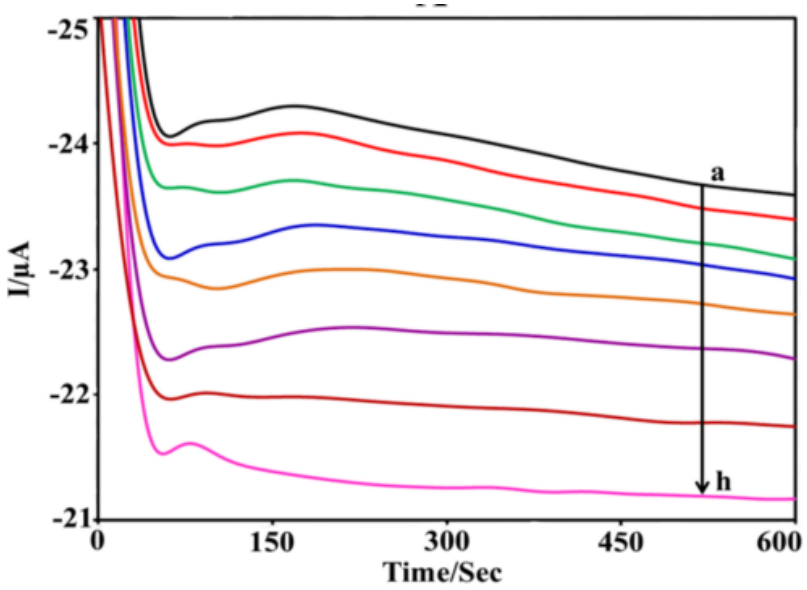
- Possono essere legati in modo irreversibile anticorpi interi, frammenti di anticorpi e piccole molecole
- In genere è necessario meno anticorpo rispetto all'adsorbimento passivo
- Si forma un legame ammidico stabile e irreversibile
- Migliore controllo sul rapporto anticorpi/particelle (difficile da ottenere nell'adsorbimento passivo a causa dei problemi di stabilità colloidale)
- Aumento fino a 20 volte della sensibilità del test
- È disponibile una chimica di legame covalente a base di NHS e COOH



SCREEN PRINTED ELECTRODE

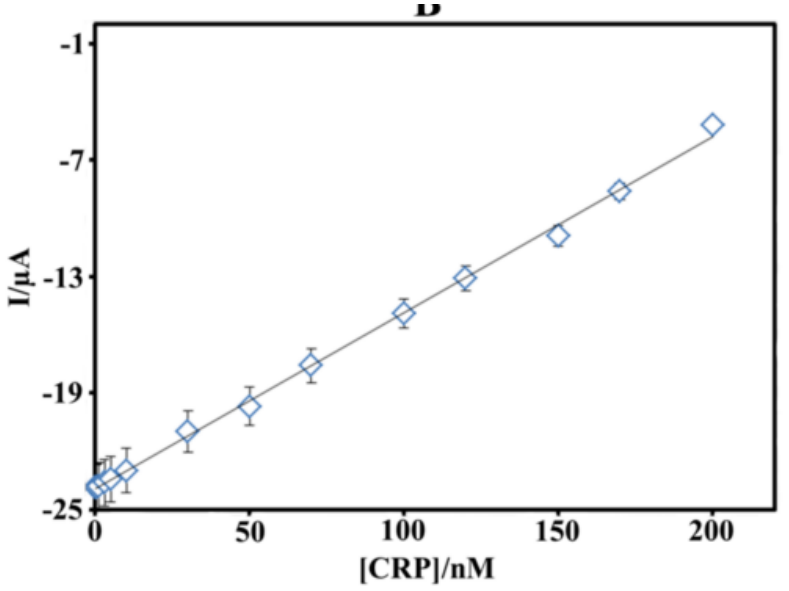
INSIDE INNOVATION





Randles-Sevcik

$$I_P = 2,686 \cdot 10^5 \cdot z^{3/2} A D^{1/2} C v^{1/2}$$



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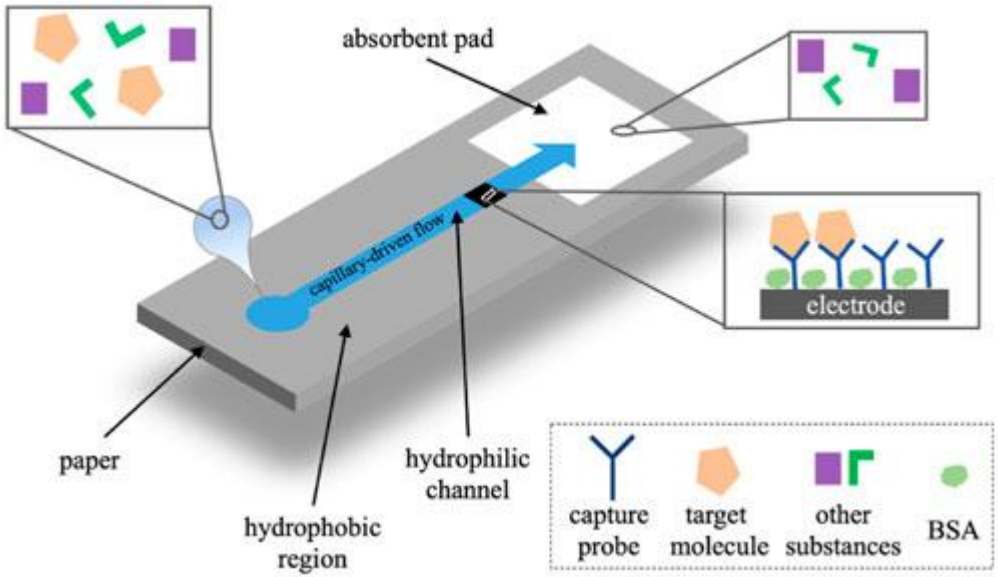
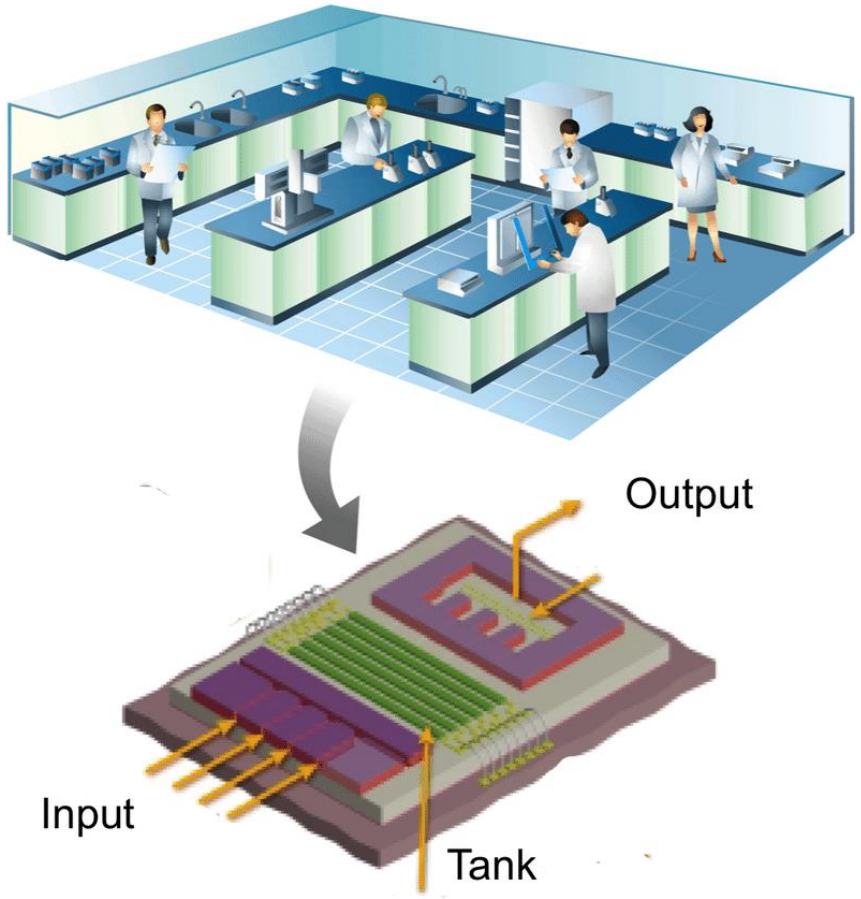
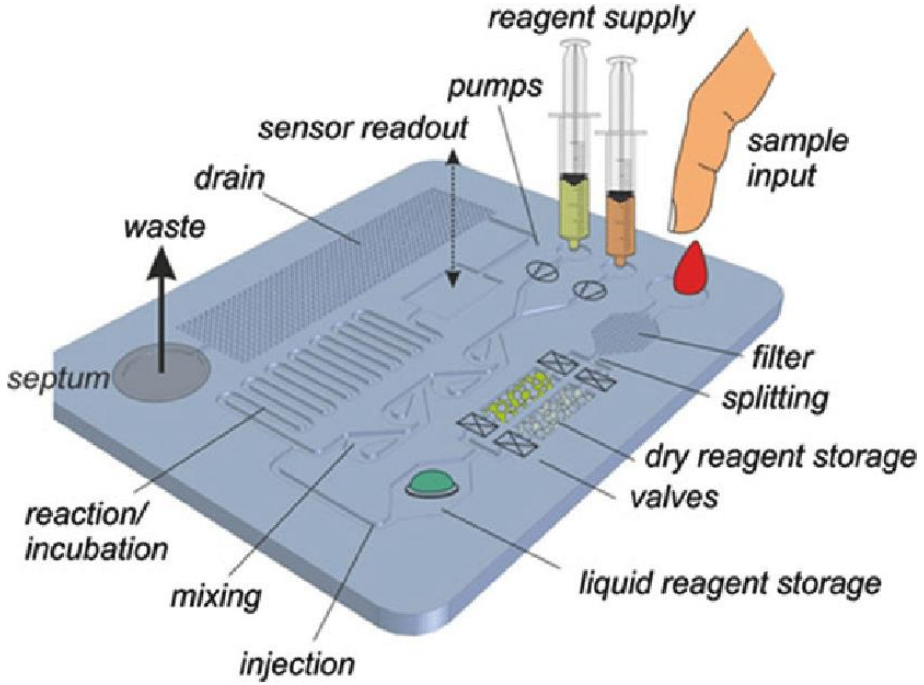
MICROFLUIDICA



LAB-ON-A-CHIP





INTELLIGENZA ARTIFICIALE

Modi in cui viene utilizzata l'AI in IVD:

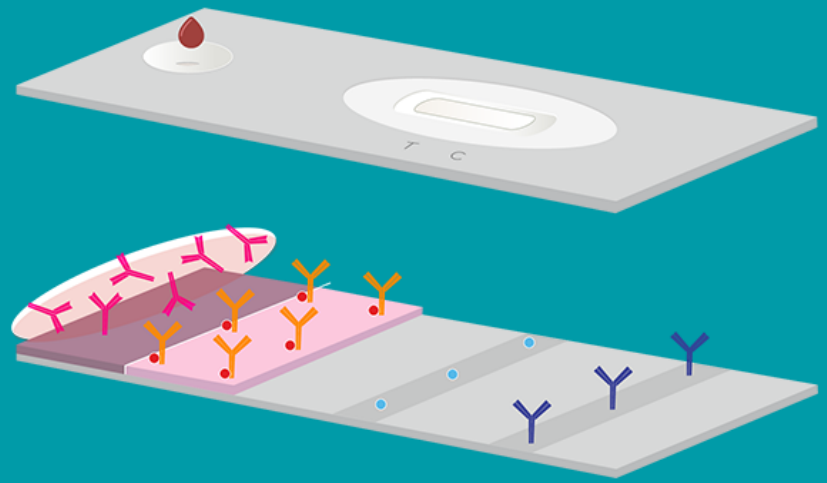
- **Machine learning**: per allenare modelli a riconoscere segnali patologici
- **Deep learning**: adatto per immagini (es. lettura automatica di test a banda, immunofluorescenza)
- **AI basata su reti neurali**: per correlare più biomarcatori insieme e formulare diagnosi predittive

Il vero potenziale è **integrare AI + diagnostica + dati clinici** per creare percorsi terapeutici personalizzati. Diagnostica predittiva, monitoraggio remoto, screening automatico: la medicina sta diventando **proattiva**, non solo reattiva.

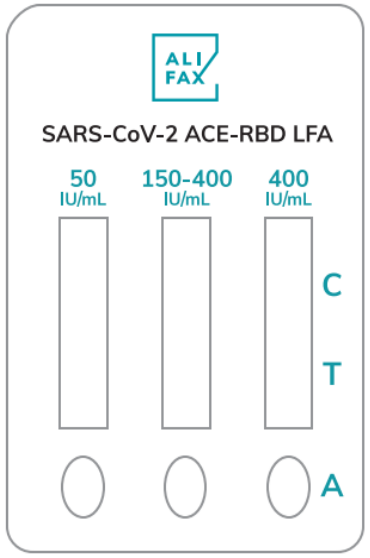
INSIDE INNOVATION

CASE STUDY #1

ANTICORPI NEUTRALIZZANTI ANTI SARS-CoV-2



CASE STUDY #1 – SARS-CoV-2 NAb



C > Control Line
T > Test Line
A > Sample & Buffer well

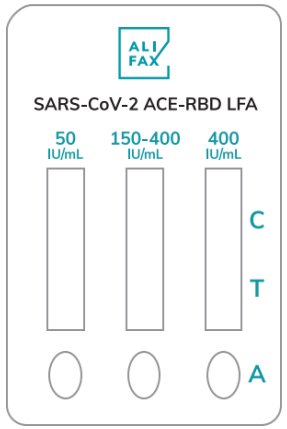
Level 1: 50 IU/mL
Level 2: 150 IU/mL
Level 3: 400 IU/mL

- Semiquantitative detection of SARS-CoV-2 neutralizing antibodies
- Figertip whole blood, serum, plasma and whole blood
- Identifies individuals with an adaptive immune response to SARS-CoV-2
- Evaluate immunity status after infection or vaccination
- The administration of the test and the interpretation of the results should be done by a trained health professional
- For Professional Use only (serum, plasma and whole blood), Point of Care Testing (fingertip whole blood)
- Not intended for self-test.

CASE STUDY #1 – SARS-CoV-2 NAb

INSIDE INNOVATION

NO indication about protection-correlated antibody titer from International Entity (WHO, CDC, NIH)



C > Control Line
T > Test Line
A > Sample & Buffer well

Level 1: 50 IU/mL
Level 2: 150 IU/mL
Level 3: 400 IU/mL

- 50 IU/mL is defined as the estimation of the protective neutralization level against COVID-19.
- 150 IU/mL defined as the minimum level of anti-SARS-CoV-2 spike glycoprotein IgG antibodies necessary to confer 6 months protection from infection.
- 400 IU/mL is defined as the peak of neutralizing antibody levels

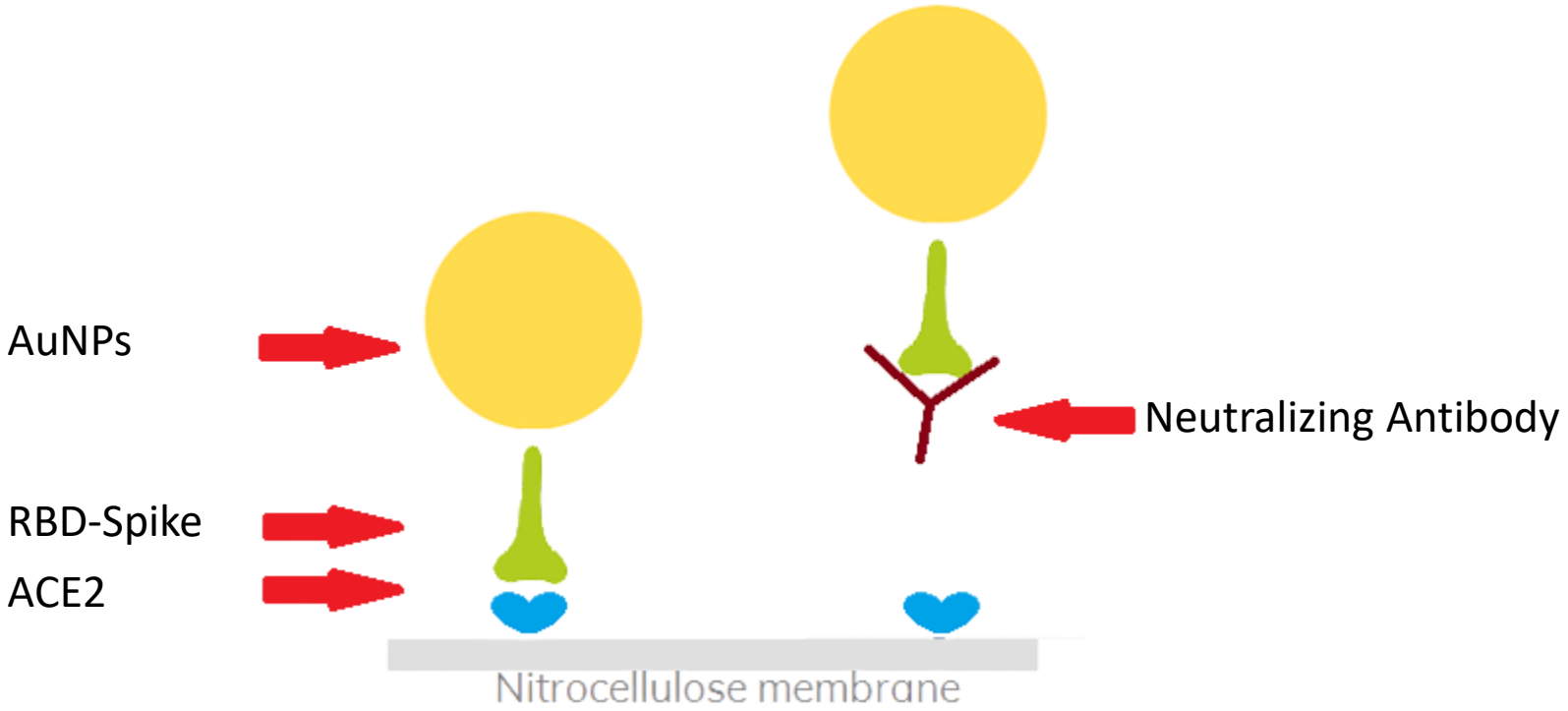
→ Khoury, D.S et al. (2021). "Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection". Nat Med 27, 1205–1211

→ Shields, A. et al, (2021). "Longitudinal Protection Following Natural SARS-CoV-2 Infection and Early Vaccine Responses: Insights from a Cohort of Community Based Dental Health Care Professionals". SSRN Electronic Journal

→ Zhu, F., et al. (2022). WHO international standard for SARS-CoV-2 antibodies to determine markers of protection. In The Lancet Microbe (Vol. 3, Issue 2)

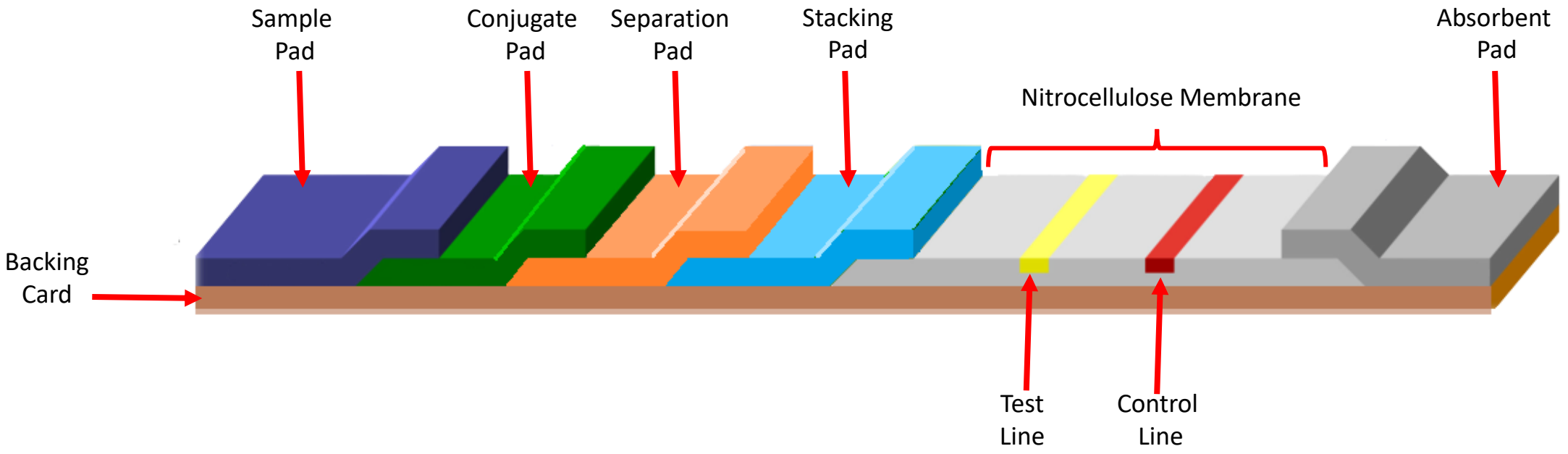
NIBSC
Confidence in Biological Medicines

CASE STUDY #1 – SARS-CoV-2 NAb



CASE STUDY #1 – SARS-CoV-2 NAb

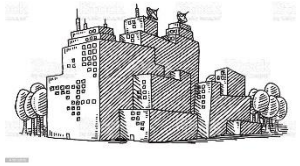
INSIDE INNOVATION



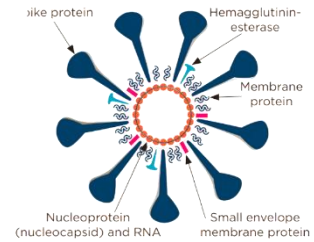
CASE STUDY #1 – SARS-CoV-2 NAb

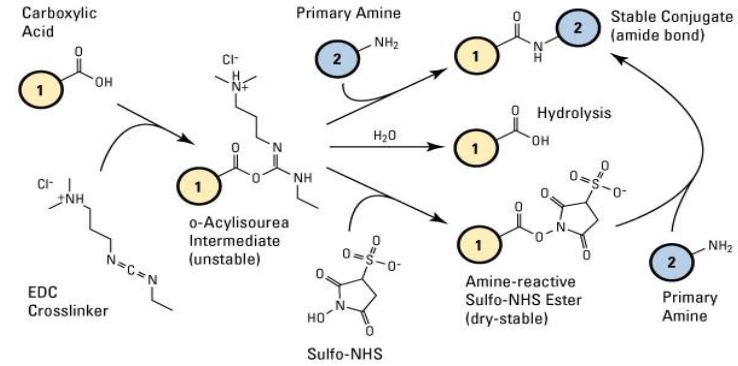
INSIDE INNOVATION

ALIFAX R&D
Basovizza (TS)



ALIFAX srl
Nimis (UD)





BIODOT

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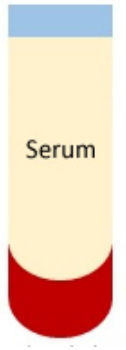
CASE STUDY #1 – SARS-CoV-2 NAb



SARS-CoV-2 ACE-RBD LFA NEUTRALIZING ANTIBODIES	ELISA – DiaPro ACE-RBD Neutralization Assay		
	Positive	Negative	Total
Positive	197	1	198
Negative	9	233	242
Total	206	234	440

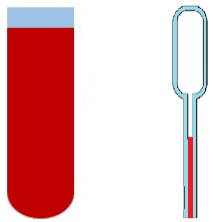
- ✓ Sensitivity: 95.6% (95% CI: 94.2%-97.1%)
- ✓ Specificity: 99.6% (95% CI: 99.1%-100.0%)
- ✓ Accuracy: 97.7% (95% CI: 97.0.%-98.4%)

CASE STUDY #1 – SARS-CoV-2 NAb

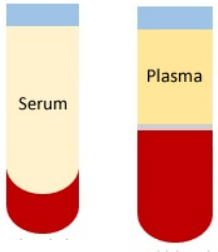
INSIDE INNOVATION

CASE STUDY #1 – SARS-CoV-2 NAb

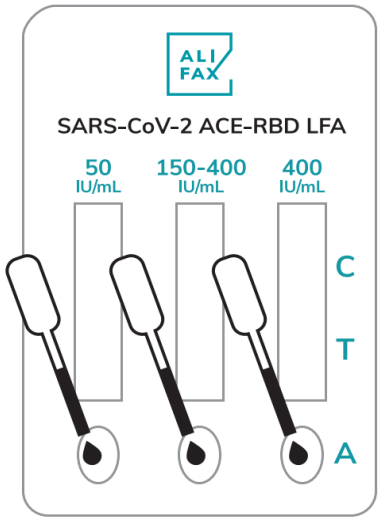
INSIDE INNOVATION

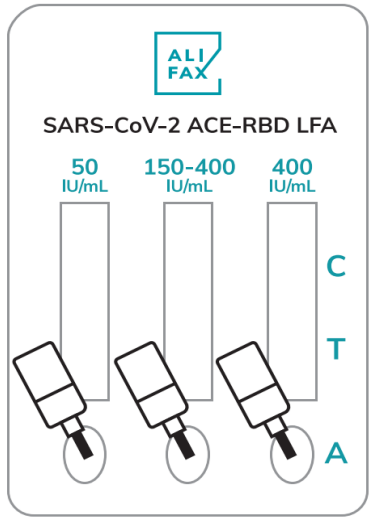


20 µL



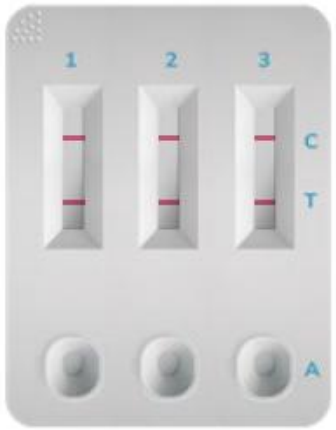
10 µL



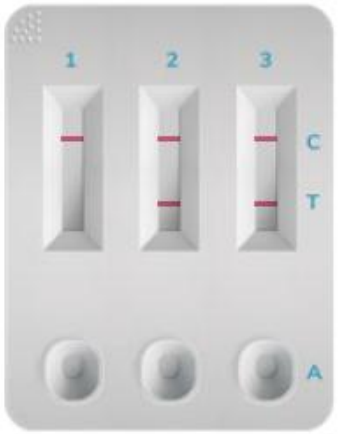


CASE STUDY #1 – SARS-CoV-2 NAb

INSIDE INNOVATION



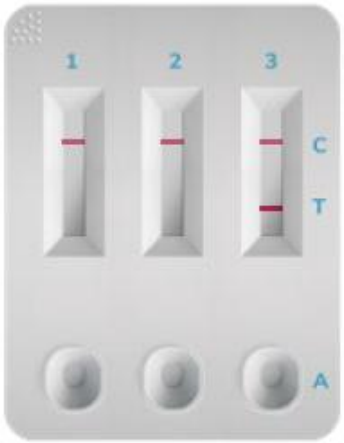
< 50 IU/mL
NAb not detected



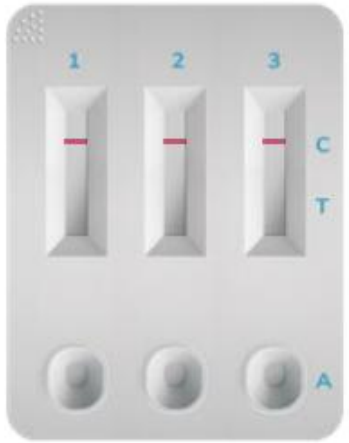
50 - 150 IU/mL
LOW NAb ntiter

No Control Line (C)

INVALID RESULT



150 - 400 IU/mL
MODERATE NAb titer



> 400 IU/mL
HIGH NAb ntiter

NO indication about protection-correlated antibody titer from International Entity (WHO, CDC, NIH)

INSIDE INNOVATION

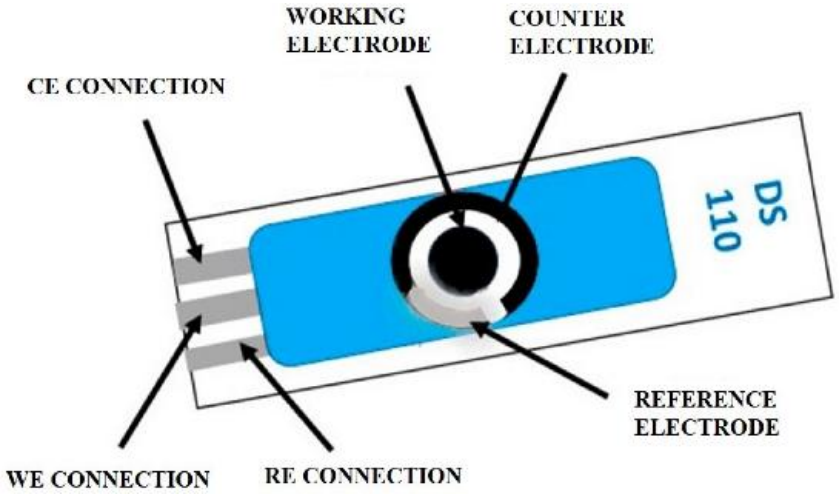
CASE STUDY #2

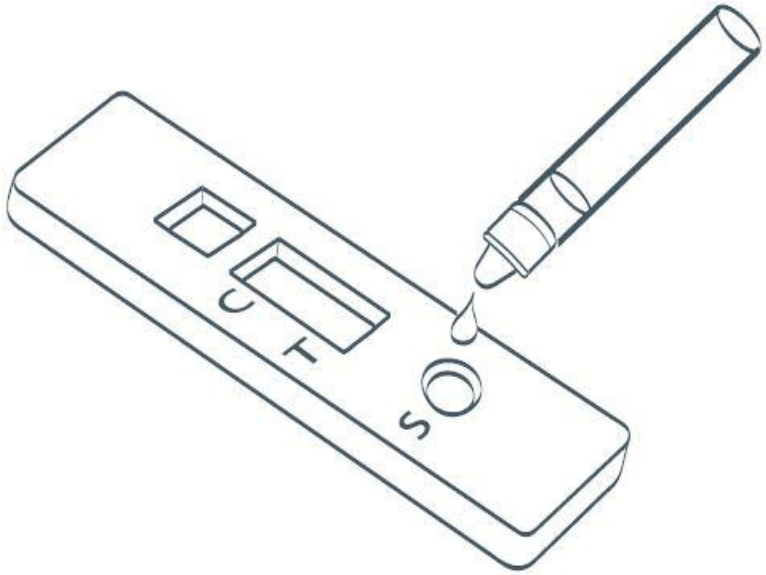
SPE x PCT

CASE STUDY #2 – SPE x PCT

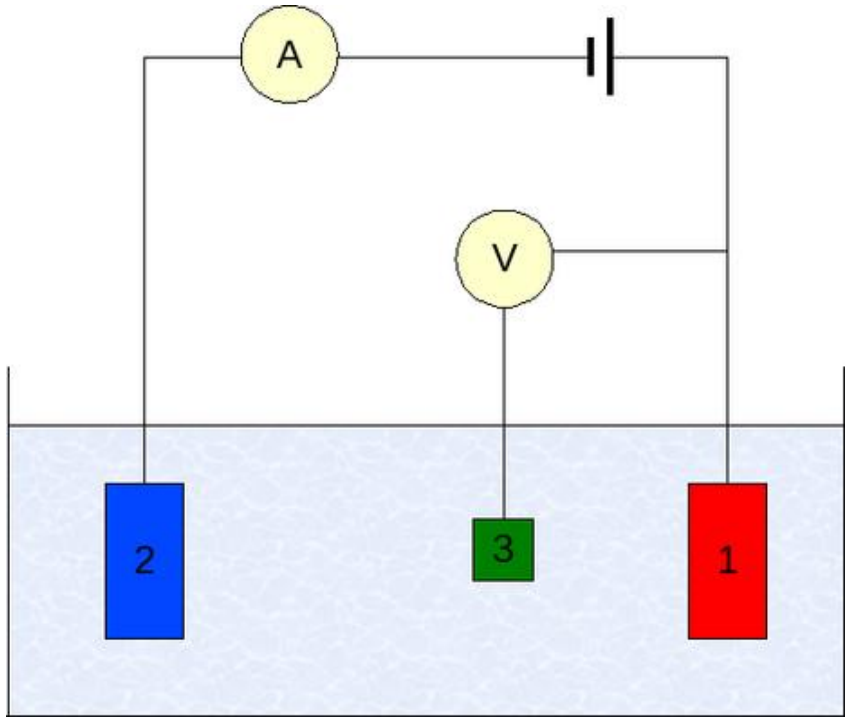
Obiettivo: studio di un biosensore elettrochimico ad impedenza per la rilevazione di Procalcitonina ed Interleuchina-6 in campioni di sangue intero/siero umani e comparazione dei risultati con un test LFA quantitativo

Tesi di laurea magistrale in Chimica Industriale anno 2023-2024





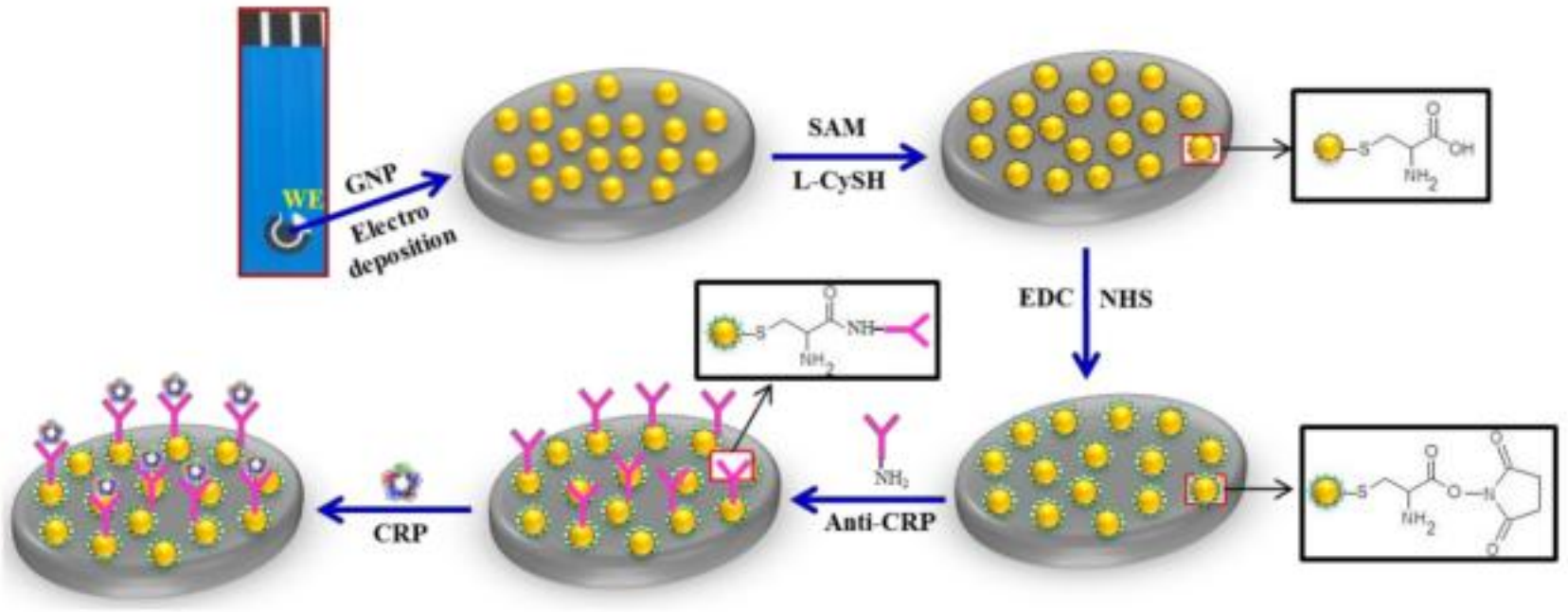
CASE STUDY #2 – SPE x PCT



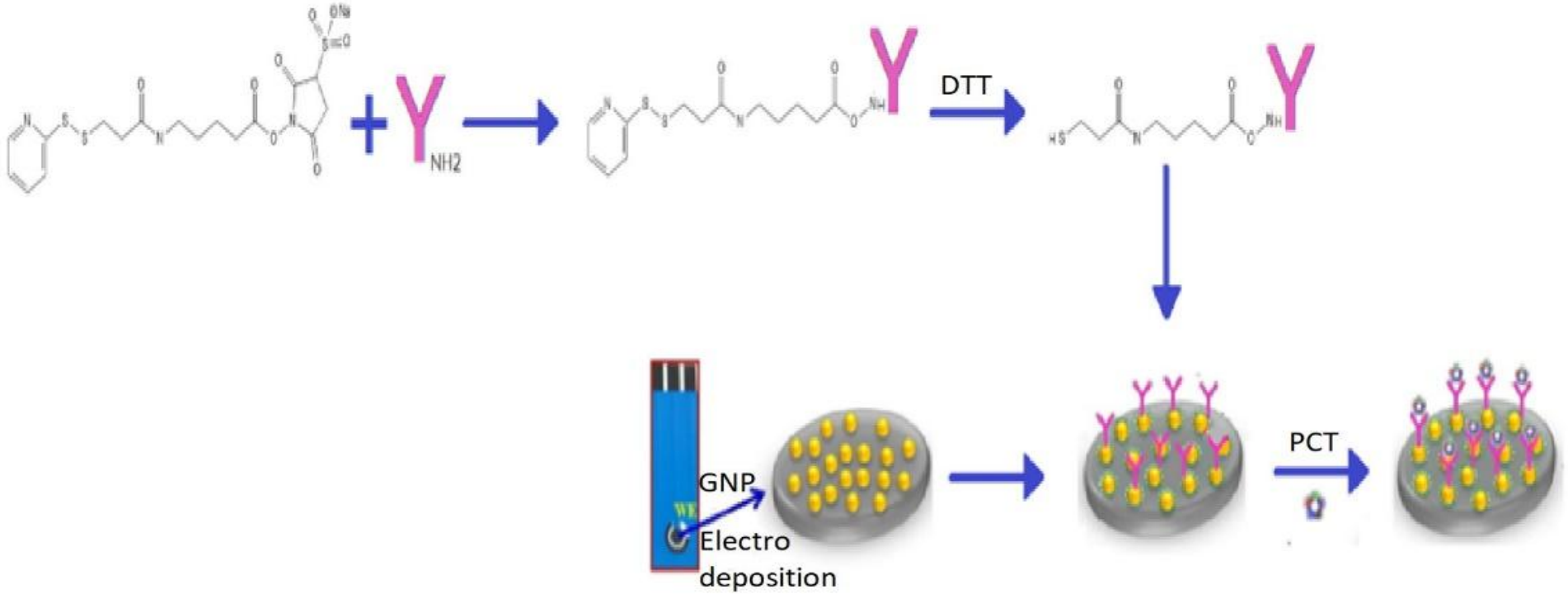
1. Si applica un potenziale tra WE e RE
2. Viene legge una corrente tra WE e CE
3. Si correla l'intensità di corrente con concentrazione di analita

LabVIEW™

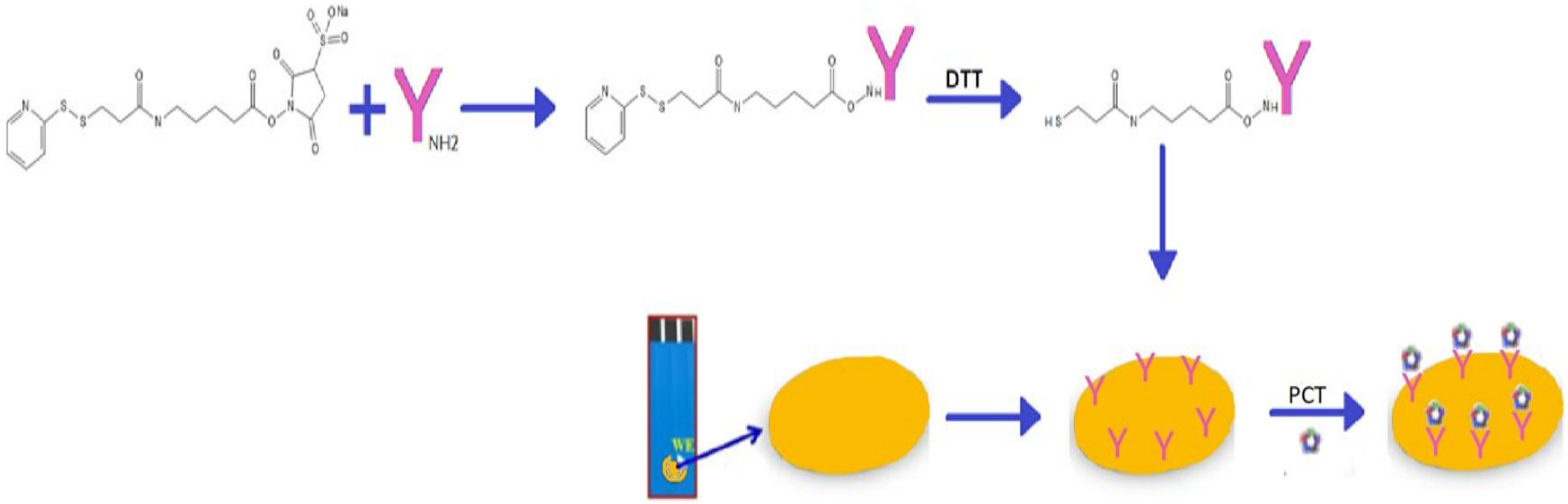
CASE STUDY #2 – SPE x PCT



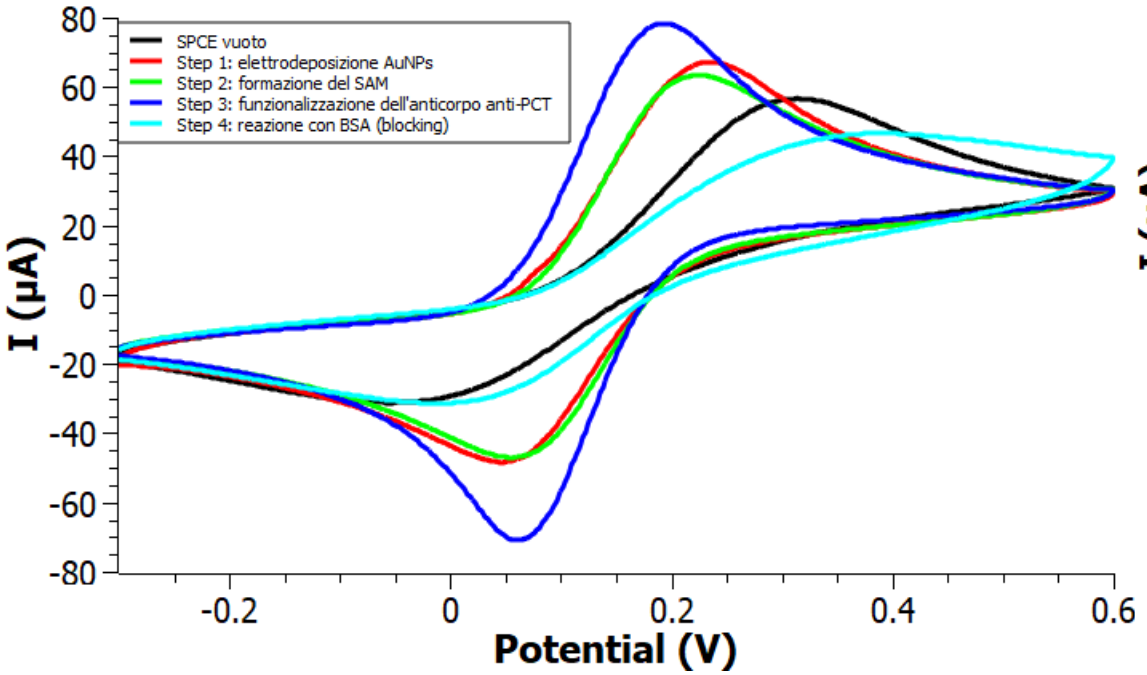
CASE STUDY #2 – SPE x PCT

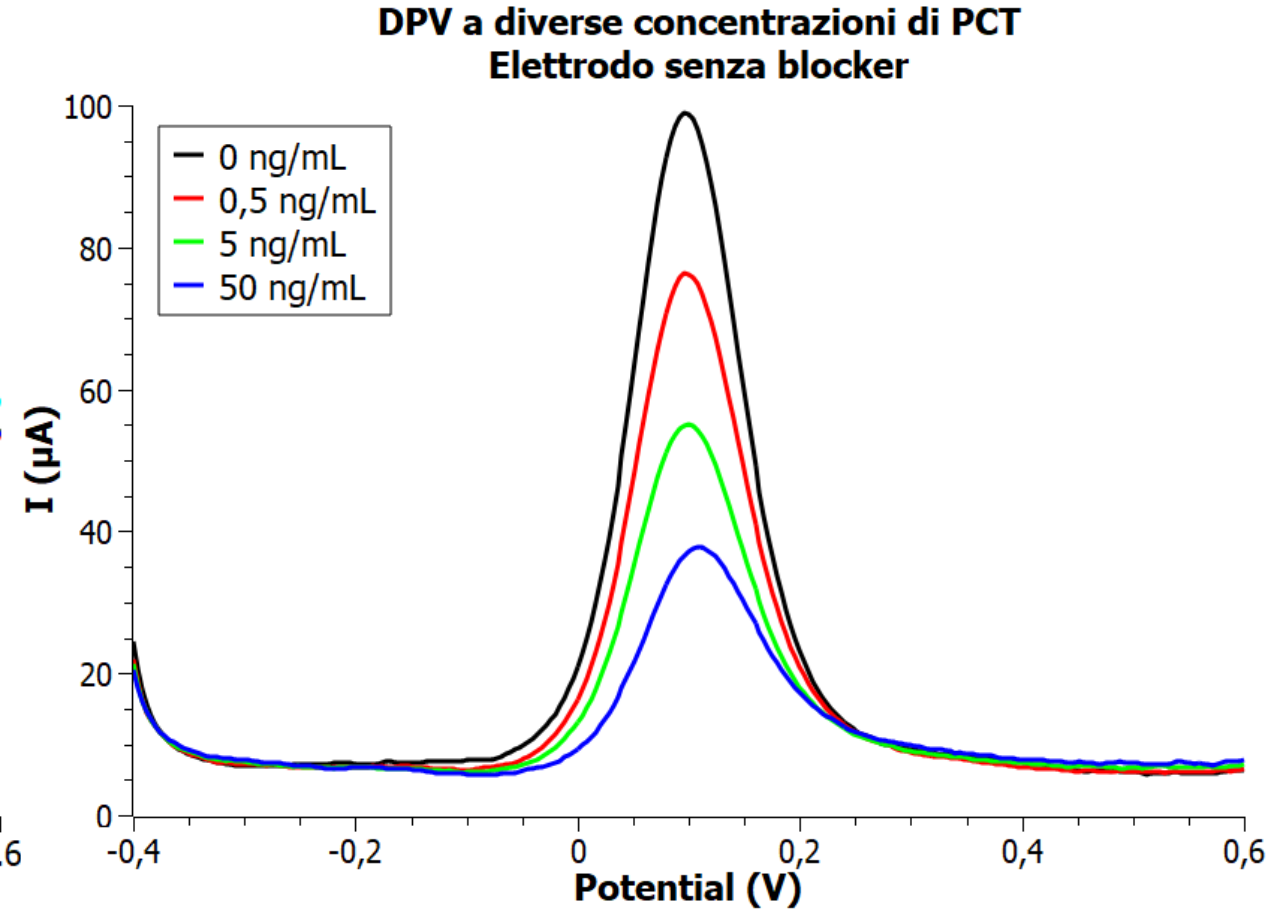


CASE STUDY #2 – SPE x PCT



CASE STUDY #2 – SPE x PCT

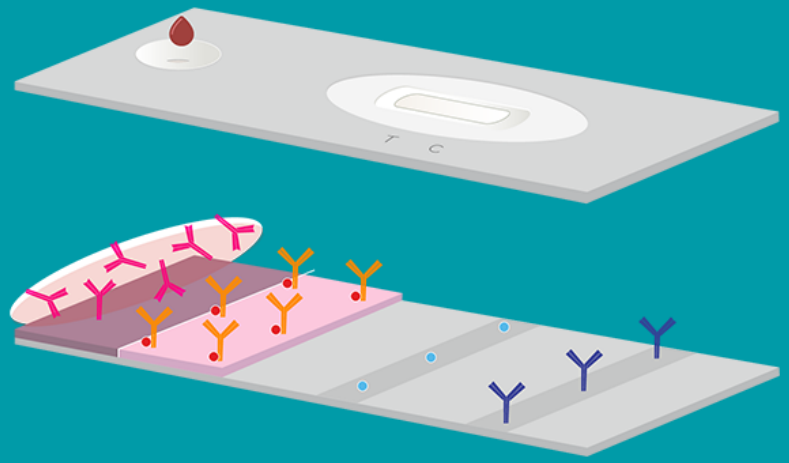


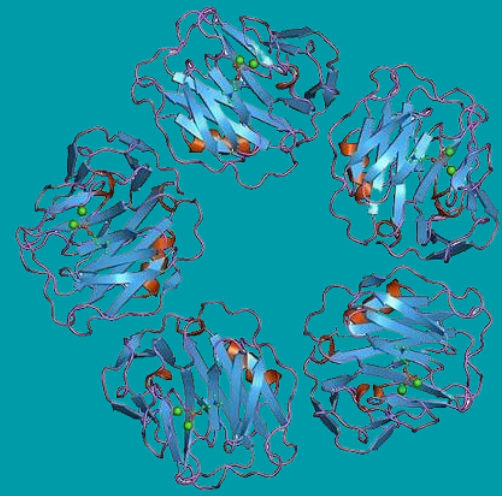


INSIDE INNOVATION

CASE STUDY #3

LFA FLUORESCENTE: PCT, CRP e IL-6

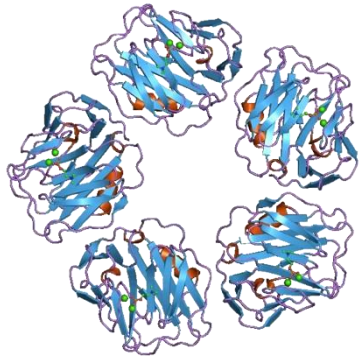




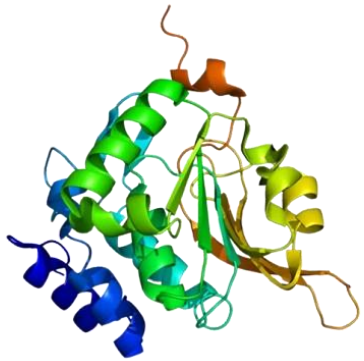
CASE STUDY #3 – LFA – CRP, PCT & IL-6

- **Fluorescent detection** → need sensitivity for PCT & IL-6
- **Multiplex LFA** → infective panel
- **Sandwich detection** → for PCT & IL-6
- **Competitive detection** → for CRP (Hook effect)

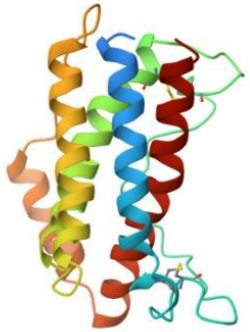
CASE STUDY #3 – LFA – CRP, PCT & IL-6



Human C-reactive protein (CRP) is one of the socalled acute phase proteins. CRP is produced in liver and its concentration in blood increases rapidly as a response to inflammation. It is routinely used as a non-specific marker of inflammation. C-reactive protein is accepted in clinical use as a major, although rather nonspecific, marker of inflammation. In generally healthy people, CRP levels are usually less than 5 mg/L. In pathology, CRP concentration has an enormous, 10,000-fold dynamic range (approximately 0.05–500 mg/L). The highest levels of CRP (above 30 mg/L) are observed in bacterial infection, such as septic arthritis, meningitis, and pneumonia.



Procalcitonin (PCT) is a small protein (~13 kDa) that is synthesized by the Ccells of the thyroid glands. It is considered to be the main marker of disorders that are accompanied by systemic inflammation and sepsi PCT is a good marker of bacterial infection because its level in the blood of normal subjects is very low and due to the fact that viral infections cause only a minor increase in PCT concentration. In addition, the diagnostic value of PCT is further supported by the close correlation between PCT concentration and the severity of inflammation s. In some cases, an increase in PCT concentration may be induced by factors independent of sepsis and infection. Surgery, polytrauma, heat shock, burn injuries, and cardiogenic shock also lead to an increase in the PCT level. Furthermore, the importance of monitoring the PCT level changes following cardiac surgery or heart transplantation for differentiating acute graft rejection from bacterial or fungal infections has been confirmed in multiple studies.

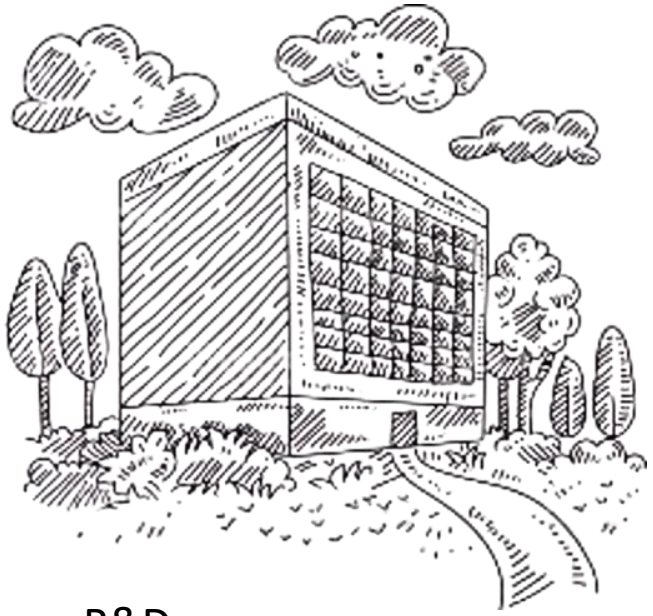


Interleukin-6 (IL-6) is a protein cytokine that was discovered in the 1980s. It is also known as B-cell stimulatory factor 2, hepatocytestimulating factor, hybridoma growth factor, or interferon (IFN)-β2. IL-6 participates in inflammation, immune response, and acts in the coordination of developmental, neuronal, and metabolic processes. IL-6 acts as a transmitter of alarm signals to the whole organism, indicating the occurrence of an emergency such as infection or tissue damage Interleukin 6 helps monitor inflammatory responses such as infection, sepsis, lupus, or rheumatoid arthritis or to evaluate diabetes, stroke, and cardiovascular disease. Baseline levels of human IL-6 in the blood are known to be in single pg per ml digits and can increase up to thousands of pg/ml upon severe sepsis

CASE STUDY #3 – LFA – CRP, PCT & IL-6

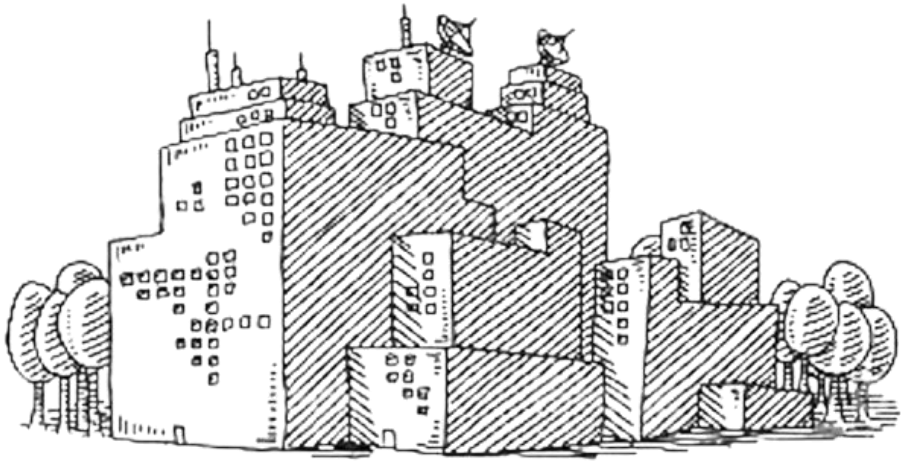
INSIDE INNOVATION

ALIFAX srl - Nimis (UD)



- R&D
- Conjugation
- Material selection
- Cassette assembly
- LFA manufacturing

ALIFAX R&D - Basovizza (TS)



- Antibody & Antigen manufacturing

ALI FAX

INSIDE INNOVATION

CASE STUDY #4

PIATTAFORMA POC-OREL

Or::el

CASE STUDY #4 – POC-OREL

The POC-OREL immunoassay represents a cutting-edge solution for the quantitative analysis of biomarkers, offering sensitivity and specificity comparable or better than traditional ELISA assays, while providing a range of unique advantages that make it an ideal choice for a wide array of clinical and diagnostic applications.

- ✓ **High Sensitivity**, comparable or better than ELISA immunoassay.
- ✓ **Multiplexing**: Detect up tp 9 analytes in one test.

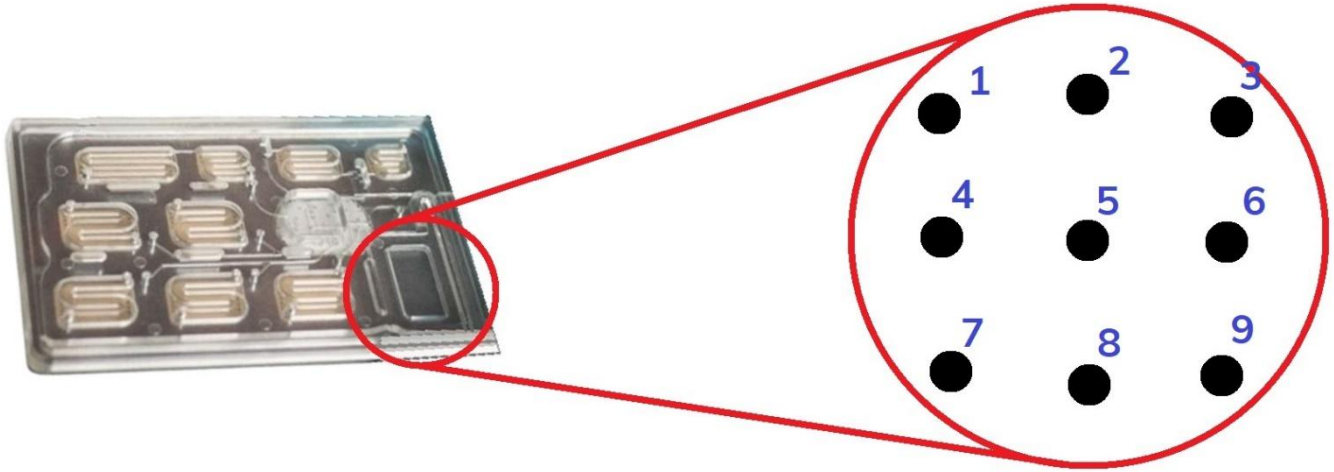
CASE STUDY #4 – POC-OREL

INSIDE INNOVATION

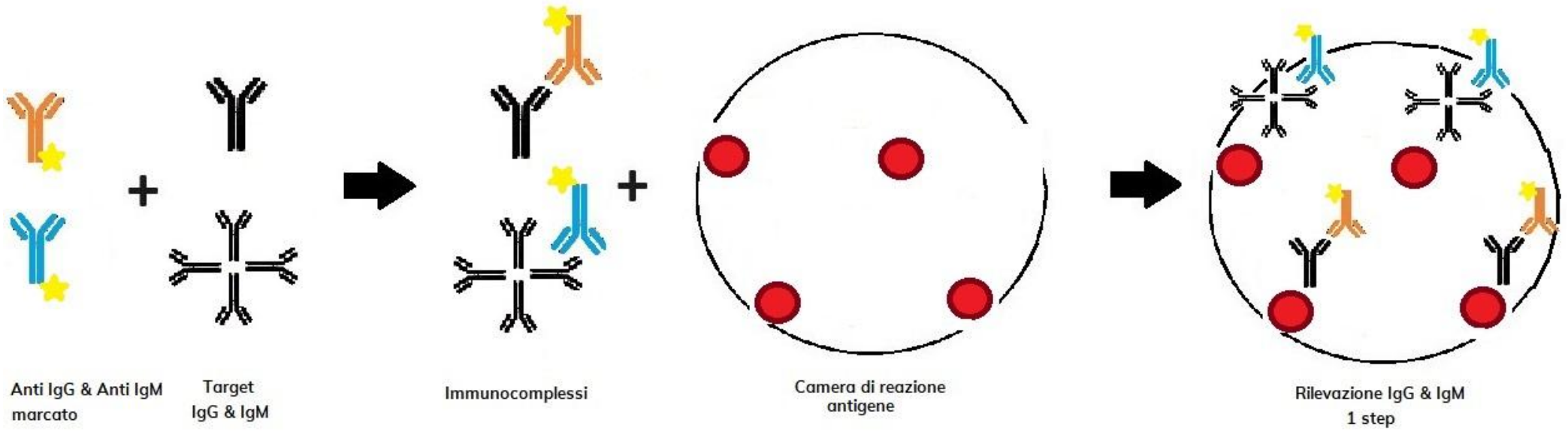
Precision	Accurate and reliable results
Sensitivity	Detect biomarkers at low concentrations
Multiplexing	Analyze up to 9 biomarkers in one test
No external preanalitical	Requires only the insertion of the sample into the cartridge
Speed	Fast results below 30 minutes
Cost-Effective	Reduced reagent and sample usage.
Versatile	Suitable with different specimens (plasma, serum, fingertip whole blood, liquor)

CASE STUDY #4 – POC-OREL

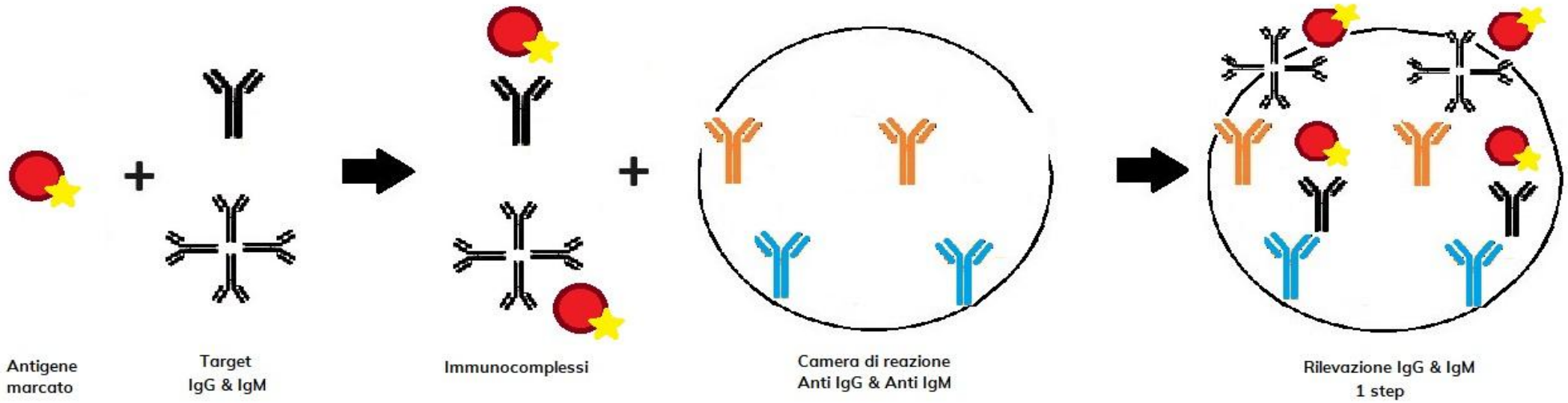
- ✓ Detect up to 9 analytes in a single test run, reducing testing time and costs.
- ✓ Perfect for complex disease diagnostics (e.g., inflammation markers, autoimmune diseases, infectious diseases).



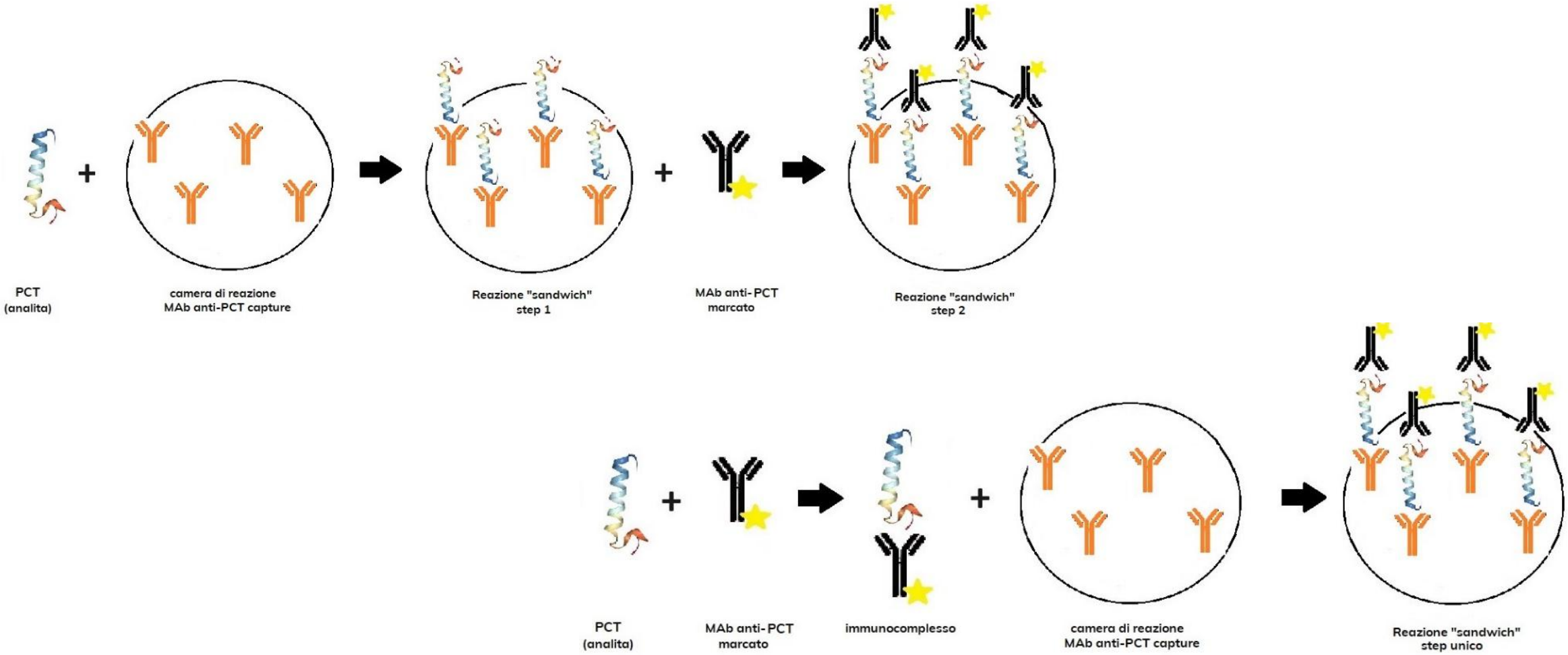
CASE STUDY #4 – POC-OREL



CASE STUDY #4 – POC-OREL



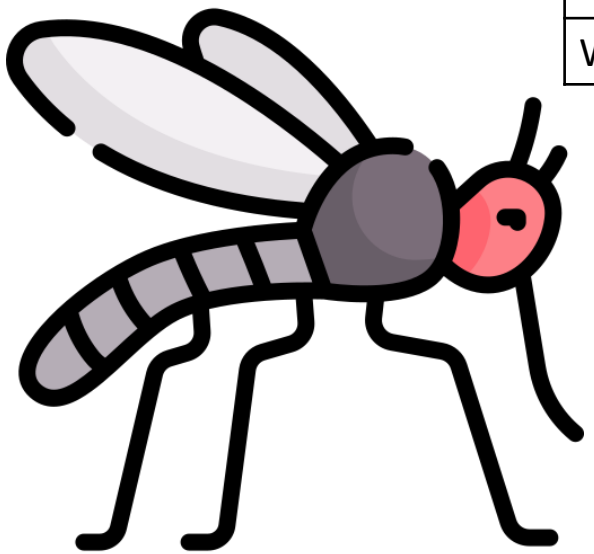
CASE STUDY #4 – POC-OREL

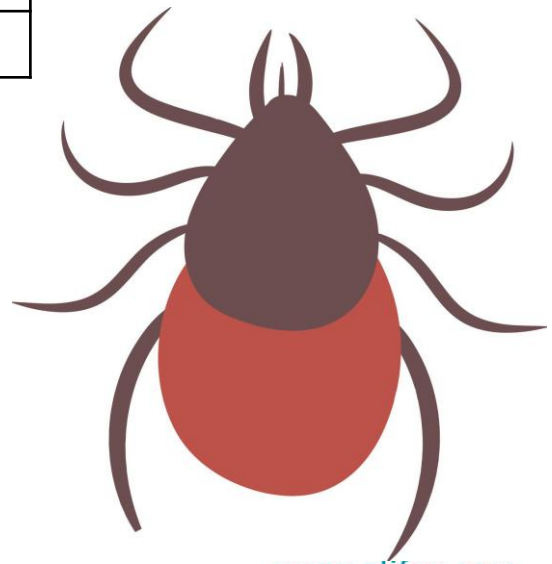


CASE STUDY #4 – POC-OREL

INSIDE INNOVATION

MOSQUITO PANEL	THICK PANEL
Zika Virus IgG	TBE IgG
Zika Virus IgM	TBE IgM
Dengue IgG	West Nile IgG
Dengue IgM	West Nile IgM
Chikungunya IgG	Toscana Virus IgG
Chikungunya IgM	Toscana Virus IgM
West Nile IgG	Borrelia Bacteria IgG
West Nile IgM	Borrelia Bacteria IgM





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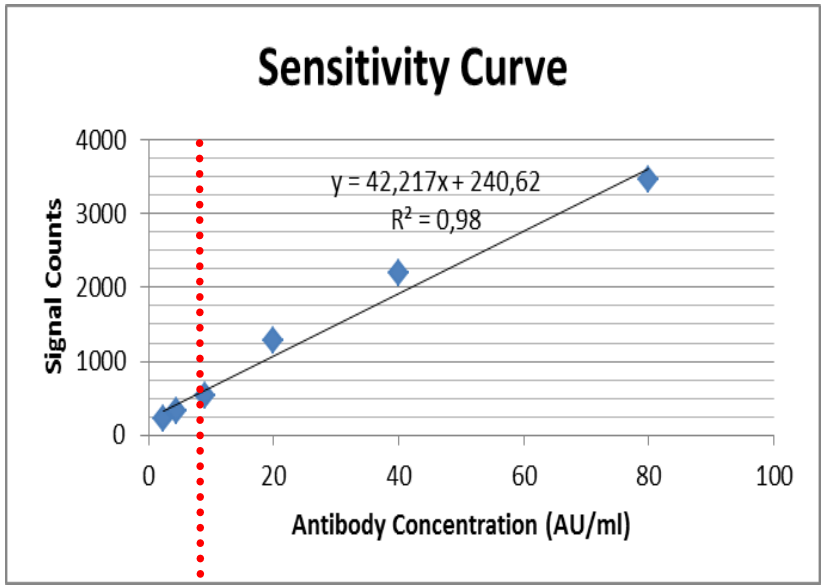
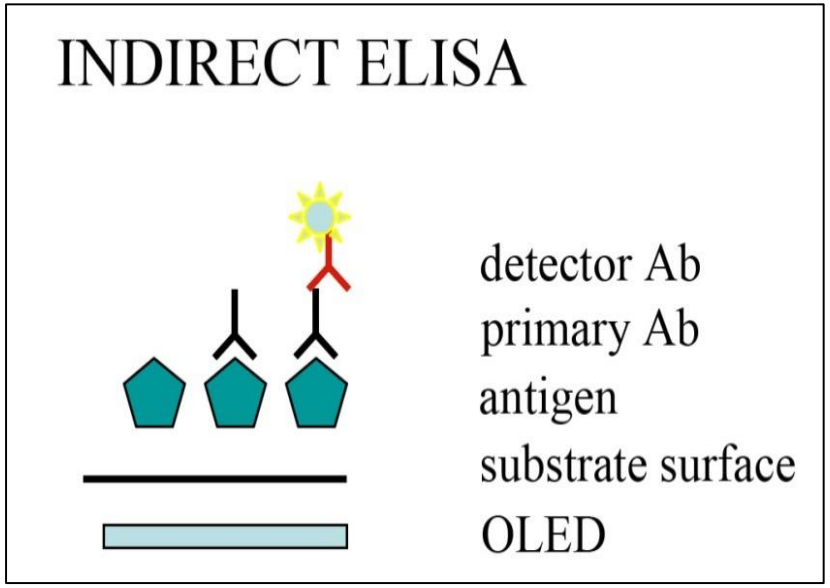
CASE STUDY #4 – POC-OREL

- ✓ Detects biomarkers at lower concentrations than traditional methods like ELISA and lateral flow assays.
- ✓ High specificity ensures minimal false positives and false negatives.



STANDARD ELISA CUT-OFF

The current ELISA cut-off concentration for the tested antibody for autoimmune disease is 9 AU/ml. With OLED device a LOD lower than 2.25 AU/ml has been obtained using human samples.



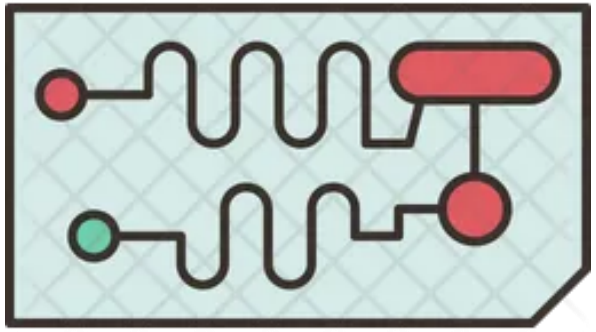
FAST, RELIABLE RESULTS

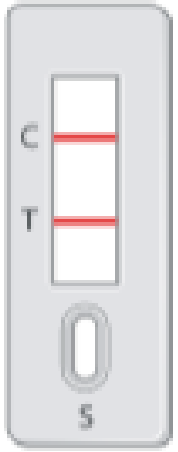
- ✓ Results ready in **under 30 minutes** —ideal for time-sensitive diagnostics.
- ✓ No manual intervention, fewer reagents, and reduced sample usage mean faster processing.

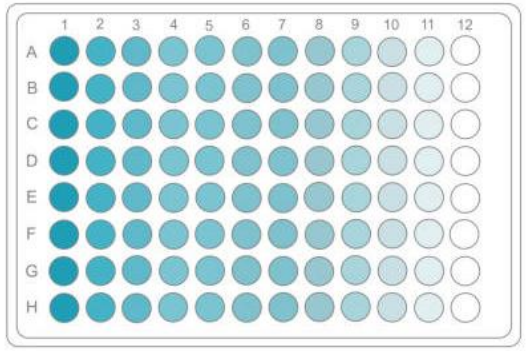
	POC-OREL	LFA	ELISA
Speed	✓	✓	✗
Sensitivity	✓	✗	✓
Point of care application	✓	✓	✗
Suitable with different specimen	✓	!	!
Multiplex	✓	!	✗
Quantification	✓	!	✓
Ease of use	✓	✓	✗

WHY POC-OREL IS BETTER THAN OTHER METHODS

- x **Traditional ELISA**: High sensitivity but slow, labor-intensive, and requires multiple steps.
- x **Lateral Flow Assays (LFAs)**: Quick, but qualitative results with lower sensitivity and multiplexing capabilities.
- ✓ **POC-OREL:** Combines the accuracy of ELISA with the speed and ease of LFAs—plus multiplexing and portability.





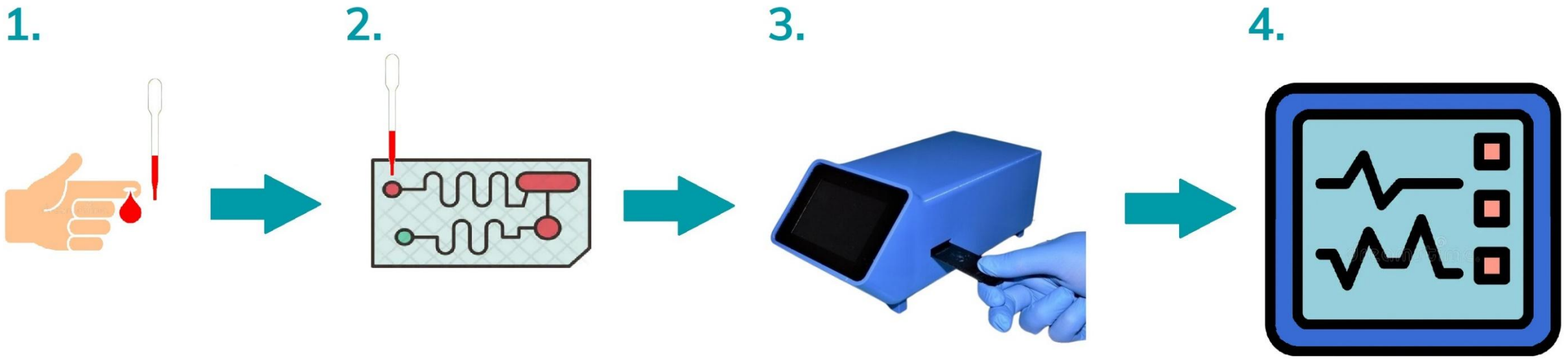


VERSATILE APPLICATION ACROSS MULTIPLE MARKERS

- ✓ Adaptable for various biomarkers (proteins, antibodies, peptides, DNA etc.)
- ✓ Strong licterature background (ELISA based)
- ✓ Suitable for clinical diagnostics, pharmaceutical research, and precision medicine.
  - **Clinical Diagnostics**: Use in hospitals, diagnostic labs, and remote healthcare settings
  - **Point-of-Care**: Ideal for on-site diagnostics in emergency rooms, clinics, or mobile health units.
  - **Pharmaceutical Research**: Biomarker monitoring and drug development studies.
  - **Precision Medicine**: Tailoring treatments based on precise, multi-biomarker data.

HOW POC-OREL ASSAY WORKS

- ✓ **Step 1**: Sample collection (e.g., blood, serum, plasma, liquor).
- ✓ **Step 2**: Sample addition in microfluidic cartridge.
- ✓ **Step 3**: Biomarker detection using advanced immunoassay techniques (15-30 minutes).
- ✓ **Step 4**: Results displayed on portable reader in quantitative form.



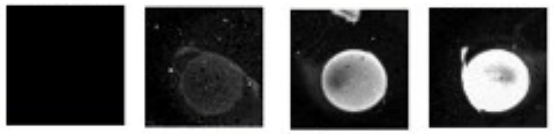
CASE STUDY #4 – POC-OREL

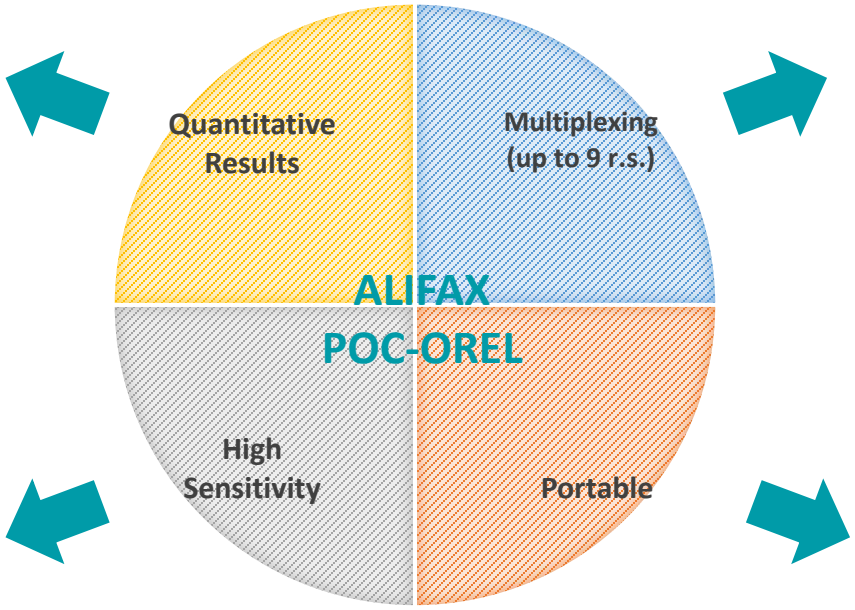
- ✓ Precise control over fluid dynamics allows for accurate assays even in small sample volumes.
- ✓ Low reagent consumption reduces overall costs and environmental impact.
- ✓ Miniaturized system provides on-site testing in resource-limited environments.

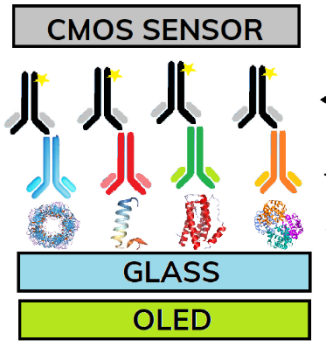
CASE STUDY #4 – POC-OREL

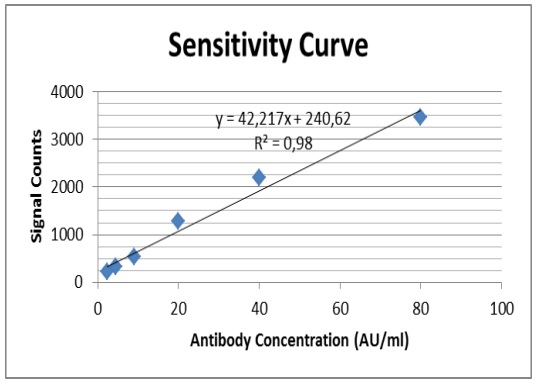
INSIDE INNOVATION

- ✓ **Innovative design** offers a competitive edge in speed, accuracy, and versatility.
- ✓ **Portability and ease of use** make it ideal for decentralized healthcare and mobile diagnostics.
- ✓ **Broad applications** in clinical diagnostics, research, and precision medicine.









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CASE STUDY #4 – POC-OREL

- ✓ POC-OREL combines precision, portability, and multiplexing in one powerful platform.
- ✓ Revolutionizing the way we diagnose diseases and monitor patient health.
- ✓ Providing fast, accurate, and comprehensive results in a variety of settings.