

ELISPOT

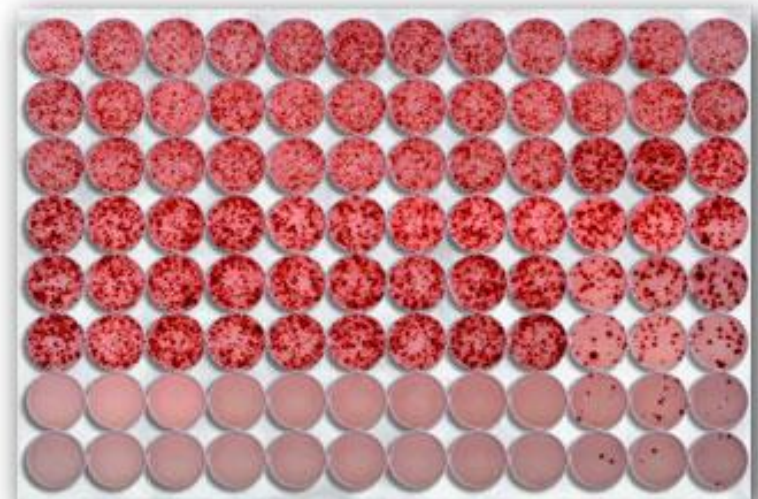
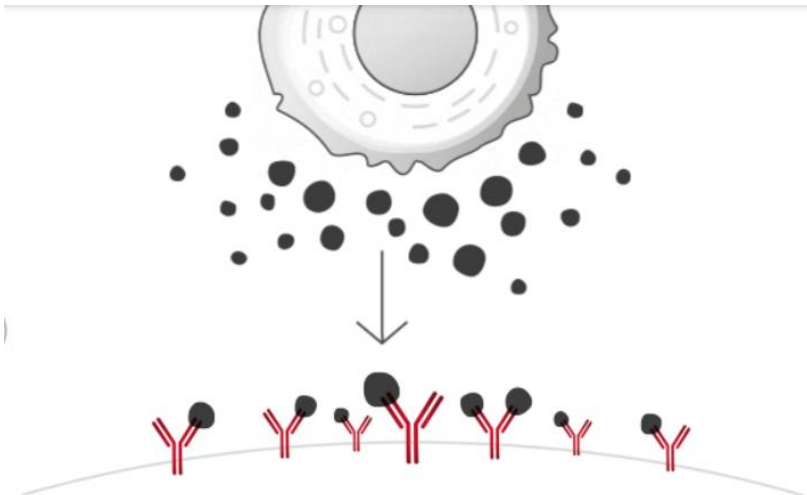
Enzyme-Linked ImmunoSpot assay

A Solid-Phase Enzyme-Linked Immunospot
(ELISPOT) Assay for Enumeration of Specific
Antibody-Secreting Cells

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Czerkinsky et al. J Immunol Methods. 1983;65(1-2):109-21



ELISPOT

Enzyme-Linked ImmunoSPOT assay

Unisce i metodi immunochimici e l'uso di colture cellulari.

Basato su ELISA, consente di **rilevare** e **contare**, a livello di **singola cellula**, la **frequenza** di **cellule positive** per la secrezione di un analita.

Vantaggio: le cellule possono essere recuperate per altri studi.

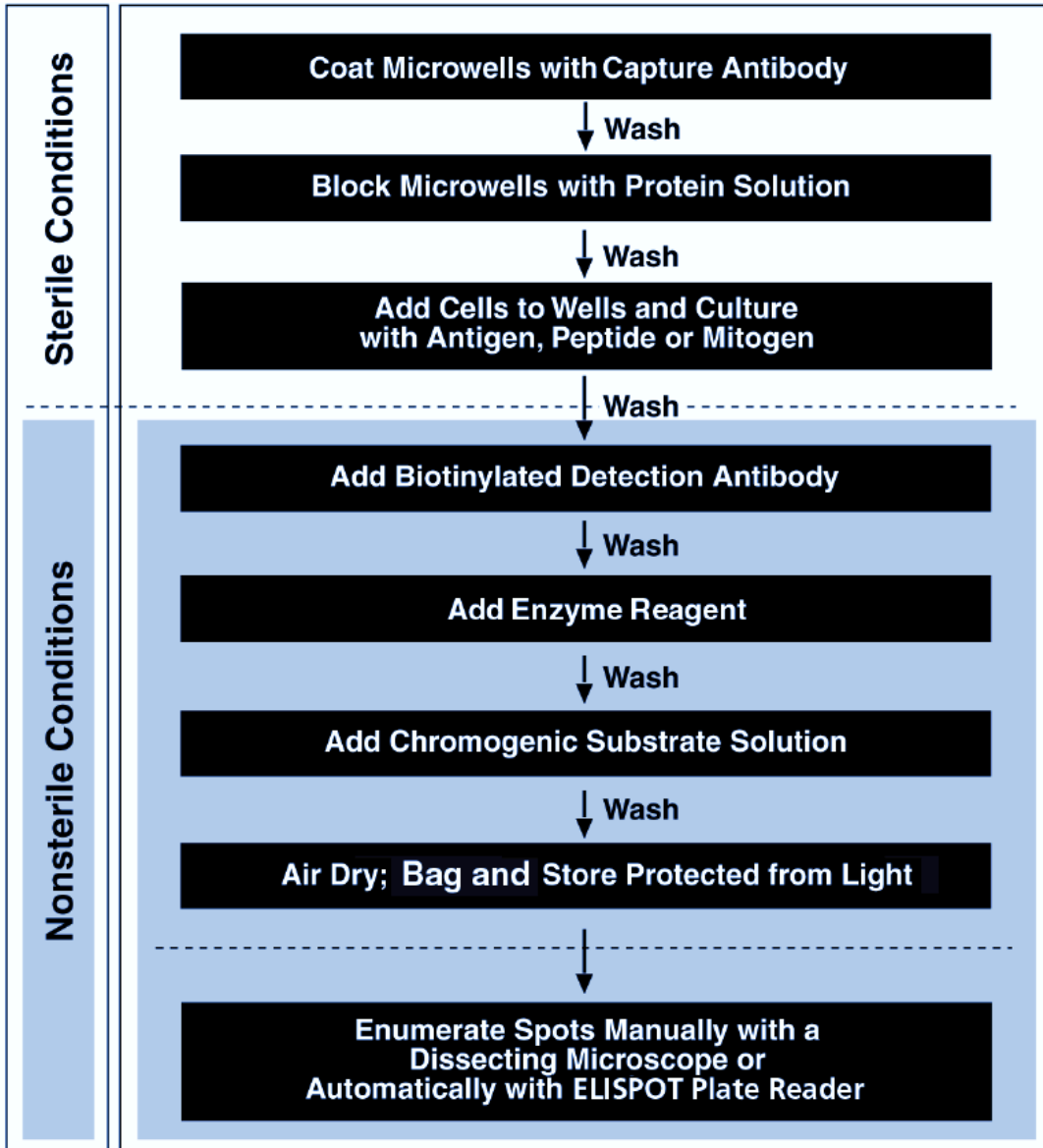
Inizialmente sviluppato per studiare la produzione e secrezione di **anticorpi** (**B-cell ELISPOT**), è stato poi adattato per rilevare la produzione di altre molecole effettrici come le **citochine** (**T-cell ELISPOT**).

Sensibilità: frequenze molto basse di cellule positive per l'analita/antigene (**fino a 1 cellula su 300.000**).

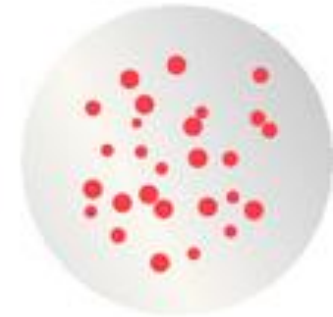
Numerose applicazioni per monitorare le risposte immuni (es. sviluppo di vaccini, allergie, malattie infettive, ecc.)

ELISPOT - Fasi sperimentali

Associa colture cellulari e metodo immunochimico

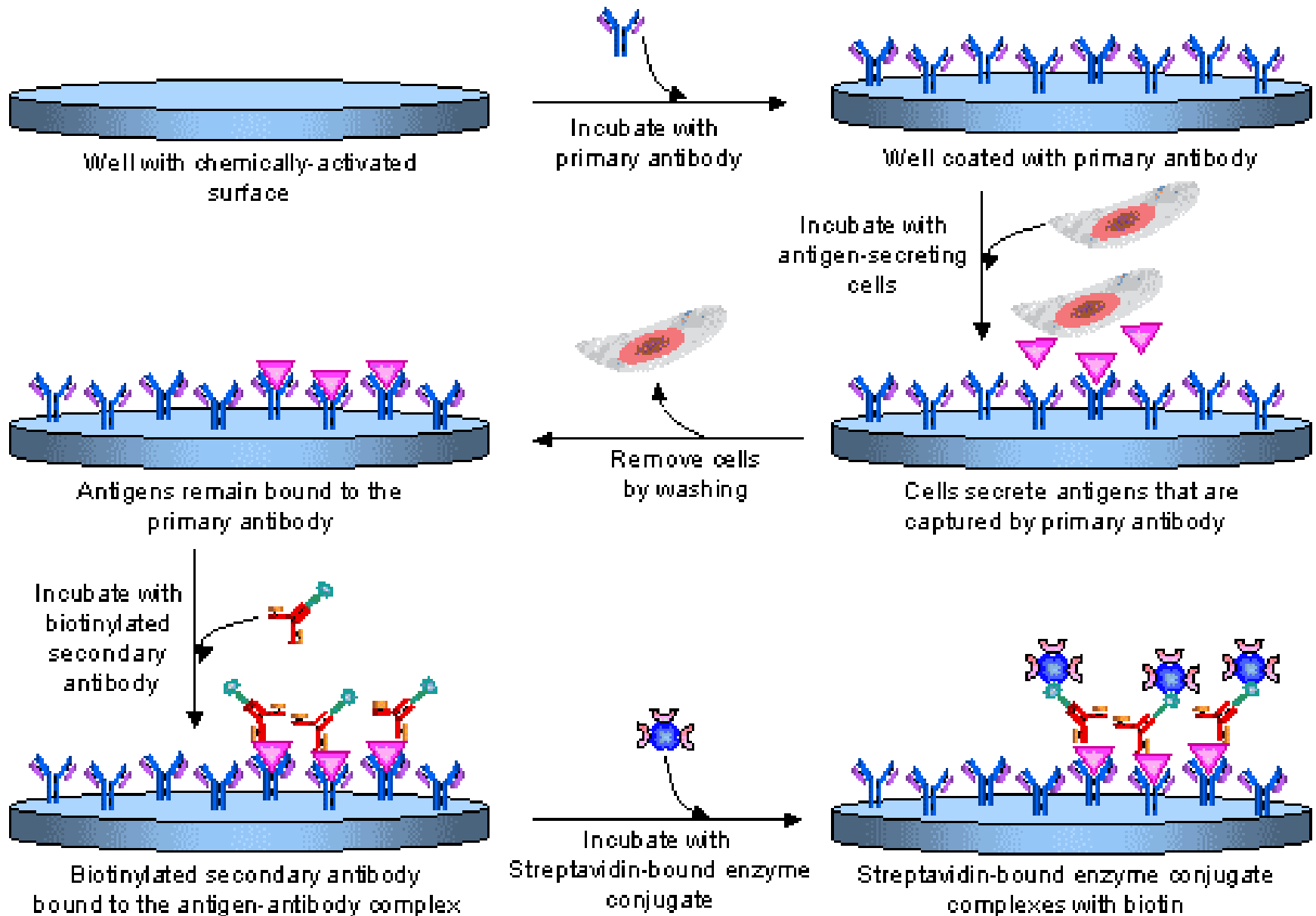


Il **risultato** è visibile come la presenza di **spot**

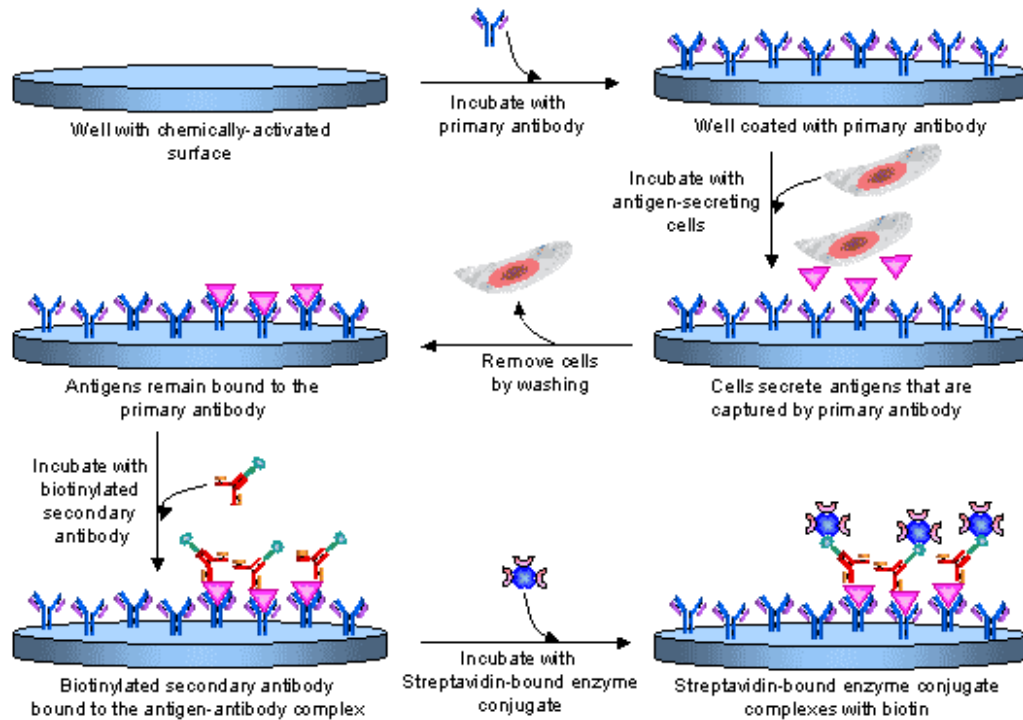


Ogni spot corrisponde ad una **singola cellula** che secerne l'analita

ELISPOT - Fasi sperimentali



ELISPOT - Fasi sperimentali



Gli analiti secreti dalle cellule (es. anticorpi o citochine) vengono immediatamente catturati, dagli anticorpi immobilizzati, dove la **concentrazione locale è elevata** → **elevata sensibilità**



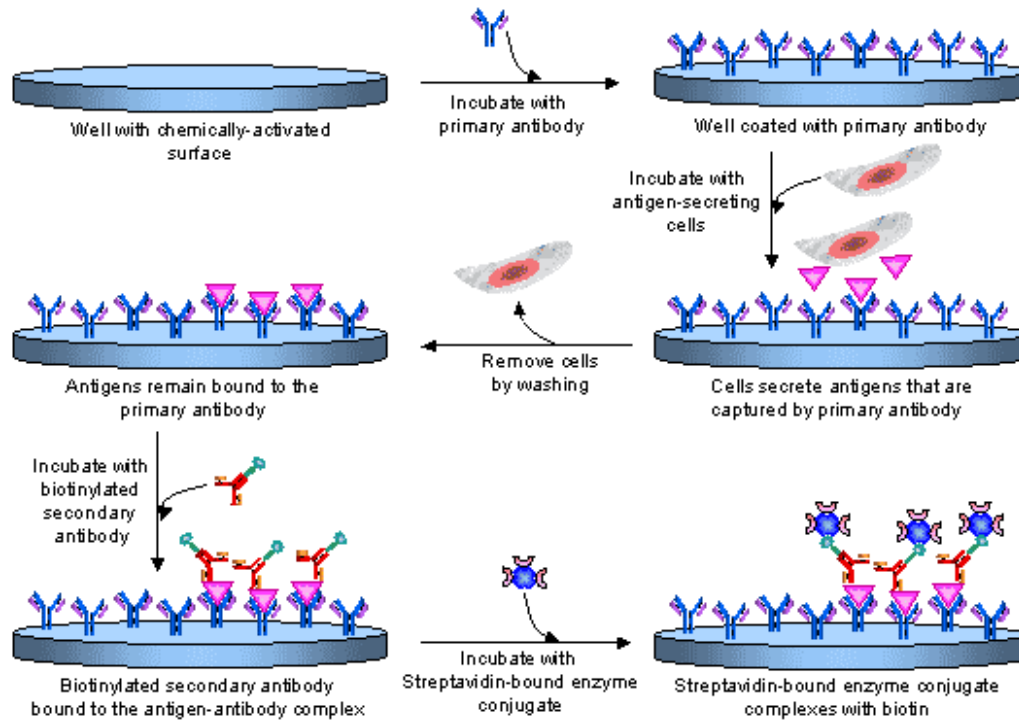
Es. campione per ELISPOT:

total peripheral blood mononuclear cells (**PBMCs**)

contenenti linfociti e monociti

Range consigliato: 10^3 - 10^6 cellule per pozzetto

ELISPOT - Fasi sperimentali



Gli analiti secreti dalle cellule (es. anticorpi o citochine) vengono immediatamente catturati, dagli anticorpi immobilizzati, dove la **concentrazione locale è elevata** → **elevata sensibilità** (*no effetto diluizione nel supernatante*)

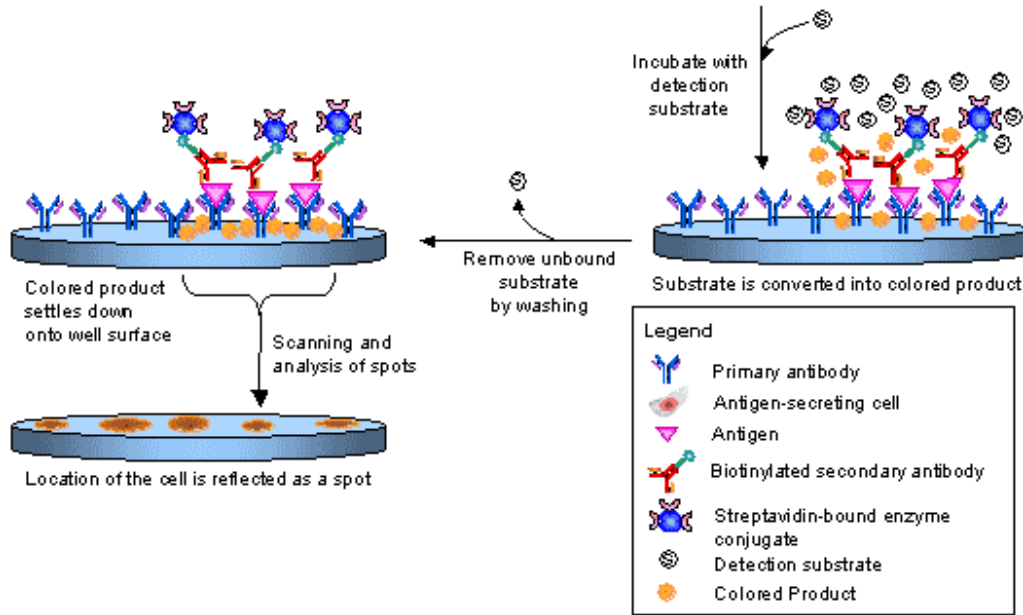
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ELISPOT - Fasi sperimentali



Gli analiti vengono rilevati dopo l'aggiunta di un substrato che, convertito in **prodotto**, precipita formando degli **spot**

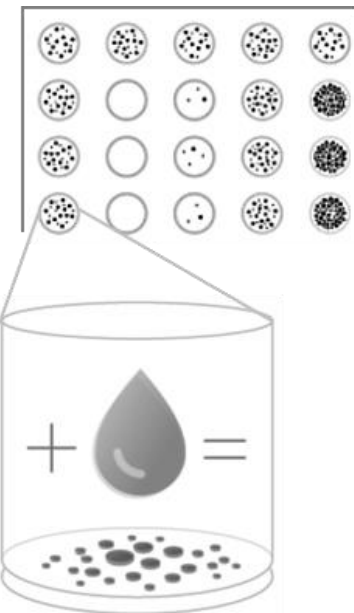


Gli **spot** vengono **contati manualmente** al microscopio o mediante **lettori di micropiastre**

1 spot = 1 cellula positiva per la secrezione dell'analita

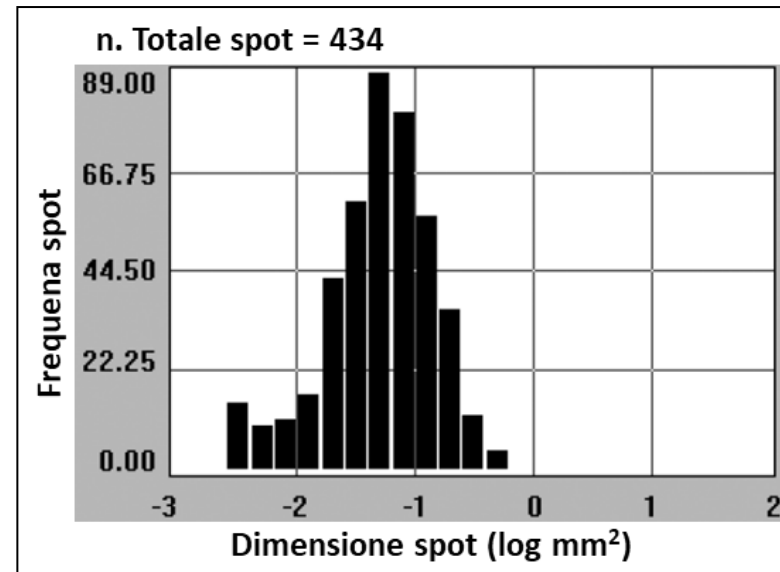
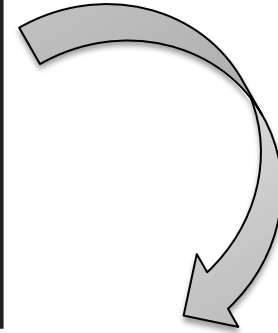
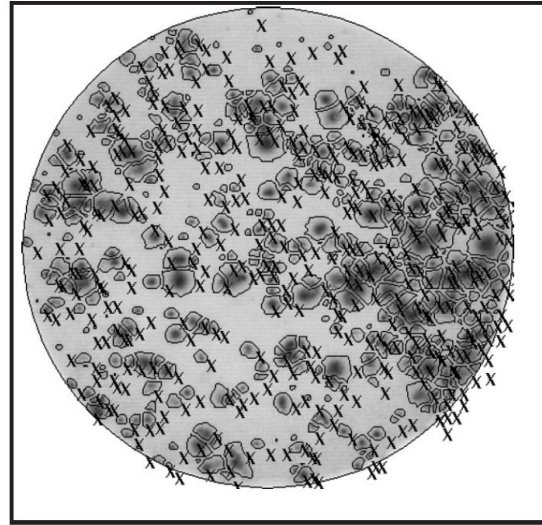
n. spot = n. cellule che secernono l'**analita**

Intensità/dimensione spot = capacità relativa di **secrezione**



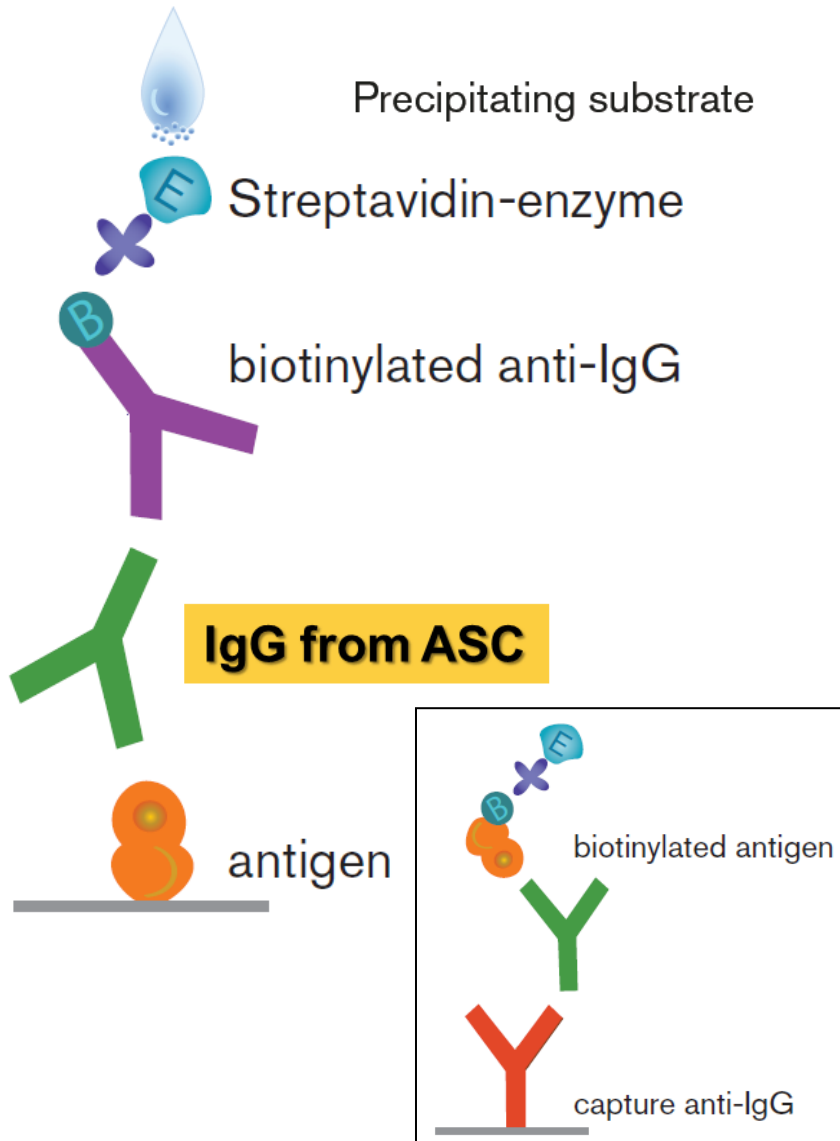
ELISPOT

Conteggio degli **spot** mediante **lettori** di **micropiastre**

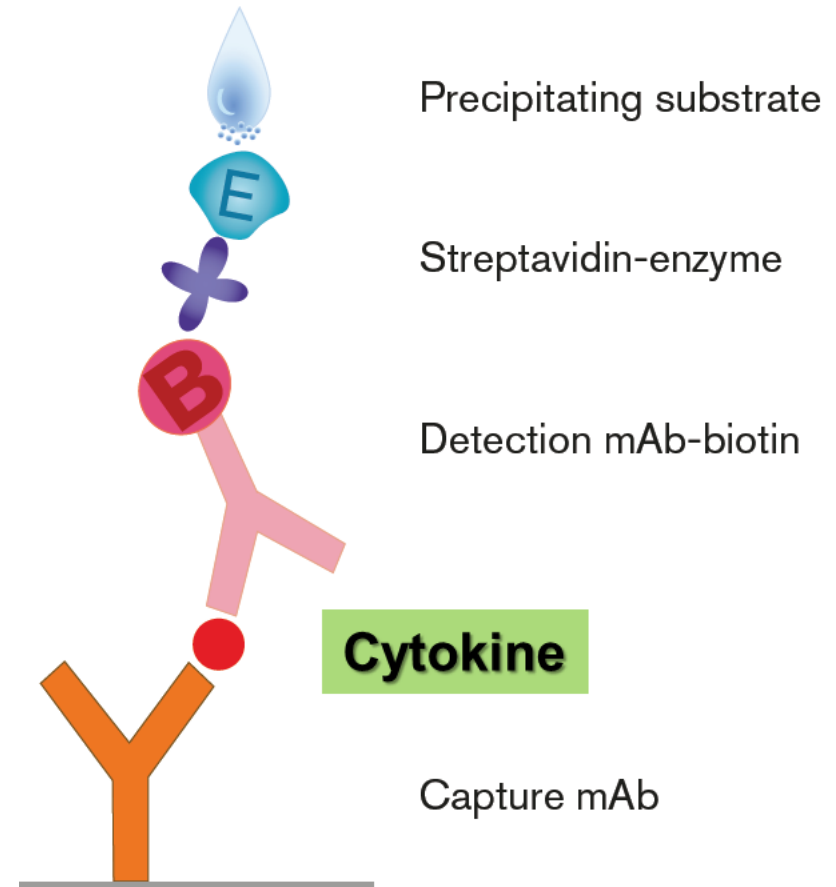


ELISPOT - esempi

B-cell ELISPOT



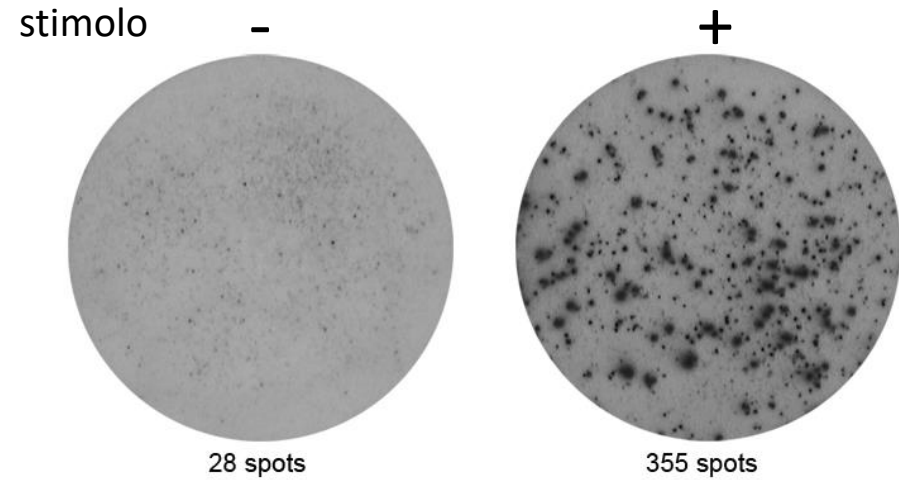
T-cell ELISPOT



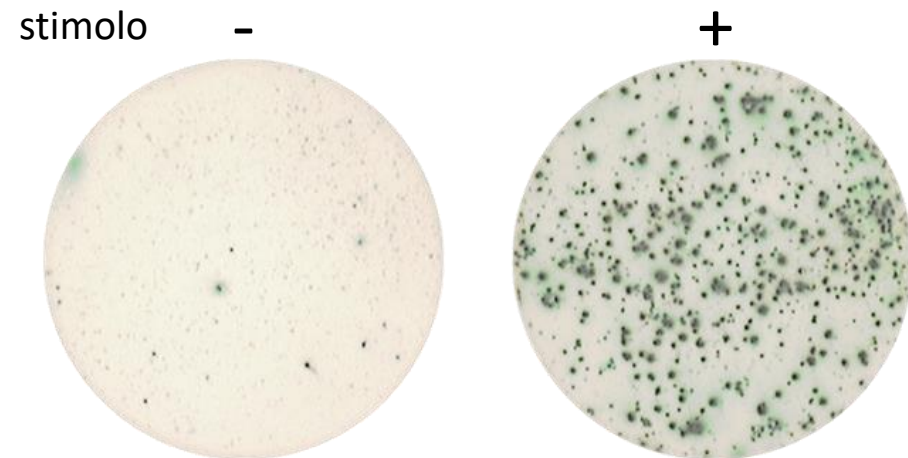
ELISPOT - esempi

B-cell ELISPOT

Human IgG (ALP)

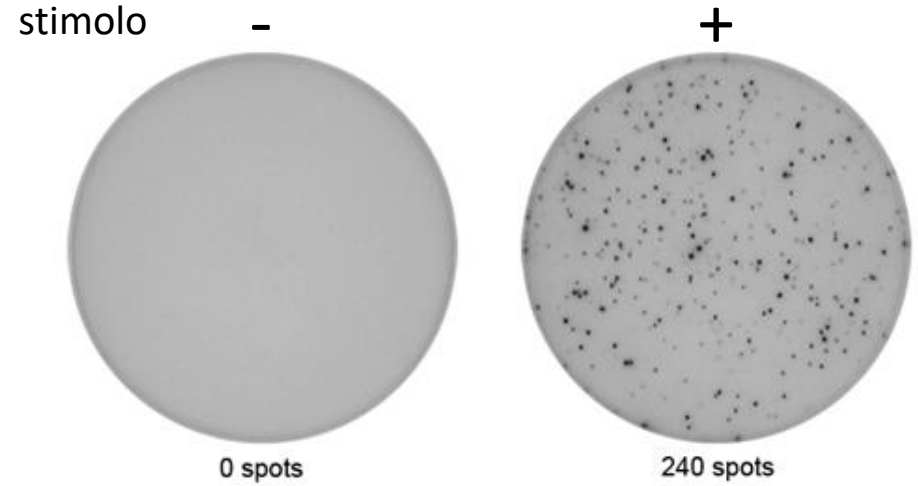


Human IgM (HRP)

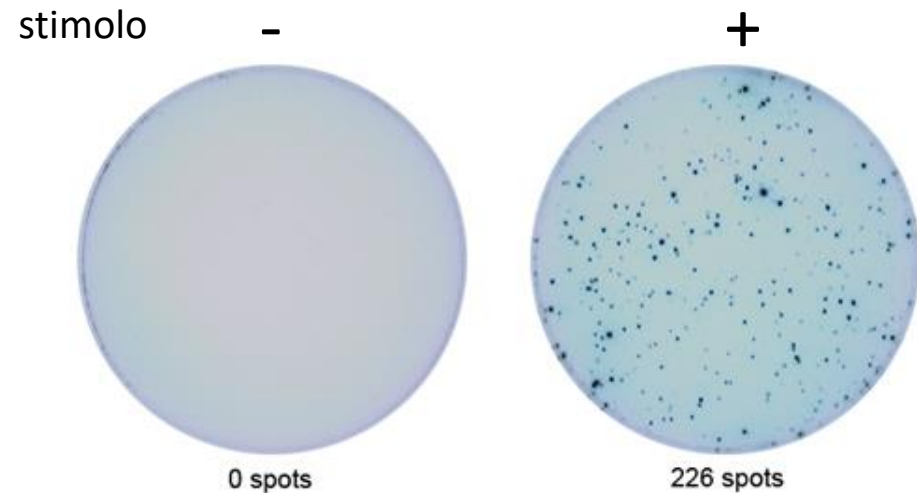


T-cell ELISPOT

Human IL-4 (ALP)

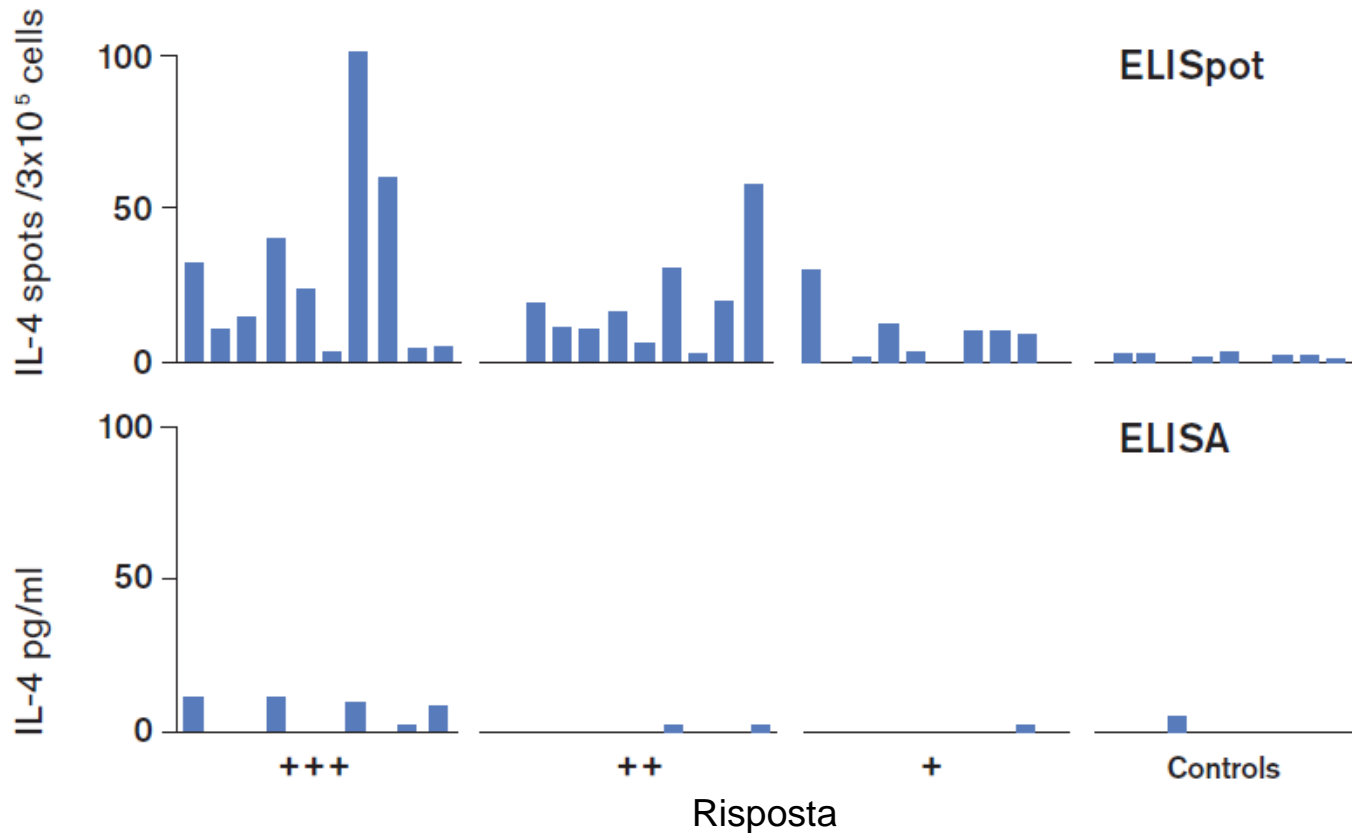


Human IL-4 (HRP)



ELISPOT vs ELISA - esempio di studio

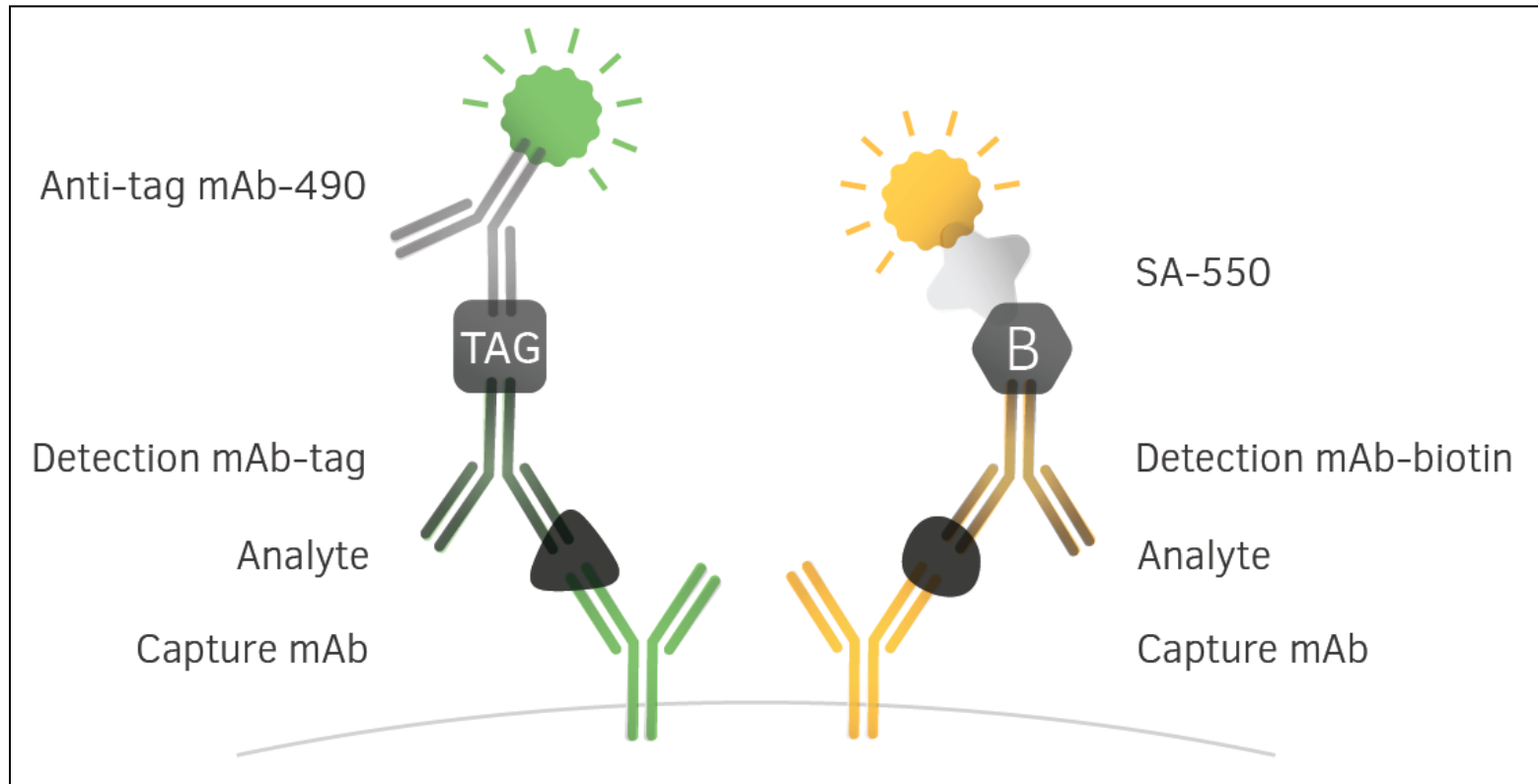
Maggiore sensibilità di un saggio ELISA



PBMC from subjects with or without contact allergy to nickel were tested for *in vitro* reactivity to nickel. The subjects displayed +++ (n=10), ++ (n=11), + (n=10) or no (controls; n=10) reactivity to nickel. PBMC were incubated with or without 50 μ M NiCl₂ for 40h and IL-4 responses were assessed in ELISpot and in ELISA (using cell culture supernatants). Detectable responses to nickel, above cut-off levels, were seen in **23 of the 31** allergic individuals when tested with **ELISpot** whereas **only 4** were defined as positive in the **ELISA**.

FLUOROSPOT

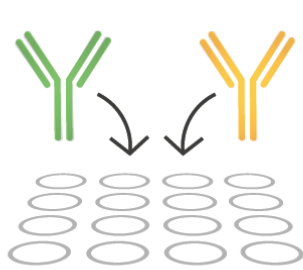
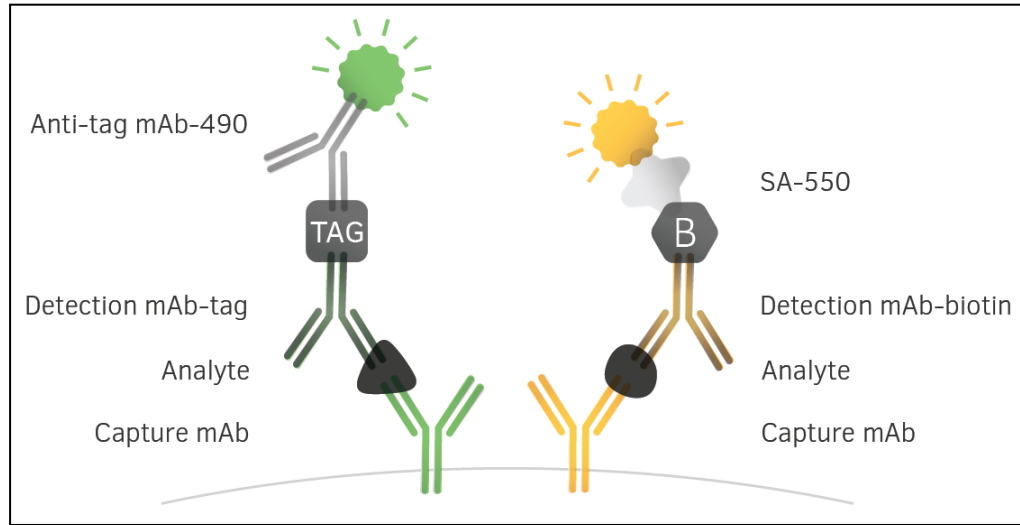
Rilevazione in **fluorescenza** che consente di valutare fino a **2 o 3 analiti** simultaneamente **nello stesso pozzetto**, e quindi la presenza di cellule aventi un **profilo di secrezione "combinato"**



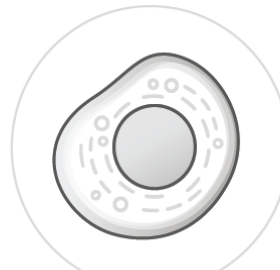
Rilevazione fino a tre analiti:

Ab biotinilati per un analita e Ab coniugati a tag per l'altro/i analita/i.

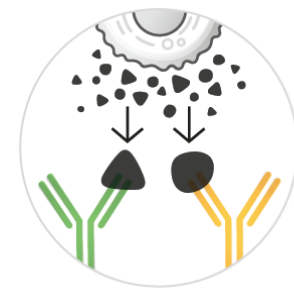
FLUOROSPOT - Fasi sperimentali



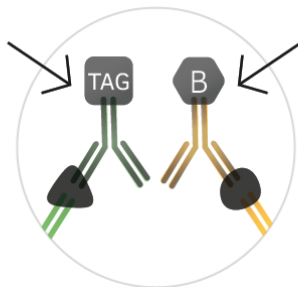
Coating



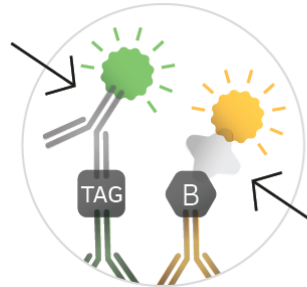
Cell incubation



Cytokine capture

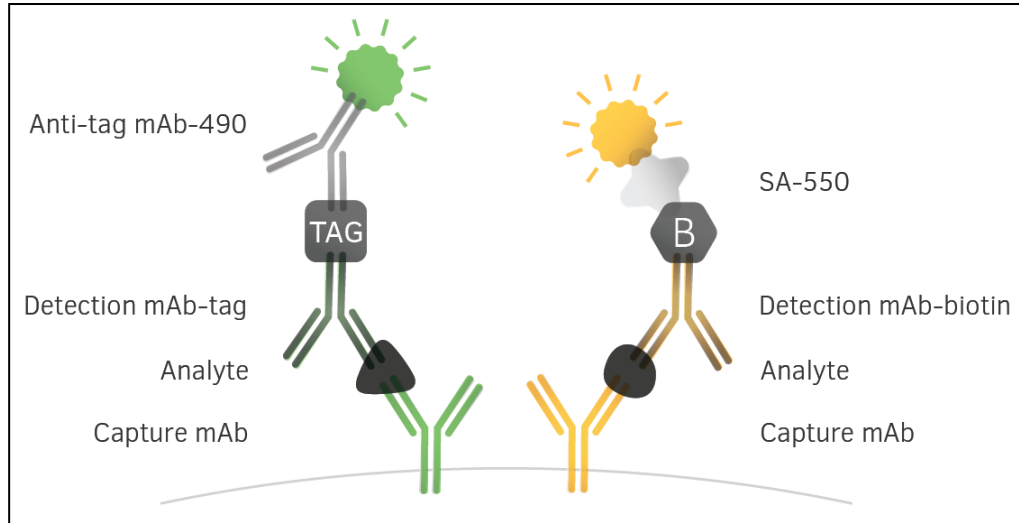


Detection antibodies



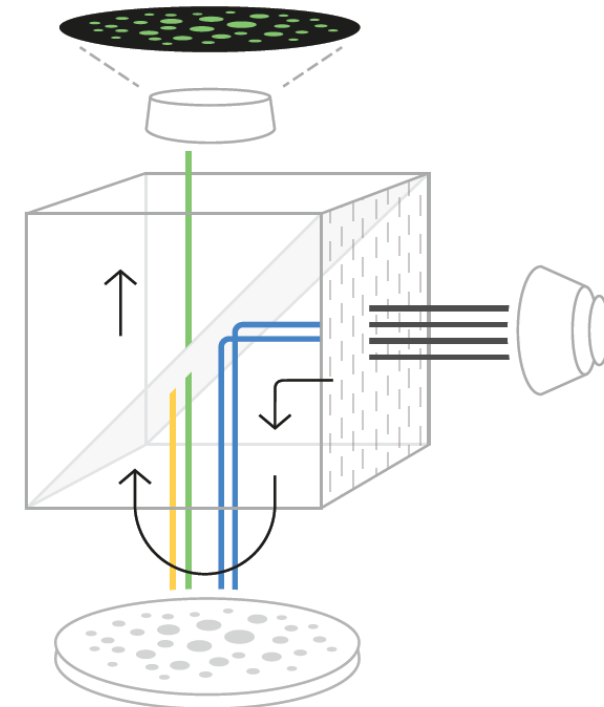
Fluorophore-labeled conjugates

FLUOROSPOT - Fasi sperimentali



Fluorophore-labeled conjugates

Analysis



Mediante i diversi filtri vengono create immagini separate e gli spot vengono contati.

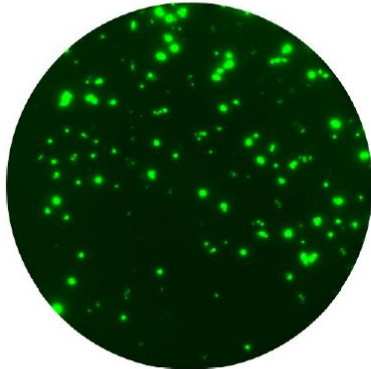
Ad ogni spot viene assegnata una coordinata

In base alle coordinate gli spot vengono analizzati e visualizzati in "overlay"

FLUOROSPOT - esempi

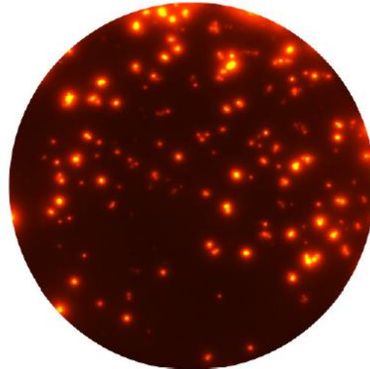
T-cell FLUOROSPOT

IFN- γ



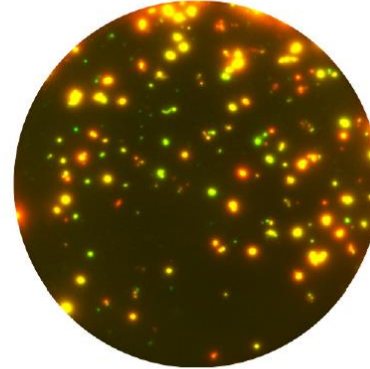
166 spots

IL-2



157 spots

Overlay

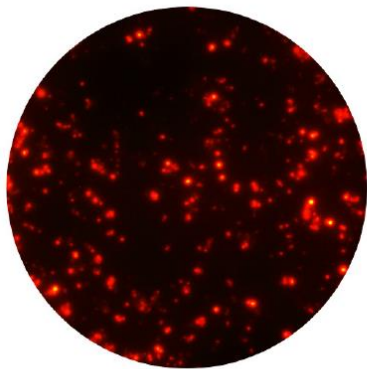


114 dual spots

IFN- γ = FITC
Cy3 = IL-2

B-cell FLUOROSPOT

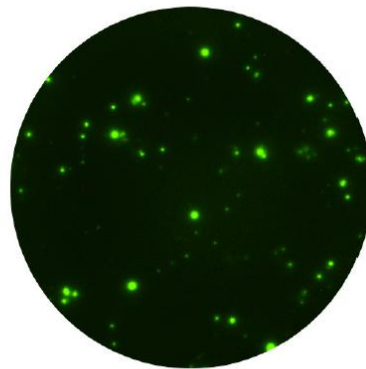
IgG



230 spots

Cy3

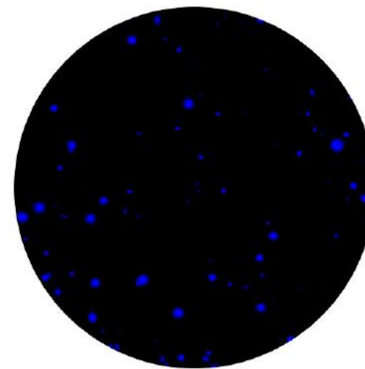
IgA



59 spots

FITC

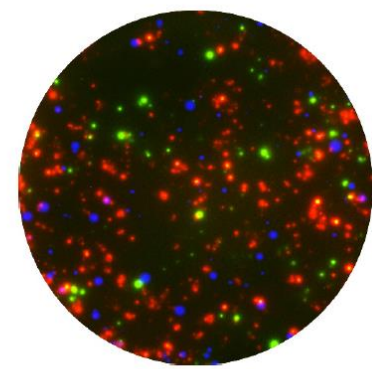
IgM



75 spots

Cy5

Overlay



0 dual/triple spots