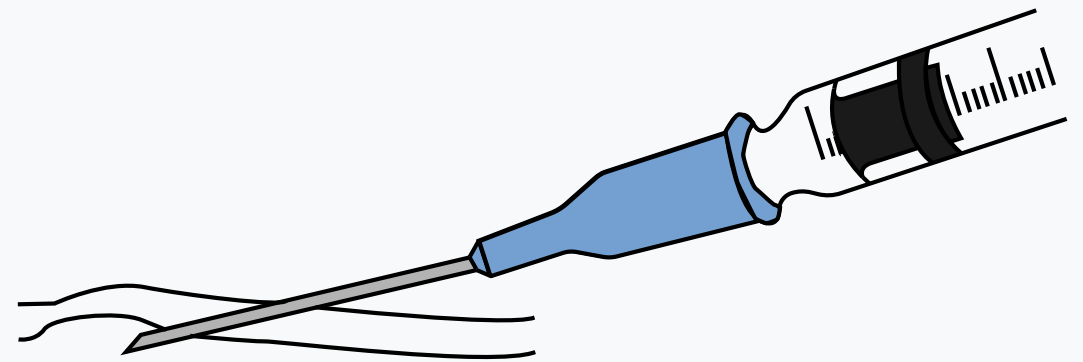


# Current State of Delayed Hypersensitivity Reactions to Medications

Matt Krantz, MD

*Assistant Professor of Medicine*

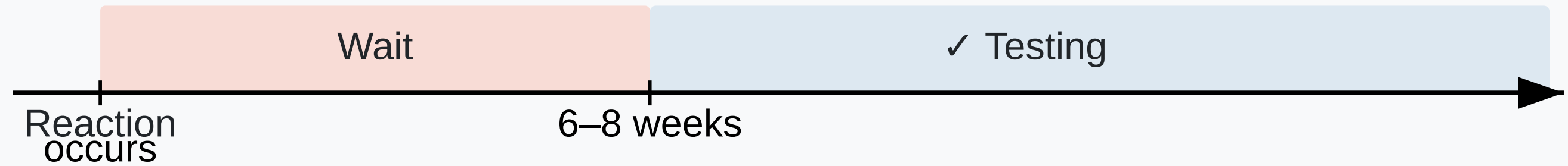
*Vanderbilt University Medical Center*



# Principles of Delayed Hypersensitivity Testing

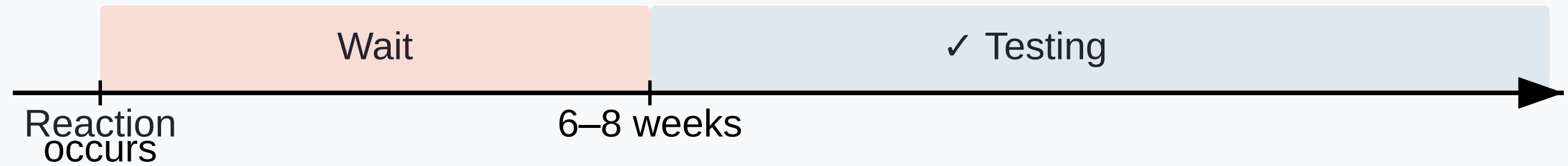
# Testing Timeline

## Non-DRESS Reactions (MPE, FDE, AGEP, SJS/TEN)

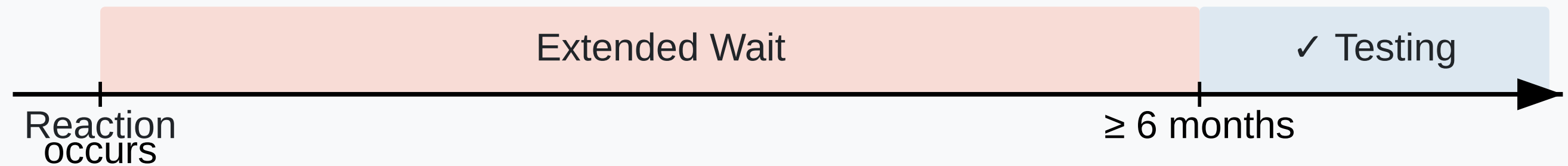


# Testing Timeline

## Non-DRESS Reactions (MPE, FDE, AGEP, SJS/TEN)



## DRESS



# Concomitant Medications

✓ Okay to Continue

- Antihistamines
- Beta-blockers

No effect on T-cell-mediated reactions

# Concomitant Medications

## ✓ Okay to Continue

- Antihistamines
- Beta-blockers

No effect on T-cell-mediated reactions

## ⚠ Must Hold or Reduce

- **Systemic Steroids**

Off  $\geq$  1 month

— or —

Prednisone equivalent

**$\leq$  10 mg/day**

Suppresses T-cell-mediated responses

# Specificity of Delayed Testing

 **No Delayed Test Achieves 100% NPV**

A negative result does not rule out delayed drug hypersensitivity

# Sensitivity of Delayed Testing

Patch Testing

40–55%

0%

50%

100%

Sensitivity

Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing

Patch Testing

40–55%

Delayed IDT

60–70%

0%

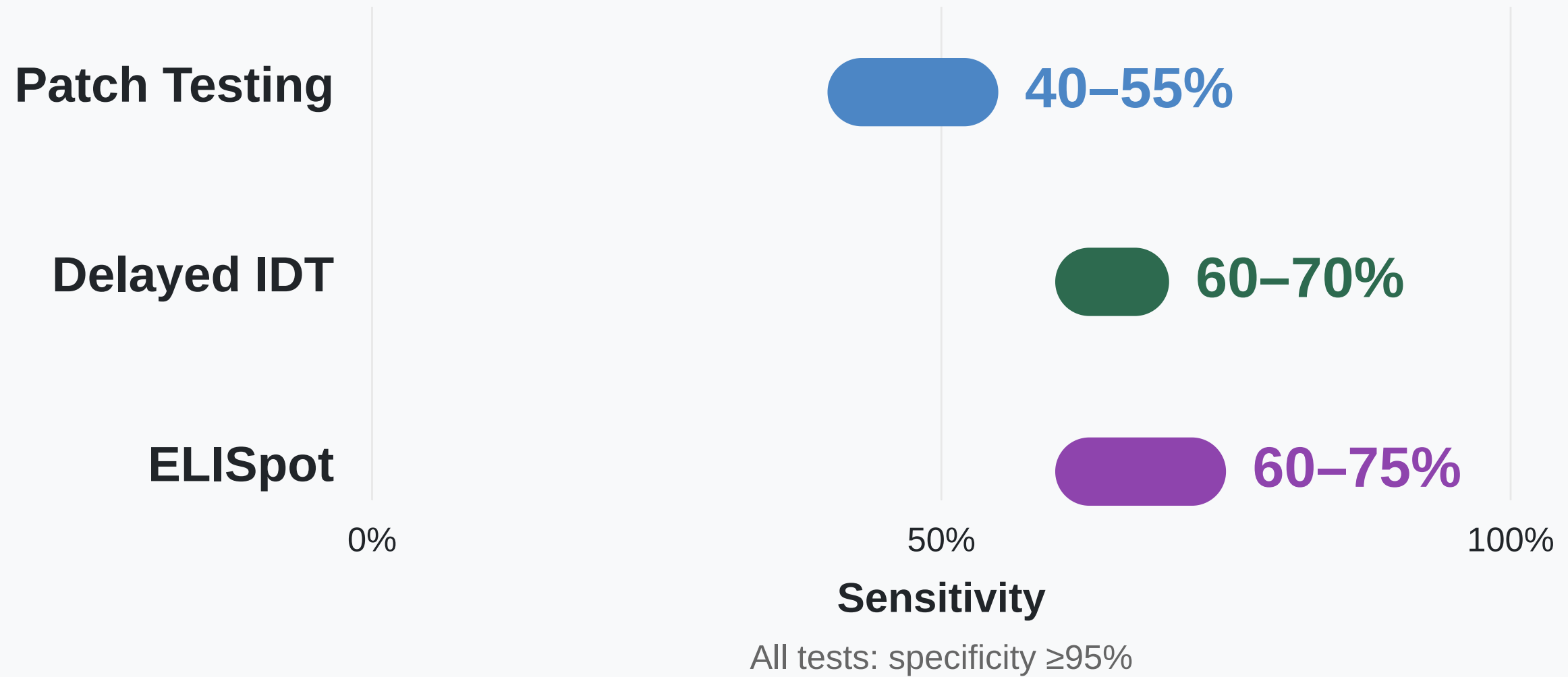
50%

100%

Sensitivity

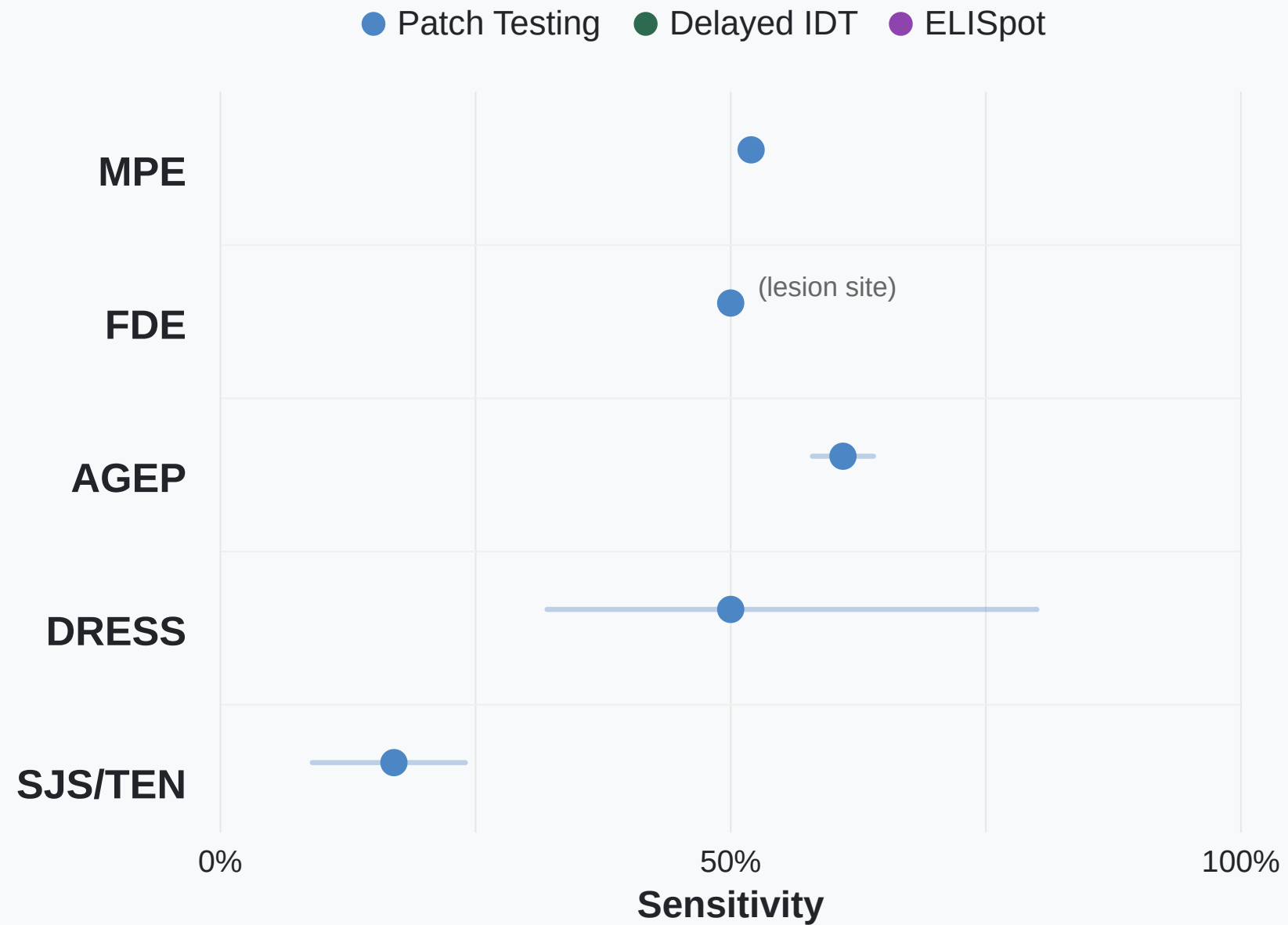
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing



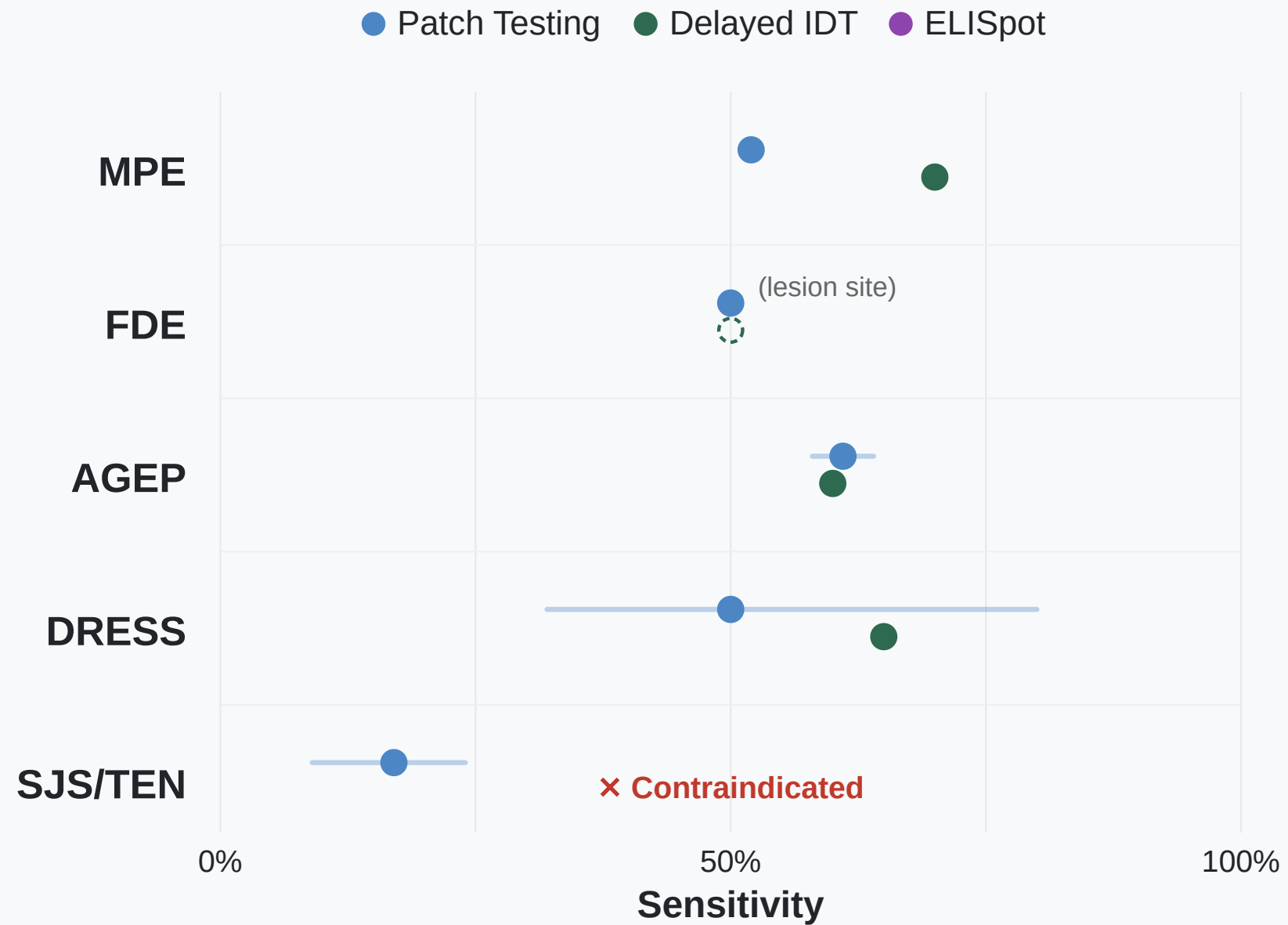
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Phenotype



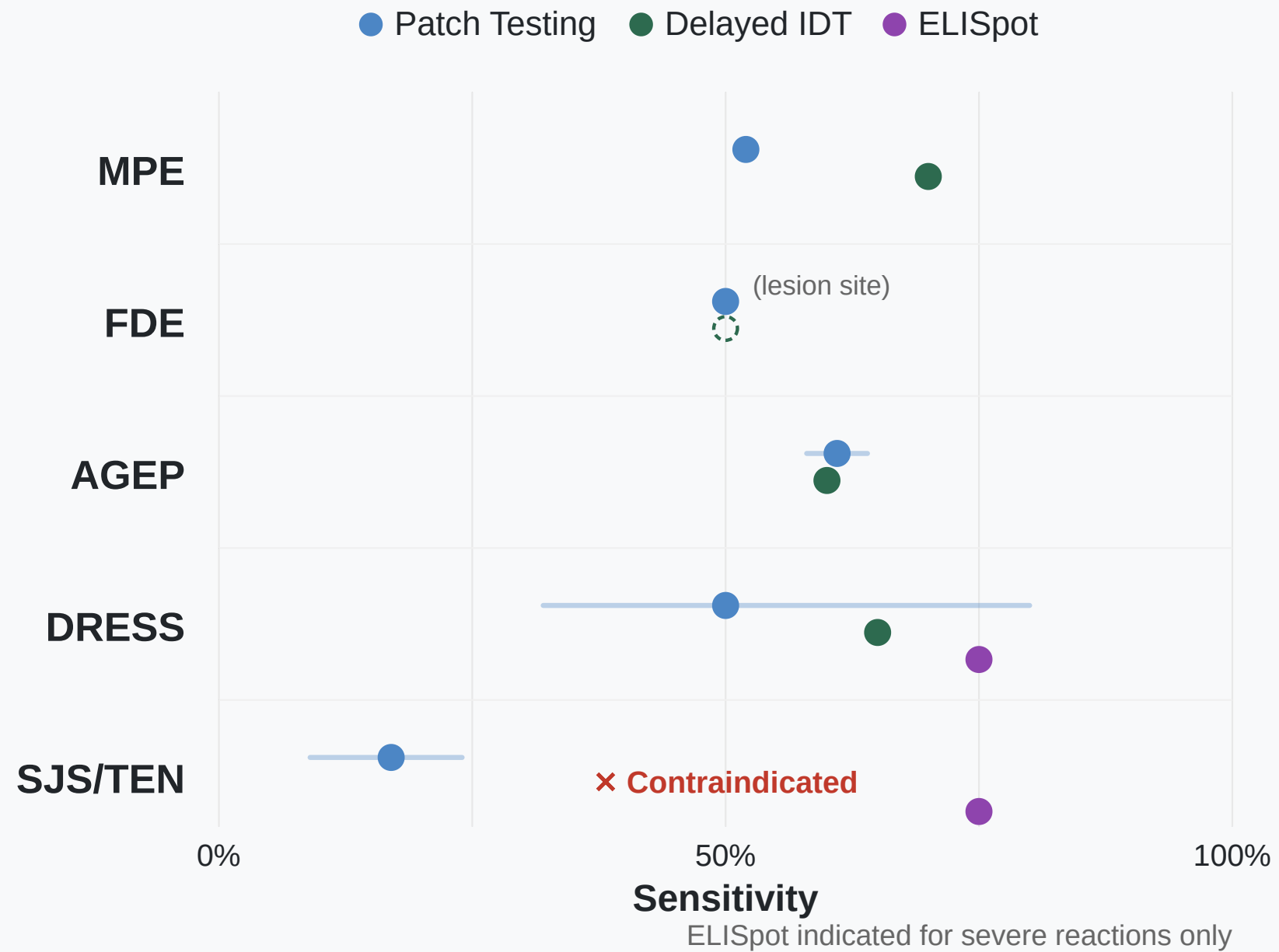
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Phenotype



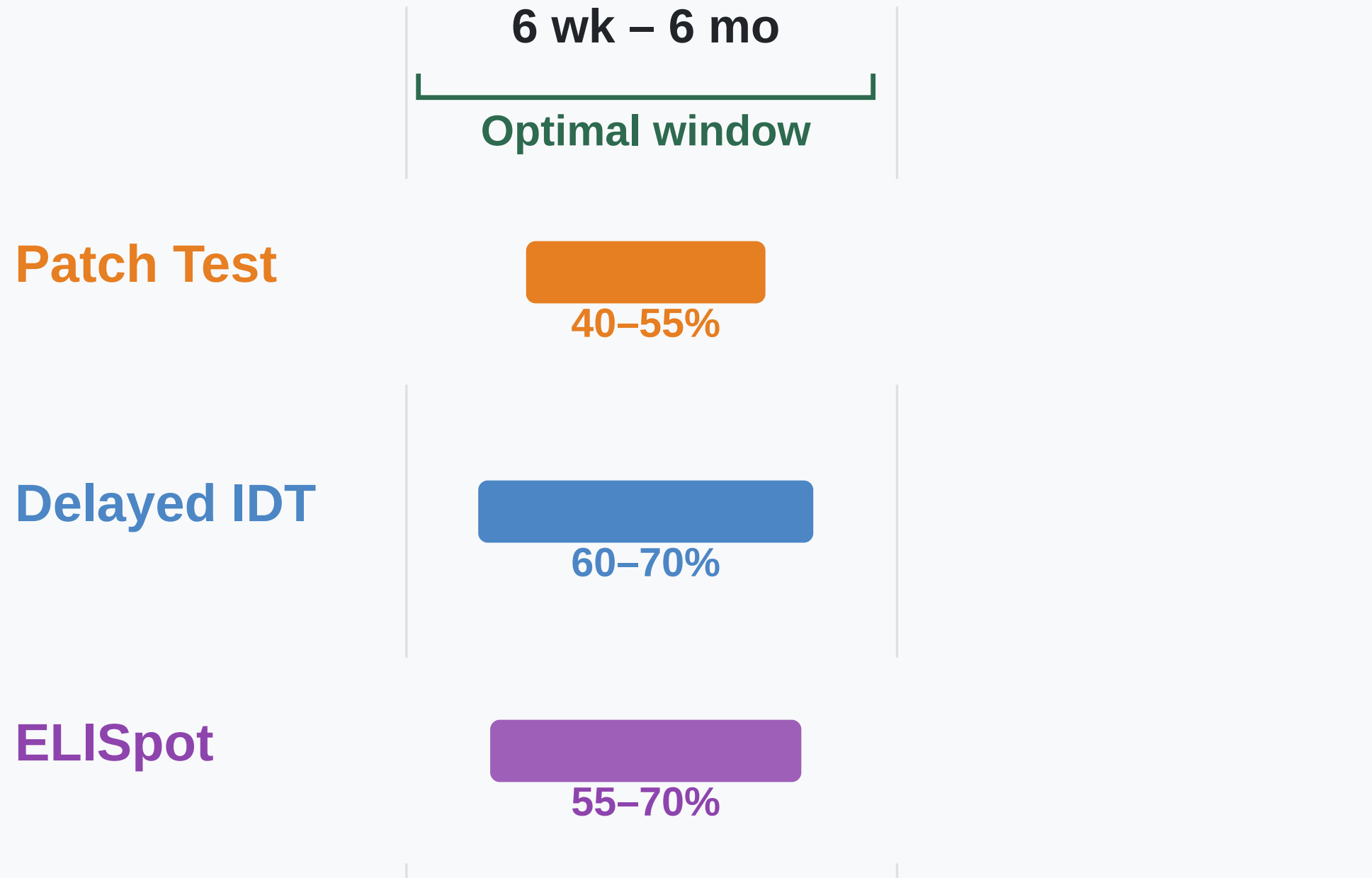
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Phenotype



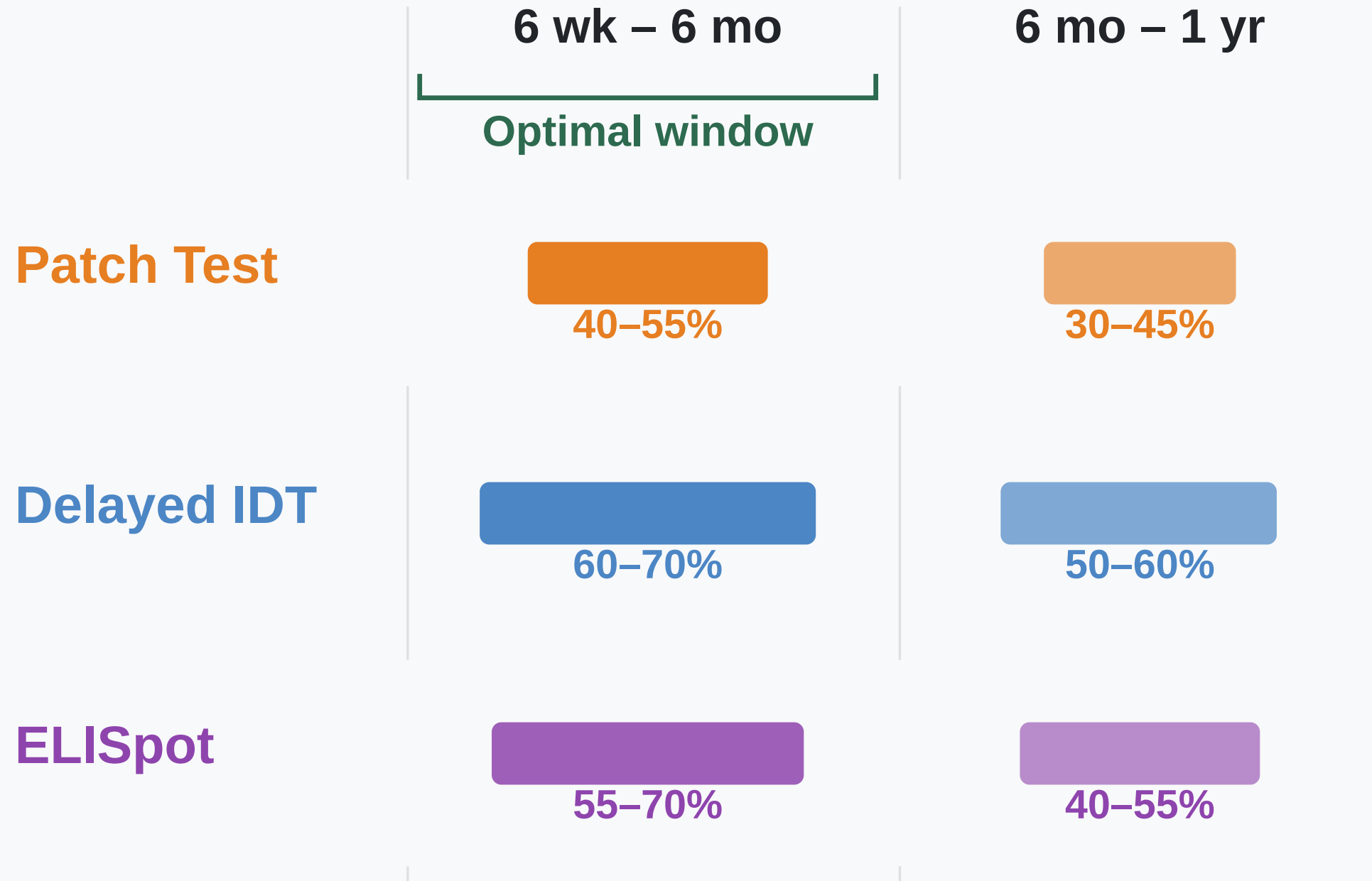
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Timing



Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Timing



Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Sensitivity of Delayed Testing by Timing



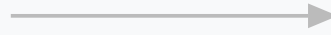
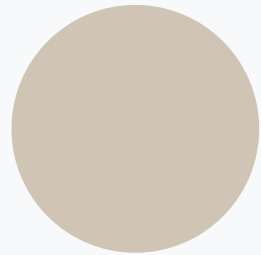
Ranges approximate — limited formal longitudinal data for delayed-type reactions

# Delayed Intradermal Testing

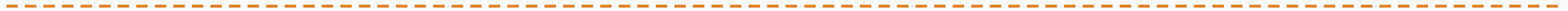
# Drug Concentrations: Immediate vs Delayed

15–20 min  
Immediate read

**Standard  
IDT conc.**  
Non-irritating  
(IgE-optimized)



**No wheal/flare**  
*e.g. vancomycin via MRGPRX2*

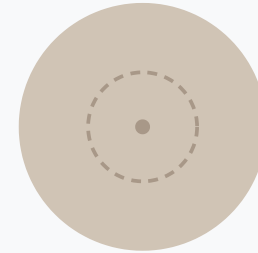
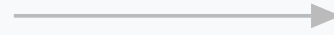
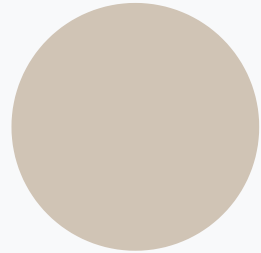


# Drug Concentrations: Immediate vs Delayed

15–20 min  
Immediate read

24–48 hr  
Delayed read

**Standard  
IDT conc.**  
Non-irritating  
(IgE-optimized)



**(-)**  
Delayed  
missed

**No wheal/flare**  
*e.g. vancomycin via MRGPRX2*

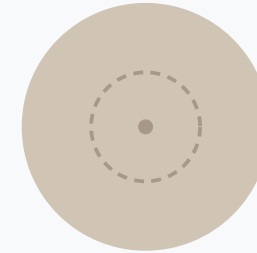
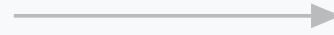
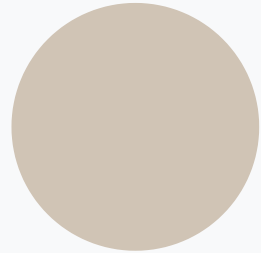
**Flat — no reaction**

# Drug Concentrations: Immediate vs Delayed

**15–20 min**  
Immediate read

**24–48 hr**  
Delayed read

**Standard  
IDT conc.**  
Non-irritating  
(IgE-optimized)



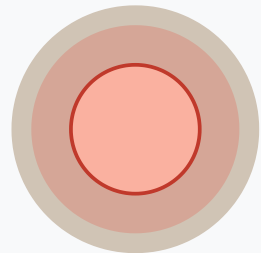
**(-)**  
Delayed  
missed

**No wheal/flare**  
*e.g. vancomycin via MRGPRX2*

**Flat — no reaction**

---

**Higher  
conc.**  
Needed for  
delayed detection



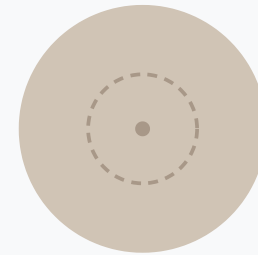
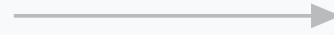
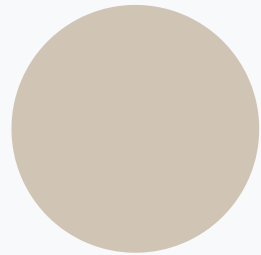
**Wheal / flare**  
*MRGPRX2 response*

# Drug Concentrations: Immediate vs Delayed

15–20 min  
Immediate read

24–48 hr  
Delayed read

**Standard  
IDT conc.**  
Non-irritating  
(IgE-optimized)

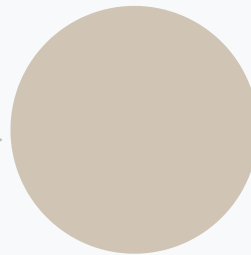
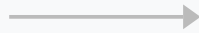
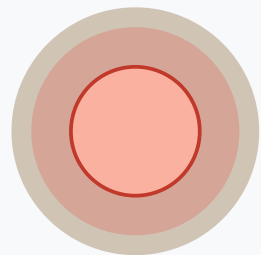


**(-)**  
Delayed  
missed

**No wheal/flare**  
*e.g. vancomycin via MRGPRX2*

**Flat — no reaction**

**Higher  
conc.**  
Needed for  
delayed detection



**Wheal / flare**  
*MRGPRX2 response*

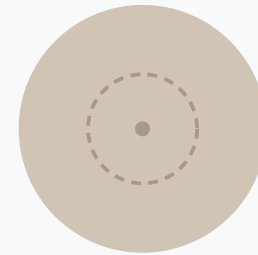
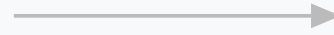
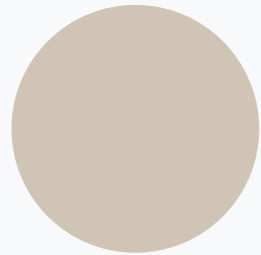
Resolves,  
then...

# Drug Concentrations: Immediate vs Delayed

15–20 min  
Immediate read

24–48 hr  
Delayed read

**Standard  
IDT conc.**  
Non-irritating  
(IgE-optimized)

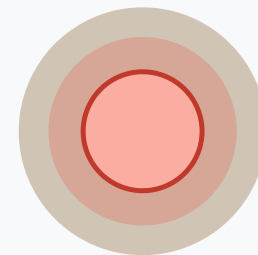
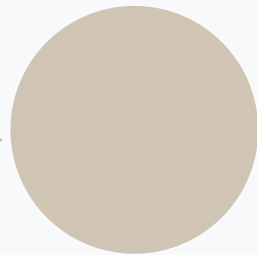
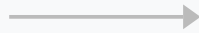
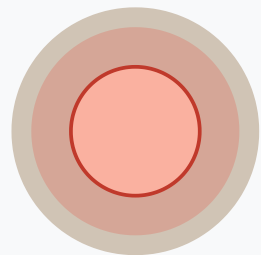


**(–)**  
Delayed  
missed

**No wheal/flare**  
*e.g. vancomycin via MRGPRX2*

**Flat — no reaction**

**Higher  
conc.**  
Needed for  
delayed detection



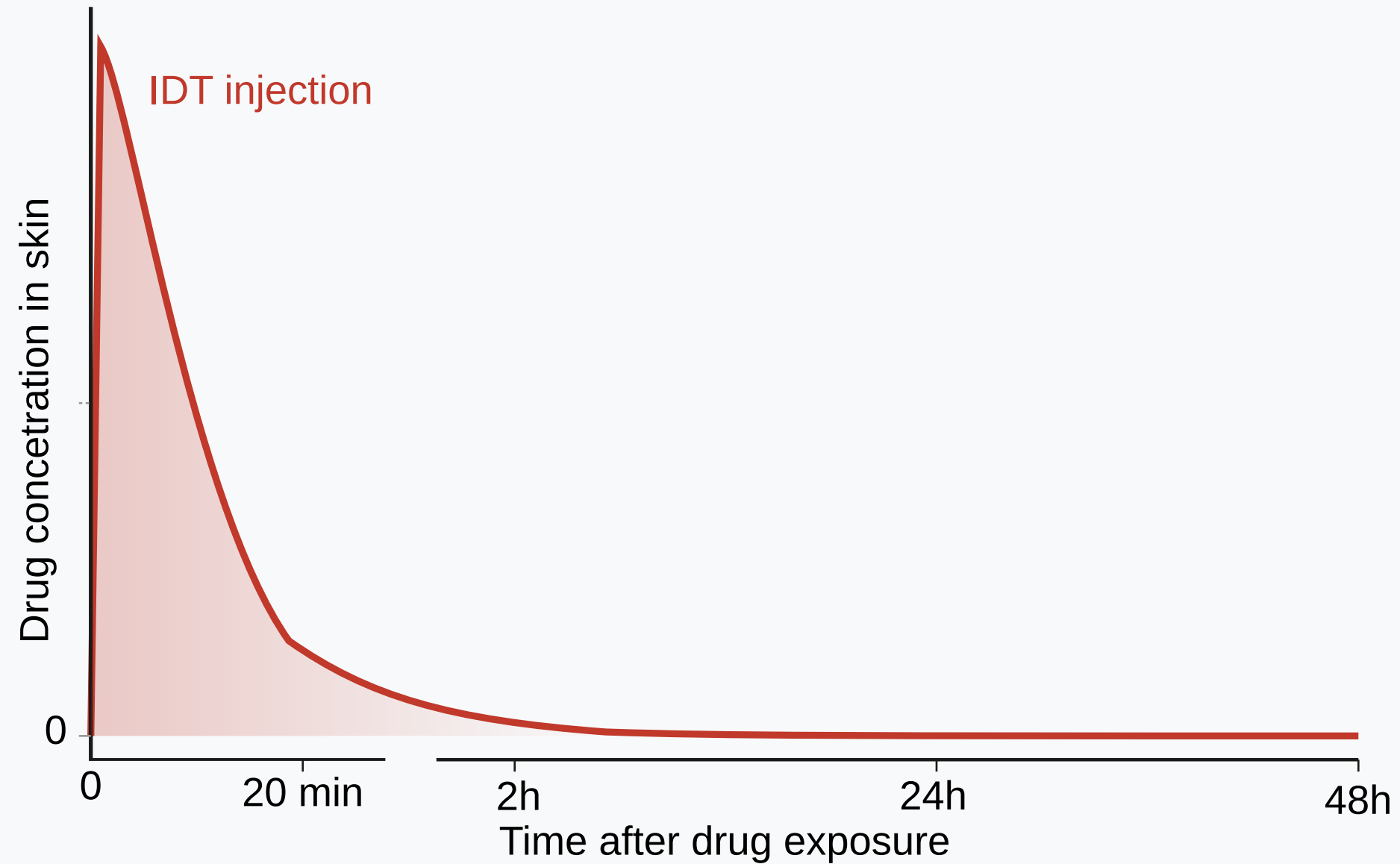
**(+)**  
Delayed  
detected

**Wheal / flare**  
*MRGPRX2 response*

Resolves,  
then...

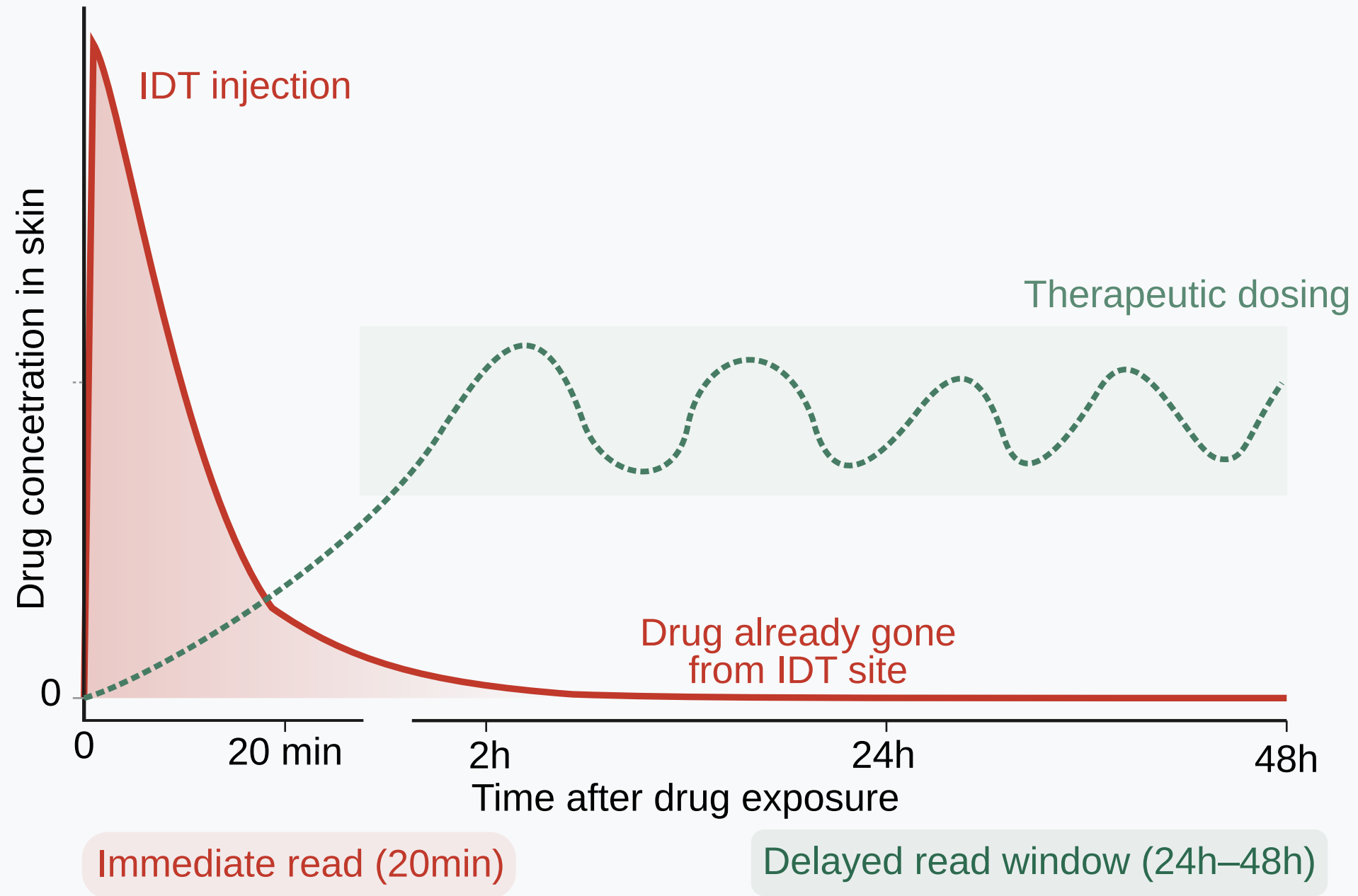
**Indurated papule**  
*T-cell mediated infiltration*

# Drug Concentrations: Immediate vs Delayed

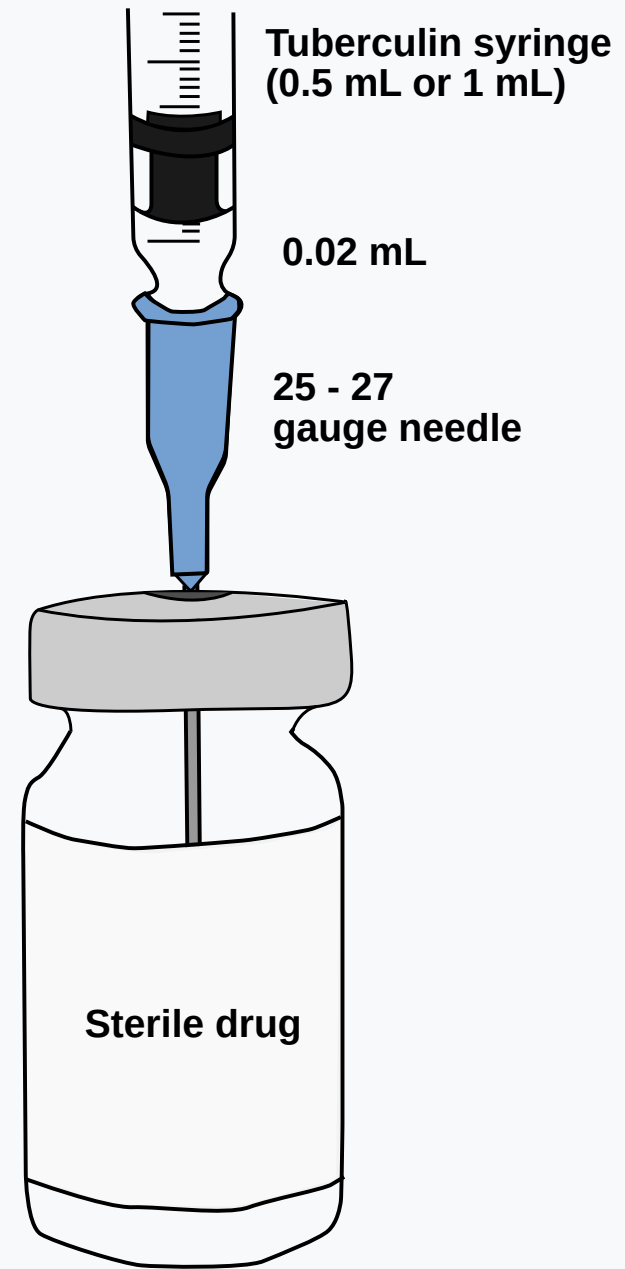


Immediate read (20min)

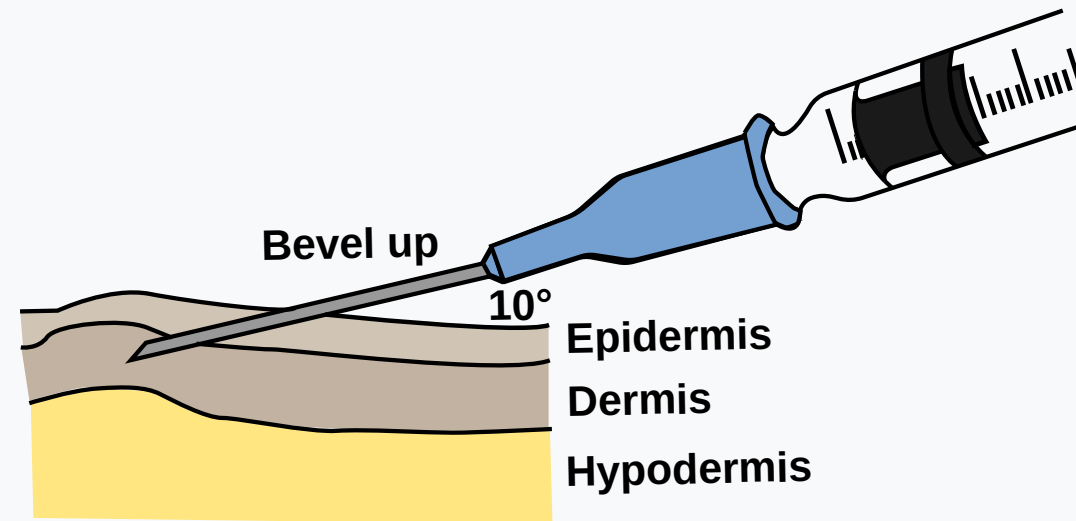
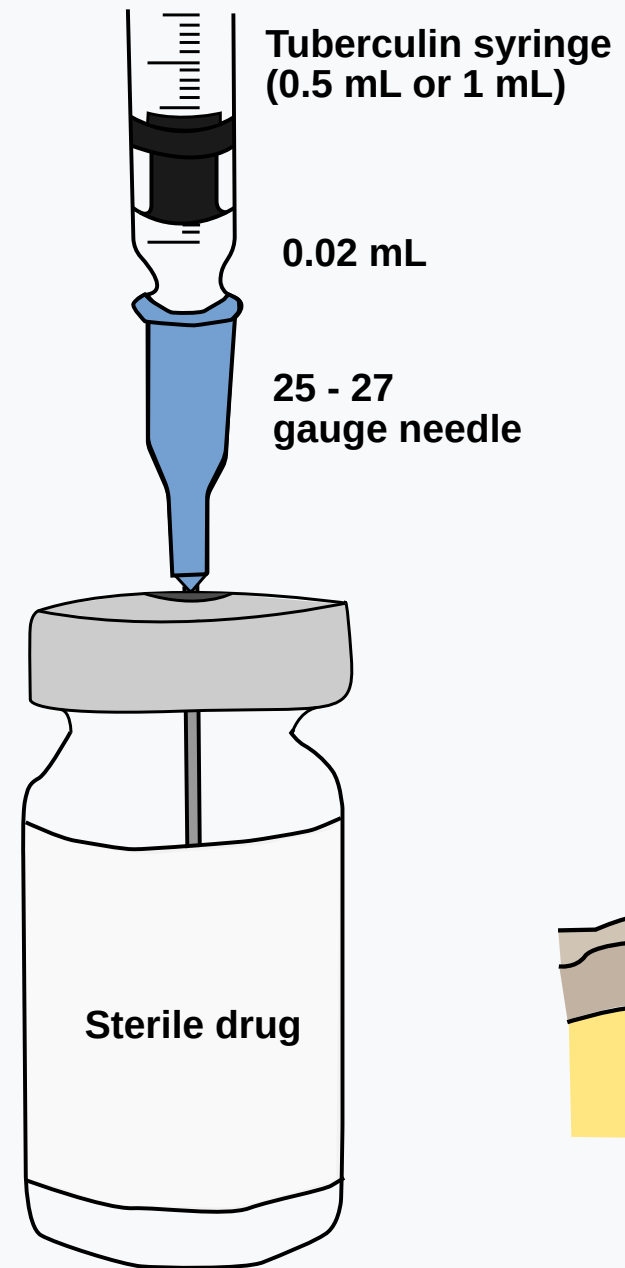
# Drug Concentrations: Immediate vs Delayed



# Intradermal Setup and Technique

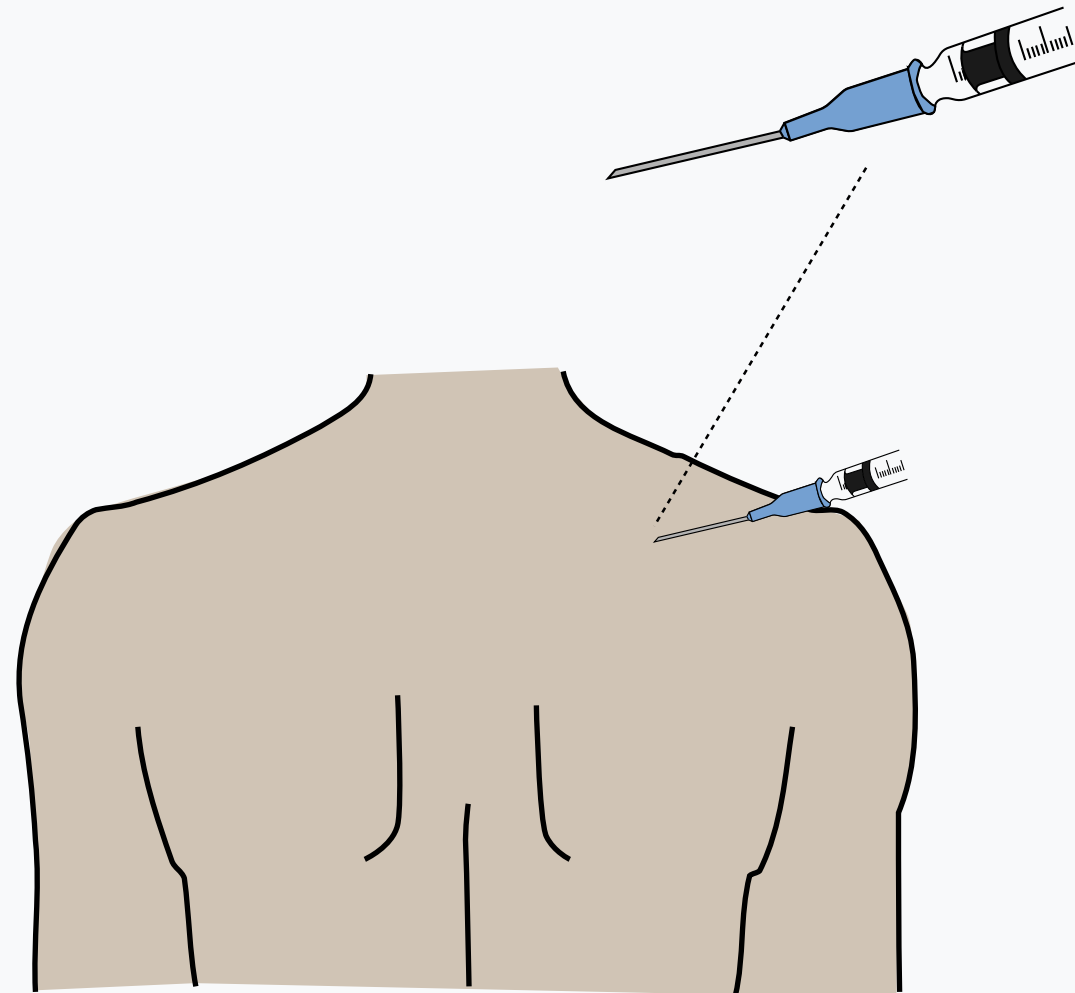


# Intradermal Setup and Technique



# Sites

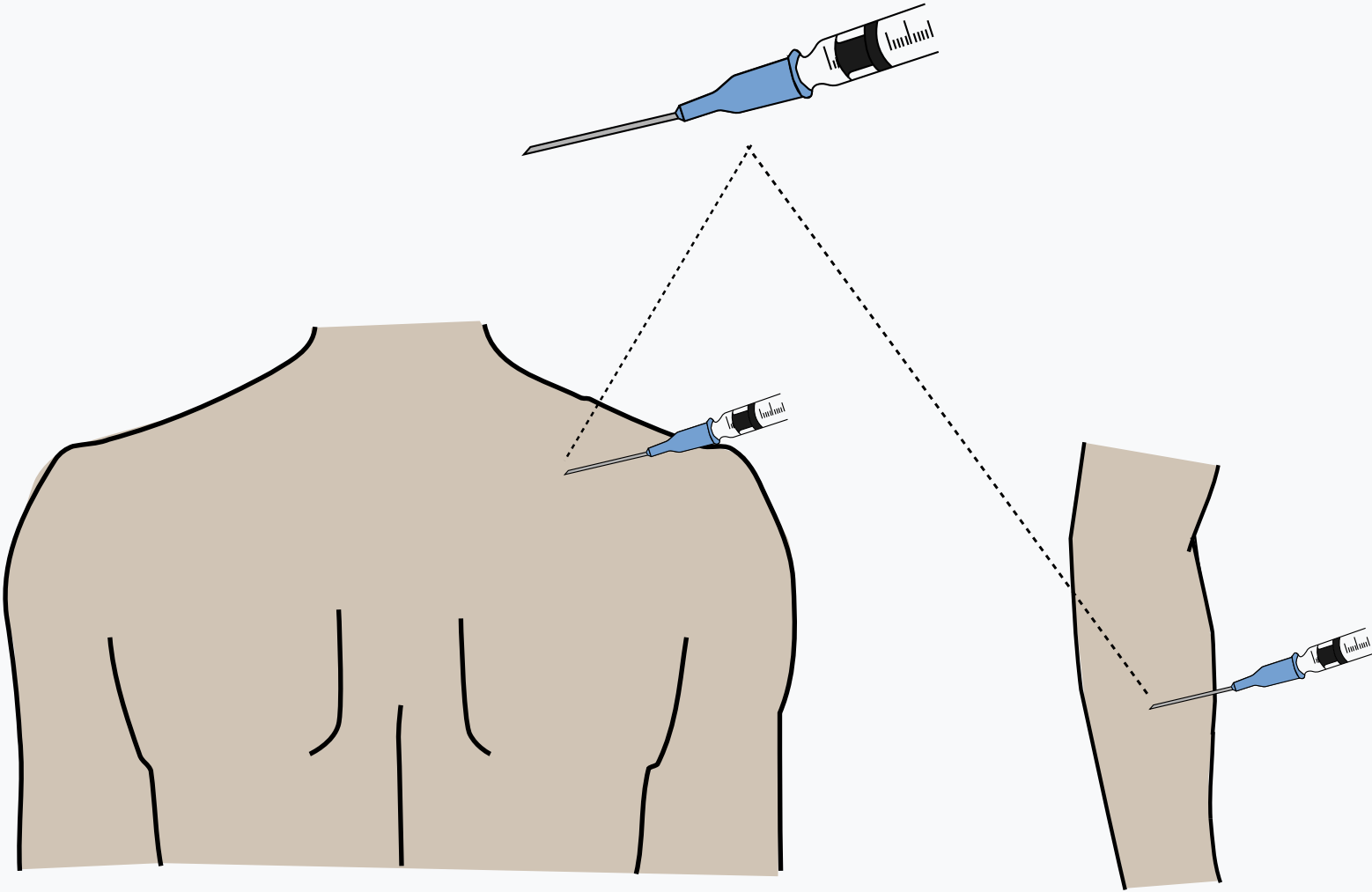
IDT injection



Back

# Sites

IDT injection

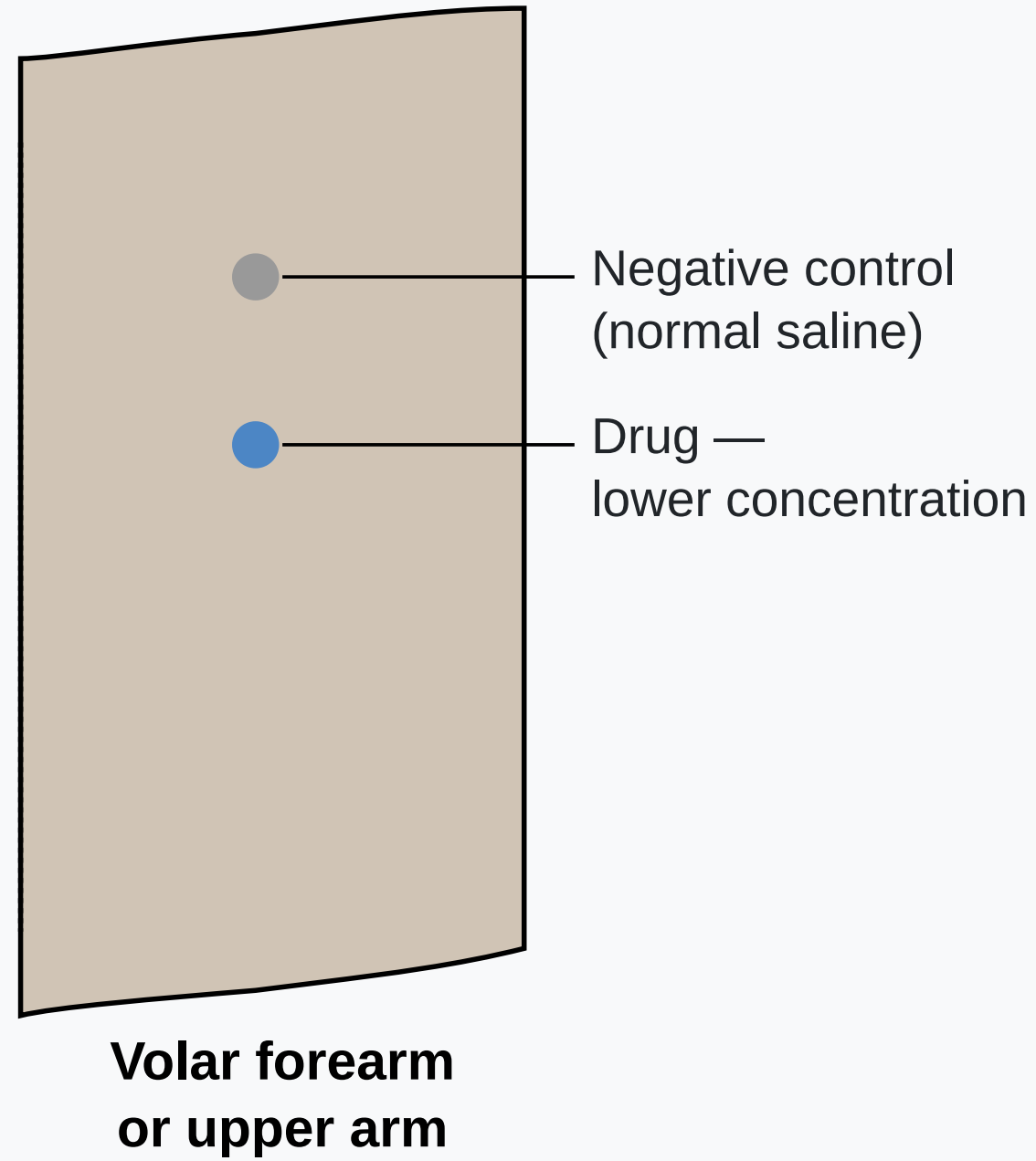


Back

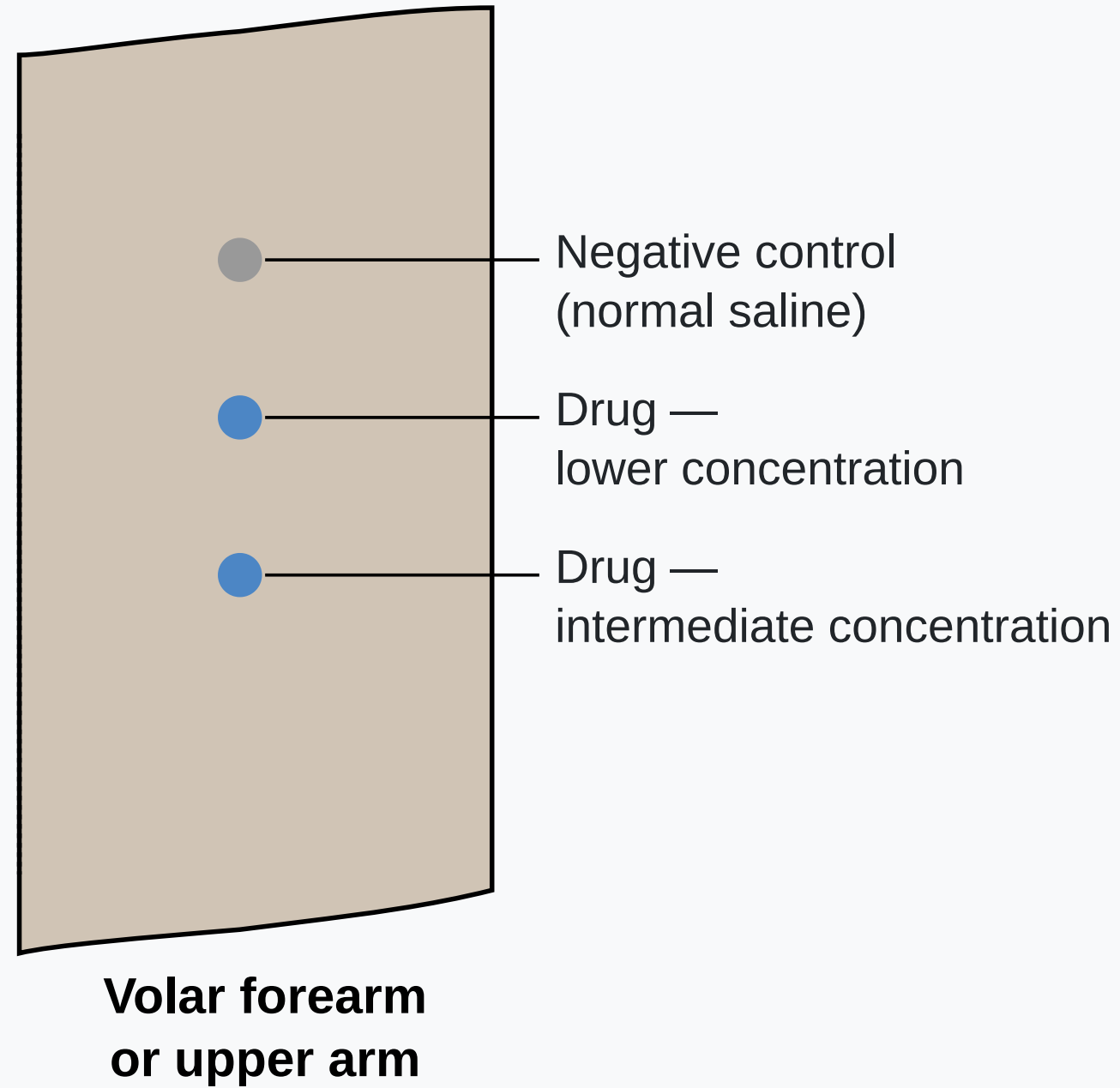
or

Volar forearm

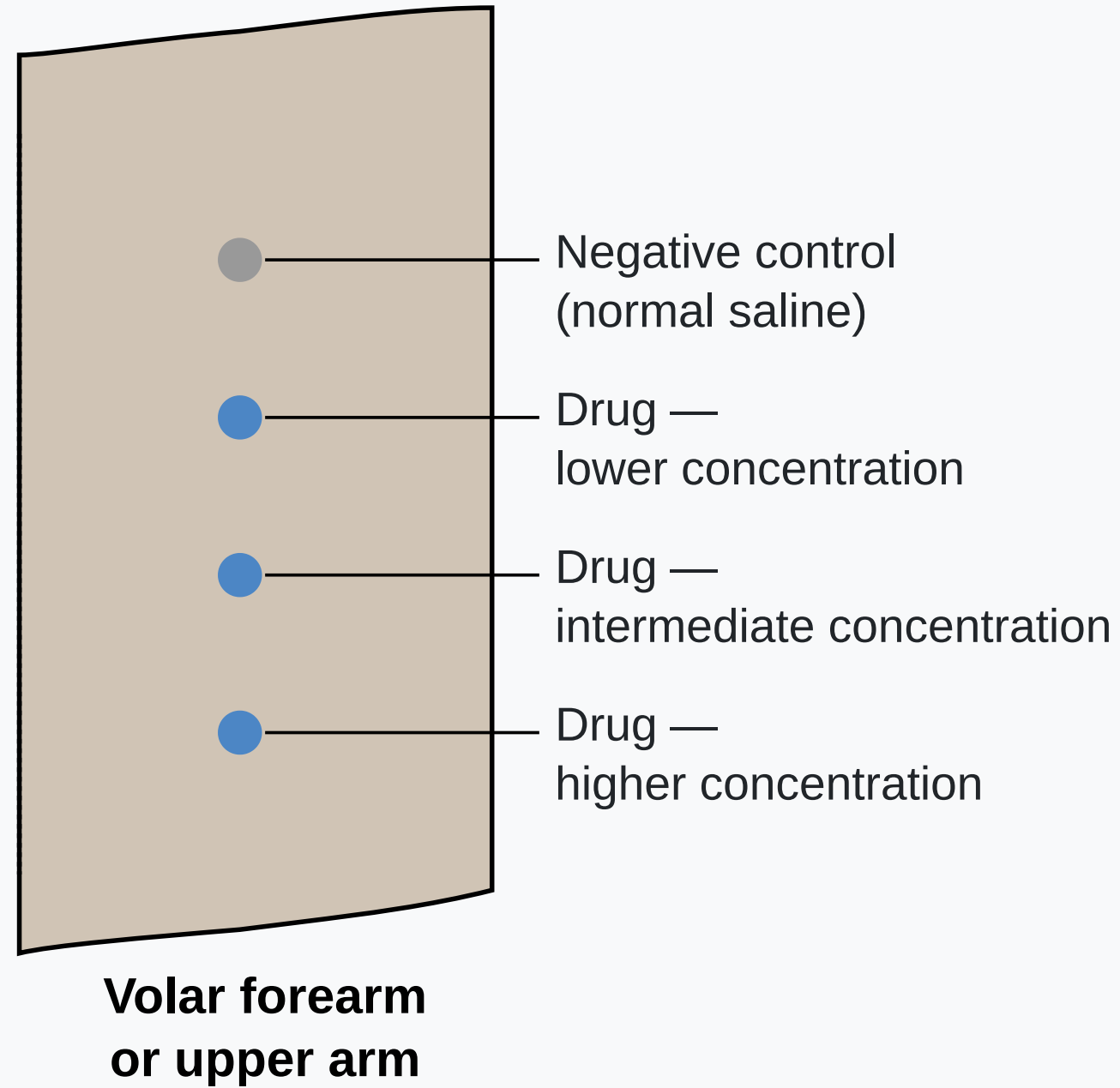
# Injection and Concentrations



# Injection and Concentrations



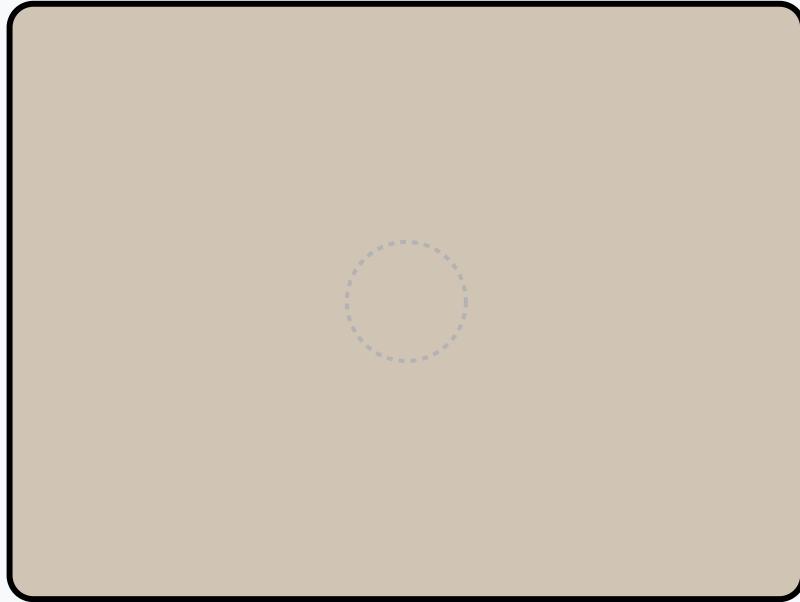
# Injection and Concentrations



# Reading

Read at 24 hours and 48 hours

Negative (-)



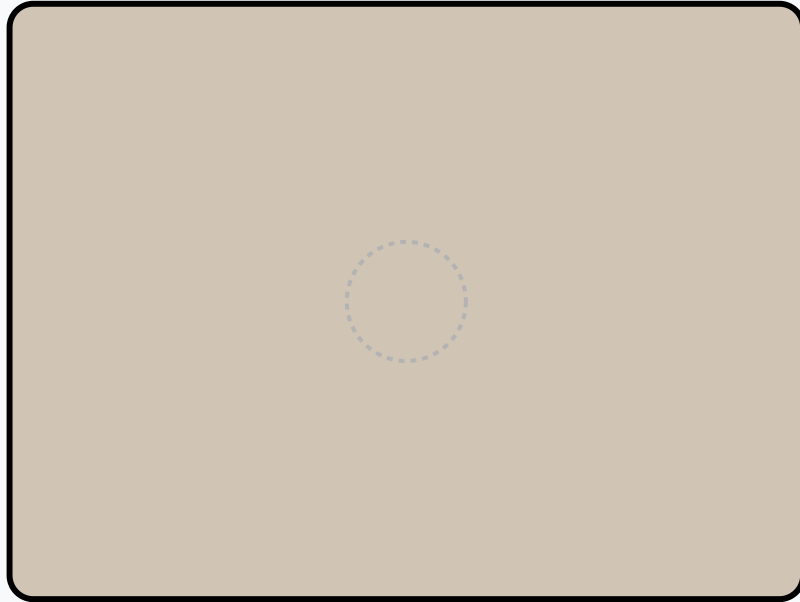
No papule

**NEGATIVE**

# Reading

Read at 24 hours and 48 hours

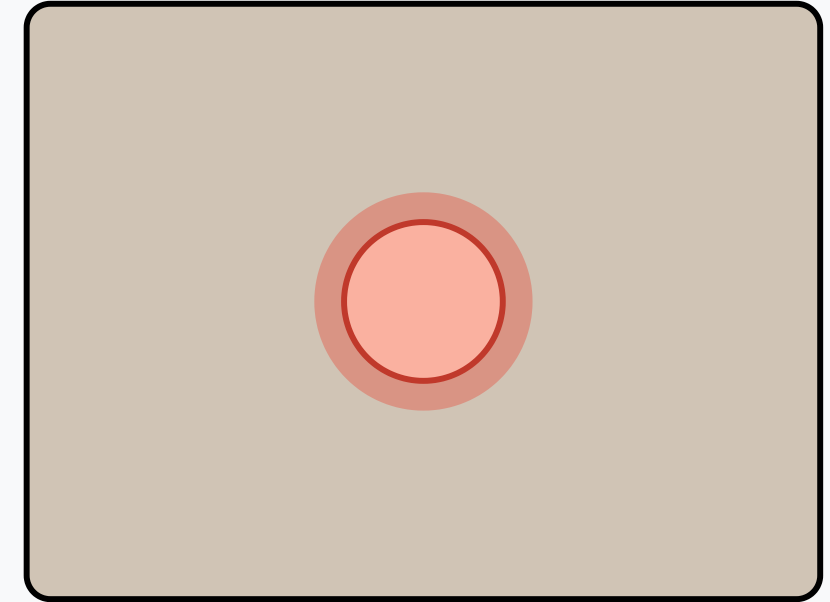
Negative (-)



No papule

**NEGATIVE**

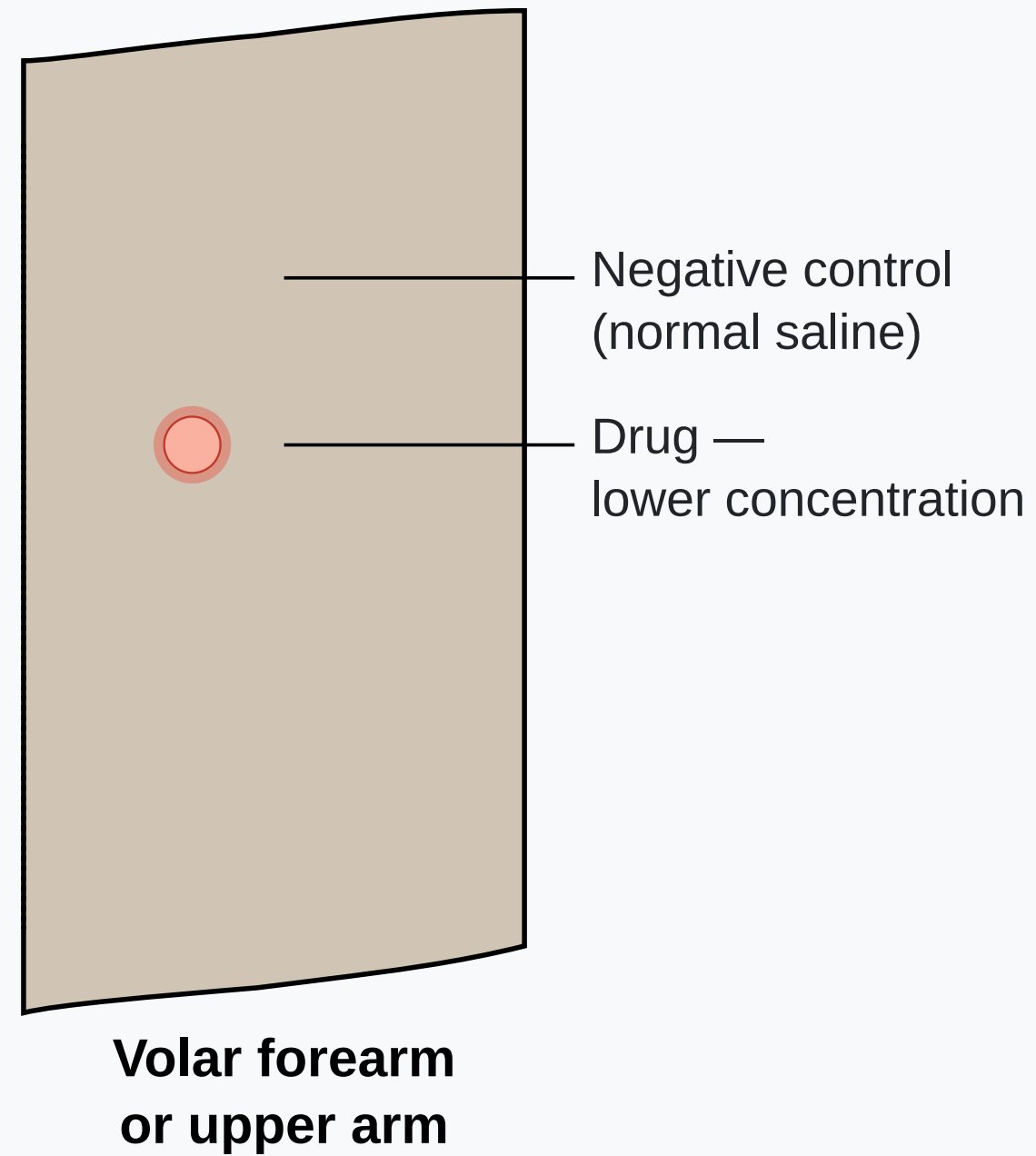
Positive (+)



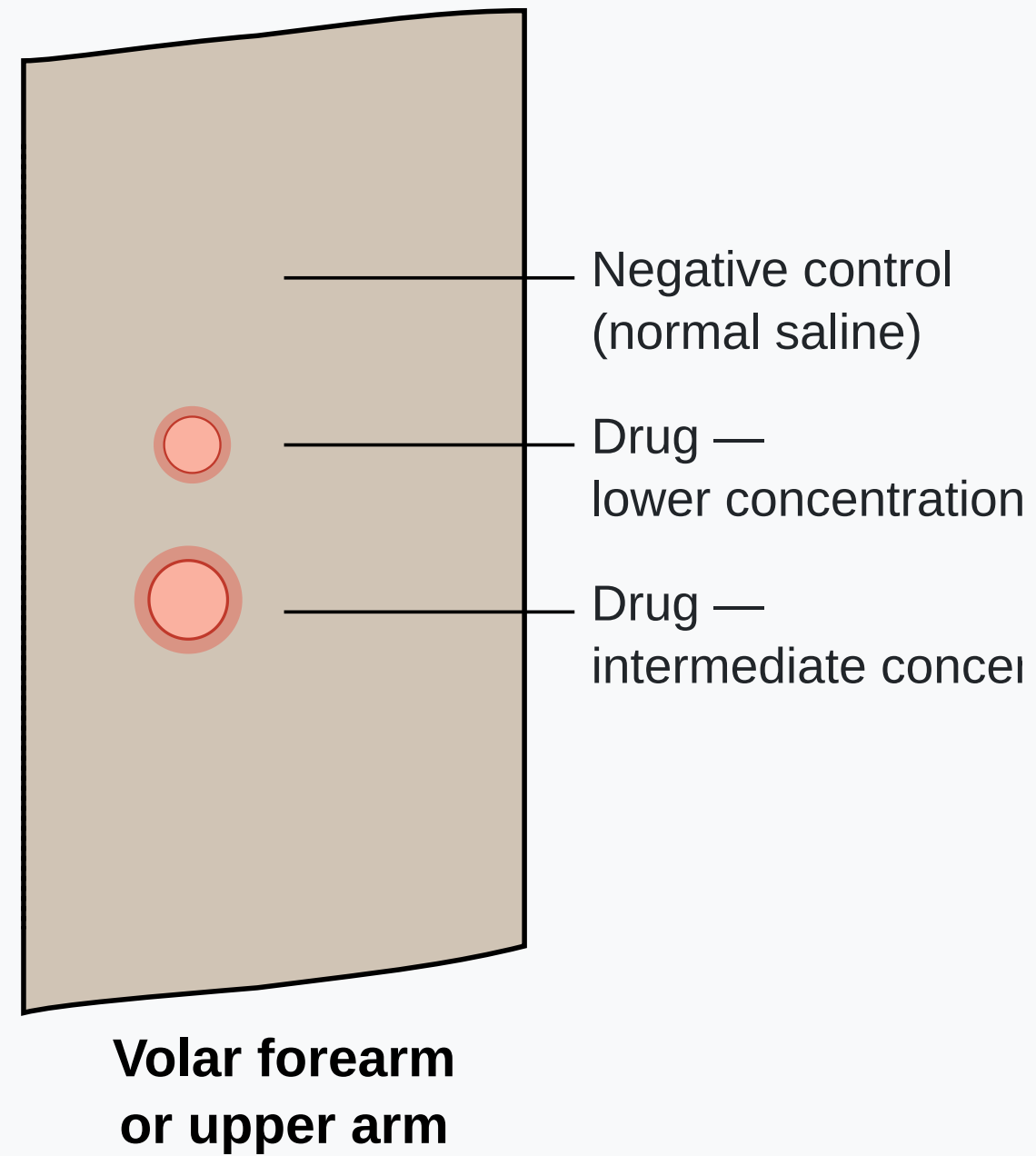
Papule present

**POSITIVE**

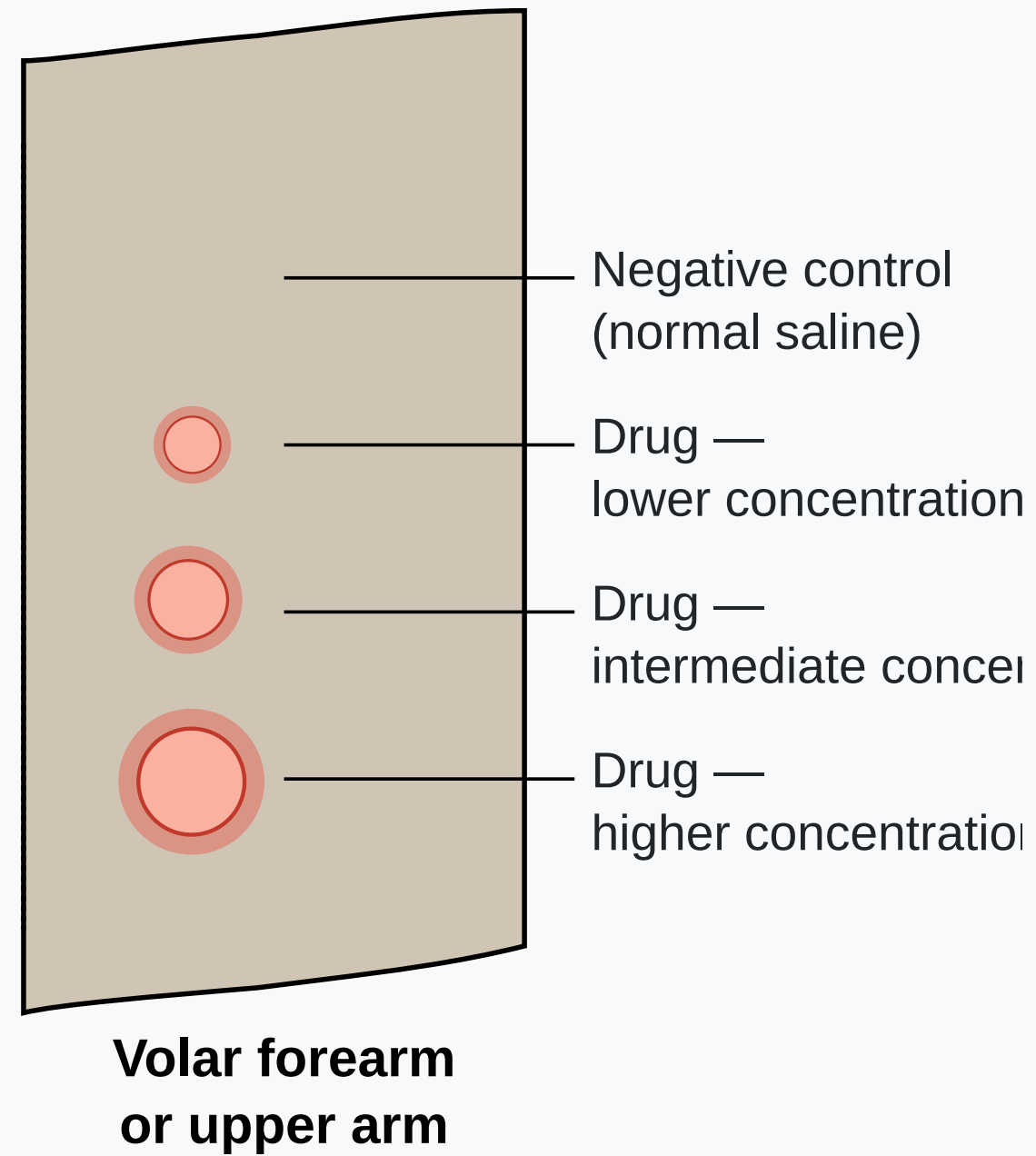
# Delayed IDT Reactions: Concentration-Dependent Response



# Delayed IDT Reactions: Concentration-Dependent Response

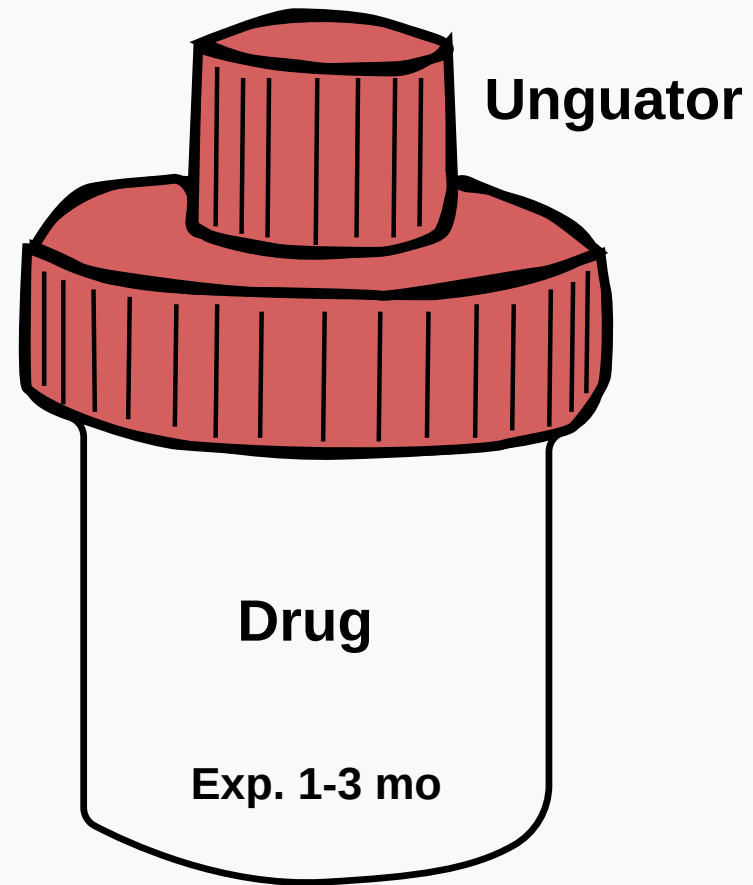


# Delayed IDT Reactions: Concentration-Dependent Response



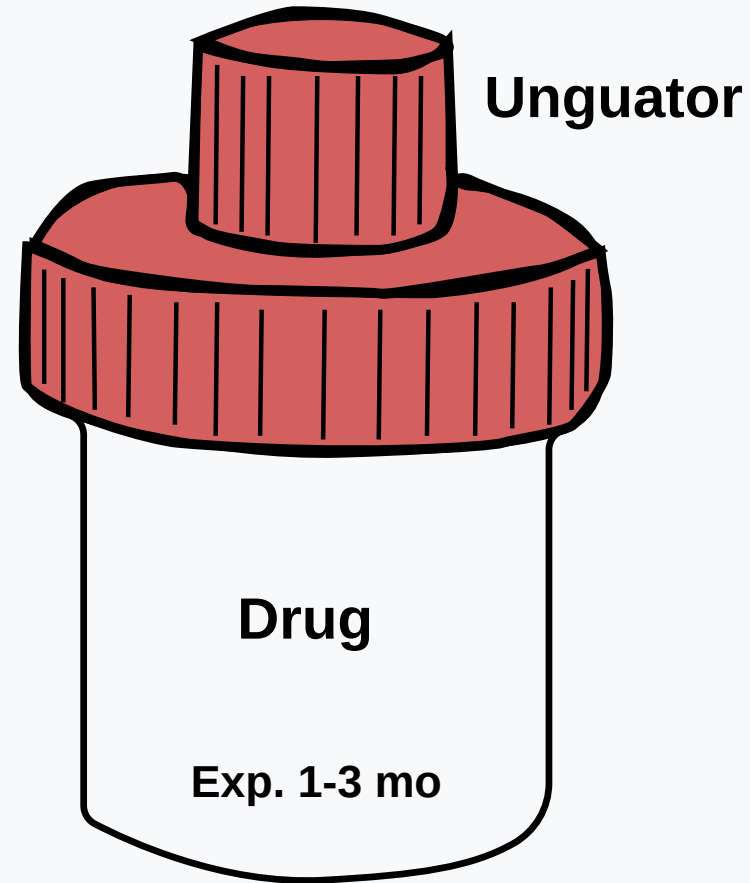
# Patch Testing

# Concentrations



**Reagent grade drug  
(1% and 10%)  
or  
Trade drug  
(10% and 30%)**

# Concentrations

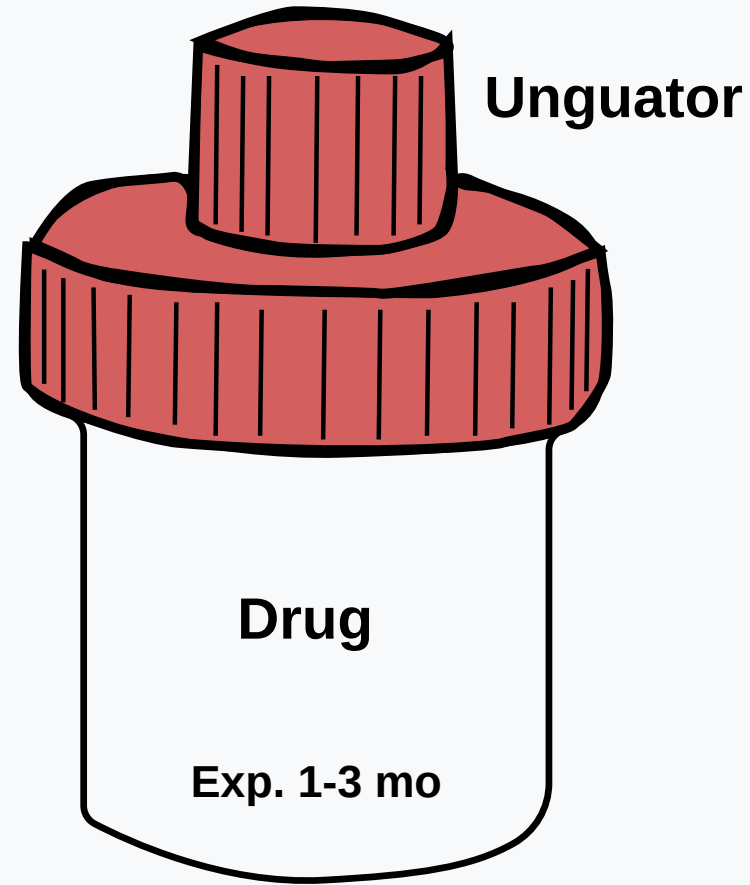


Reagent grade drug  
(1% and 10%)  
or  
Trade drug  
(10% and 30%)



Vehicle and  
Negative control

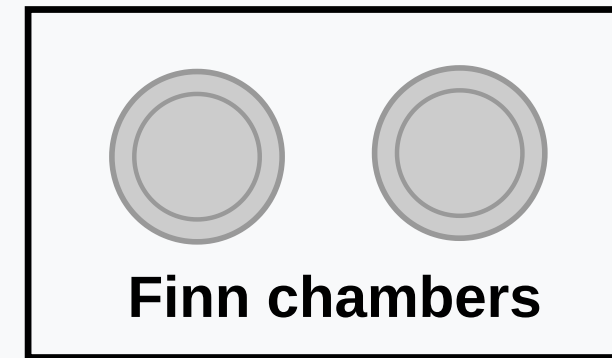
# Concentrations



Reagent grade drug  
(1% and 10%)  
or  
Trade drug  
(10% and 30%)



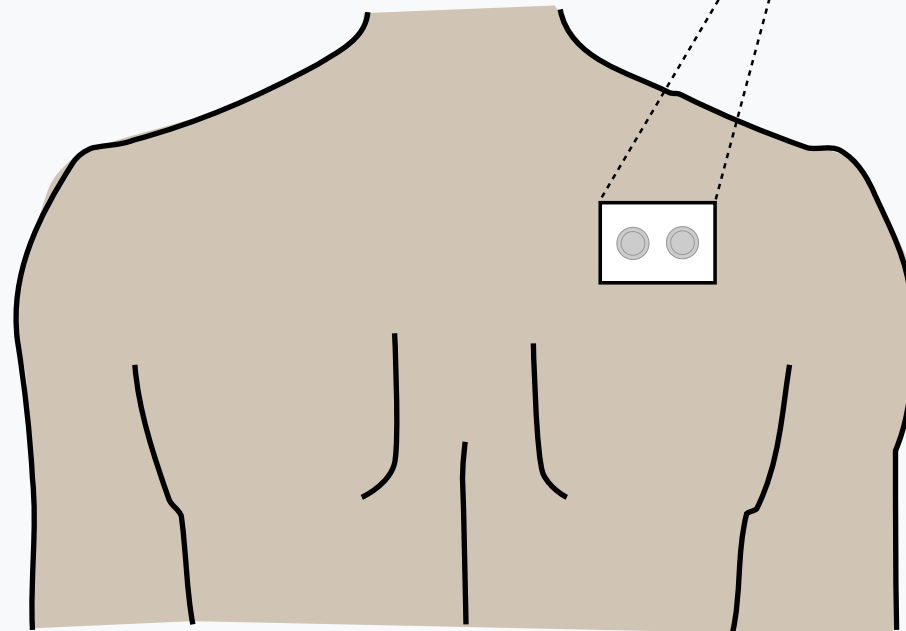
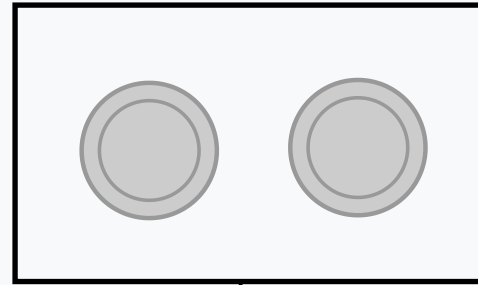
Vehicle and  
Negative control



Scanpor tape

# Sites

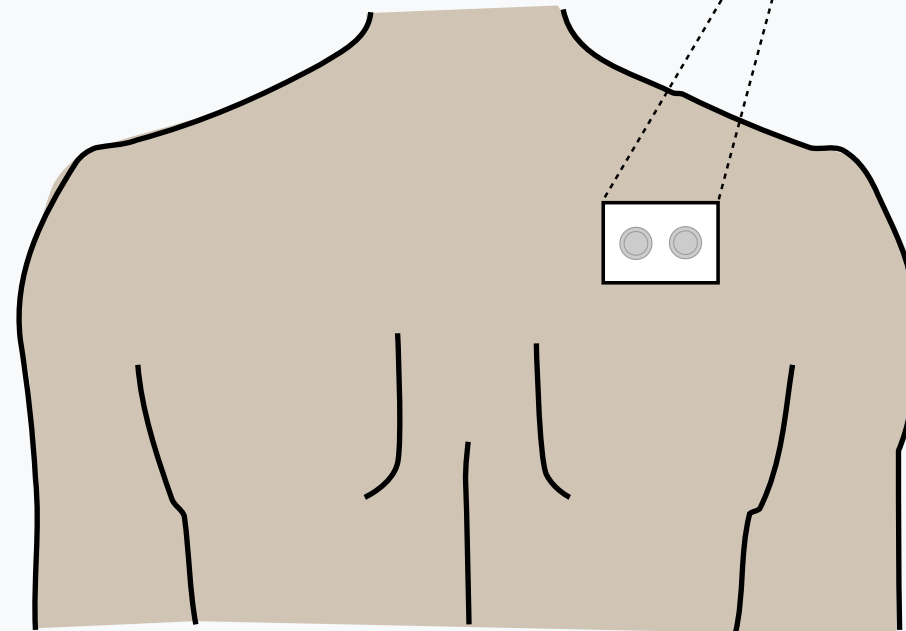
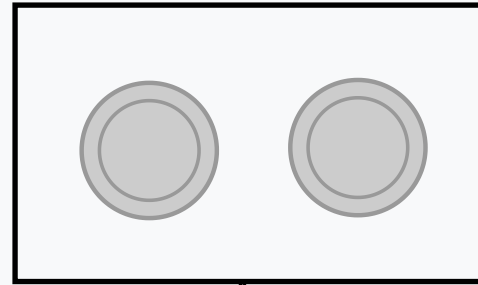
Patch placement



Back

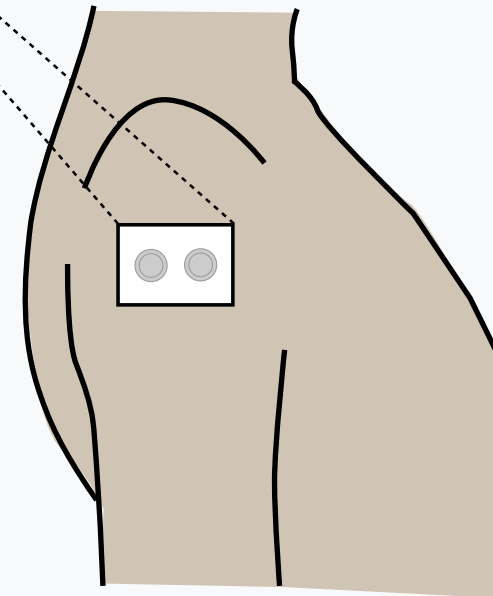
# Sites

Patch placement



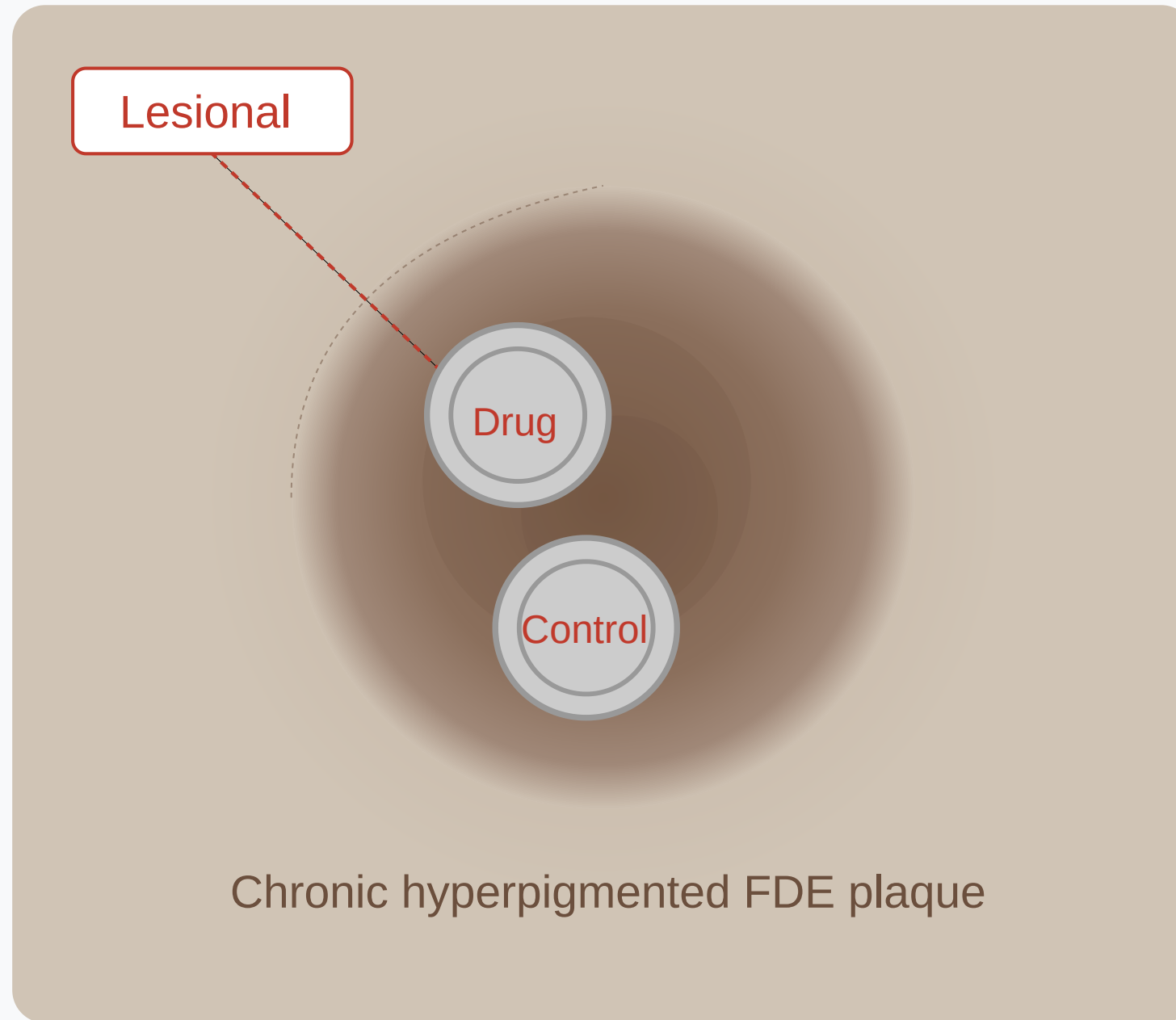
Back

or

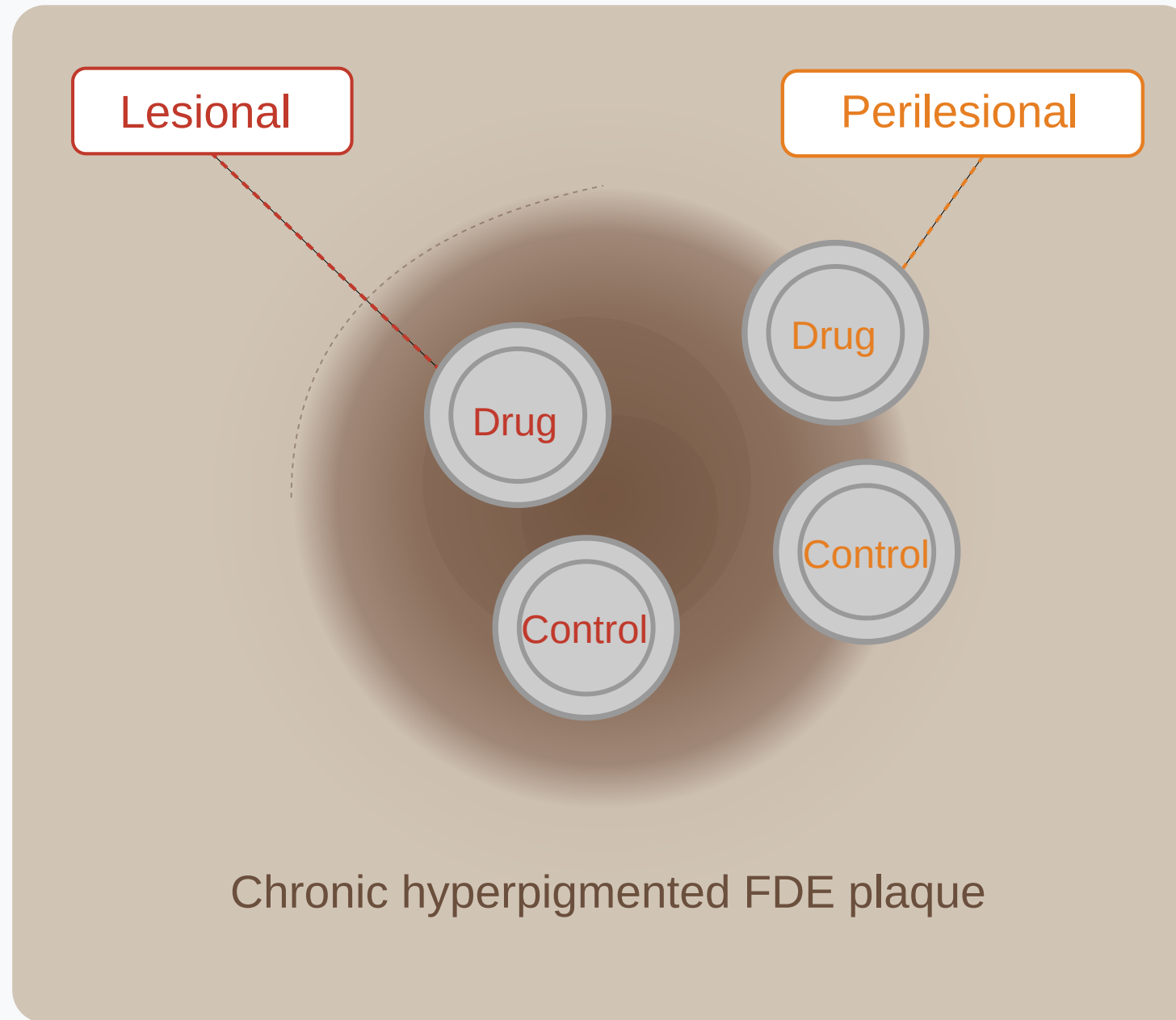


Upper arm

# Fixed Drug Eruption

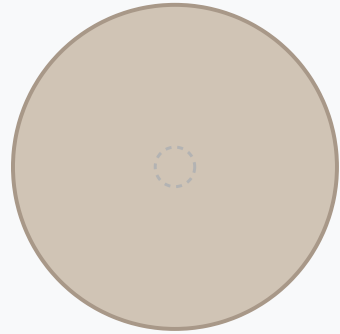


# Fixed Drug Eruption



# Interpretation

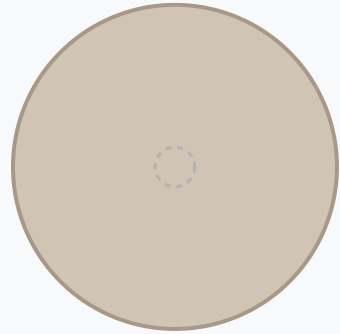
–  
Negative



No reaction

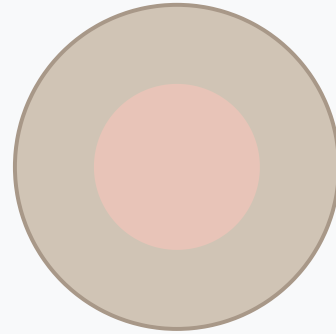
# Interpretation

–  
Negative



No reaction

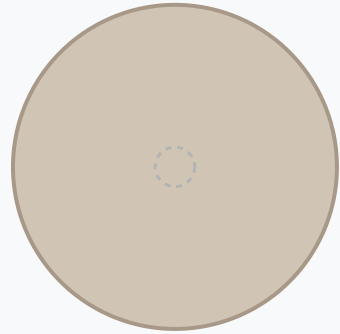
?+  
Doubtful



Faint erythema  
No infiltration

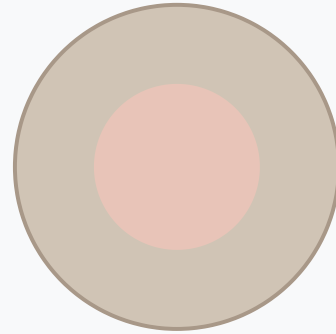
# Interpretation

**-**  
Negative



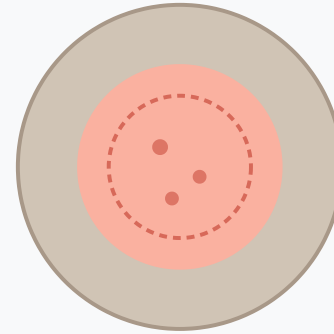
No reaction

**?+**  
Doubtful



Faint erythema  
No infiltration

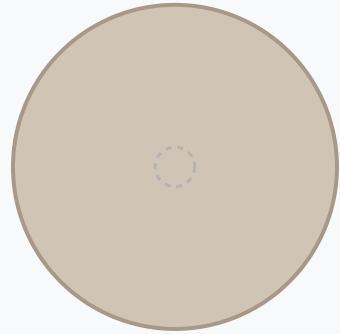
**+**  
Weak positive



Erythema  
Infiltration  
± papules

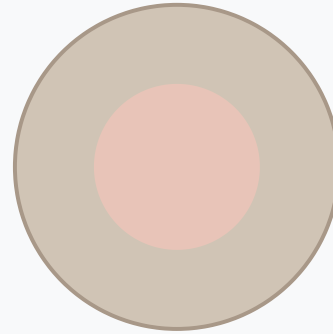
# Interpretation

**-**  
Negative



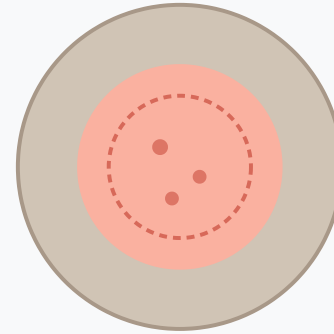
No reaction

**?+**  
Doubtful



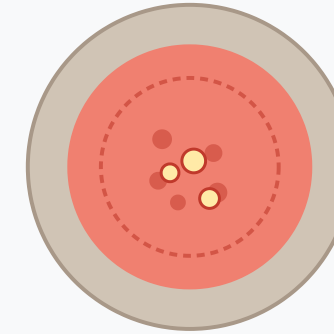
Faint erythema  
No infiltration

**+**  
Weak positive



Erythema  
Infiltration  
± papules

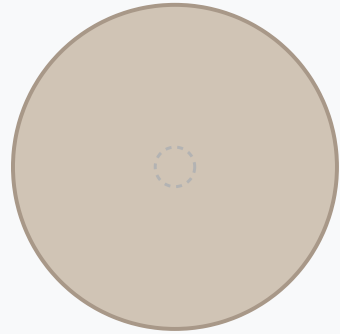
**++**  
Strong positive



Erythema, infiltration  
Papules  
Vesicles

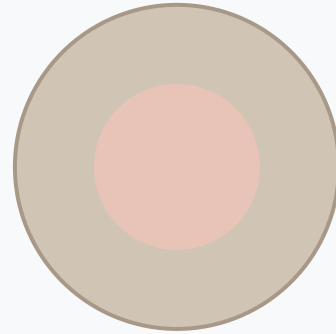
# Interpretation

–  
Negative



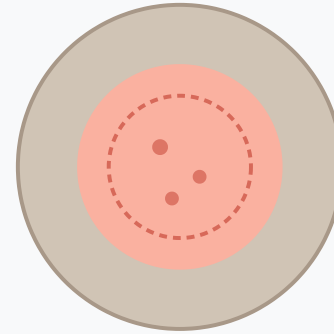
No reaction

?+  
Doubtful



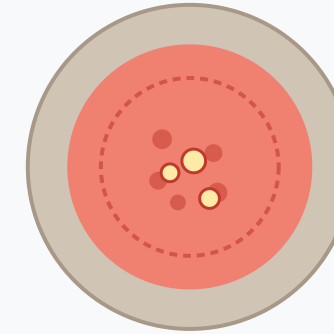
Faint erythema  
No infiltration

+  
Weak positive



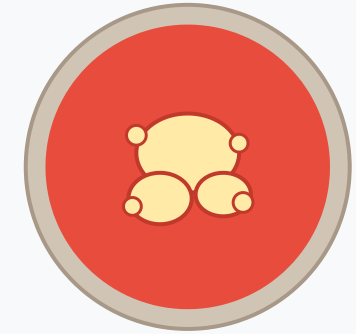
Erythema  
Infiltration  
± papules

++  
Strong positive



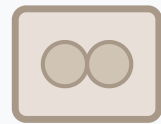
Erythema, infiltration  
Papules  
Vesicles

+++  
Extreme



Intense erythema  
Coalescing vesicles  
or bullous

# Cumulative Detection of Positive Reactions

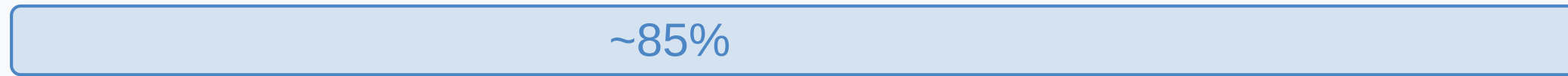


Place patches

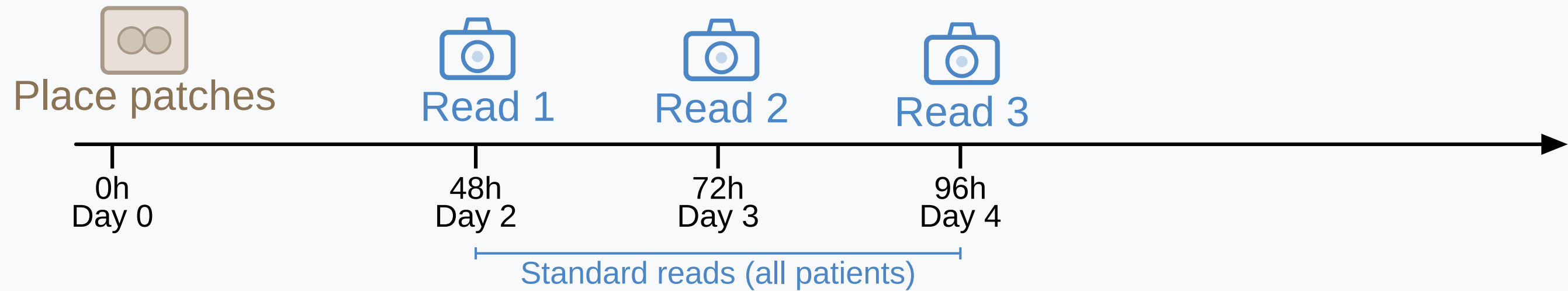


# Cumulative Detection of Positive Reactions

Among drug patch tests that will ultimately be positive, % detected by each read

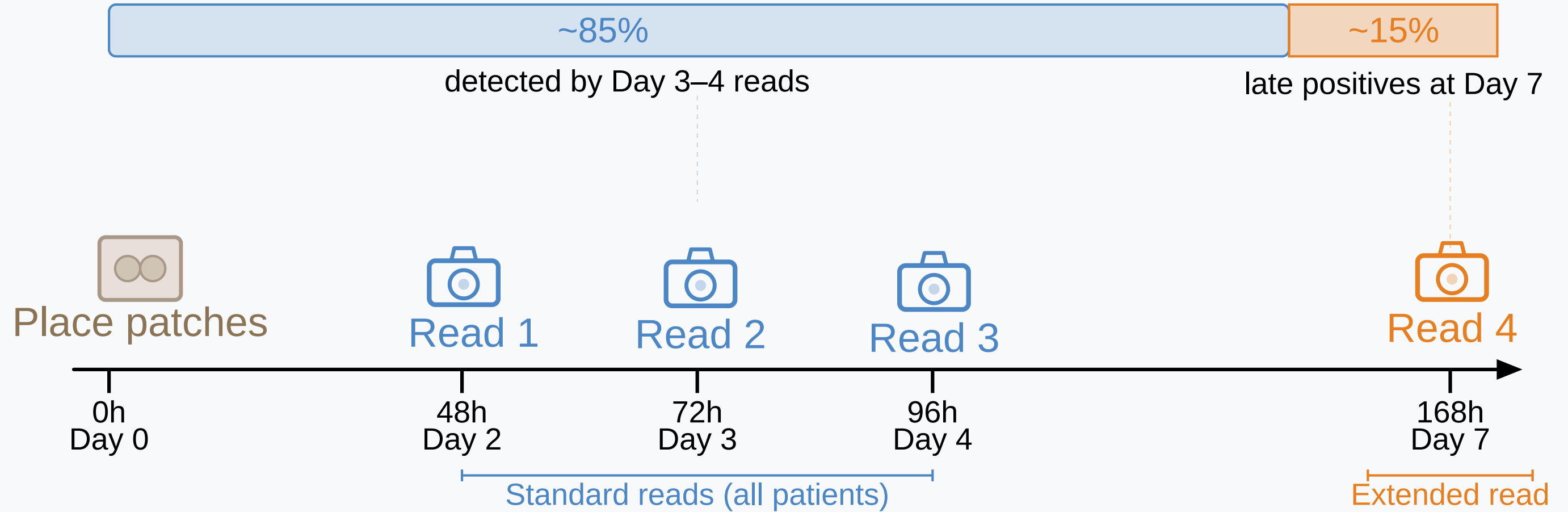


detected by Day 3–4 reads

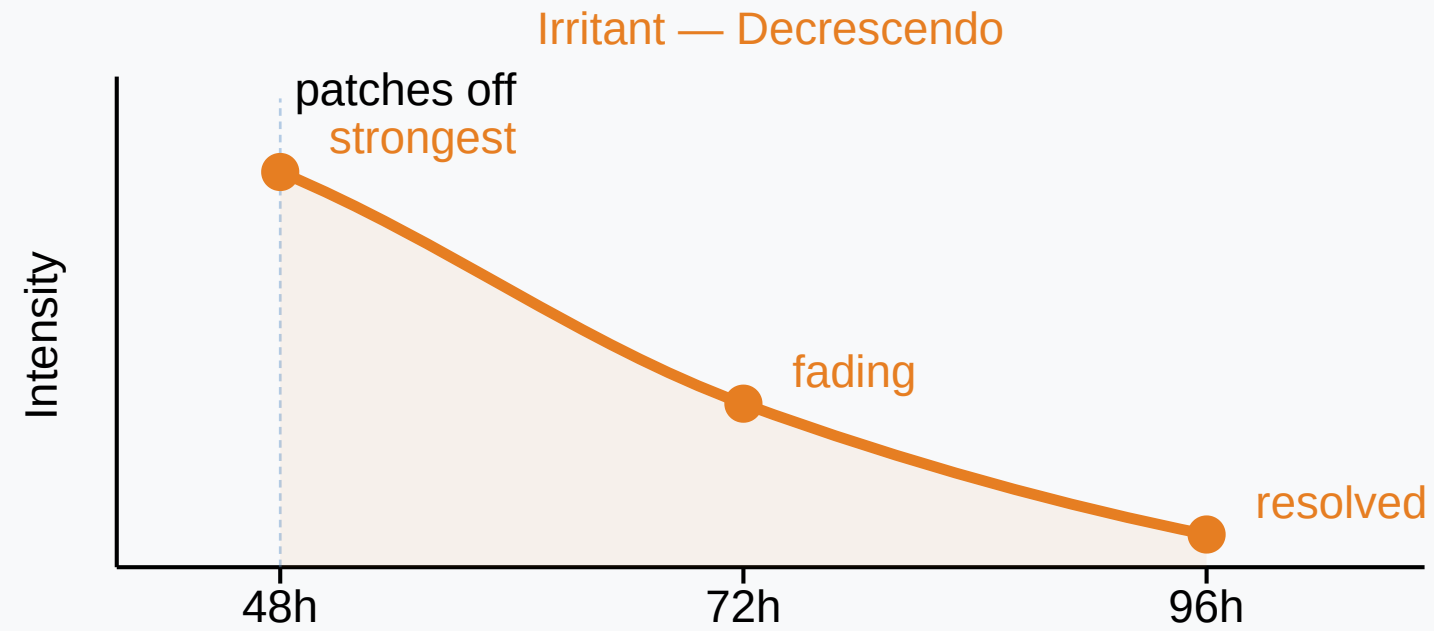


# Cumulative Detection of Positive Reactions

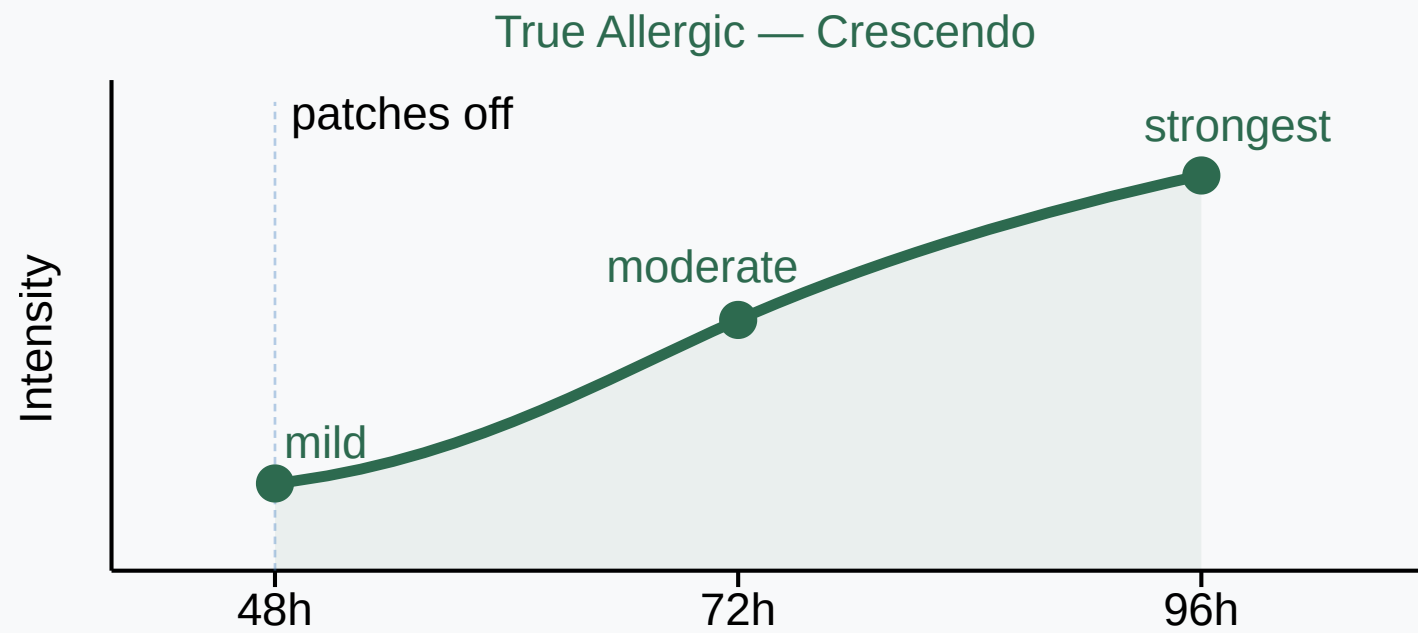
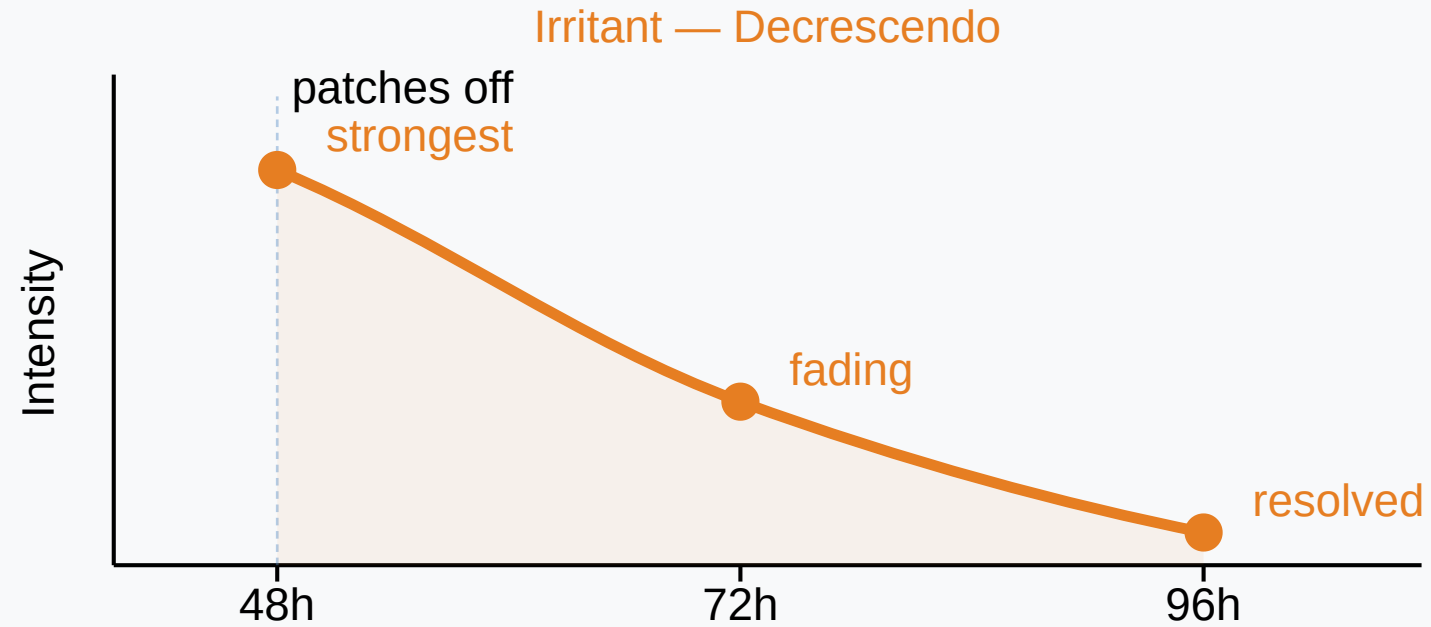
Among drug patch tests that will ultimately be positive, % detected by each read



# Patch Testing: Allergic vs Irritant reactions



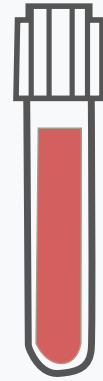
# Patch Testing: Allergic vs Irritant reactions



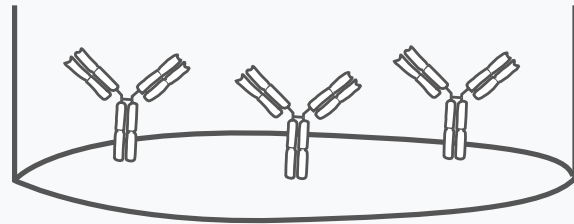
# ELISpot Testing

# ELISpot Steps

## Day 1



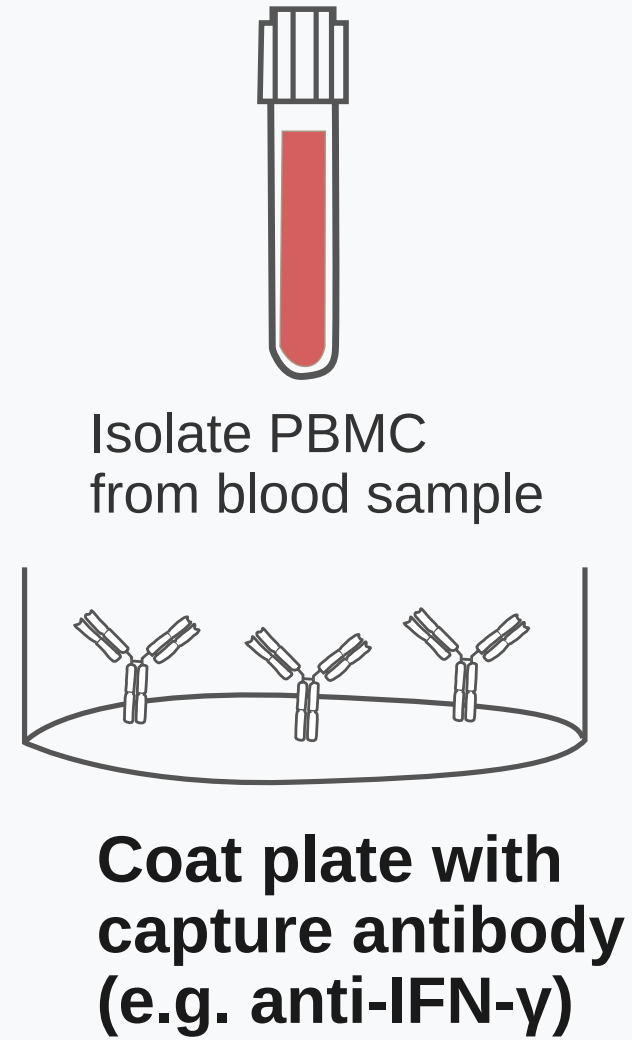
Isolate PBMC  
from blood sample



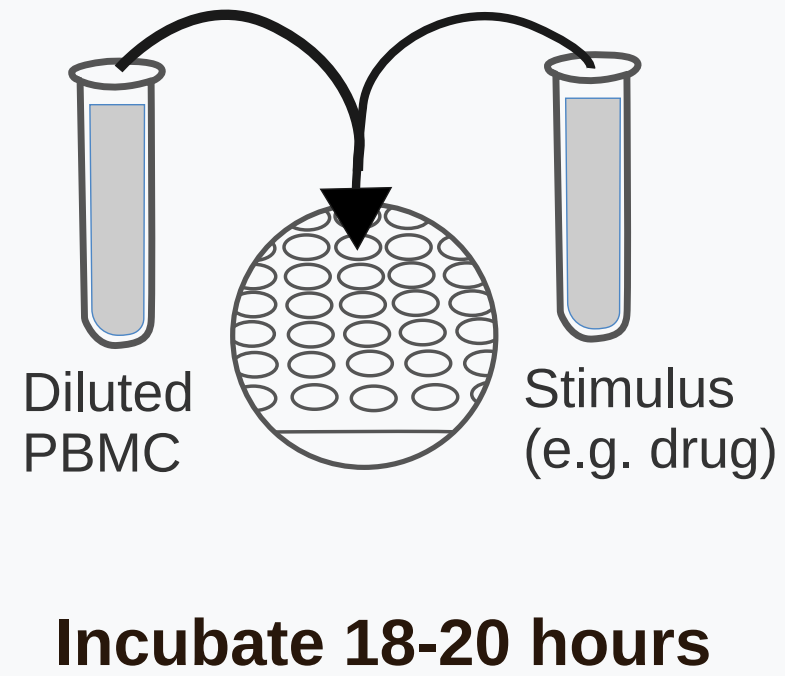
**Coat plate with  
capture antibody  
(e.g. anti-IFN- $\gamma$ )**

# ELISpot Steps

**Day 1**

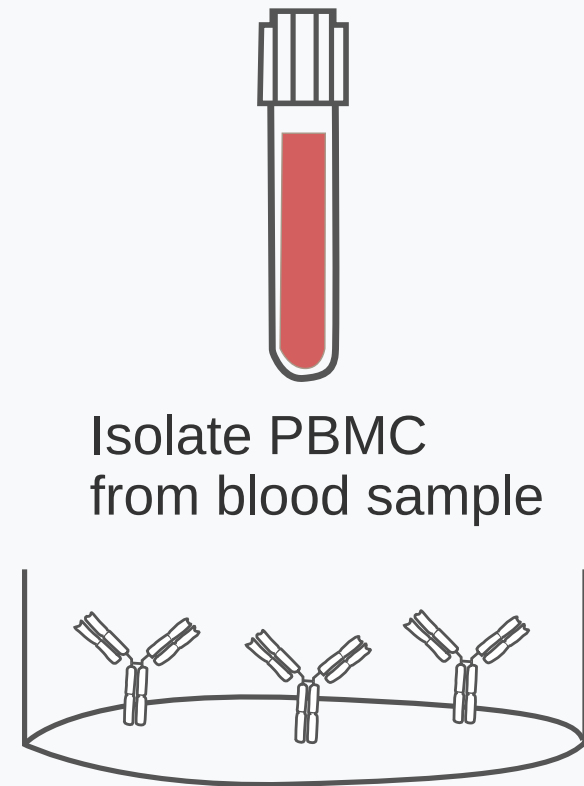


**Day 2**

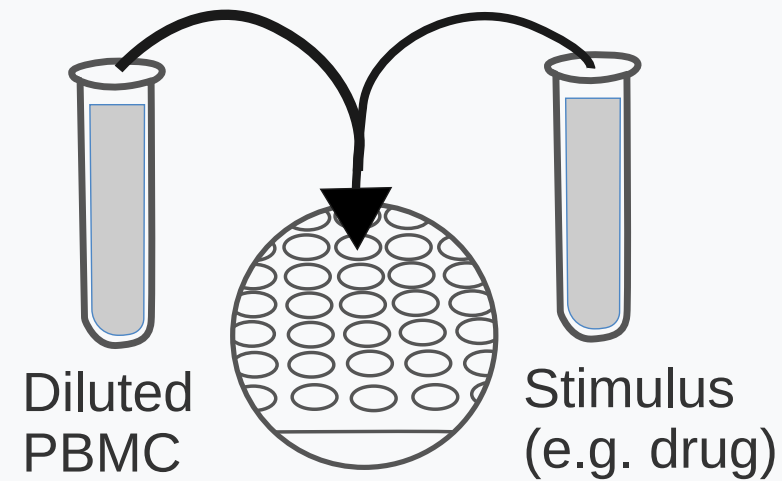


# ELISpot Steps

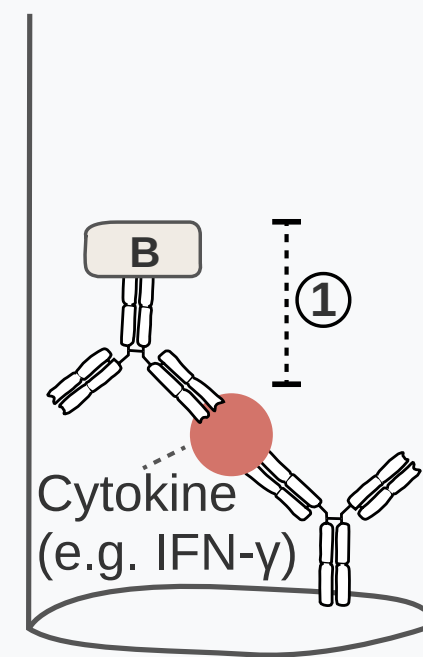
**Day 1**



**Day 2**

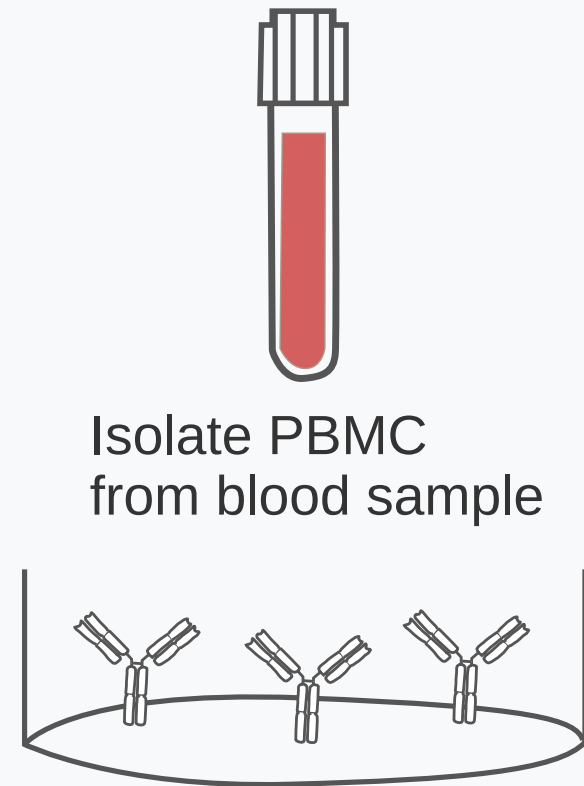


**Day 3**

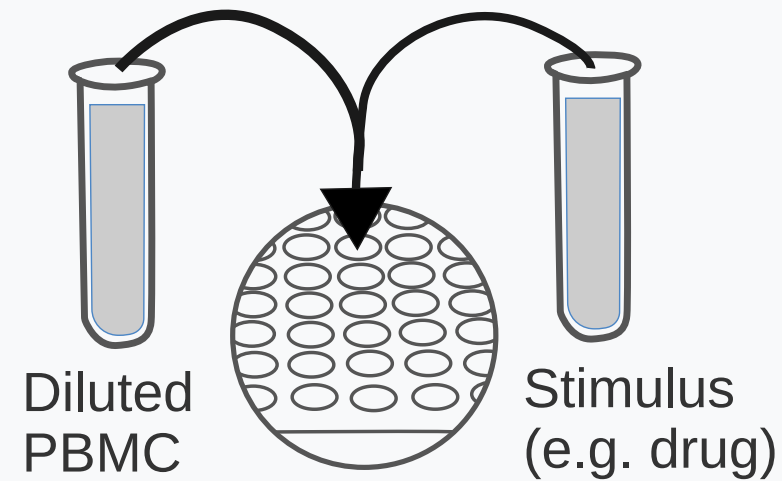


# ELISpot Steps

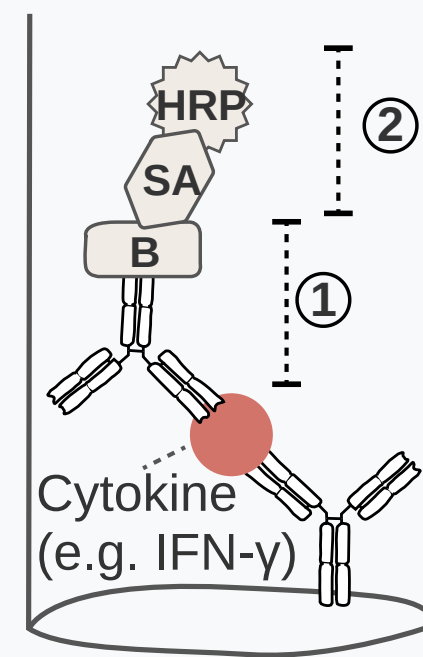
**Day 1**



**Day 2**



**Day 3**

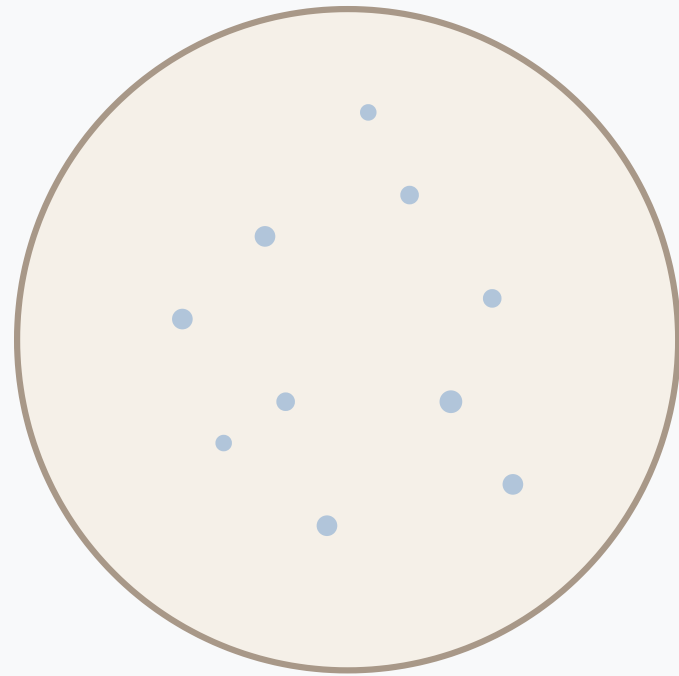


- ① Biotinylated detection antibody (B)
- ② Streptavidin (SA)-HRP conjugate



# ELISpot Plate Incubation

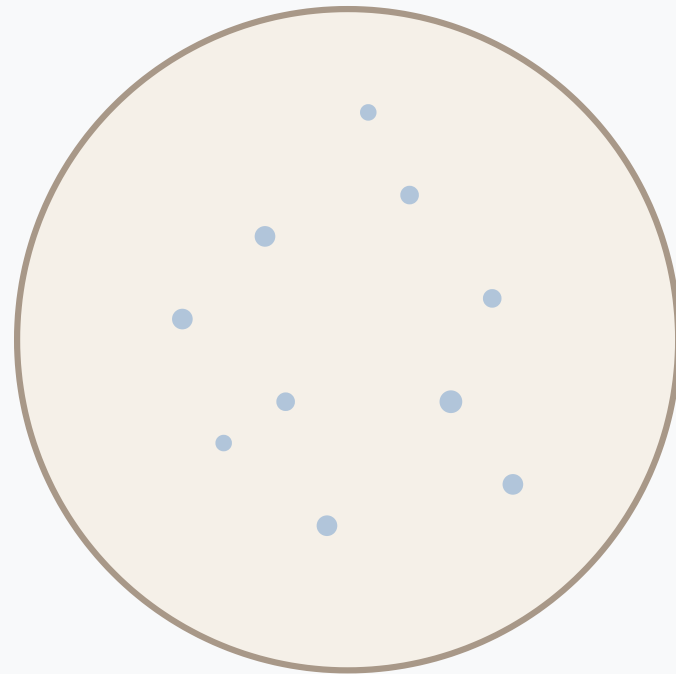
Negative Control  
(cell media only)



10 SFU  
Background

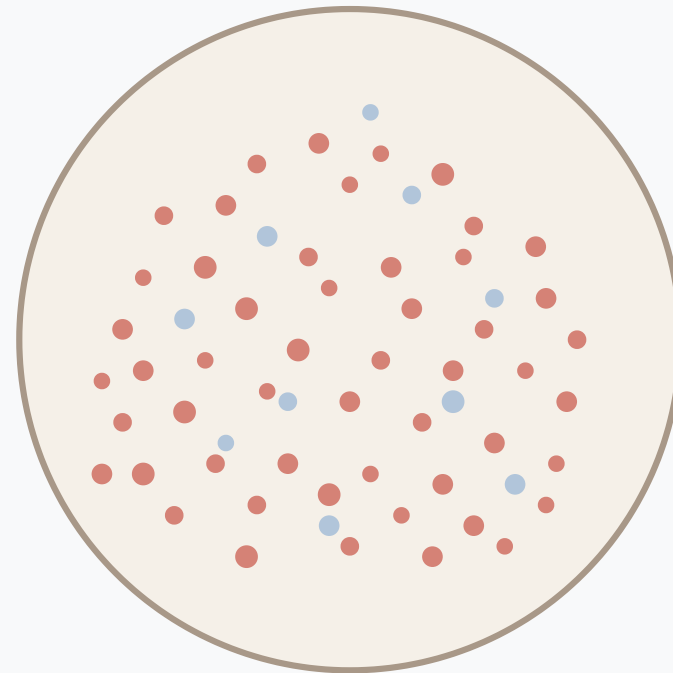
# ELISpot Plate Incubation

Negative Control  
(cell media only)



10 SFU  
Background

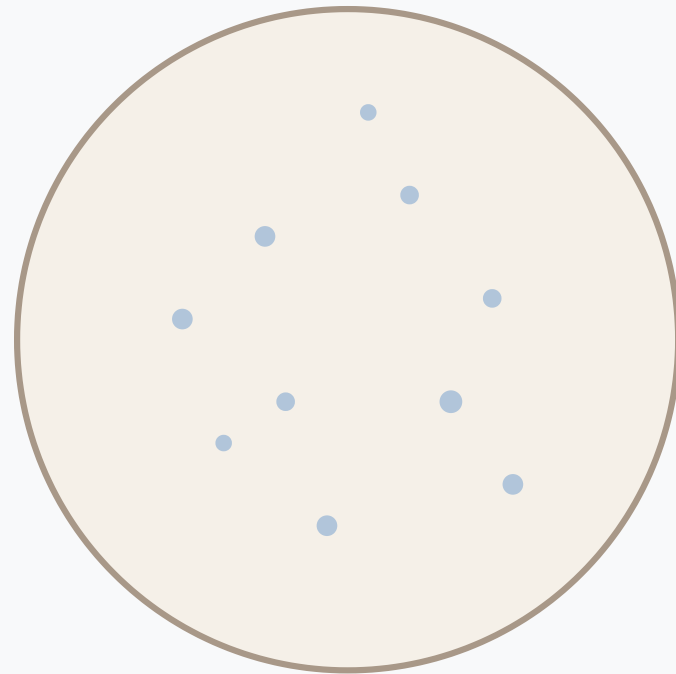
Drug Well  
(raw count)



110 SFU  
Raw count

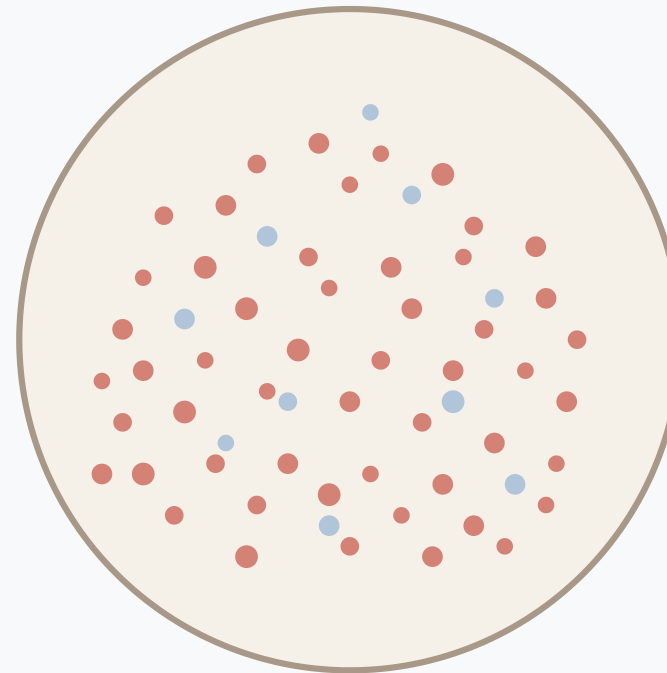
# ELISpot Plate Incubation

Negative Control  
(cell media only)



10 SFU  
Background

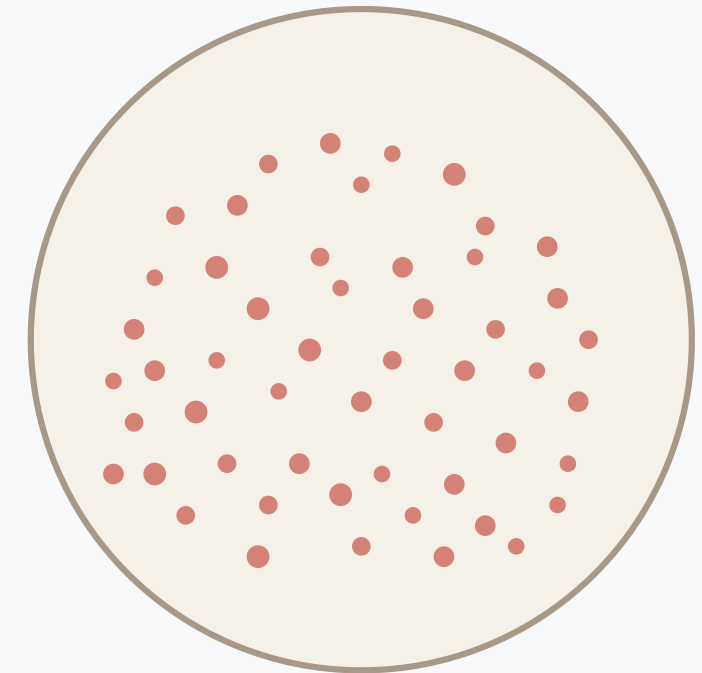
Drug Well  
(raw count)



110 SFU  
Raw count

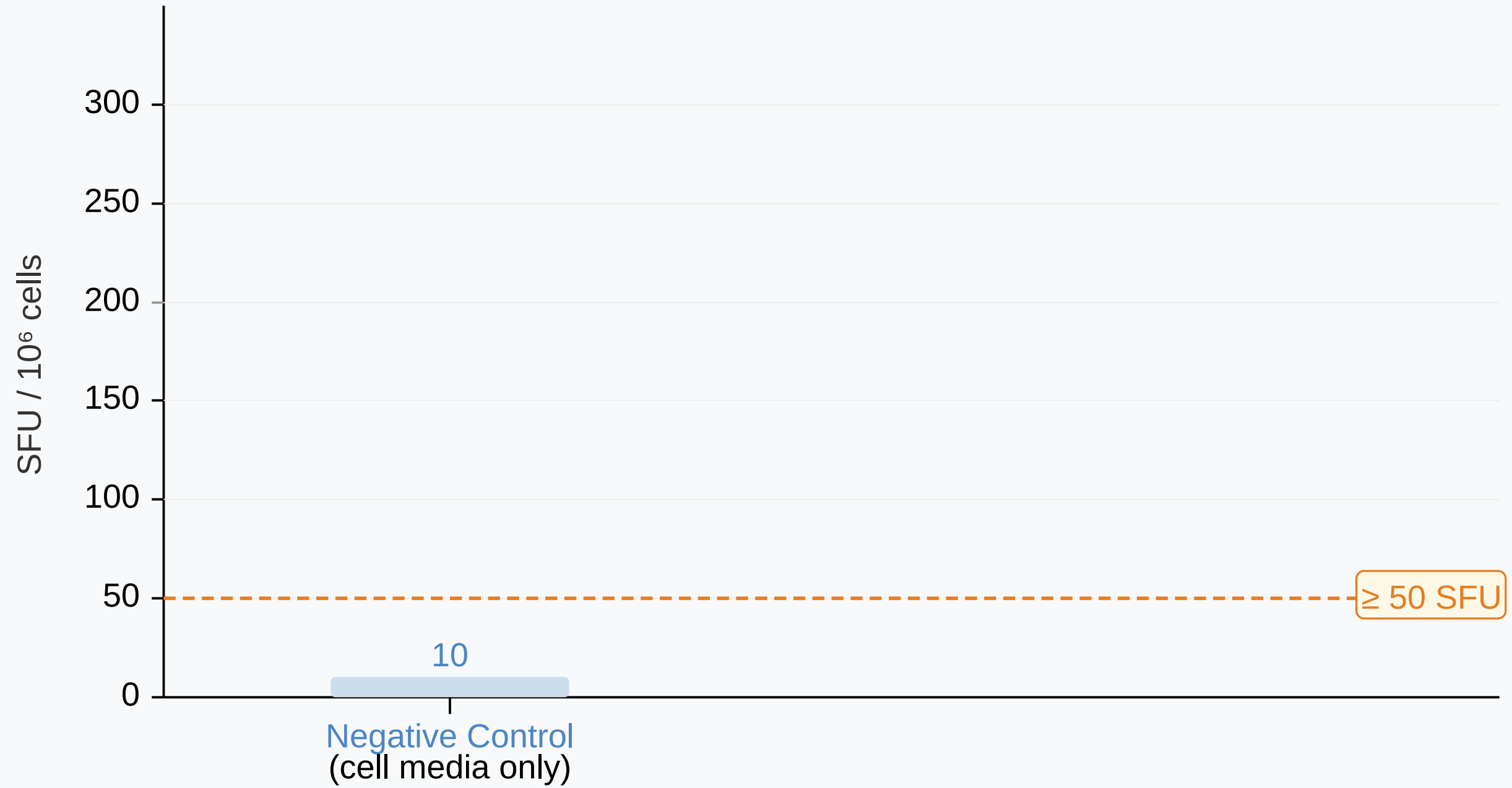
- 10 SFU  
subtract  
background

Drug Well  
(background removed)

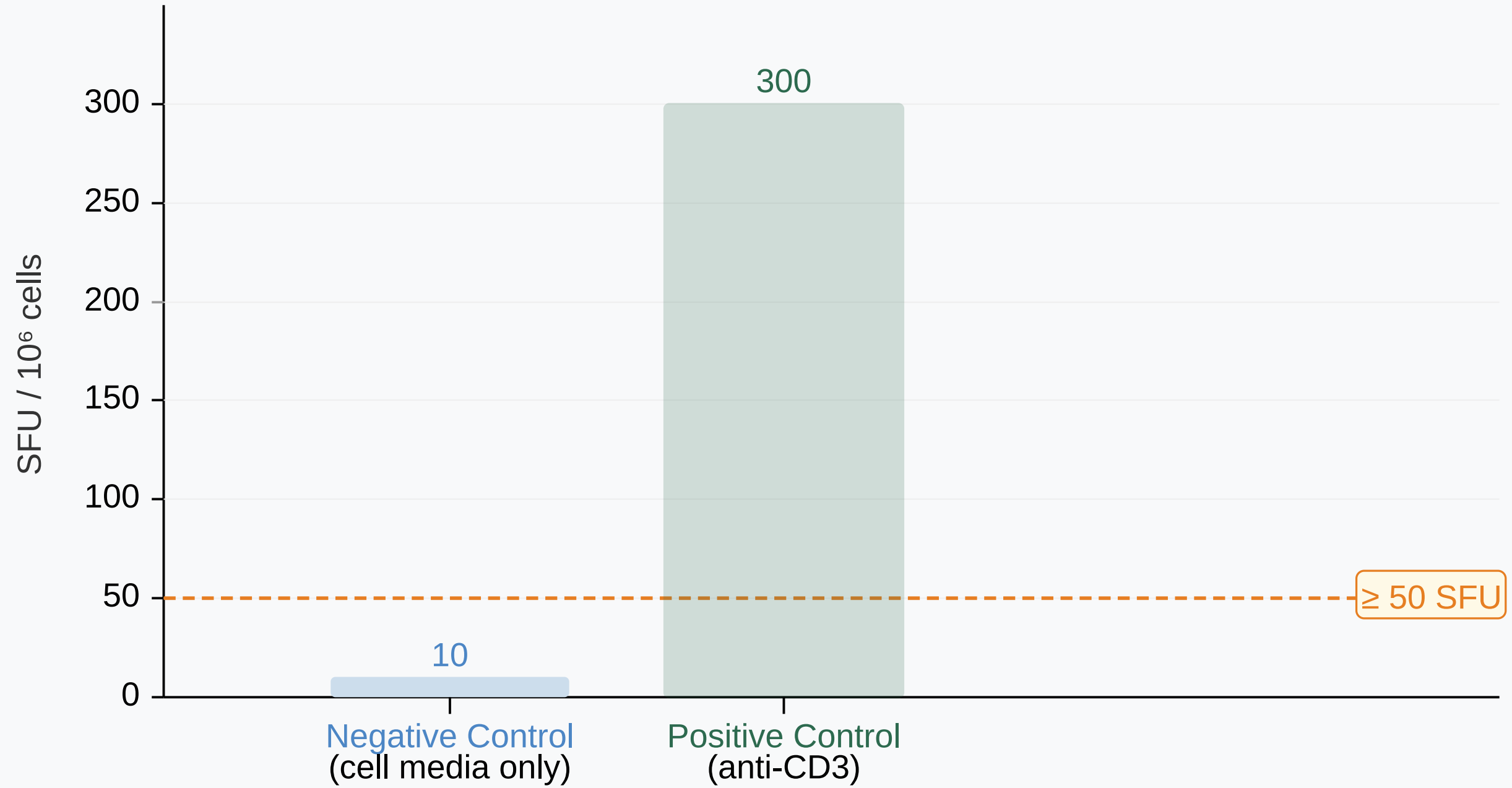


100 SFU  
Drug-specific response

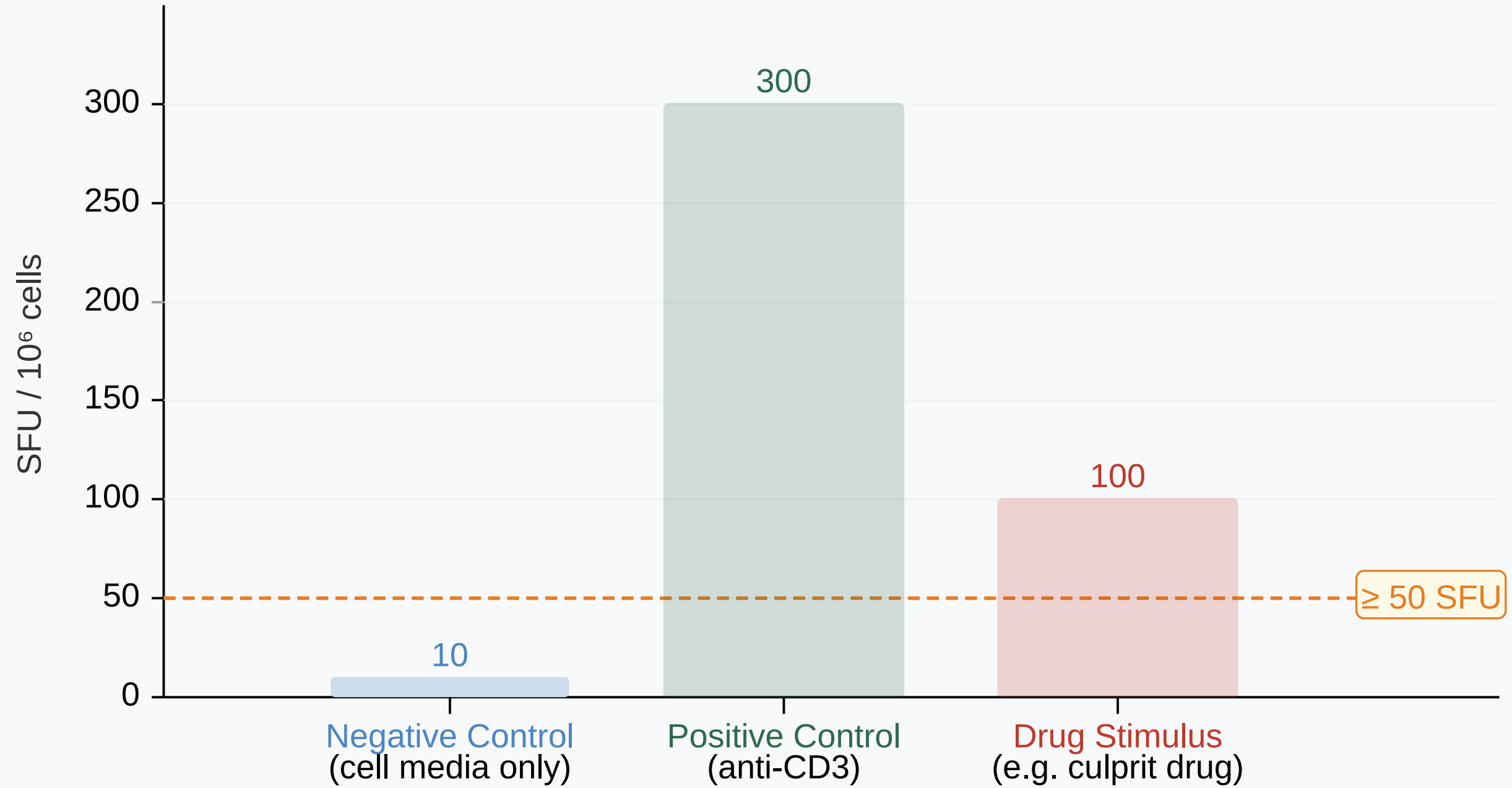
# ELISpot Interpretation



# ELISpot Interpretation



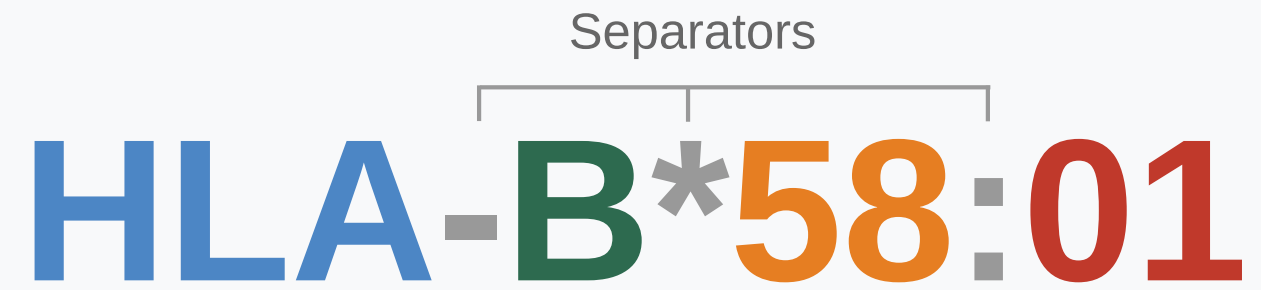
# ELISpot Interpretation



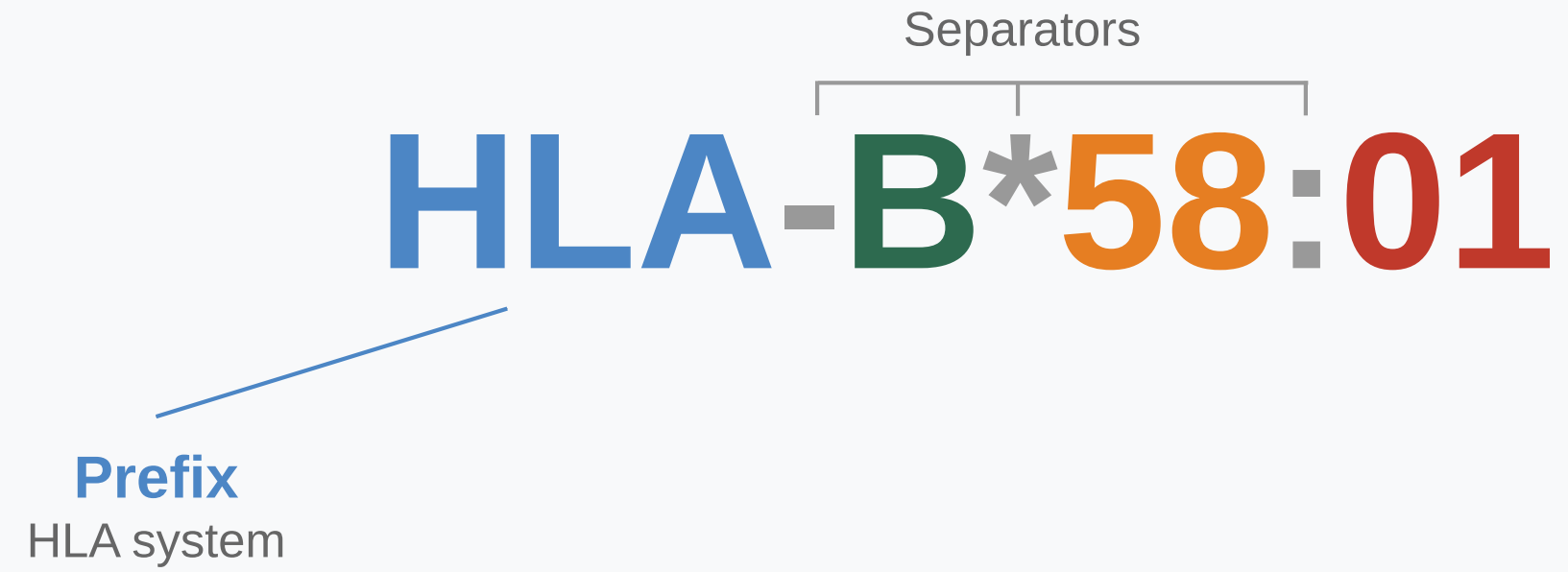
# HLA Naming

Separators

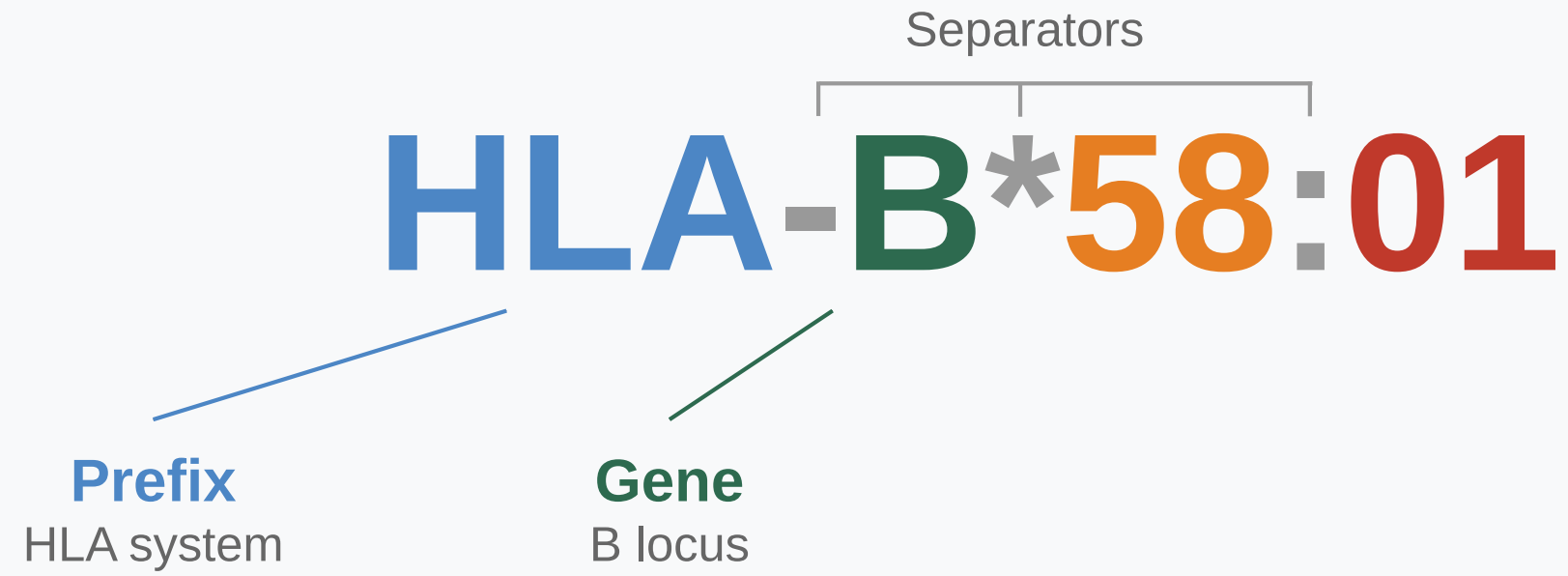
HLA-B\*58:01

The diagram shows the HLA name "HLA-B\*58:01" with a bracket above the characters "B\*", "58", and ":" labeled "Separators". The characters are color-coded: "HLA" is blue, "B" is green, "\*" is grey, "58" is orange, ":" is grey, and "01" is red.

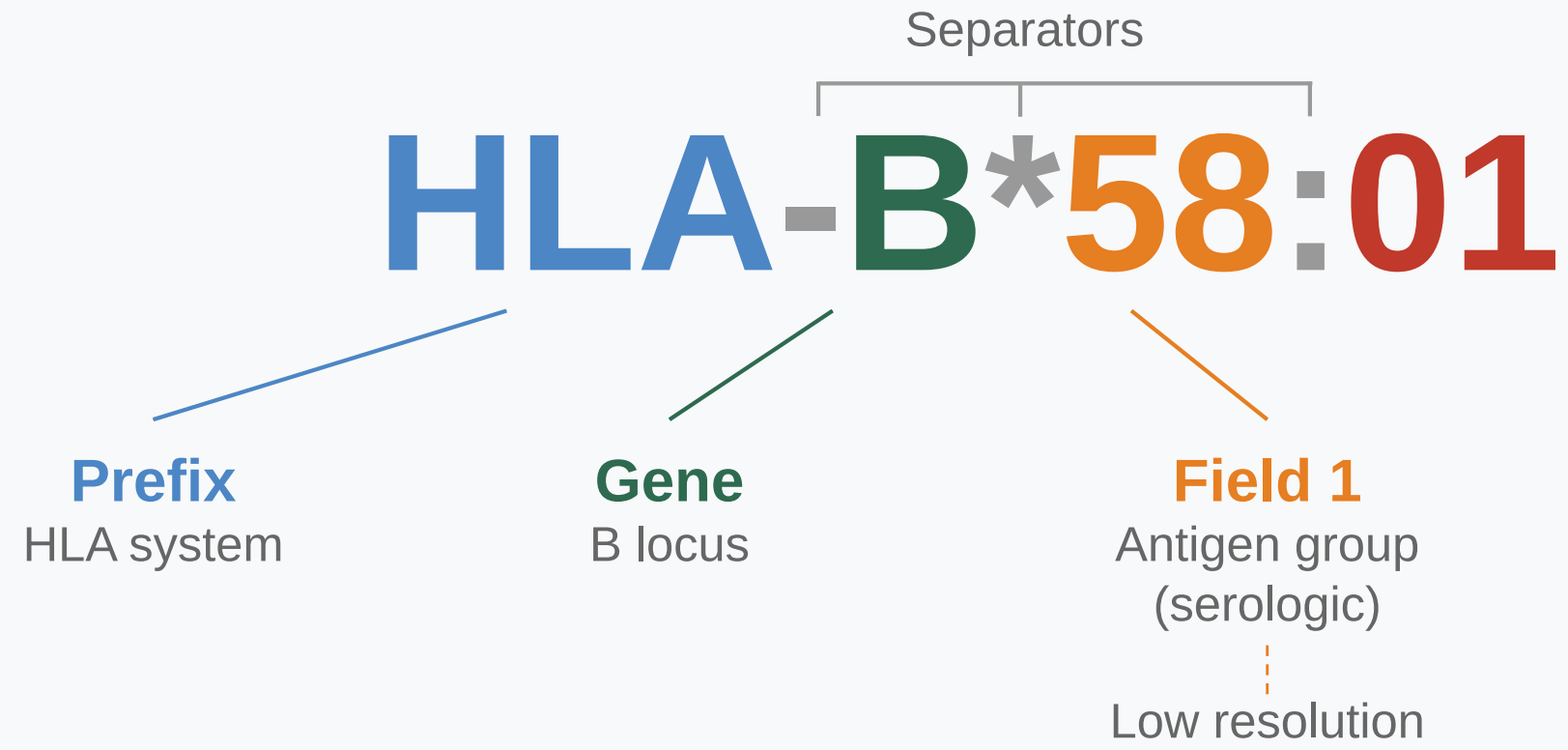
# HLA Naming



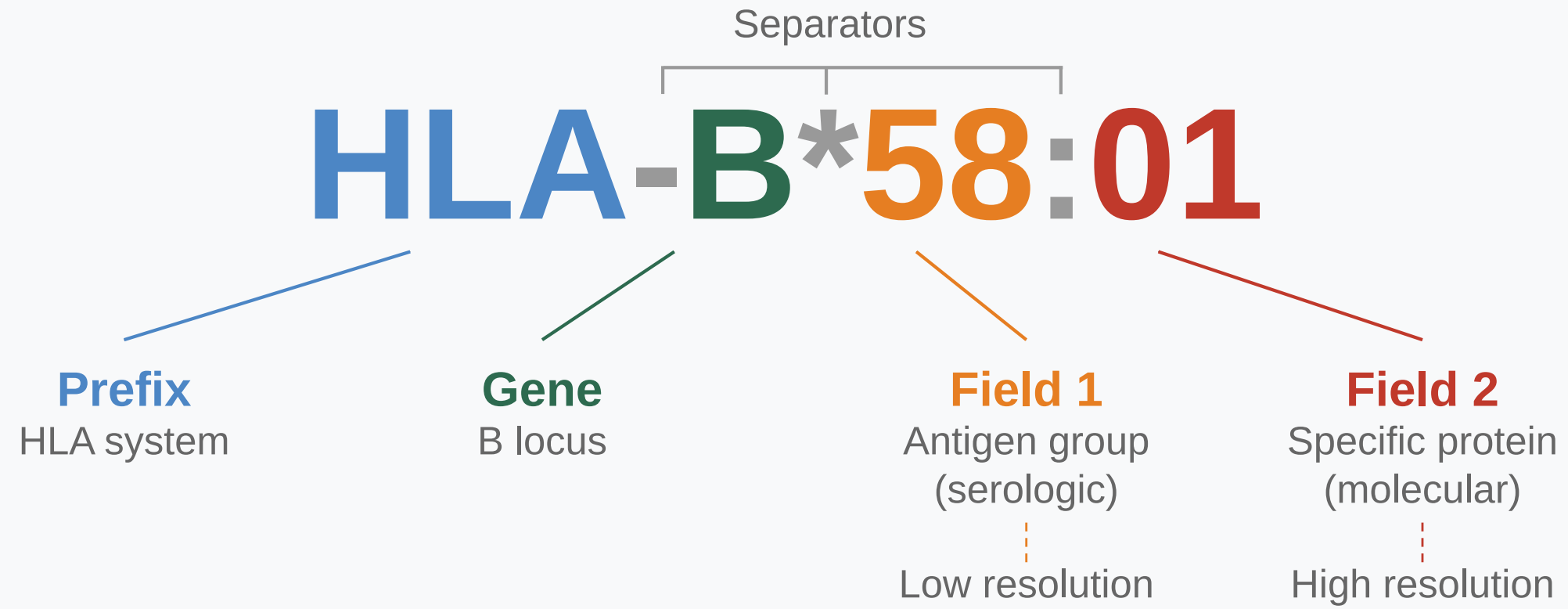
# HLA Naming



# HLA Naming



# HLA Naming



# Serologic Typing

## Complement-Dependent Cytotoxicity (CDC) Assay

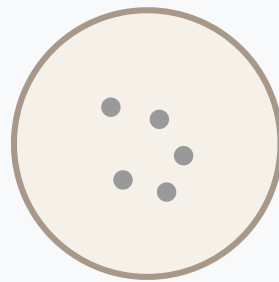
Serologic typing → Field 1 (antigen group)

1 Patient lymphocytes

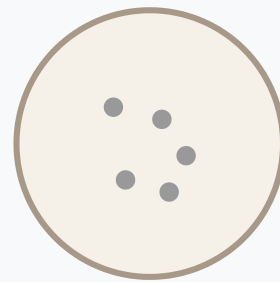
2 Add typing antisera

3 Add complement + dye

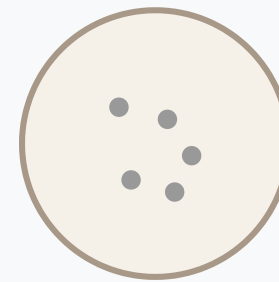
Anti-B7



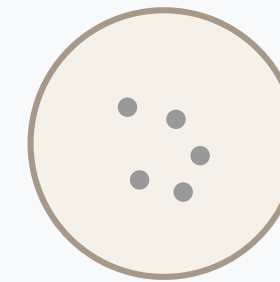
Anti-B27



Anti-B44



Anti-B57



# Serologic Typing

## Complement-Dependent Cytotoxicity (CDC) Assay

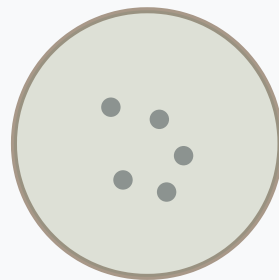
Serologic typing → Field 1 (antigen group)

1 Patient lymphocytes

2 Add typing antisera

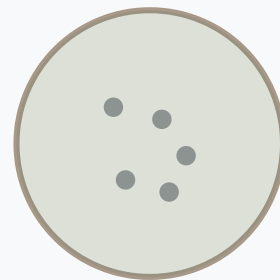
3 Add complement + dye

Anti-B7



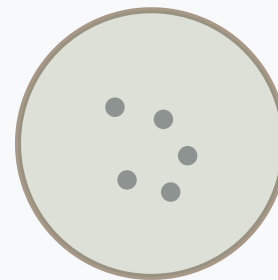
-  
Alive

Anti-B27



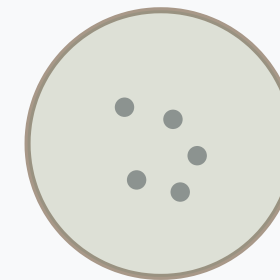
-  
Alive

Anti-B44



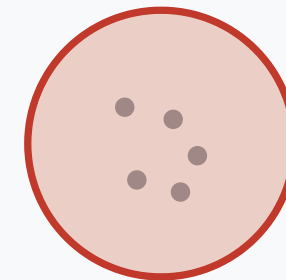
-  
Alive

Anti-B57



-  
Alive

Anti-B58



+  
Dead

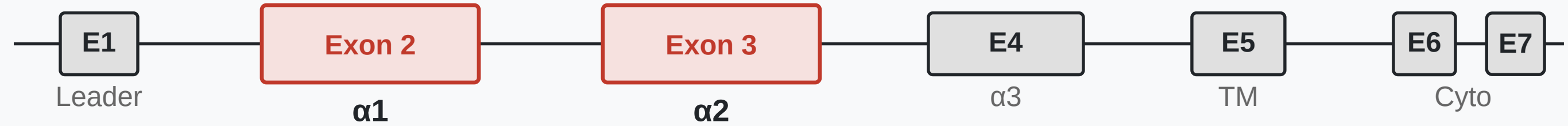
Patient is **B58** → Field 1 resolved

HLA-B\***58**:\_\_

Antibodies define antigen group but cannot distinguish alleles within a group

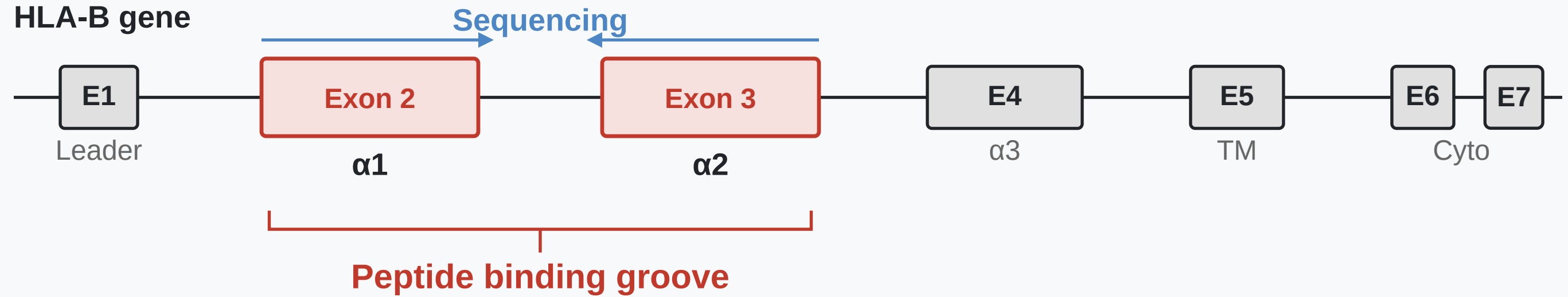
# Sequencing-Based Typing

HLA-B gene

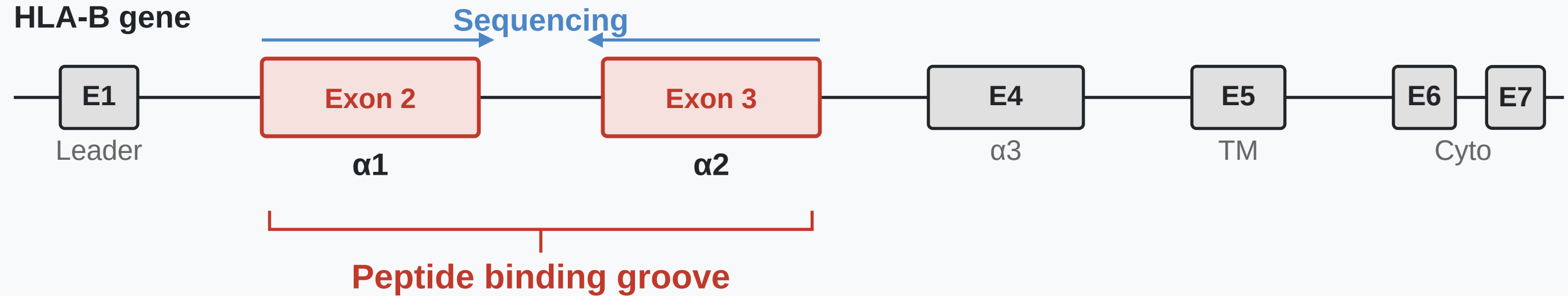


# Sequencing-Based Typing

HLA-B gene



# Sequencing-Based Typing



**HLA-B\*58:01**

...Asp-114, Trp-147...

Allopurinol hypersensitivity

VS

**HLA-B\*58:02**

...different residues...

Not associated

Same antigen group, different amino acid sequence → different clinical significance

# HLA Testing

**High-Resolution Sequencing**  
PCR sequencing of Exon 2 & 3

**Single-Allele Assay**  
PCR with allele-specific primers

# HLA Testing

## High-Resolution Sequencing

PCR sequencing of Exon 2 & 3

RESOLUTION

**4-digit (2-field)**

HLA-B\*57:01, B\*58:01, B\*15:02 ...  
Determines zygosity

## Single-Allele Assay

PCR with allele-specific primers

RESOLUTION

**Present or Absent**

Is HLA-B\*58:01 here? Yes / No

# HLA Testing

## High-Resolution Sequencing

PCR sequencing of Exon 2 & 3

### RESOLUTION

#### 4-digit (2-field)

HLA-B\*57:01, B\*58:01, B\*15:02 ...  
Determines zygosity

### USE CASE

**Transplant typing**  
**Research**

## Single-Allele Assay

PCR with allele-specific primers

### RESOLUTION

#### Present or Absent

Is HLA-B\*58:01 here? Yes / No

### USE CASE

#### Pharmacogenomics

HLA-B\*58:01 → allopurinol  
HLA-B\*57:01 → abacavir

# HLA Testing

## High-Resolution Sequencing

PCR sequencing of Exon 2 & 3

### RESOLUTION

#### 4-digit (2-field)

HLA-B\*57:01, B\*58:01, B\*15:02 ...  
Determines zygosity

### USE CASE

**Transplant typing**  
**Research**

### COST / TIME

\$\$\$ · Weeks

## Single-Allele Assay

PCR with allele-specific primers

### RESOLUTION

#### Present or Absent

Is HLA-B\*58:01 here? Yes / No

### USE CASE

#### Pharmacogenomics

HLA-B\*58:01 → allopurinol  
HLA-B\*57:01 → abacavir

### COST / TIME

\$ · Hours to Days

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

★ [Universal pre-prescription screening](#)

13

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

★ [Universal pre-prescription screening](#)

13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

# Strong HLA Class I associations with SCAR

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B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

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13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

## Dapsone

B13\*01 · DRESS / SJS-TEN · PPV 7.8%

84

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

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B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

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13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

## Dapsone

B13\*01 · DRESS / SJS-TEN · PPV 7.8%

84

## Nevirapine

C\*04:01 · SJS/TEN · PPV 2.5%

200

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

★ [Universal pre-prescription screening](#)

13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

## Dapsone

B13\*01 · DRESS / SJS-TEN · PPV 7.8%

84

## Nevirapine

C\*04:01 · SJS/TEN · PPV 2.5%

200

## TMP-SMX

B\*13:01 · DRESS / SJS-TEN · PPV 3–4%

211

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

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13

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A\*32:01 · DRESS · PPV 20%

75

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84

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C\*04:01 · SJS/TEN · PPV 2.5%

200

## TMP-SMX

B\*13:01 · DRESS / SJS-TEN · PPV 3–4%

211

## Allopurinol

B\*58:01 · DRESS / SJS-TEN · PPV 3%

250

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

★ [Universal pre-prescription screening](#)

13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

## Dapsone

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84

## Nevirapine

C\*04:01 · SJS/TEN · PPV 2.5%

200

## TMP-SMX

B\*13:01 · DRESS / SJS-TEN · PPV 3–4%

211

## Allopurinol

B\*58:01 · DRESS / SJS-TEN · PPV 3%

250

## Carbamazepine

B\*15:02 · SJS/TEN only · PPV 3%

1,000

# Strong HLA Class I associations with SCAR

Number Needed to Test to Prevent 1 Case

## Abacavir

B\*57:01 · Abacavir HSR · PPV 55% · 100% NPV

★ [Universal pre-prescription screening](#)

13

## Vancomycin

A\*32:01 · DRESS · PPV 20%

75

## Dapsone

B13\*01 · DRESS / SJS-TEN · PPV 7.8%

84

## Nevirapine

C\*04:01 · SJS/TEN · PPV 2.5%

200

## TMP-SMX

B\*13:01 · DRESS / SJS-TEN · PPV 3–4%

211

## Allopurinol

B\*58:01 · DRESS / SJS-TEN · PPV 3%

250

## Carbamazepine

B\*15:02 · SJS/TEN only · PPV 3%

1,000

## Carbamazepine

A\*31:01 · DRESS >>> SJS-TEN · PPV <0.5%

5,000

# HLA Testing

## ? Multiple Suspects

Patient with DRESS on concurrent antibiotics

- **Piperacillin-tazobactam**
- **Vancomycin**
- **Cefepime**
- **Metronidazole**

Which drug caused it?

# HLA Testing

## ? Multiple Suspects

Patient with DRESS on concurrent antibiotics

- **Piperacillin-tazobactam**
- **Vancomycin**
- **Cefepime**
- **Metronidazole**

Which drug caused it?

## HLA-A\*32:01 Positive

HLA result narrows causality:

Piperacillin-tazobactam

▶ **Vancomycin** — Most probable culprit

Cefepime

Metronidazole

HLA-A\*32:01 strongly associated with vancomycin-induced DRESS

Low population PPV → high clinical utility for adjudication

# Conclusions

## Valuable but Limited

Delayed testing aids  
**diagnosis, causality,  
and risk stratification**

but no test achieves  
100% sensitivity or NPV

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**diagnosis, causality,  
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## Sensitivity Varies

Depends on  
**modality**  
**phenotype**  
**timing from reaction**

# Conclusions

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Delayed testing aids  
**diagnosis, causality,  
and risk stratification**

but no test achieves  
100% sensitivity or NPV

## Sensitivity Varies

Depends on  
**modality**  
**phenotype**  
**timing from reaction**

## Unmet Need

Skin test concentrations  
for delayed reactions  
need to be  
**validated and standardized**

# Extra Slide

- Extraslide