



Sanquin

HEALTH SOLUTIONS

# Development of a novel drug-tolerant ADA assay: the acid-dissociation lanthanide-fluorescence immunoassay (ALFIA)

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**For Life.**



# Anti-drug antibodies (ADA)

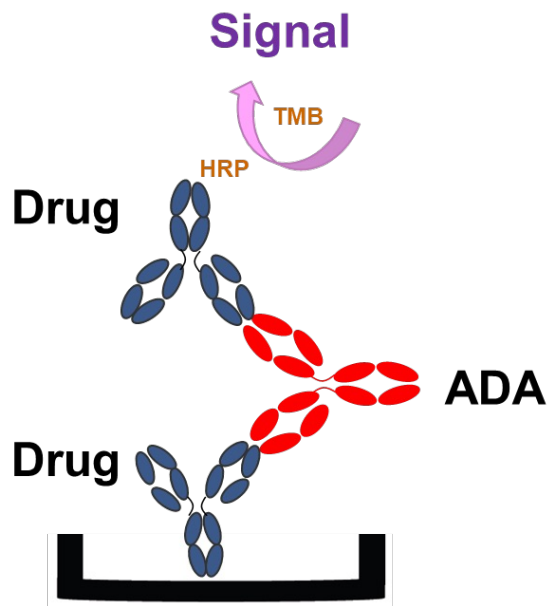
- Therapeutic monoclonal antibodies can be immunogenic

ADA formation decreases efficacy of the therapy:

- Blocks drug – target interaction (neutralisation)
  - Increased clearance
- 
- ADA in some cases linked with serious adverse events



# ADA assays – Bridging formats

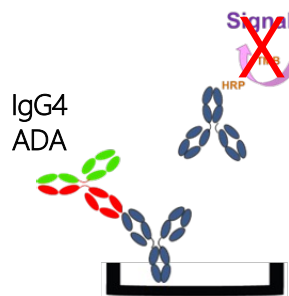


## PROS

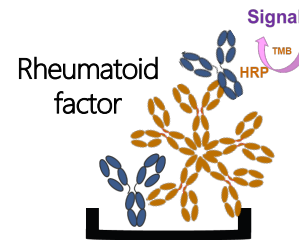
- Easy handling
- Sensitive, large dynamic range (MSD)

## CONS

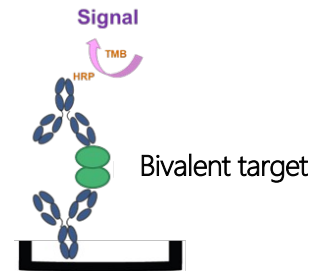
- No detection of IgG4
- False positives due to rheumatoid factor or bivalent target



false negative



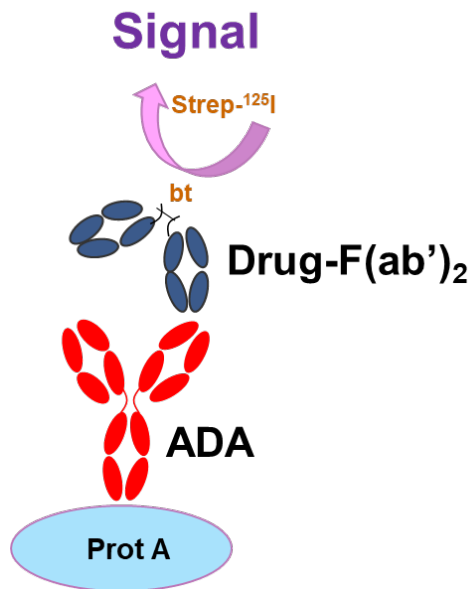
false positive



false positive

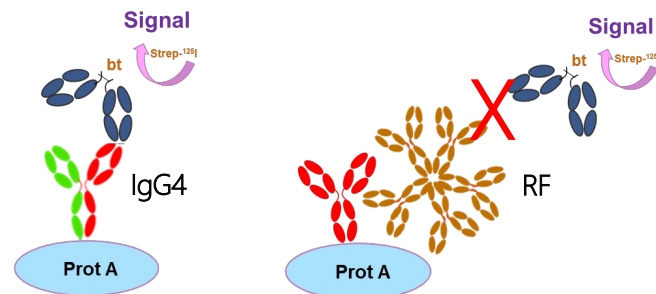


# ADA assays - antigen binding test (ABT)



## PROS

- IgG4 detection
- No false positives due to rheumatoid factor

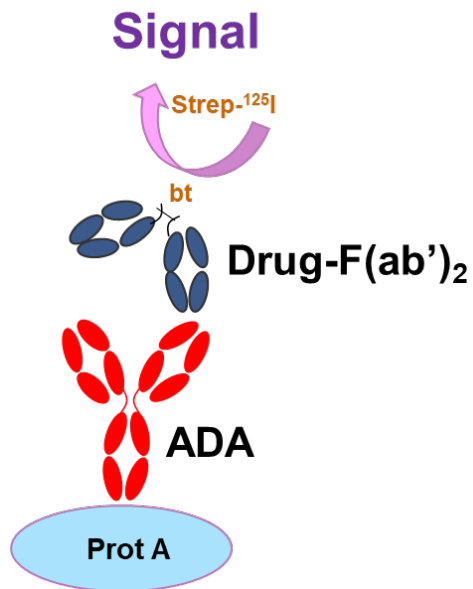


## CONS

- No/low detection of IgD, IgE, IgM, IgA (protein A specific)



# ABT format: practical drawbacks



Labour intensive

- Individual tubes
- Manual washing steps

Radioactive detection reagent

- Specialised facilities needed
- RA decay requires frequent labeling of the drug

Goals: 1) increase throughput of the assay  
2) replace radioactive detection reagent

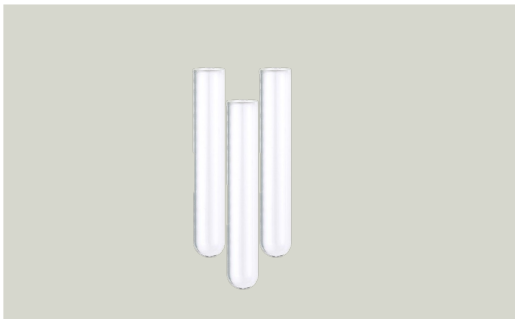
Adalimumab: ADL

Anti-drug antibodies: ADA / anti-ADL



# Increase throughput of the ABT

tubes



Individual tubes  
Manual washing (centrifuge, remove buffer,  
add buffer)  
Current assay volume: ~800  $\mu\text{L}$

filterplate



96-well filterplate  
Vacuum washing (apply vacuum, add buffer)  
Max assay volume: ~250  $\mu\text{L}$



# (poly-)HRP as detection reagent

Replace RA detection with colorimetric detection:

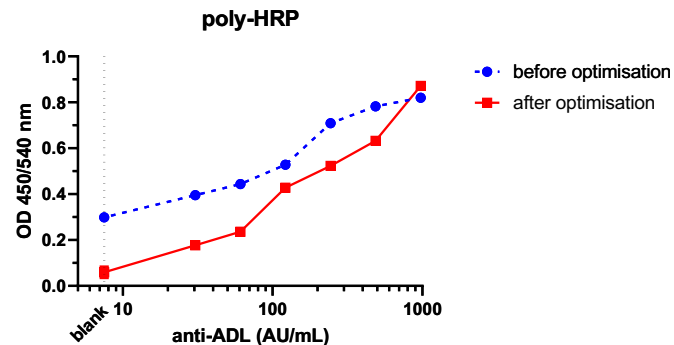
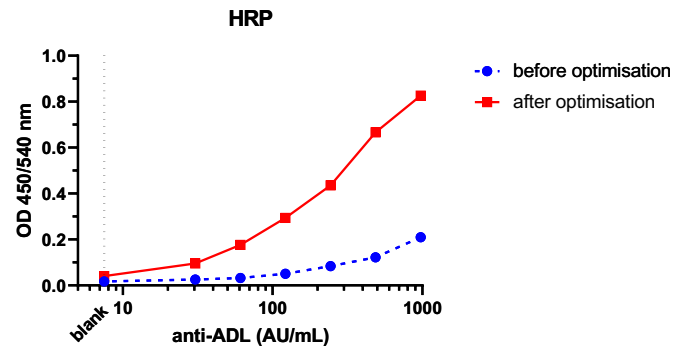
- HRP/poly-HRP + TMB

Optimisation steps:

- beads
- sample
- drug-F(ab')<sub>2</sub>-bt
- strep-(p)HRP
- TMB
- colouring time

Proof of principle:

- 1) ABT can be performed in filterplates
- 2) RA detection can be replaced with colorimetric detection, but results in a low dynamic range





# Europium as detection reagent

Replace RA detection with fluorescent detection:

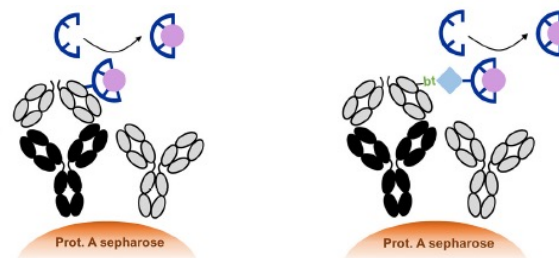
- europium

Time resolved fluorescence: low background

Release of europium in solution: easy transfer to receiver plate

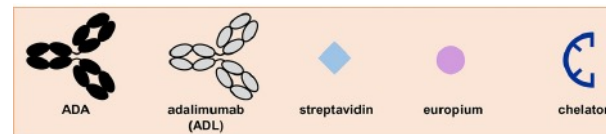
Direct and indirect detection

- Direct: drug-F(ab')<sub>2</sub>-eu
- Indirect: drug-F(ab')<sub>2</sub>-bt + strep-eu



direct

indirect

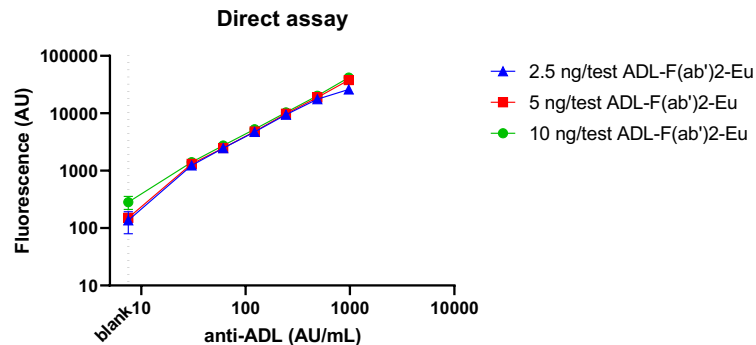
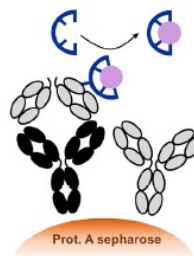




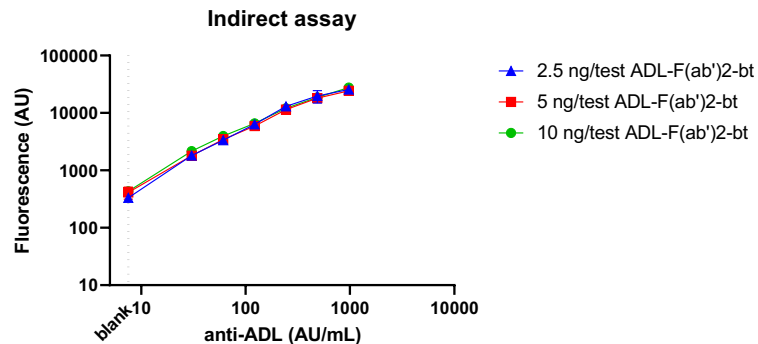
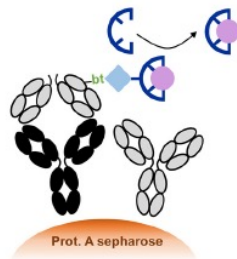


# Europium succesfull as detection reagent

direct

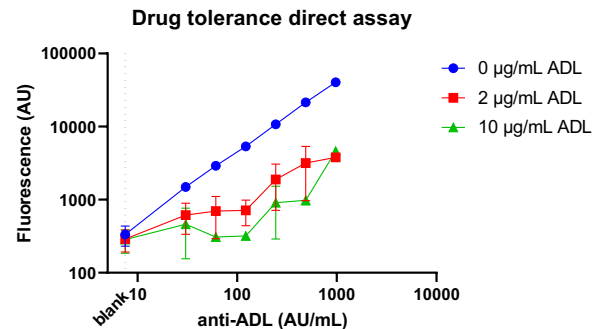
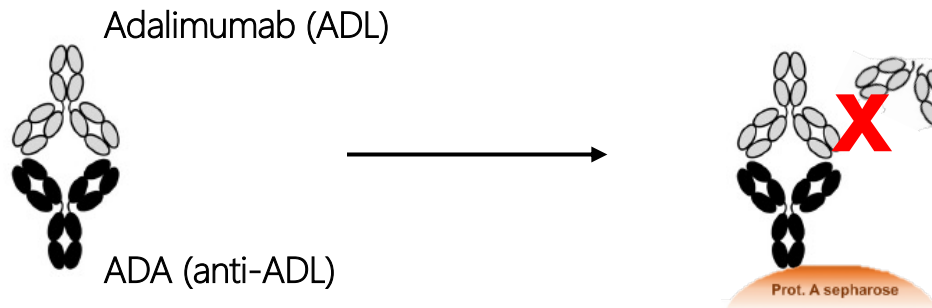


indirect

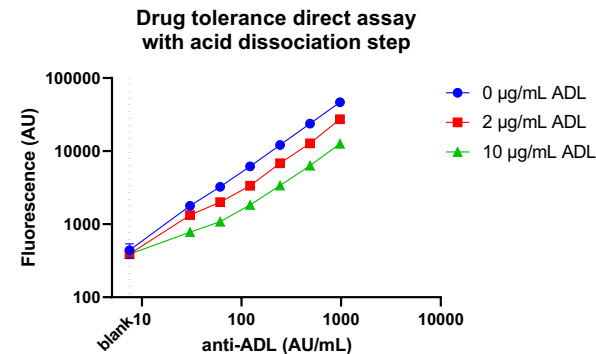
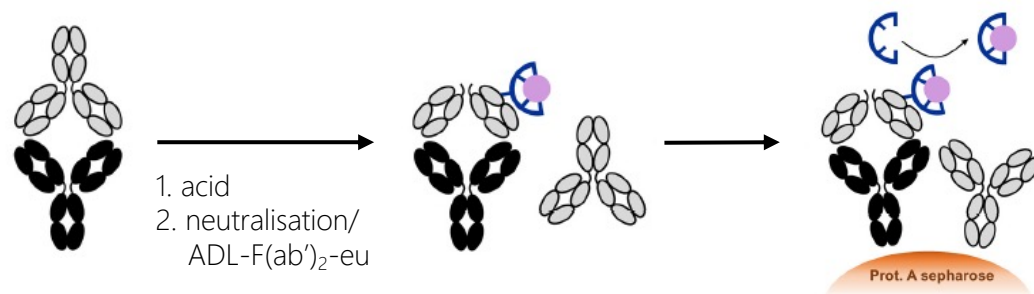




# Drug tolerance



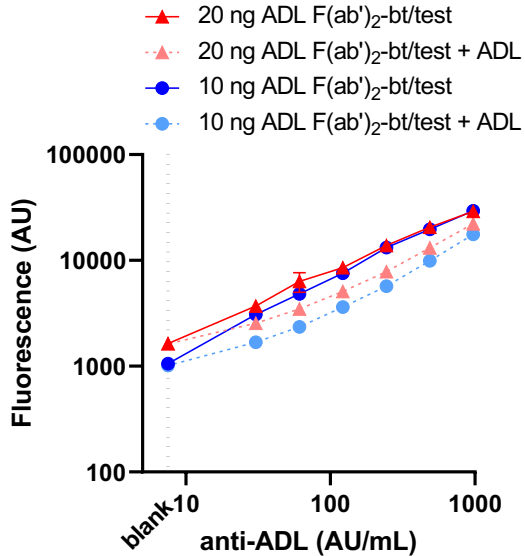
## Acid dissociation step increases drug tolerance



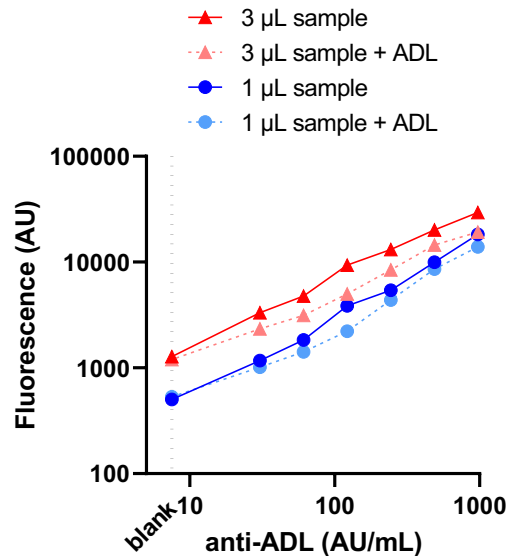


# Optimisation steps

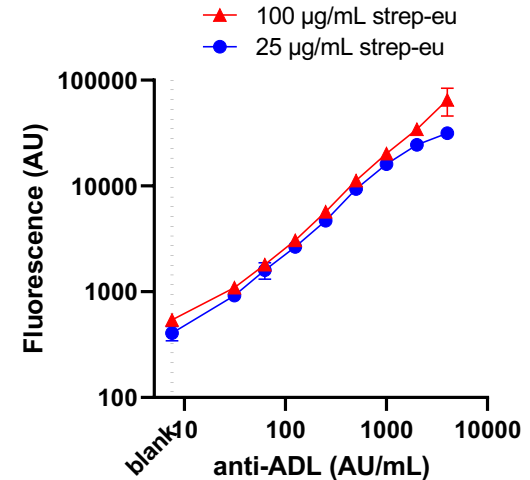
Increasing ADL-F(ab')<sub>2</sub>-bt/eu concentration increases drug tolerance



Decreasing the amount of sample and beads decreases background

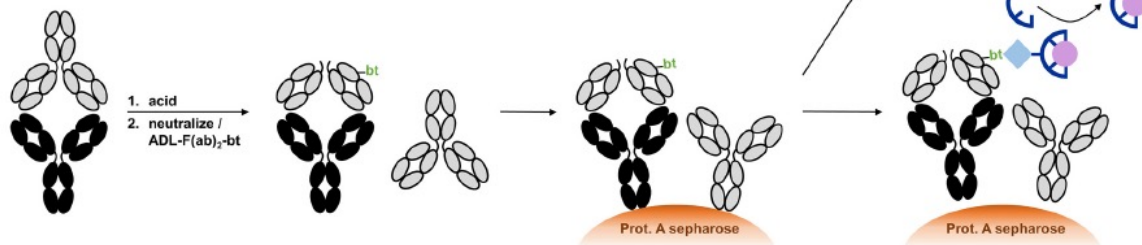
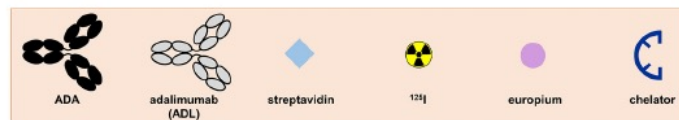


Large dynamic range can be achieved





# ALFIA vs ARIA



ARIA

ARIA

- tubes
- RA detection

indirect  
ALFIA

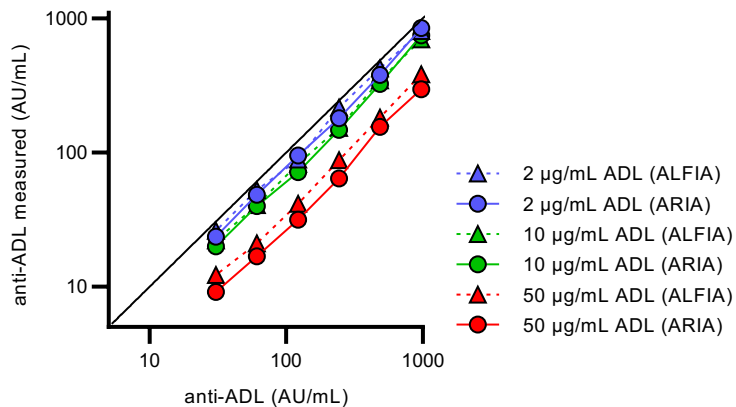
ALFIA

- filterplate
- eu detection



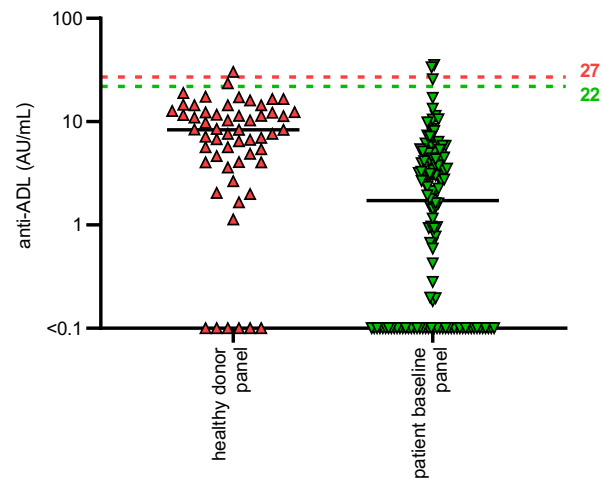
# ALFIA vs ARIA

## Drug tolerance



ARIA and ALFIA have similar drugtolerance

## Limit of detection (LOD)



LOD ARIA: 30 AU/mL

LOD ALFIA: 27 AU/mL

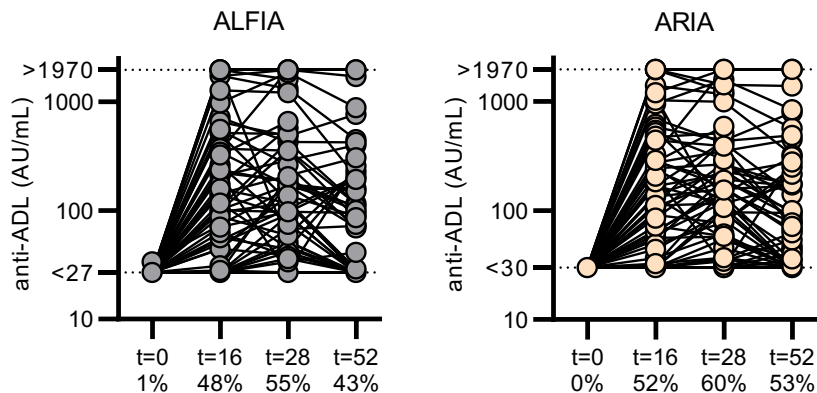
ARIA and ALFIA have similar limits of detection



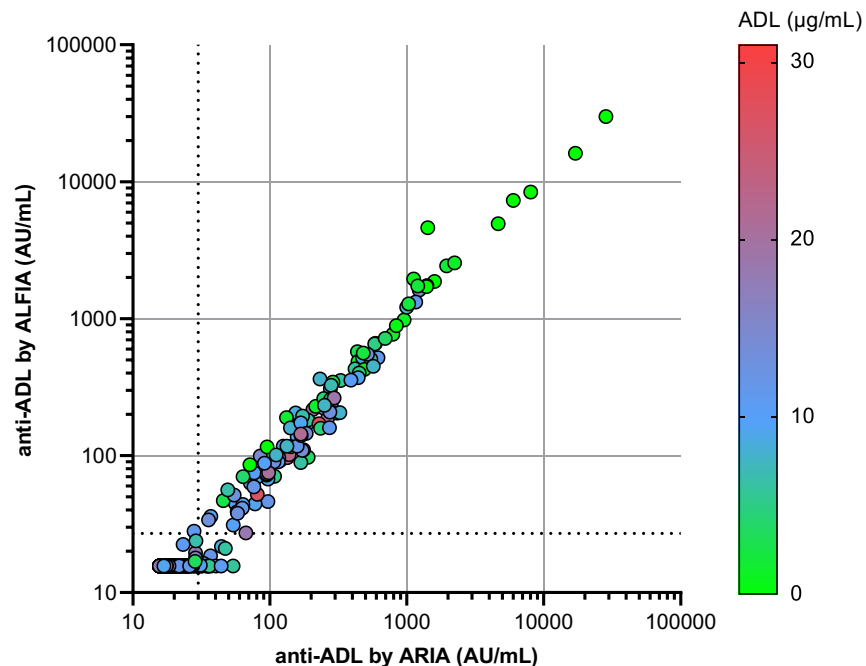
# ALFIA vs ARIA

Measurement of ADA response in 94 adalimumab-treated patients

- similar ADA response, ~60% of patients ADA+



- high correlation between ALFIA and ARIA ( $r=0.93$ ,  $p \leq 0,0001$ )





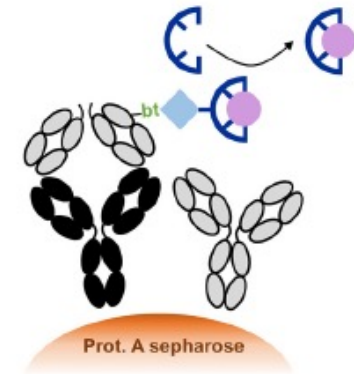
# Conclusions

We developed a novel drug-tolerant ADA assay: ALFIA

Similar sensitivity, drug tolerance and overall performance to the ARIA

Performance in 96-well filterplates enables easy handling and washing and allows for further automation using liquid handling robots

Fluorescent detection makes this assay suitable for implementation in any common laboratory



ALFIA

van Strien J, Dijk L, Atiqi S, Schouten R, Bloem K, Wolbink GJ, Loeff F, Rispens T. Drug-tolerant detection of anti-drug antibodies in an antigen-binding assay using europium chelate fluorescence. *J Immunol Methods*. 2023 Mar;514:113436. doi: 10.1016/j.jim.2023.113436. Epub 2023 Jan 28. PMID: 36716916.



# Acknowledgements

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