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Comparison of immunoassay technologies for the detection of anti-drug antibodies against biotherapeutics

DELFIA, AlphaLISA and Gyros

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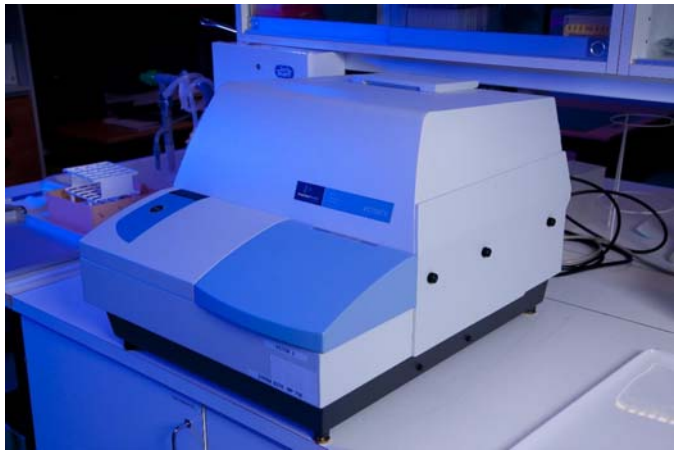
SYRINX BIOANALYTICS

DELFIA

- **D**issociation-**E**nhanced **L**anthanide **F**luorescent **I**mmuno**a**ssay
- Time resolved fluorescence detection (Europium label)
- 96-well plate format
- Victor^{2}V and Victor^{3}V multilabel readers, AutoDELFIA

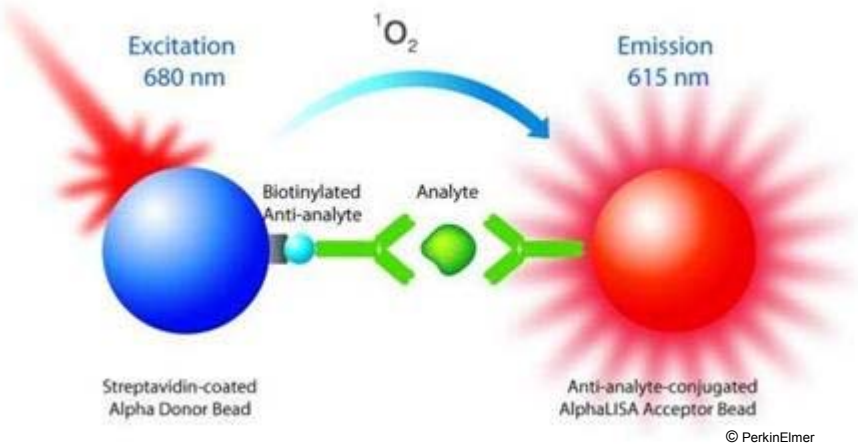


Gyros technology

- Automated immunoassay platform
- Prompt fluorescence detection (Alexa label)
- Gyrolab Bioaffy CDs (112 columns)
- Gyrolab xP workstation

AlphaLISA

- **A**mplified **L**uminescent **P**roximity **H**omogeneous **A**ssay
- Bead based assay with fluorescence detection (acceptor beads containing Europium)
- Proximity enables energy transfer from donor to acceptor beads
- 384-well plate format
- EnVision multilabel reader

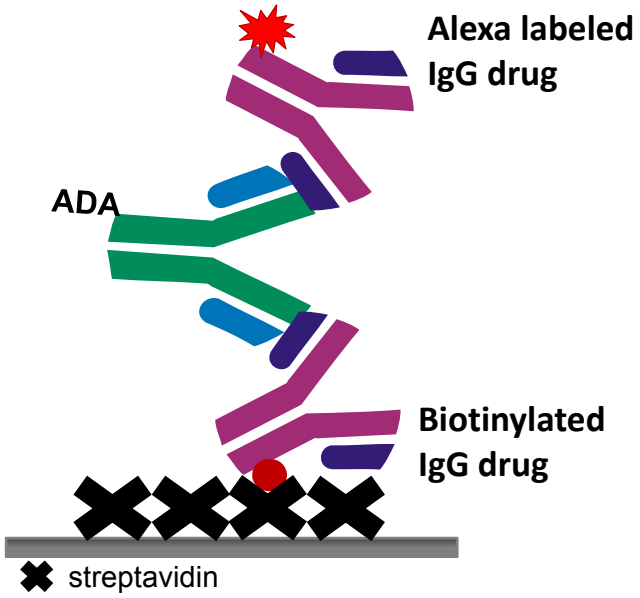


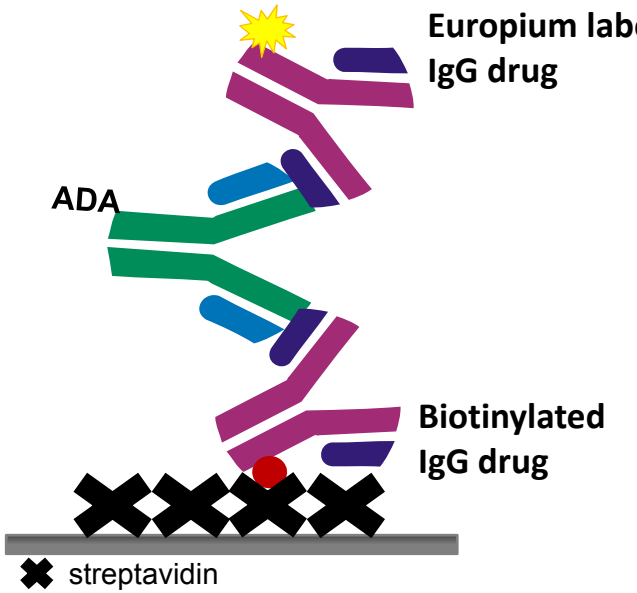
Comparison of different technologies

- Most method parameters are directly dependent on the drug molecule and positive control antibody
- Data created with one drug molecule/control antibody is valid only for these molecules

⇒ Same drug molecule and control antibody should be used when comparing methods for immunogenicity assessment

Gyros vs. DELFIA

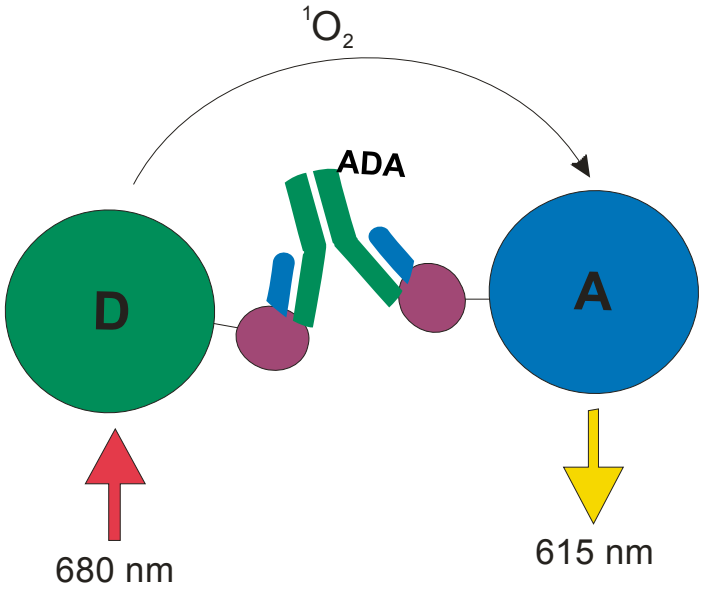


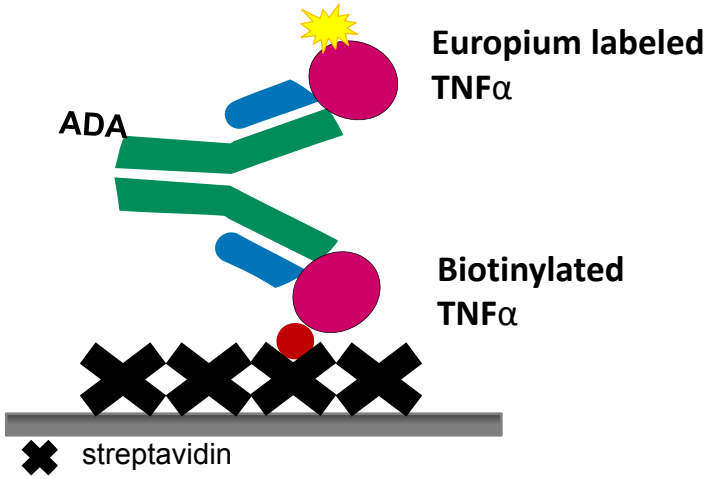


- Gyros:
- Reagents and samples are added in separate steps
- Spinning and washing of columns after each step (total analysis time 55 min)

- DELFIA:
- Reagents and samples are added in separate steps
- Incubation and washing of wells after each step (3 hour incubations in total)

AlphaLISA vs. DELFIA

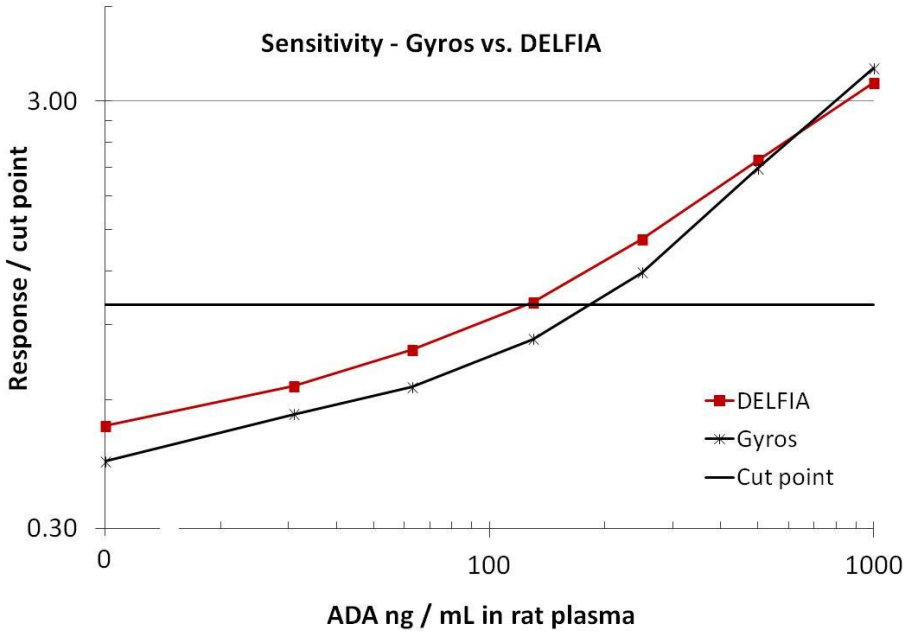


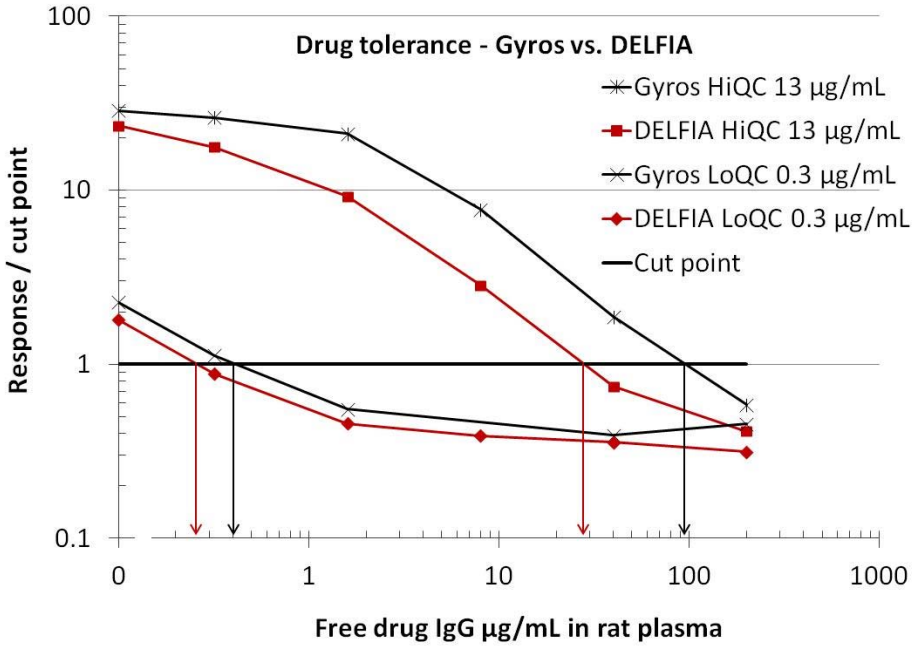


- Reagents and samples are incubated in an uncoated well in one step (1 hour)
- TNFα conjugated beads are brought into proximity by anti-TNFα antibodies (ADA)
- Signal is measured after the incubation step without washing

- Reagents and samples are incubated in an uncoated well in one step (1 hour)
- ADA-drug complexes are immobilized on the streptavidin plate in a second step (1 hour)
- Streptavidin wells are washed before the measurement

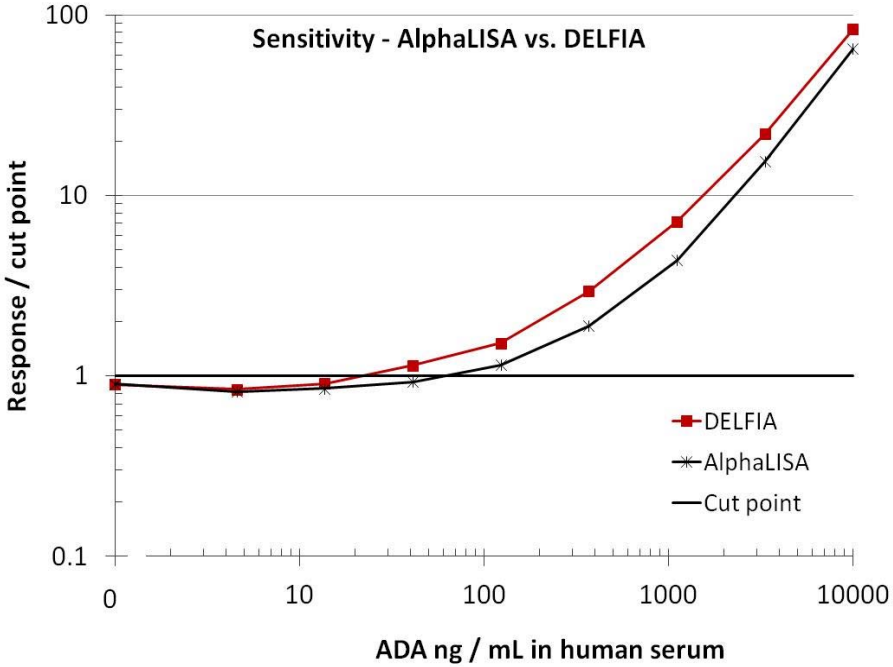
Gyros vs. DELFIA

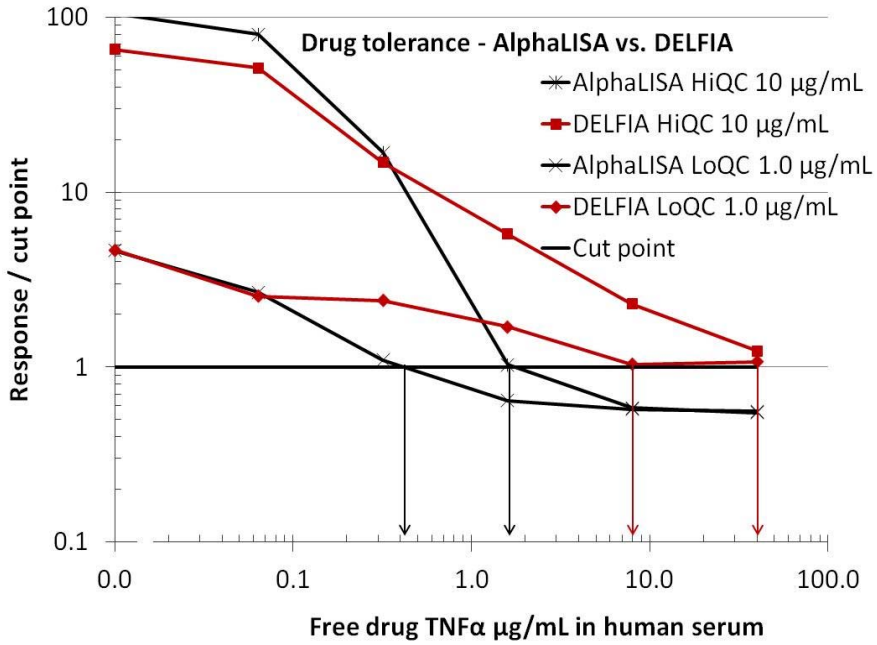




- Very similar sensitivities (130-200 ng/mL)
- Gyros tolerates 2-3 fold more free drug

AlphaLISA vs. DELFIA





- Very similar sensitivities (25-60 ng/mL)
- DELFIA tolerates 20-30 fold more free drug

Method comparison

Method comparison	**Gyros IgG**	**DELFIA IgG**	**AlphaLISA TNFα**	**DELFIA TNFα**
Assay format	Heterogeneous	Heterogeneous	**Homogeneous**	**Heterogeneous**
Wash steps	3	3	**0**	**1**
Analysis time/CD or plate	**55 min**	**4 hours**	1.5 hours	2.5 hours
Sample volume (duplicates)	**4 µL**	**50 µL**	**10 µL**	**50 µL**
Capture reagent ng/well	**49**	**100**	**214**	**15**
Detection reagent ng/well	**6.7**	**50**	**214**	**15**
Drug tolerance HiQC	100 µg/mL	30 µg/mL	**1.6 µg/mL**	**40 µg/mL**
Drug tolerance LoQC	0.40 µg/mL	0.25 µg/mL	**0.40 µg/mL**	**8.0 µg/mL**
Sensitivity	200 ng/mL	130 ng/mL	60 ng/mL	25 ng/mL
Intra assay precision	6.0 – 17.1 %	0.4 – 3.1 %	3.3 – 7.4 %	3.4 – 11.5 %
Inter assay precision	12.1 – 35.9 %	5.3 – 17.9 %	5.6 – 7.8 %	3.9 – 11.5 %
Hook effect	NO	NO	YES	YES
Detection of low affinity antibodies	NO	NO	**YES**	**NO**
Automation	YES	NO (possible)	NO (possible)	NO (possible)

Conclusions

- Tested methods had similar sensitivities, some difference in drug tolerance
- Several parameters affect the method selection
- None of the methods is better than the others in terms of all parameters
- Selection of the method is a compromise between different parameters

Thank you!

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