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Impact of ADA interference in PK assays:

Gyrolab immunoassay utilizing acid
dissociation as a potential solution for
reducing ADA interference

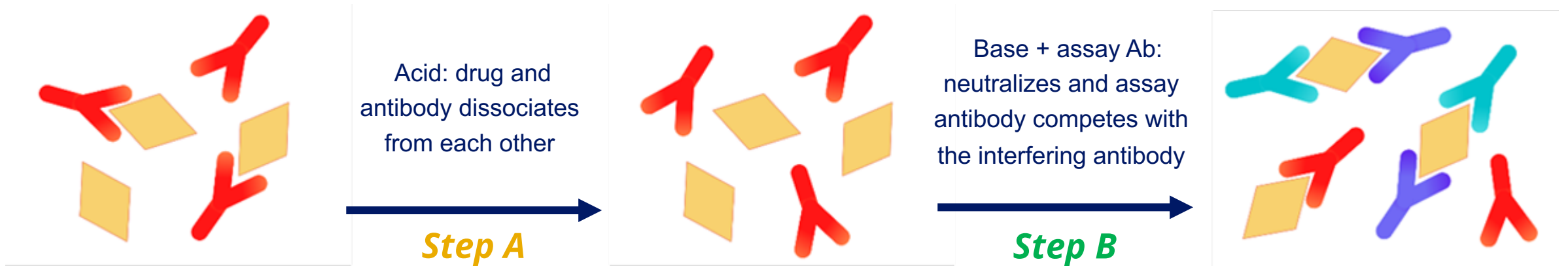
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- **Gyrolab mixing CD**
- **Gyrolab Mix vs interference**
- **Comparison of methods on model samples**
- **Comparison of methods on clinical samples**
- **Summary**

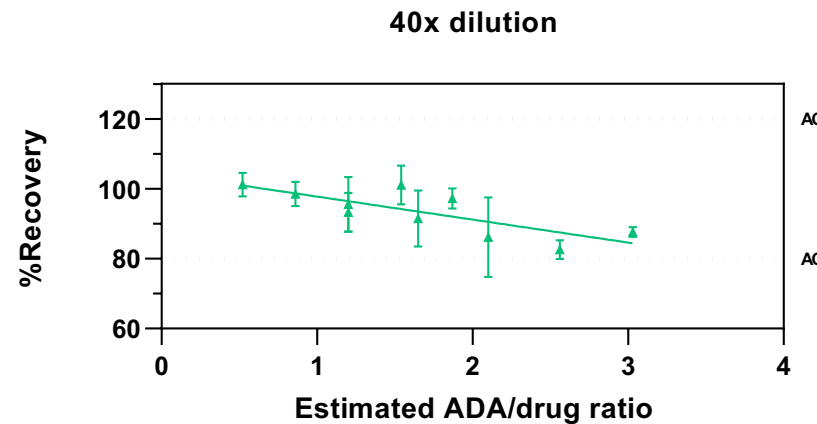
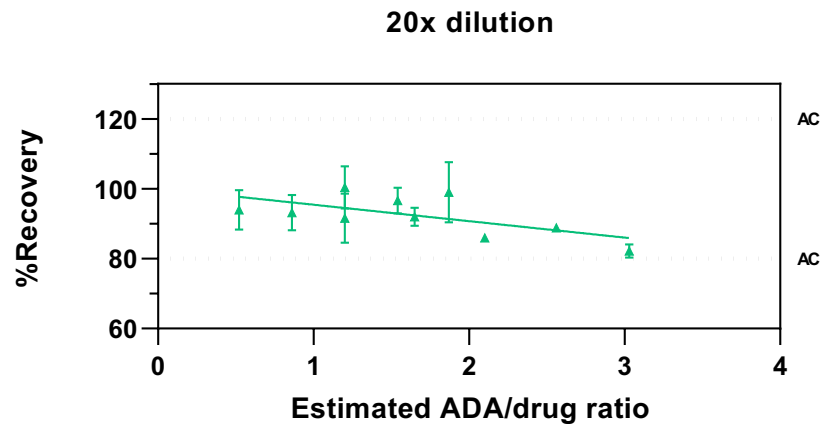
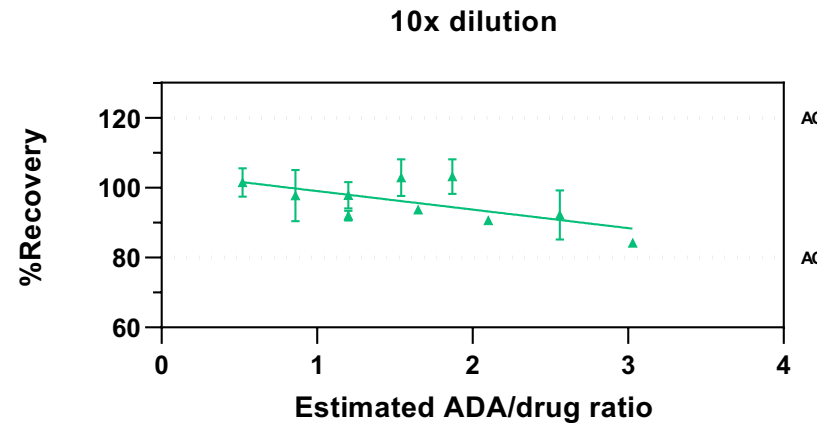
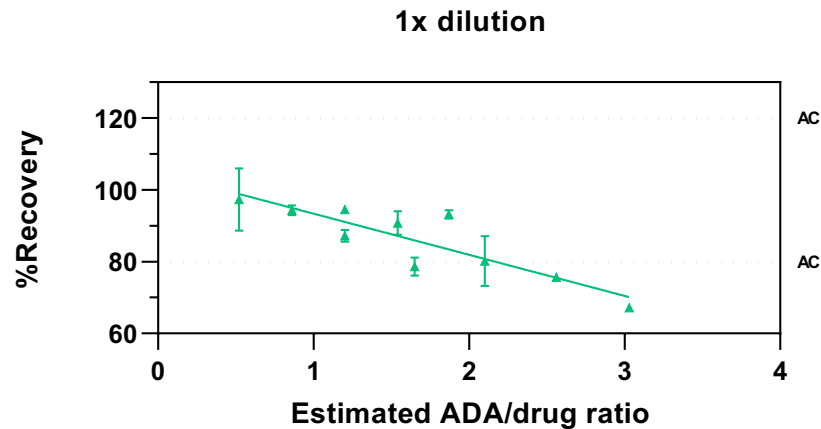
Gyrolab mixing CD



Developed method for quantification of an insulin analogue:

- Optimal *step B* pH is ~5.2
- Precision: CV < 7%, Accuracy: RE < ±5%
 - Range: 0.2-25 nM
- 10 µL sample needed

Gyrolab Mix vs interference



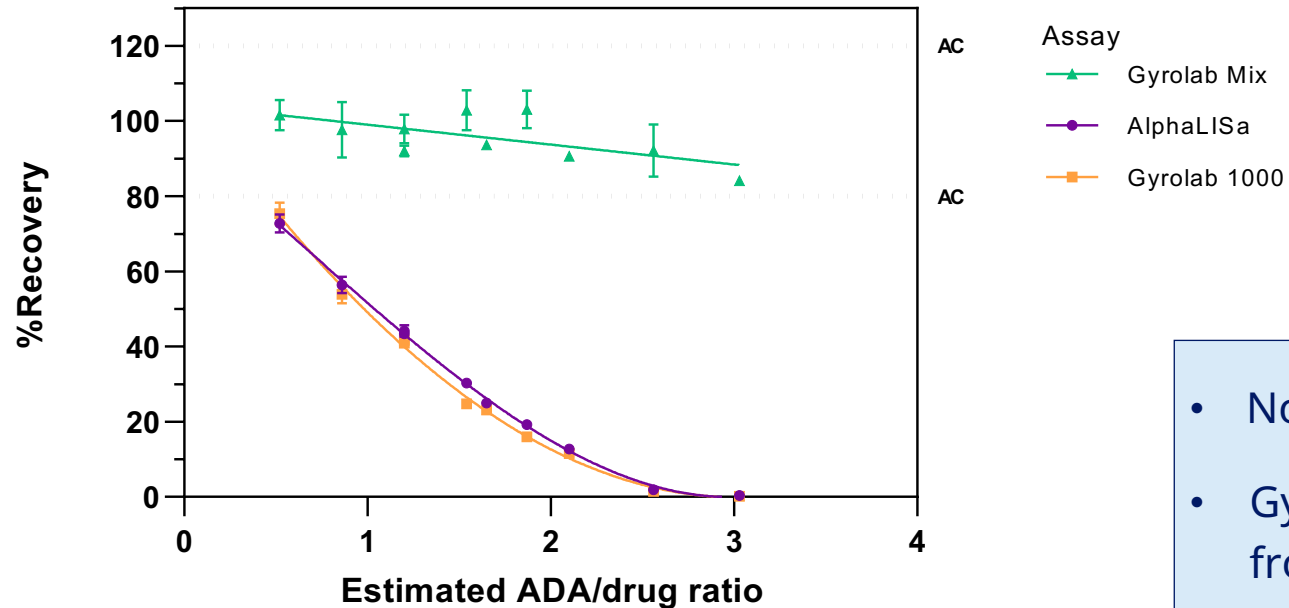
AC: Acceptance criteria +/- 20%

MRD= minimal required dilution

- All results are normalized to their negative control
- Additional to 10x MRD: 1-40x dilutions of model samples in serum

- 1x dilution on Gyrolab mix: up to 1.5 ADA/drug ratio within acceptance criteria
- From 10x dilution all results within acceptance criteria
- Original method: 10 MRD → could be improved

Model samples – comparison of 3 assays

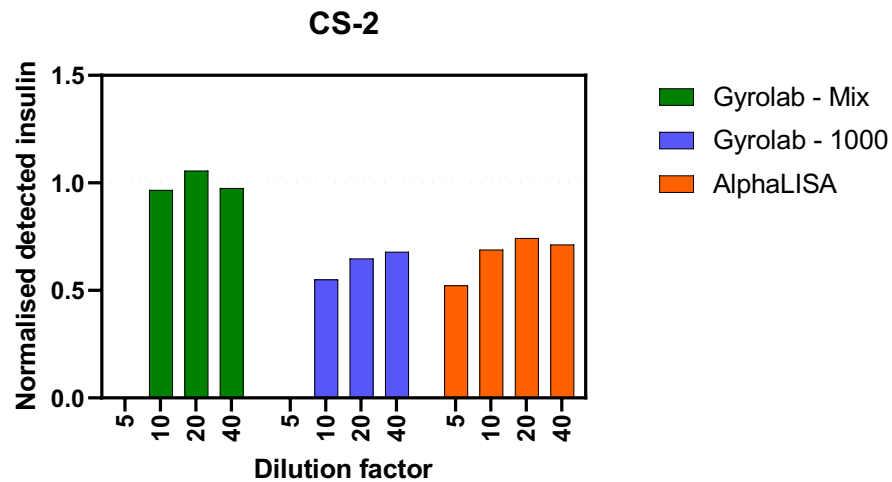
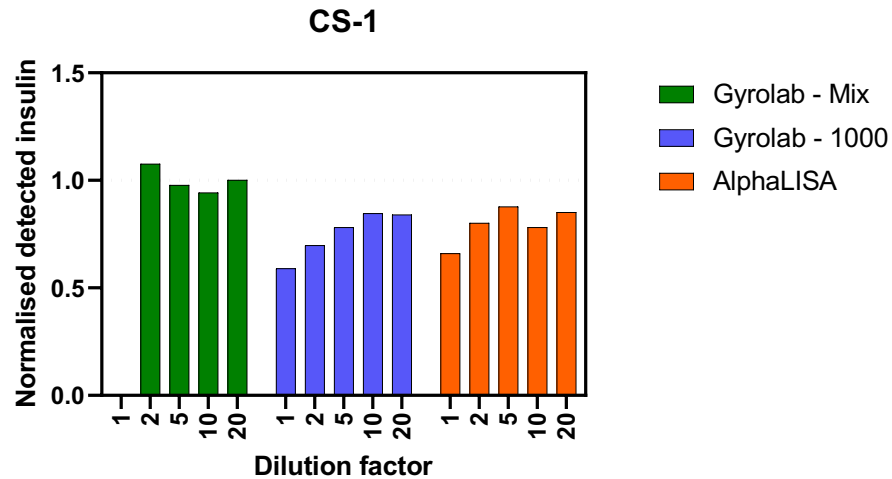


AC: Acceptance criteria +/- 20%

- All results are normalized to their negative control
- 10x diluted Gyrolab Mix results are portrayed

- No difference between Gyrolab and AlphaLISA
- Gyrolab Mix 10x dilution: interference removed from all the samples (up to 3 ADA/drug ratio)
- Increased %CV but still within acceptable range

Clinical Samples



- Two clinical sample pools (CS-1, CS-2, suspected to contain interference) were tested in dilution factor 1-40 on three assays
- Assumption: Gyrolab Mix produces reliable results: data normalized to Gyrolab Mix

- None of the methods produce as high results as Gyrolab Mix
- Dilution helps: both on Gyrolab 1000 & AlphaLISA
- Dilution has no effect on Gyrolab mix method
→ 😊 assumption is reassured
- Possibly the original method (AlphaLISA) underestimates the detected insulin levels under specific circumstances (interfering ADA)

Summary:

- No difference between Gyrolab and AlphaLISA regarding interference removal
- Gyrolab Mixing CD: suitable for PK assay
- MRD should be optimized for interference removal
- Gyrolab Mix method (10x in serum) removed interference up to 3:1 ADA to drug ratio (from >95% to 16%)
- Clinical samples show different results on Mix and alternative methods → they are susceptible to ADA interference without pre-treatment
- Future plan: alternative methods (LC-MS), optimize current conditions (MRD, acid/base pair), investigate different sample pre-treatments (heat treatment, precipitation, salt treatment)



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