Sample pre-treatment, drug tolerance and sensitivity in an antibody analysis assay

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Guideline Requirements for antibody assays

EMA 2017: Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins:

- Screening assays should be **sensitive**. A low false positive rate is desirable but **false negative results are unacceptable**

FDA 2016 Draft: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products - Guidance for Industry

- Assay sensitivity: FDA recommends that screening ADA assays achieve a **sensitivity of at least 100 nanograms per milliliter (ng/mL)**

USP 1106 2017: Immunogenicity Assays – Design and Validation of Immunoassays to Detect Anti-Drug Antibodies

- When products have long terminal half-lives scientists should develop ADA assays that are **capable of detecting ADA in the presence of product** levels that are expected to be **present in patient test samples**.

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ADA screening RIA method (original assay) -

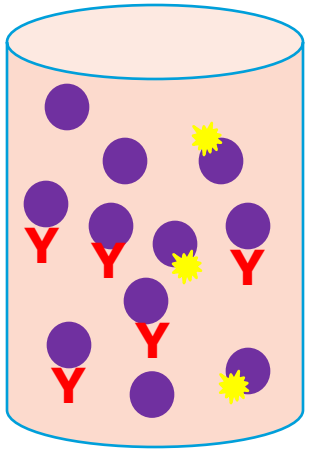
Parameter	Result
Sensitivity	< 100 ng/ml ADA
Sensitivity in presence of drug	5 nM dru 50

Insufficient Drug tolerance

ate

Consequences of insufficient drug tolerance

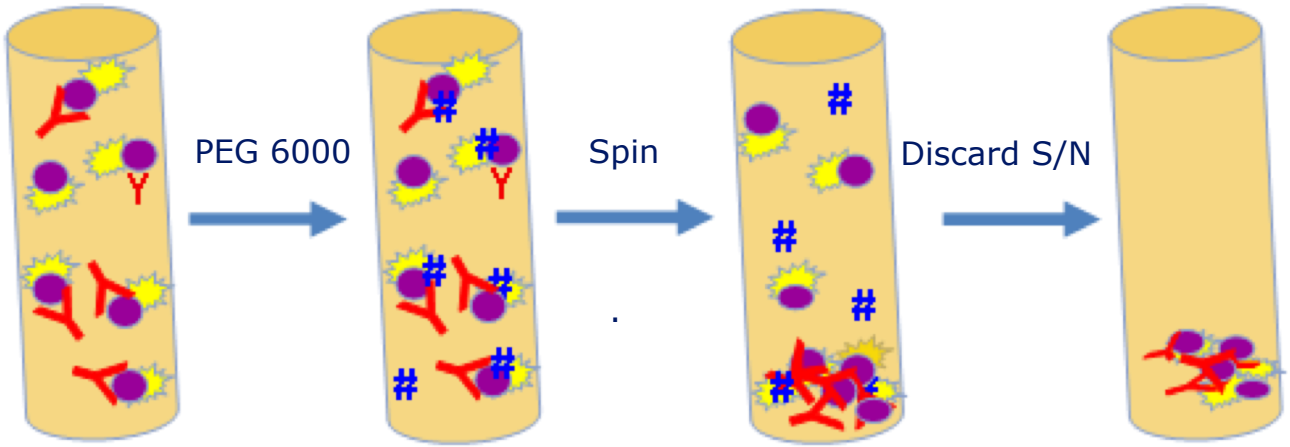
- Too much drug in sample
  - Risk of false negative samples during treatment
- Pre-treatment
  - Pre-treatment with PEG6000
  - Pre-treatment with PEG6000 + Glycine-HCl, pH 3



Y = ADA,
● = Drug
● = ^{125}I labelled drug

ADA radioimmuno assay - principle

Y = ADA
# = PEG-6000/γ-globuline
= ^{125}I-labelled drug



Sample containing antibodies binding to labelled drug

Precipitation of drug-antibody complexes

Supernatant: unbound drug - discarded

Precipitate: All antibodies bound to radioactive drug

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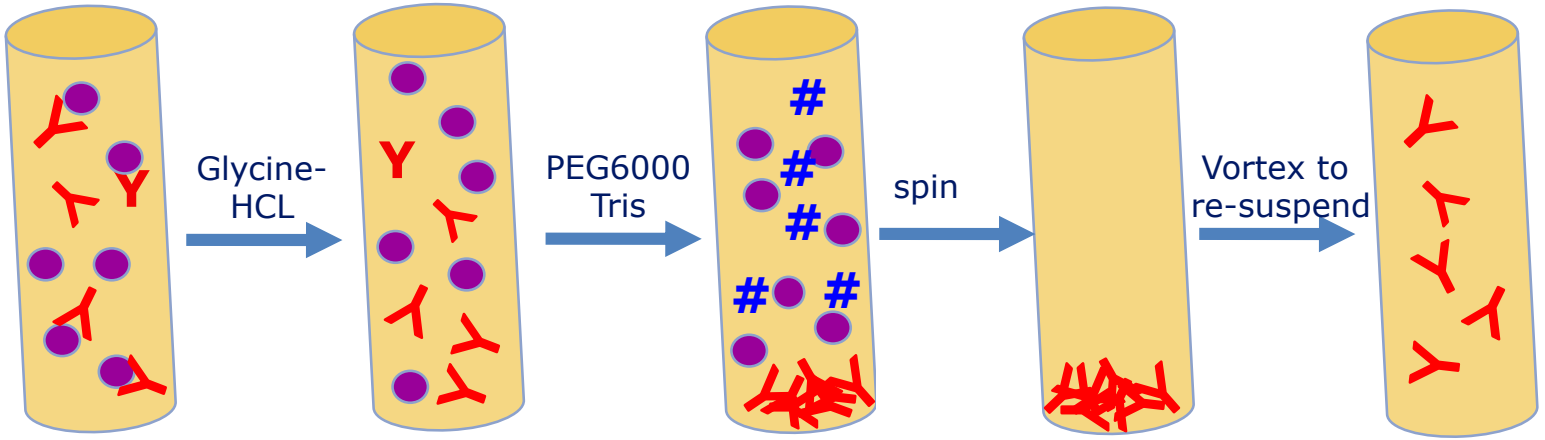
Pre-treatment protocol with glycine-HCl and PEG6000

This work is based on the mAb which gave the best sensitivity in the original antibody RIA

Y = ADA
# = PEG-6000/γ-globuline
● = Drug



Sample with ADA and excess drug

Dissociation of ADA-Drug

Precipitation of ADA

ADA without drug in precipitate

Sample with ADA in buffer

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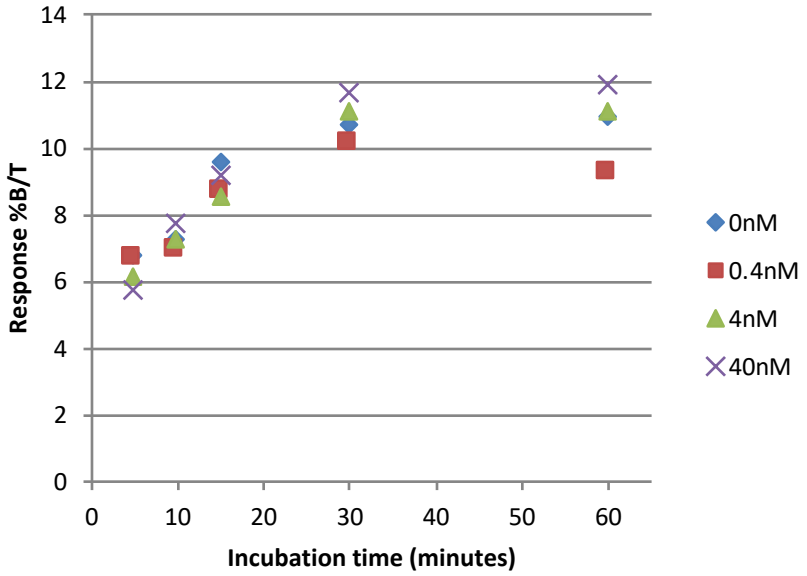
Incubation with Glycine-HCl: time and non specific binding correlates positively

- Glycine-HCl, pH3
  - Mix with sample and incubate 5, 10, 15, 30, 60 mins
- Non specific Background:
  - Samples W/O ab
- Drug interference samples:
  - 40 nM, 4 nM, 0.4 nM, 0 nM

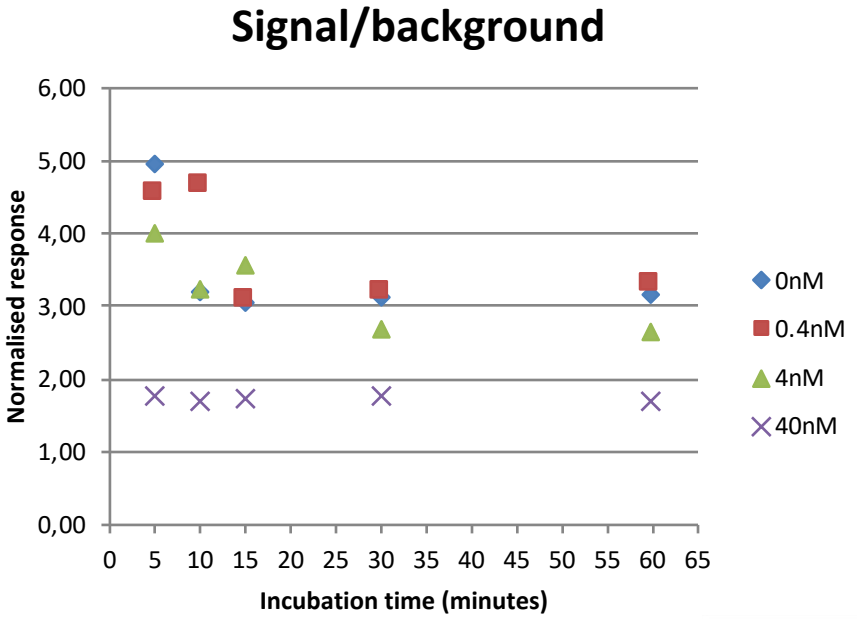
- Increased background with increased incubation time irrespective of drug levels



Increased incubation leads to lower signal/ background ratio

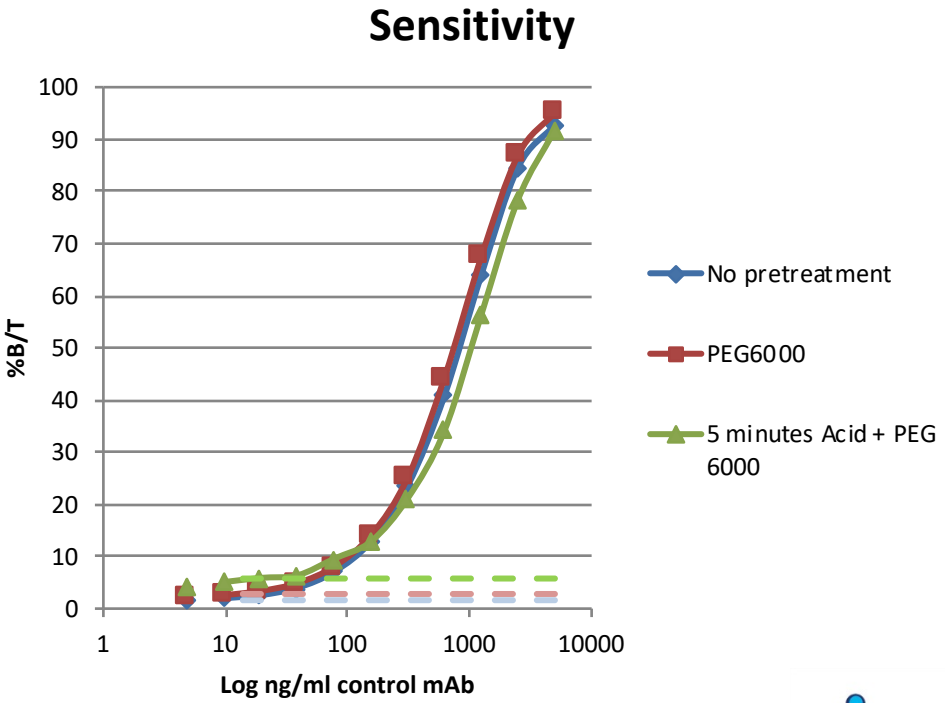
- In the presence of antibody the response is not increased to the same degree as the background
- Sensitivity will decrease due to higher background

- 5 minutes pre-treatment is preferable



Pre-treatment does not alter sensitivity significantly

- Sensitivity samples
  - 2 fold titration of ab starting at 5 µg/ml
  - Cut point based on 20 individual sera
  - Sensitivity 5-20ng/ml



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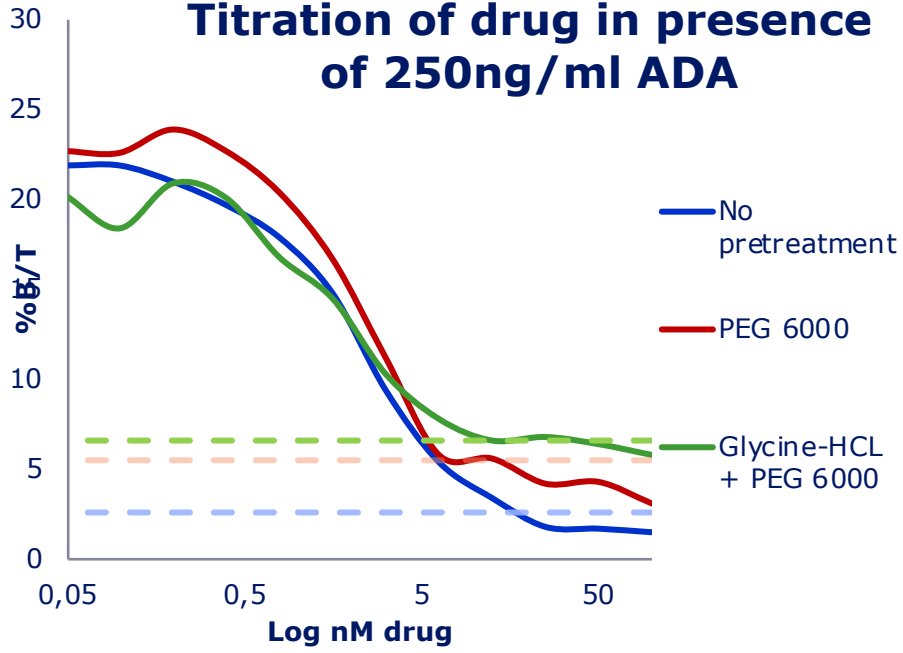
Pre-treatment with Glycine-HCl and PEG6000 increases drug tolerance

250ng/ml Ab	%B/T		
Drug nM	No pre-treatment	PEG6000 alone	Acid+ PEG6000
100 nM	1.5	3.1	5.8
50 nM	1.7	4.3	6.4
25 nM	1.8	4.2	6.8
12.5 nM	3.4	5.6	6.6
6.3 nM	5.4	5.8	7.8
3.1 nM	9.4	11.2	10.3
1.6 nM	14.8	16.7	14.5
0.78 nM	17.9	20.4	16.8
0.39 nM	19.7	22.7	20.1
0.20 nM	21	23.9	20.9
0.10 nM	21.9	22.6	18.4
0.05 nM	21.9	22.7	20.2
0 nM	22.5	23	18.7



Cut-point: Mean QC neg + NF

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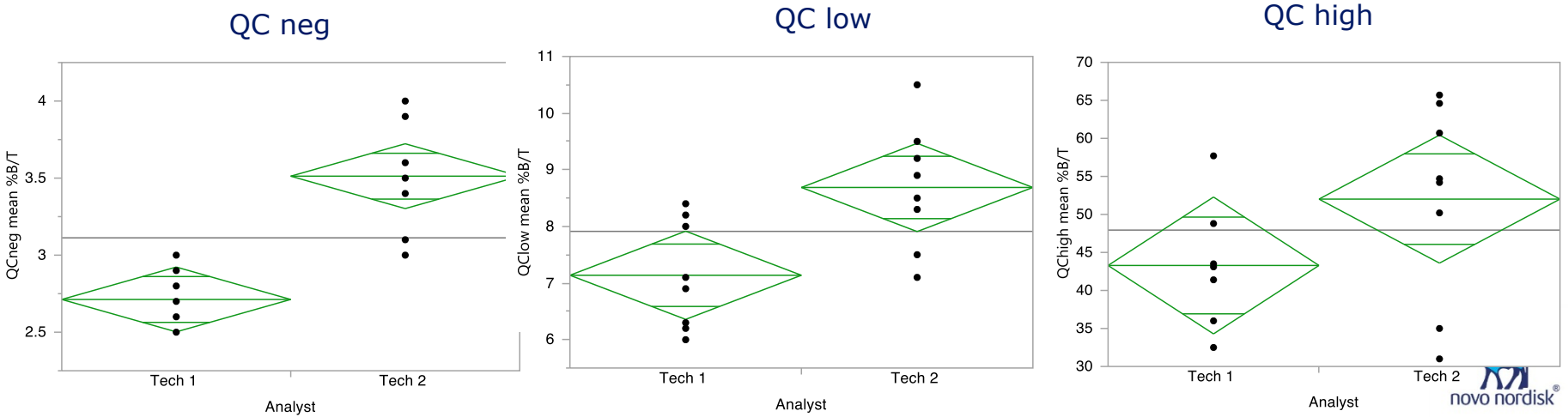
Vortexing important for sensitivity

- Short 'vortexing' at low speed leads to deterioration in sensitivity < 10 sec
- Longer 'vortexing' at max speed leads to improved sensitivity > 10-20 sec.
- Sensitivity Tech 1: 65ng/ml, Tech 2: 30 ng/ml



Improved drug tolerance using pre-treatment with Glycine-HCl and PEG 6000

	Original assay	Assay with pre-treatment
Sensitivity in absence of drug	< 100 ng/ml	< 100 ng/ml
Sensitivity in presence of drug	5 nM: 120 ng/ml 50 nM: 2100 ng/ml	5 nM: 170 ng/ml 25 nM: 250 ng/ml 100 nM: 500 ng/ml

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What has been achieved

- Application of pre-treatment step to remove excess drug
- Identification of important steps for sensitivity and drug tolerance
- Sensitivity in absence of drug retained
- Improved sensitivity in presence of steady state drug levels
- Ensures valid Ab data during treatment

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