Pre-existing antibodies and the multi-tiered assay approach

Experience with the FDA

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Outline

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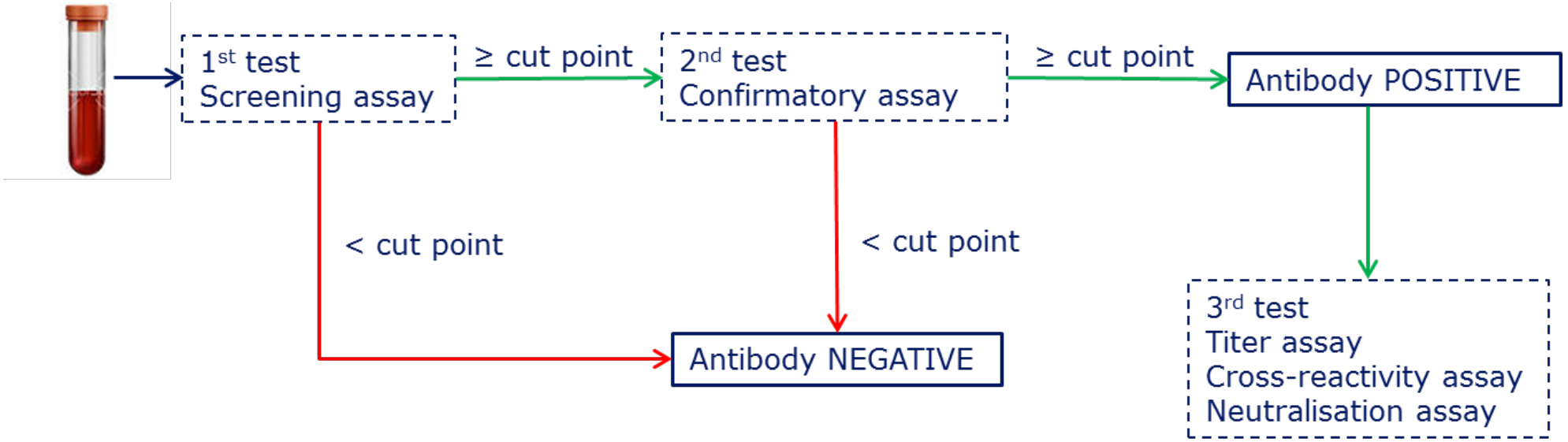
Regulatory expectations - multi-tiered assay



Regulatory expectations – evaluate cut points

- The sponsor should evaluate the appropriateness of the cut points selected in the study using the baseline samples from the study
  - Recommended that means and variances of the individual sera from the validation and at baseline from the clinical study are compared using statistics
  - If there is a significant difference in the variance, then a study-specific cut point is needed

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Focus of my talk

- How to determine an appropriate screening cut point when there are a lot of pre-existing antibodies?

Outline

1 Regulatory expectations

2 **Pre-existing insulin antibodies in diabetic patients**

3 Strategies for handling pre-existing antibodies

4 Proposal – "false positives in screening and removal by confirmation"

5 Summary

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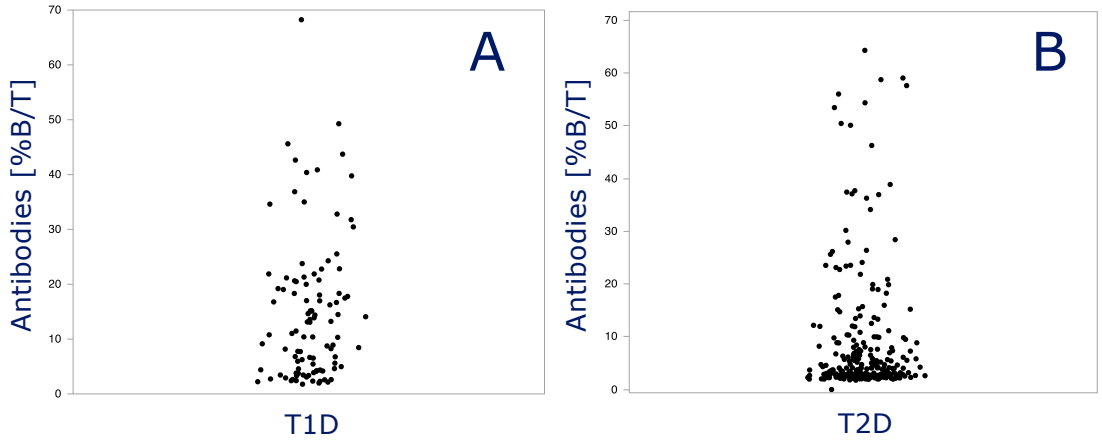
Pre-existing insulin antibodies in diabetic patients



Trial	Patients	Pre-dose samples (baseline) with insulin antibodies
A	T1D	**YES** > 70%
B	T2D	**YES** > 50%

T1D = Type 1 diabetes
T2D = Type 2 diabetes

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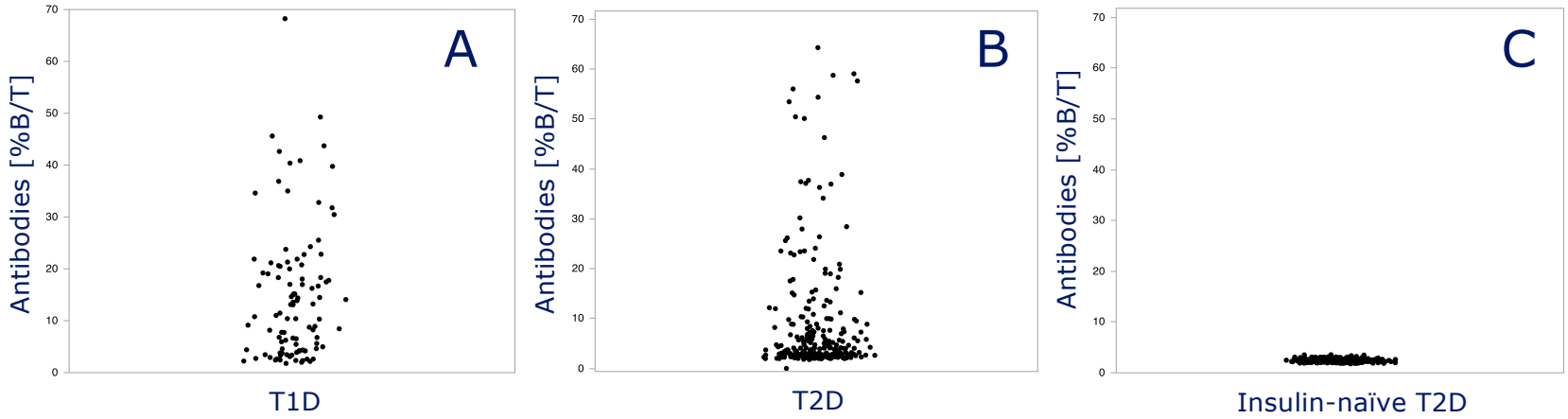
Pre-existing insulin antibodies in diabetic patients



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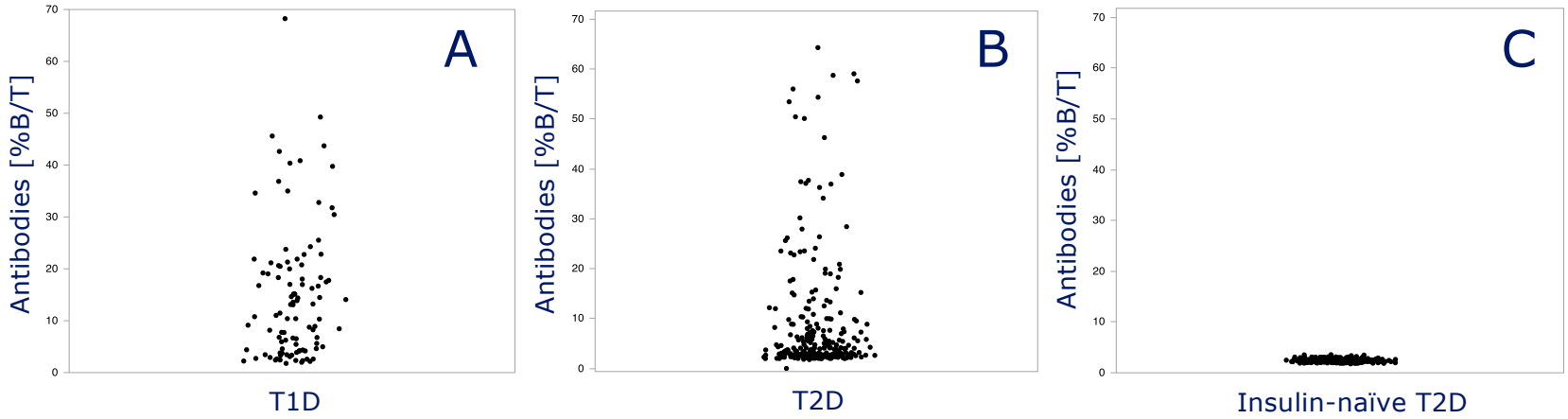
C	Insulin-naïve T2D	**NO** - expected to be antibody negative because patients have not received exogenous insulin

Pre-existing insulin antibodies in diabetic patients



Trial	Patients	Pre-dose samples (baseline) with insulin antibodies
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C	Insulin-naïve T2D	**NO** - expected to be antibody negative because patients have not received exogenous insulin
D	*Insulin-naïve T1D*	*Does not exist because all diagnosed T1D patients require insulin treatment and pre-existing insulin autoantibodies is part of the disease symptoms*

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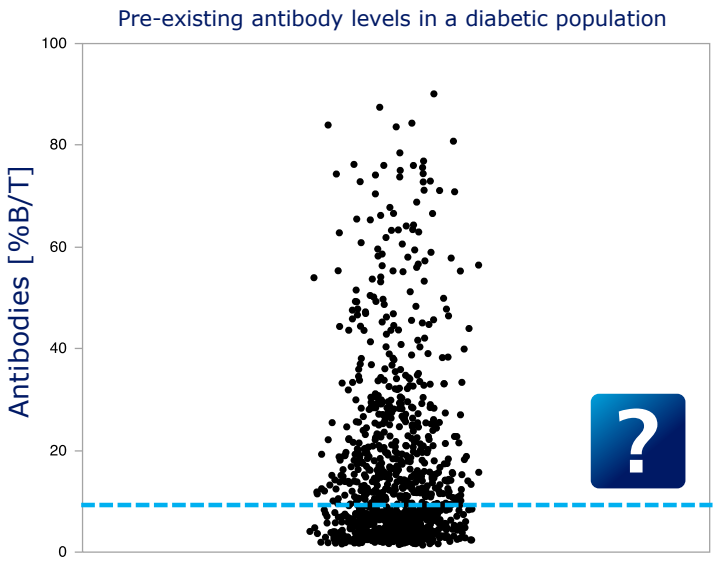
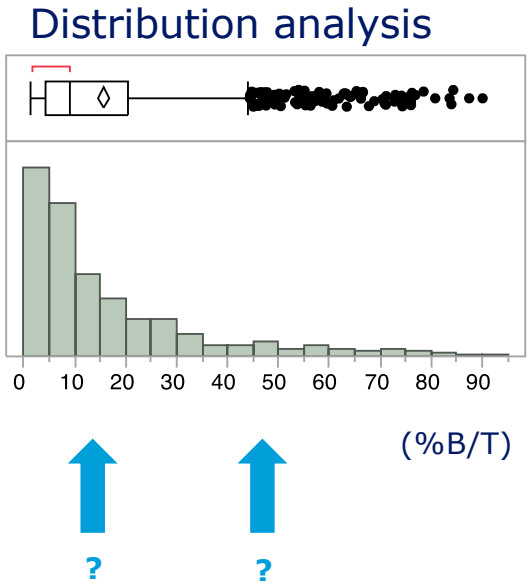
How to determine cut points based on baseline samples?

- The high frequency of pre-existing insulin antibodies in diabetic patients makes it difficult to set the cut point





How to define which samples are truly negative for antibodies?

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Strategies for handling pre-existing antibodies

- Creation of a pseudo antibody negative population
  - Spike drug into samples to abolish the antibody signal
  - Use these values for cut point determination
- Removal of antibody positive samples using Gaussian mixture modelling
  - Use 2 population modelling to identify which baseline samples are of lowest antibody reactivity
  - Use these samples for cut point determination
- Removal of antibody positive samples until a normally distributed antibody negative subpopulation is identified
  - Use remaining antibody negative samples for cut point determination
- Removal of antibody positive samples by identifying them through
  - A characterization assay, e.g. immunodepletion or
  - A confirmatory assay
  - Use remaining antibody negative samples for cut point determination

Key references:
Xue et al. 2017 in publication
Kumar et al. 2016 The AAPS Journal DOI: 10.1208/s12248-016-0011-2

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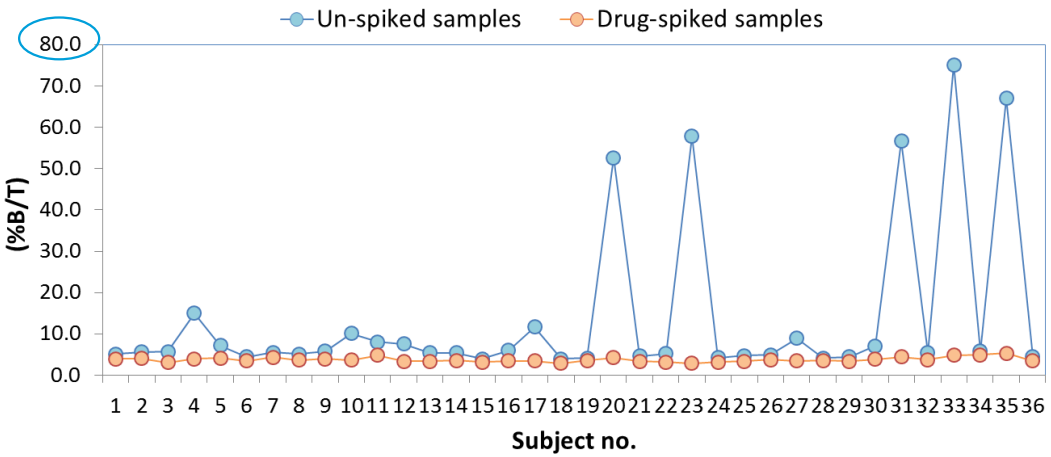
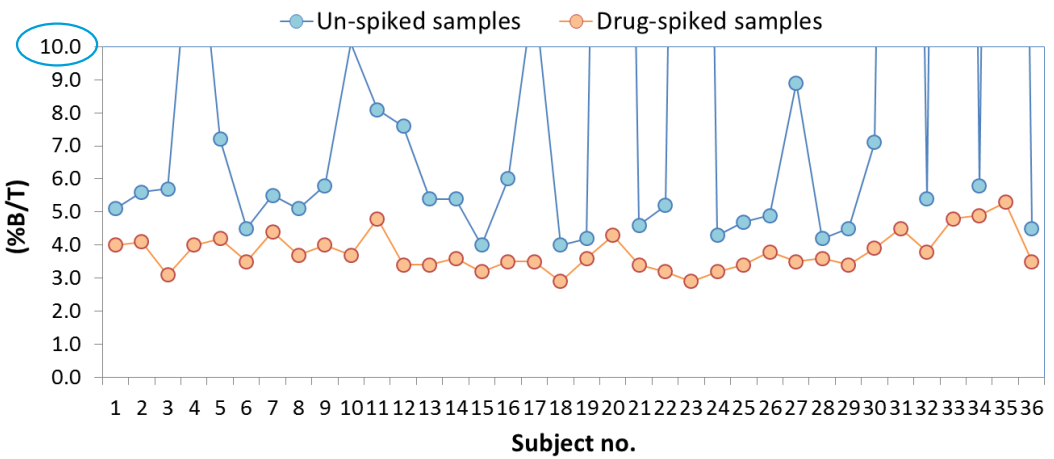
Creation of a pseudo antibody negative population

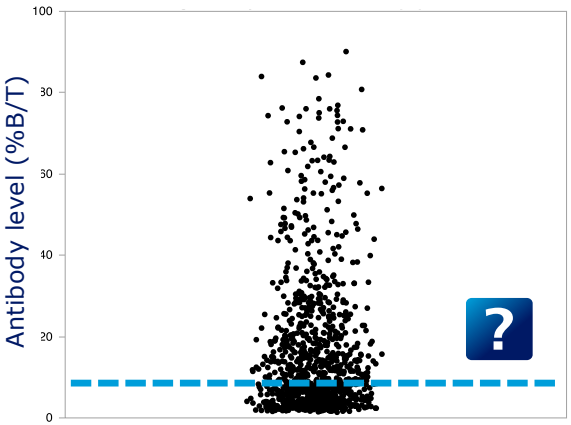
- Challenge: drug-spiked samples give a lower assay signal than un-spiked samples
- Consequence: screening cut point will be set too low



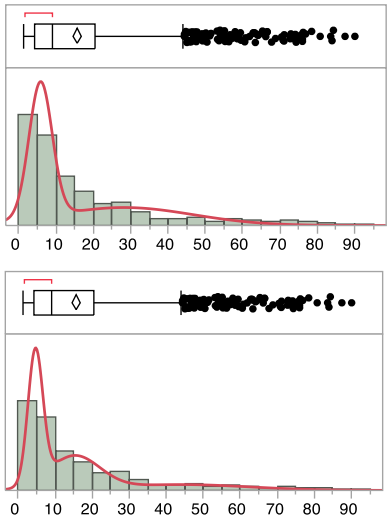


Removal of antibody positive samples using Gaussian modelling

- Challenge: difficult to apply when results are not clearly divided into 2 populations
- Consequence: screening cut point may be set too high



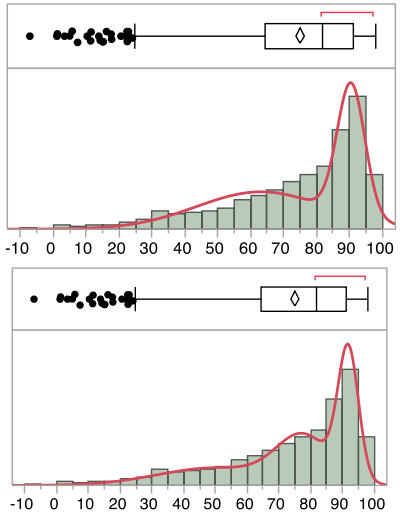
Screening results



Fitted:
2 normal mixtures

3 normal mixtures

Confirmatory results



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Available cut point parameters

Assay validation (pre-study)

- Cut points determined using healthy sera
  - Screening assay - normalisation factor (NF) used for floating cut point
  - Confirmatory assay – fixed cut point

Options when analysing clinical samples (in-study)

- Use validation parameters
  - Screening assay - validation NF
  - Have to show appropriateness – how? false positive rate

- Use baseline samples for cut point determination
  - How?

confirmatory assay

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Evaluation of cut point – "false positives in screening"

Analyse baseline samples

- Analyse around 500 samples 1X in: screening confirmatory
- 70% of pre-existing antibodies -> 30% negative = 150 samples

Determine screening cut point[1]

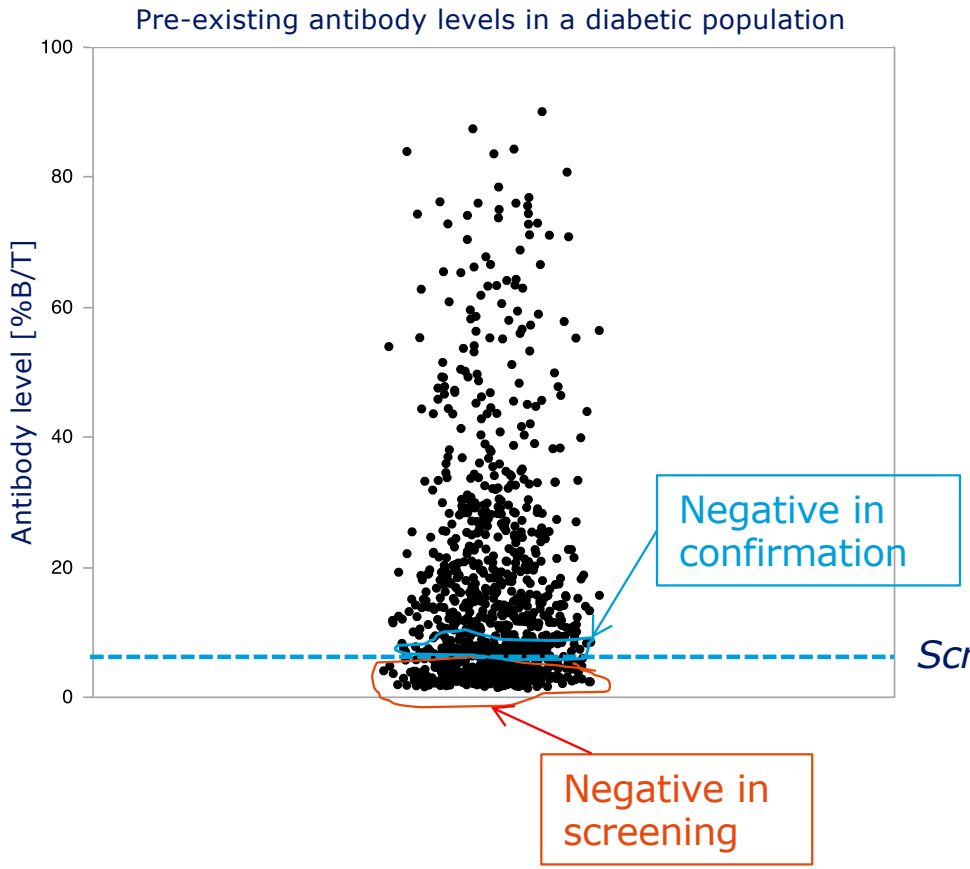
- Use validation NF
- Use negative control from validation

Evaluate screening cut point

- Calculate false positive rate
- Target range around 2-11%[2]

[1]*Shankar et al. 2008.* J Pharm Biomed Anal 48(5): 1267-1281.
[2]*Amaravadi et al. 2015. Bioanalysis* 7(24), 3107–3124

False positive rate and pre-existing antibodies



Validation parameters used:

- Validation NF for floating screening cut point
- Fixed confirmatory cut point

False positive rate defined as:

$$\frac{Negative\ in\ confirmation}{(Negative\ in\ confirmation) + (Negative\ in\ screening)}$$

Evaluation of cut point – "false positives in screening"

Analyse baseline samples

- Analyse around 500 samples in:
  screening
  confirmatory
- 70% of pre-existing antibodies -> 30% negative = 150 samples

Determine screening cut point[1]

- Use validation NF
- Use negative control from validation

Evaluate screening cut point

- Calculate false positive rate
- Target range around 2-11%[2]

- False positive rate is acceptable
  Screening cut point is ok 
- False positive rate is not acceptable 

[1]*Shankar et al. 2008.* J Pharm Biomed Anal 48(5): 1267-1281.
[2]*Amaravadi et al. 2015. Bioanalysis* 7(24), 3107–3124

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Determination of study-specific cut point – "removal by confirmation"

Evaluate the confirmatory results

- Look at confirmatory results for all baseline samples
- Remove all samples that are confirmed positive

Determine study-specific screening cut point

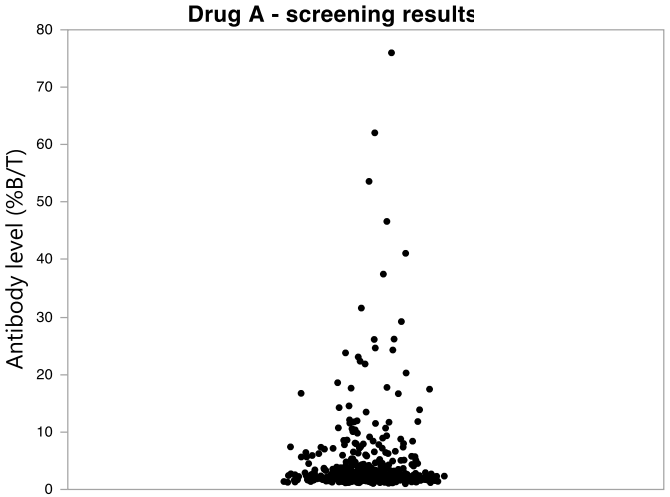
- Identify remaining antibody negative samples
- Dependent on number of negative samples: analyse in 3-6 independent screening assays with 2 analysts
- Determine study-specific NF and screening cut point

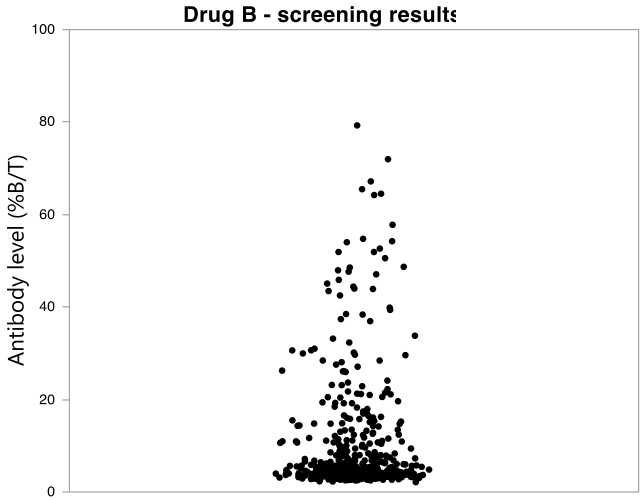
Evaluate screening cut point

- Calculate false positive rate based on screening results for the antibody negative samples
- Target range around 2-11%

Clinical cases – "false positives in screening"

Insulin drug	Baseline samples	Baseline samples < screening cut point	Baseline samples ≥ screening cut point and confirmed negative	False positive rate	% pre-existing antibodies
A	512	340	12	**3.4%** ☑	31%
B	526	253	41	**13.9%** ☑	44%





☑ Validation NF can be used

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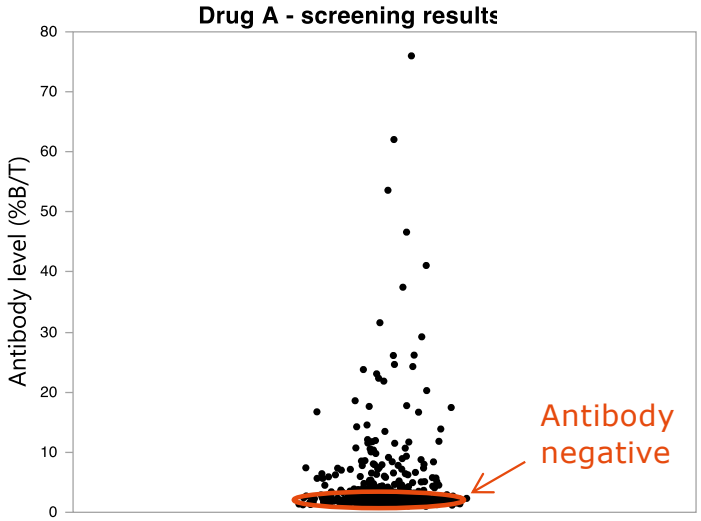
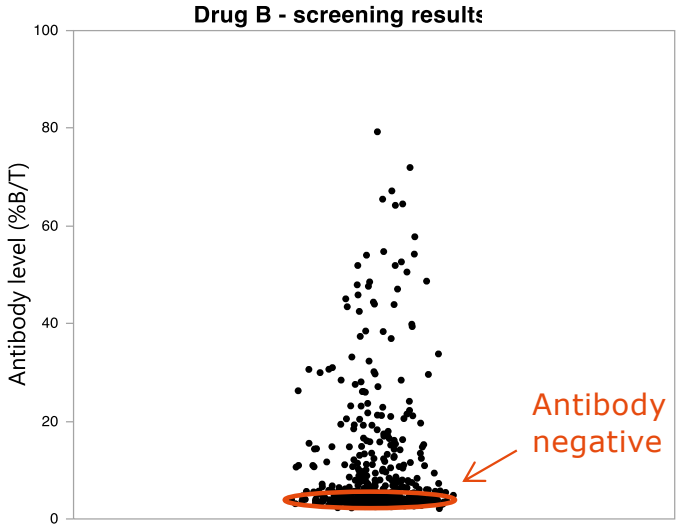
Clinical cases – "removal by confirmation"

Insulin drug	Baseline samples	Baseline samples ≥ confirmatory cut point	Baseline samples < confirmatory cut point
A	512	204	**308**
B	526	269	**257**





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Summary

The proposed strategy meet the overall regulatory expectations

- Multi-tiered assay approach ☑
- Evaluation of cut points ☑
  - Appropriateness of validation NF is evaluated by the false positive rate
  - If study-specific NF is required, antibody negative baseline samples may be identified using the confirmatory results
- Clinical impact?

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Acknowledgement

- Helene Solberg, Team Manager
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- Tony O'Connor, Senior Scientist

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Questions and feedback 

Suggestions and input from the regulators and industry

are highly appreciated!