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Determination of ADA in IFNβ treated MS patients

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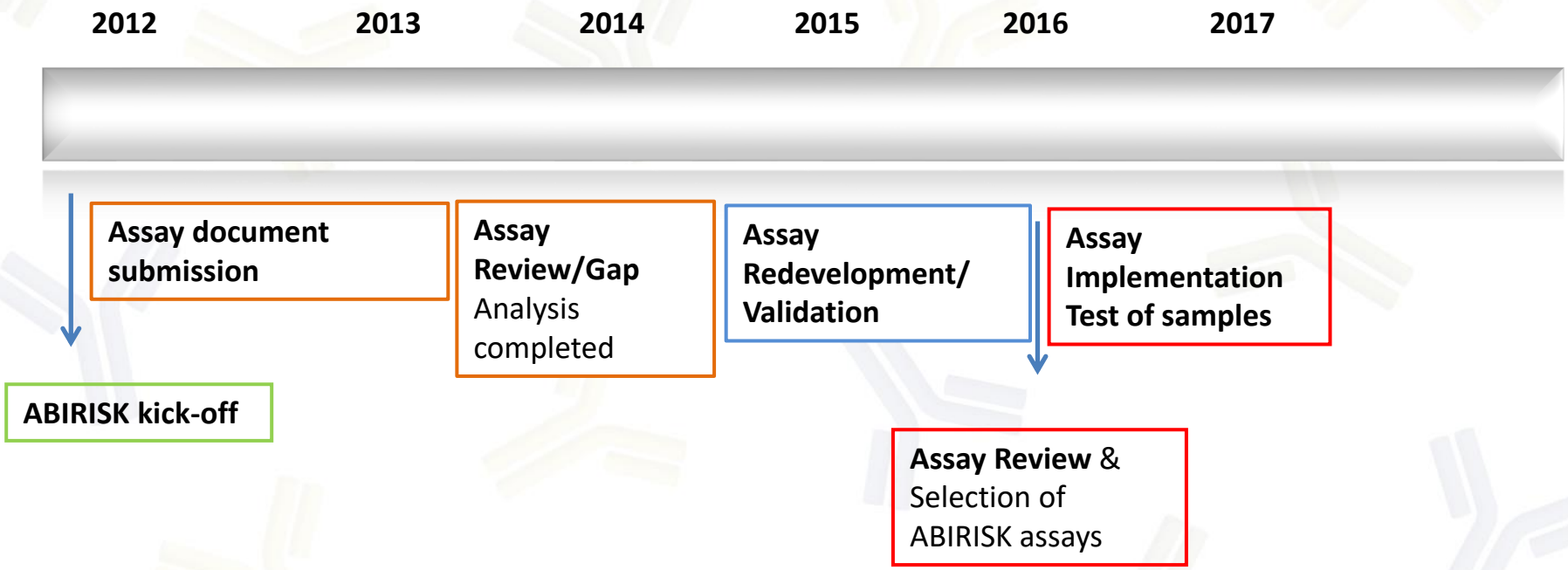
ADA assays for interferon beta in ABIRISK



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Assays used for IFNβ

- ELISA for binding ADA (bADA)
- Bioassays for neutralizing ADA (nADA)
  - Cytopathic effect (CPE)
  - MxA protein assay (MPA)
  - MxA gene expression assay (MGA)
  - Luciferase (LUC)
  - iLite® (Biomonitor)

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In clinical routine before ABIRISK

- No ADA screening analysis (except in Munich, Düsseldorf and Innsbruck)
- No pharmacokinetics (PK) analysis
- Use only NAb analysis with bioassays
  - → Start with screening for positivity (identifies 20-30% positive)
  - → Do titration of positive samples

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Feedback from EFPIA review

- None of the assays were up to current standards
  - Matrix
  - Cut-point design

➢ **decided that a re-development and re-validation of the assays were needed**

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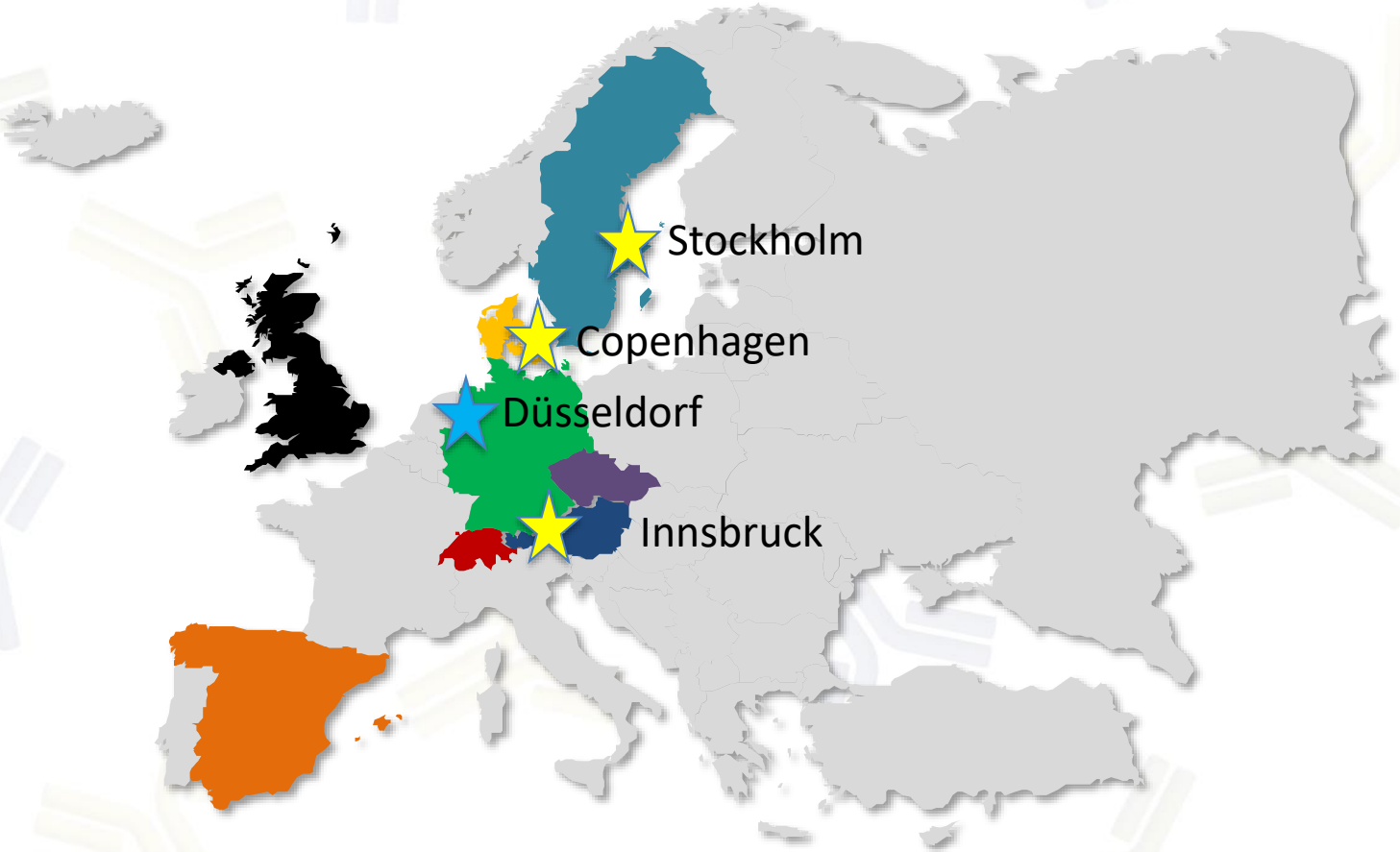
Development and validation of ADA and nADA assays



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- **ADA Assay**
  - New bridging assay was developed, central lab Düsseldorf

- **Neutralizing ADA Assay**
  - Luciferase assay in Copenhagen, Innsbruck and London was compared to iLite in Stockholm

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Results

frontiers in Neurology

METHODS
published: 06 July 2017
doi: 10.3389/fneur.2017.00305

Development and Validation of an Enzyme-Linked Immunosorbent Assay for the Detection of Binding Anti-Drug Antibodies against Interferon Beta

Kathleen Ingenhoven[1], Daniel Kramer[2], Poul Erik Jensen[3], Christina Hermanrud[4], Malin Ryner[4], Florian Deisenhammer[5], Marc Pallardy[6], Til Menge[1], Hans-Peter Hartung[1], Bernd C. Kieseier[1], Elisa Bertotti[7], Paul Creeke[8], Anna Fogdell-Hahn[4] and Clemens Warnke[1,9] On Behalf of the ABIRISK Consortium[†]*

OPEN ACCESS

Edited by:
Richard Weissert

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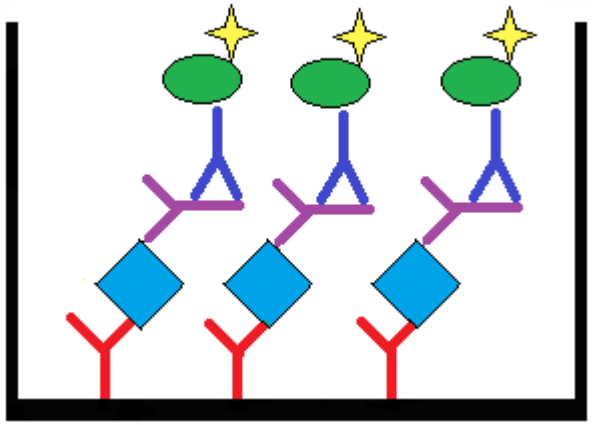
Change of format for the ADA assay

Indirect ELISA



Horseradish peroxidase (HRP)

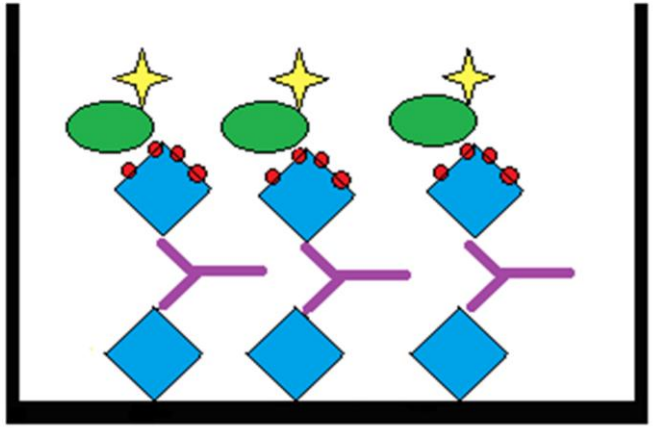
pAb anti-human HRP conjugated

Sample antibodies

IFN-ß-1a (Avonex®)

Monoclonal antibody (mAb) rabbit anti-IFN-ß

Bridging ELISA



Streptavidin labelled HRP

Biotin labelled IFN-ß-1a (Avonex®)

Sample antibodies or controls

IFN-ß-1a (Avonex®)

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Results

Journal of Immunological Methods 430 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Development and validation of cell-based luciferase reporter gene assays for measuring neutralizing anti-drug antibodies against interferon beta

Christina Hermanrud [a], Malin Ryner [a], Thomas Luft [b], Poul Erik Jensen [c], Kathleen Ingenhoven [d], Dorothea Rat [e], Florian Deisenhammer [b], Per Soelberg Sørensen [c], Marc Pallardy [f], Dan Sikkema [g], Elisa Bertotti [h], Daniel Kramer [e], Paul Creeke [i], Anna Fogdell-Hahn [a,*], on behalf of the ABIRISK consortium:

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nADA (NAb assay)

	Luc Innsbruck	Luc Copenhagen	iLite
Cut-point type - Value	Floating	Floating	Floating
Normalisation Factor (NF)	556.81	4532	35014
Specificity assay cut-point	1.32	1.3	1.23
Sensitivity (LOD)	1520 ng/mL	814 ng/mL	320 ng/mL
Inter-Run precision for the LPC (CV%)	49.9	56.6	24.7
Inter-Run precision for the HPC (CV%)	47.7	59.2	19.0
Inter-Run precision for the NC (CV%)	21.0	28.6	34.8
LPC Upper/Lower acceptance criteria	0-967	0-27158	17690-102357
HPC Upper/Lower acceptance criteria	0-304	0-16577	16618-56192
NC Upper/Lower acceptance criteria	853-3501	5898-44833	17013-229200
Recovery (Matrix interference)	LPC =87-108 % HPC=80-116 %	LPC = 85 – 116% HPC = 81 – 124%	LPC = 86 – 109 % HPC= 87 – 113 %
Drug tolerance	100 IU/mL for LPC >1000 IU/mL for HPC	500IU/mL for LPC > 1000IU/mL for HPC	not done

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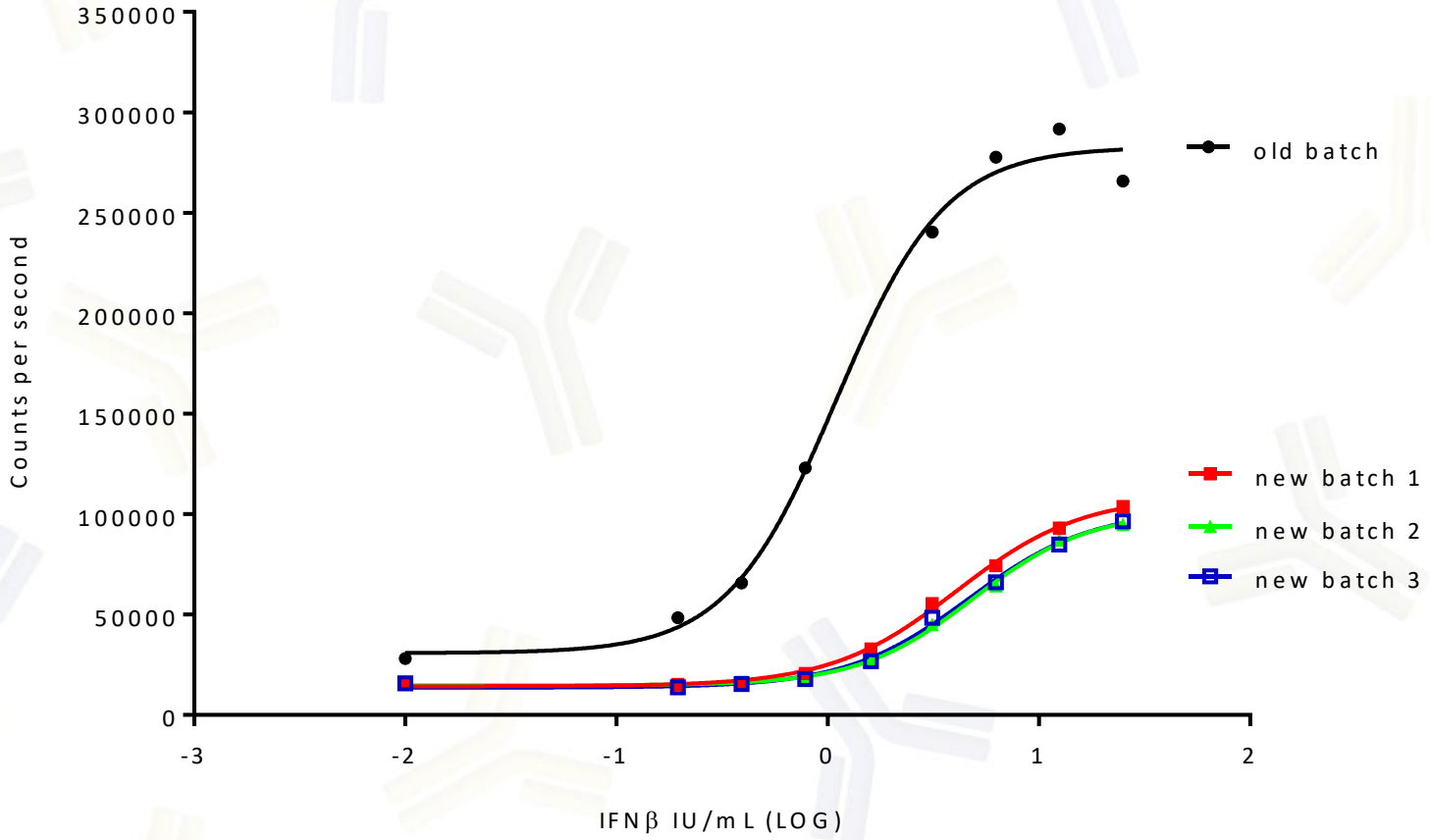
iLite batch variation



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Higher sensitivity in new NAb assay compared to old NAb assay

- **32.7%** positive in Sweden vs **44.6%** in Copenhagen (n=507)
- 86% samples were confirmed negative and positive
- 13% negative in Sweden became positive in Copenhagen
- 1% positive in Sweden became negative in Copenhagen

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Comparing new NAb assay with new ADA assay using the human mAbs positive controls

Name of MAb(s)	NAb titers ng/ml	ADA titers ng/ml
1.06	385	3.18
1.53	330	0.35
1.54	354	0.86
1.62	336	0.42
1.71	860	3.63
Mix 1 (06,53,54,62,71)	300	1.29
Mix 2 (06,53,71)	850	6.24
Mix 3 (06,54,62)	330	3.82
Mix 4 (53,71)	673	7.35

ADA assay is 100x more sensitive than the NAb assay

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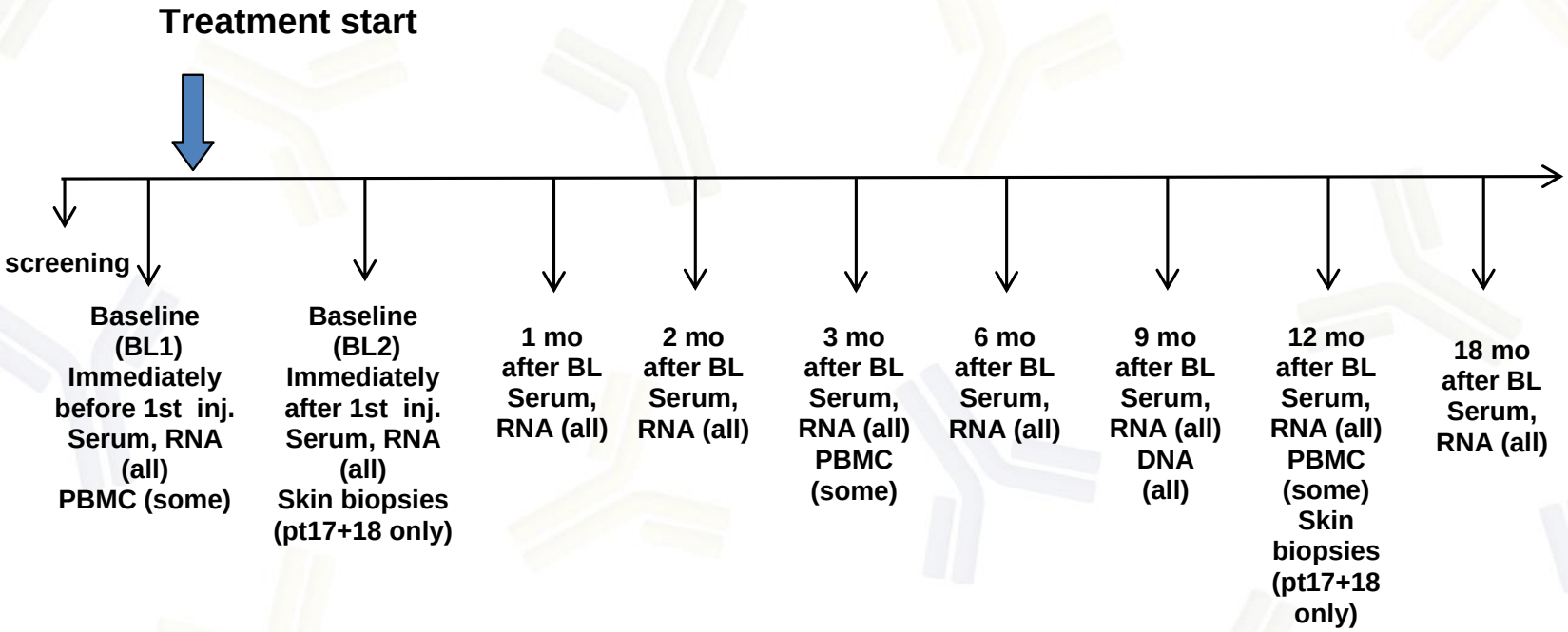
Comparing assays using the ABIRISK prospective study of IFNβ treated MS patients



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nADA (NAb assay)

- Copenhagen was selected as central lab nADA analysis
- All prospective samples were run on both the ELISA and the NAb assay

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NAb assay had a higher sensitivity than the ADA assay when clinical samples were tested

	ELISA	NAb assay
IFNβ-1a (Avonex)	4%	17%
IFNβ-1a (Rebif)	21%	28%
IFNβ-1b (Betaferon/Extavia)	59%	96%

- Nabs were detected earlier (month 2) than ADA (month 3)

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Why and how?

- Matrix effect?
- Dynamic range of ADA assay?

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Recalculating positive patients with a titre over the threshold 320 U/mL

- The number of persistently NAb positive patients (n=26, 27%) = confirmed ADA positive
- number of transiently positive patients was reduced to none

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Conclusions

- Test assays in parallel
- For clinic use most sensitive and then set clinical threshold, then use easiest and cheapest assay
- For research you want to know about all ADA, maybe especially the low affinity early ADA

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