PK SCIENCES
Clinical Bioanalytics and Regulatory Science

Acceptance range of titer positive control in clinical ADA assays: practical examples

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11h EBF Open Symposium "Raise the Anchor – Set Sail for Science"
Session "Immunogenicity 2"
Friday, November 23rd, 2018

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Agenda

1. Establishment of the titer range
   - MSD Example
   - FACS Example -> in backup section
2. Adaptation of the titer range -> Critical reagents
   - ELISA Example
   - FACS Example
3. Discussion:
   - Pivotal for gene therapy (and all molecules with pre-existing antibodies) projects
   - Can titer assays be avoided?

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Topic relevance

Many «classical» biologics (*e.g.* humanized/fully human monoclonal Abs, immune suppressants) are lowly immunogenic / have a low immunogenicity

=>ADA assessments do not require an extensive titration step

New entities (*e.g* nanobodies, replacement proteins, gene therapies) have a highest humoral immunogenicity potential - sometimes including the presence of pre-existing antibodies

=> «revival» in the need / interest for titer assays

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Establishment of PC titer ranges

Titer was defined as the reciprocal dilution calculated with the intercept method

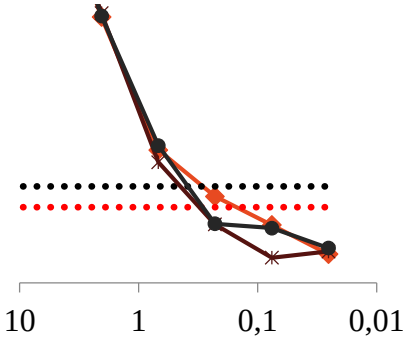
- intercept of the linear regression of the two titration points which produce assay signals directly above and below the TCP, with the TCP
- TCP setup

Assessment of the precision of titer values:

- Minimum Significant Ratio MSR = $10^{2*\sqrt{2}*SD}$
- Inter-run coefficient of variation

Titer Positive Control acceptance ranges were calculated:

- From titer data from validation
- with different methods to investigate the impact on titer range calculation



n: mean of the titer values

M: Median of titer values

$n * \sqrt{MSR}$ to $n/\sqrt{MSR}$

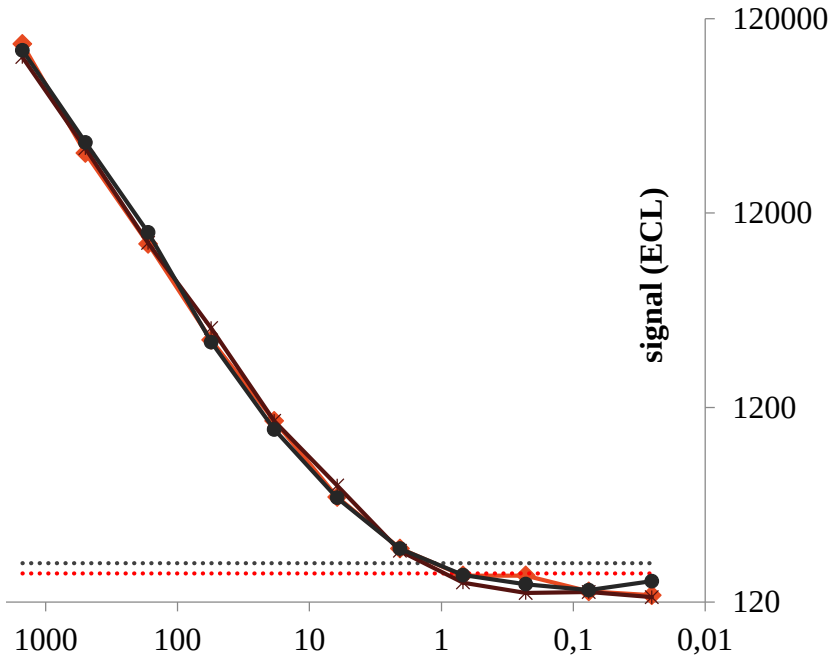
$n * MSR$ to n/MSR

$n \pm 3.09SD$

$M * 2$ to $M/2$

$M \pm 1$ dilution step

Example 1: MSD ADA assay
Influence of the titer cut point



Run	TCP = SCP	TCP = mean NC + 3.09pSD*
33	2100	1576
	1840	1363
	2130	1589
34	6080	1950
	1930	1433
	2070	1539
35	n.d.	1501
	1690	1189
	2950	1811
38	3960	1901
	1840	1350
	1930	1567
Median Titer (M)	2070	1553
Mean Titer (n)	2593	1564
Precision (%)	51	15
MSR ($10^{2*\sqrt{2}xSD}$)	3.07	1.51

** Wakshull et al., (2011) Proposal for a new protein therapeutic immunogenicity titer assay cutpoint*

Titer curves close-up at the intercept

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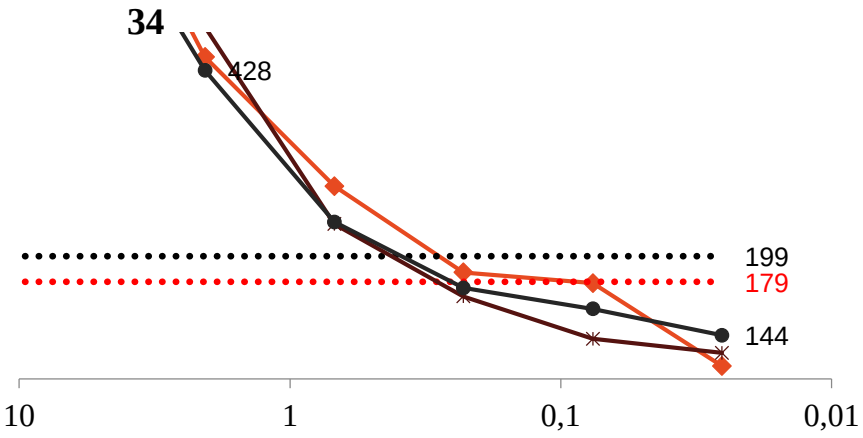

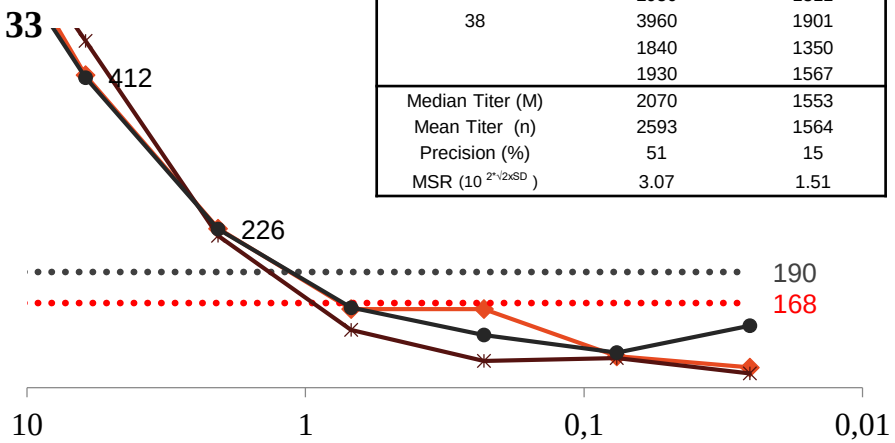

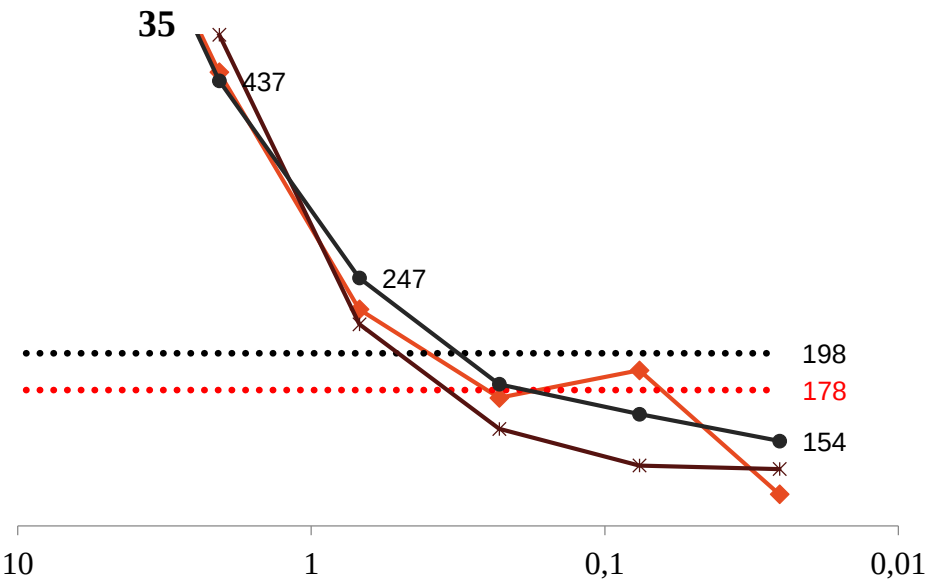

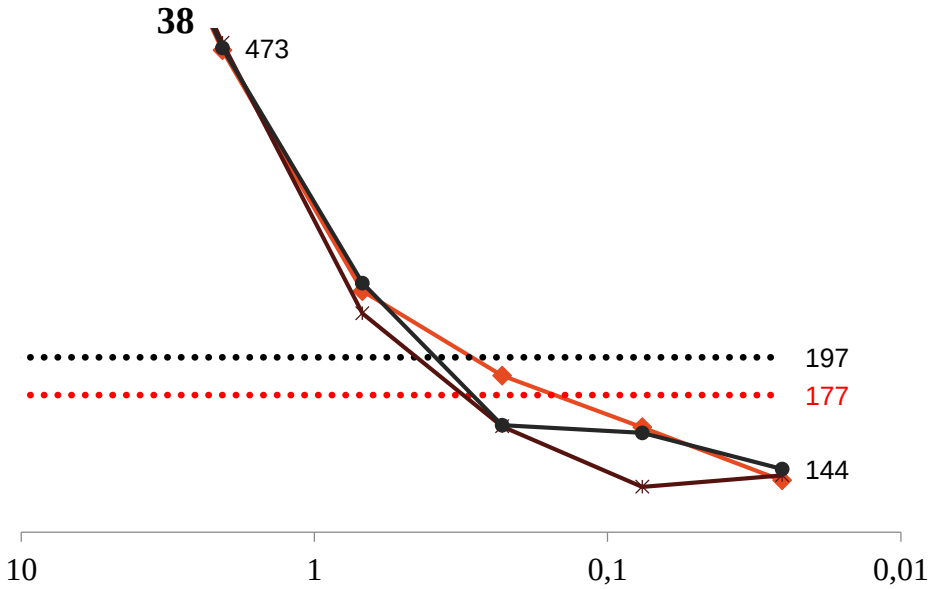

Minimal difference in CP values, maximal impact on titer precision

Run	TCP = SCP	TCP = mean NC + 3.09pSD*
33	2100	1576
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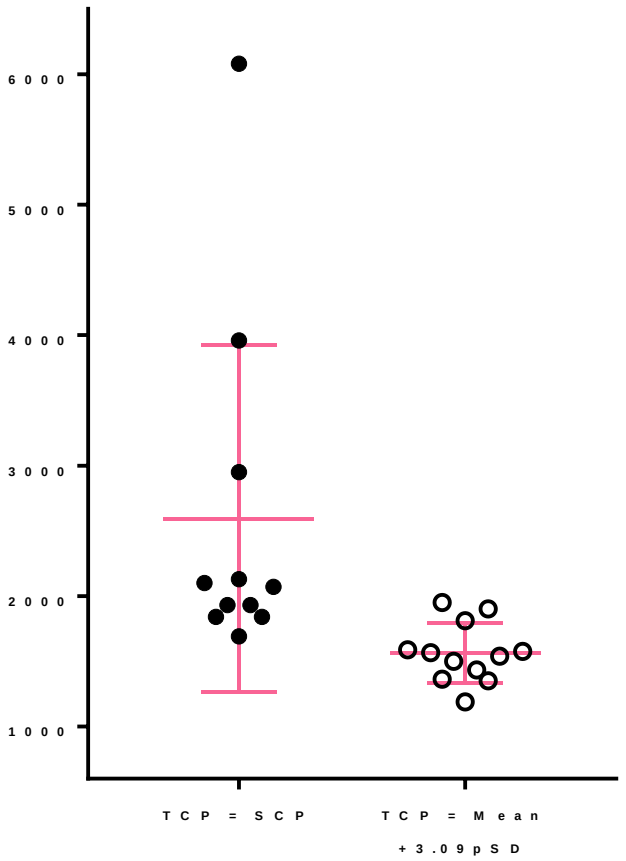

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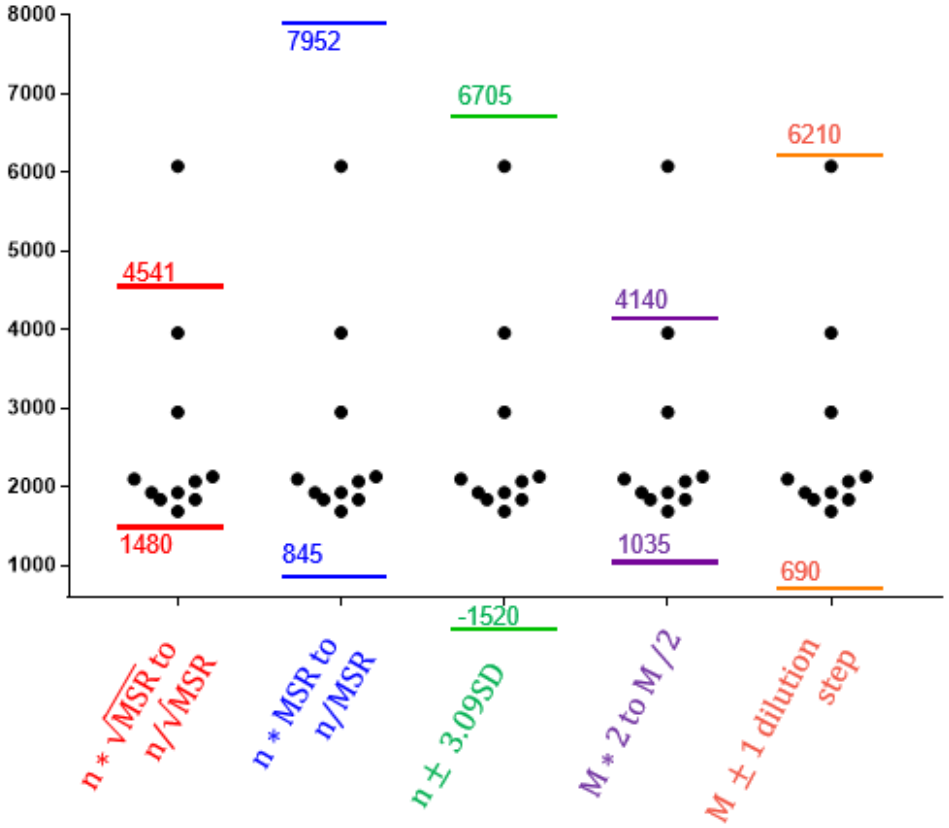
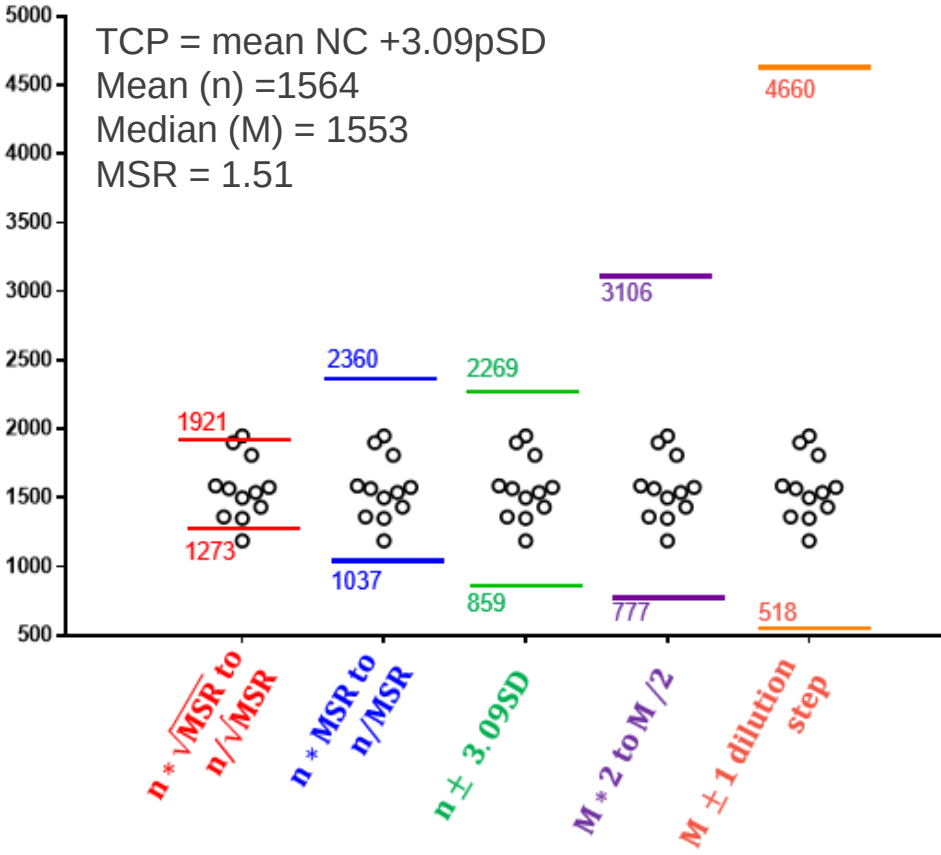
Graphical representation of the various acceptance ranges



TCP = SCP
mean (n) = 2593
Median (M) = 2070
MSR = 3.07



Just a question

Acceptance criteria for titer assays by using the range of titrated positive control must be carefully designed during assay validation, e.g. assessed on different days, at least 12 titer curves, 2 analysts

-> But is this even enough?

Assay validation also occurs during sample analysis -> inclusion of the results of the SSCs obtained during the first clinical studies to refine the threshold?

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Conclusion

- The titer assay needs to be well controlled to ensure that titer data are comparable between samples within a study but also between studies of a certain biotherapeutic project
- The decision about TPC range method should be based on the needed precision during study sample measurement and depends on:
  - ADA incidence,
  - correlation of titer results with clinical outcome & safety,
  - frequency of pre-existing ADAs...

-> Information ultimately ends up in the drug label

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Part II

Adaptation of the titer range -> Critical reagents

- ELISA Example
- FACS Example

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Adaption of titer range: ELISA

- Assay transfer: use of new reagent lot of ADP (Anti-Dig-POD Fab fragment)
- Validated TPC range : 26.5 – 59.6

1.

ADP dilution used during validation

	1:10000	
	OD	DF
TPC01	0.277	1.00
TPC02	0.202	1.50
TPC03	0.146	2.25
TPC04	0.110	3.38
TPC05	0.088	5.06
TPC06	0.073	7.59
TPC07	0.063	11.39
TPC08	0.056	17.09
TPC09	0.056	25.63
TPC10	0.049	38.44
TPC11	0.054	57.67
TPC12	0.046	86.50
TPC13	0.047	129.75

2.

After change of ADP lot TPC range was no more met

y	x	log x
0.054	57.665	1.7609
0.046	86.498	1.937
	slope	-0.0491
	intercept	0.1408
CP	0.048	
Titer	78.321	

3.

ADP was newly diluted

		1:5000			1:7500	
TPC:		OD	DF		OD	DF
	TPC01	0.238	1.00	TPC01	0.232	1.00
	TPC02	0.203	1.50	TPC02	0.204	1.50
	TPC03	0.155	2.25	TPC03	0.154	2.25
	TPC04	0.118	3.38	TPC04	0.115	3.38
	TPC05	0.091	5.06	TPC05	0.092	5.06
	TPC06	0.077	7.59	TPC06	0.077	7.59
	TPC07	0.067	11.39	TPC07	0.065	11.39
	TPC08	0.062	17.09	TPC08	0.062	17.09
	TPC09	0.056	25.63	TPC09	0.054	25.63
	TPC10	0.053	38.44	TPC10	0.050	38.44
	TPC11	0.050	57.67	TPC11	0.048	57.67
	TPC12	0.043	86.50	TPC12	0.047	86.50
	TPC13	0.0488	129.75	TPC13	0.046	129.75

4.

TPC range was met again & new ADP dilution was used in the assay

	y	x	log x	y	x	log x
	0.053	38.443	1.5848	0.050	38.443	1.5848
	0.050	57.665	1.7609	0.048	57.665	1.7609
		slope	-0.0142		slope	-0.0085
		intercept	0.0754		intercept	0.0633
	CP	0.051		CP	0.048	
	Titer	43.353		Titer	55.776	

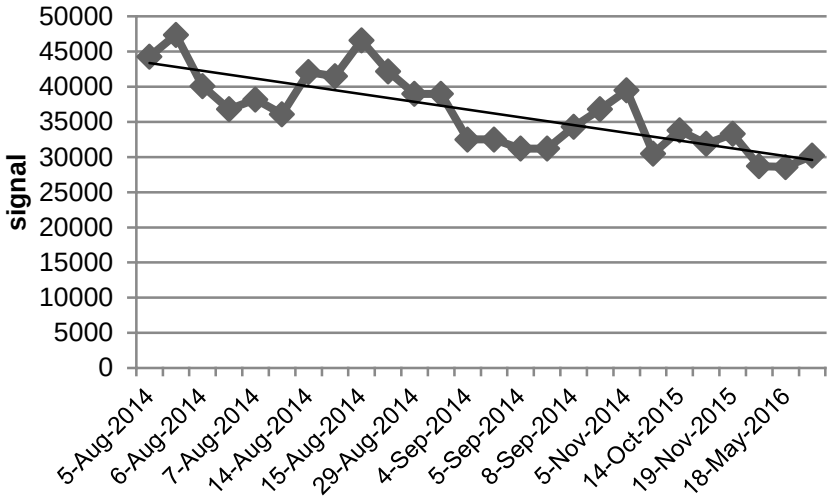
⇒ assay signals could be maintained by changing the concentration of the detection antibody

⇒ No need to adjust TPC acceptance range

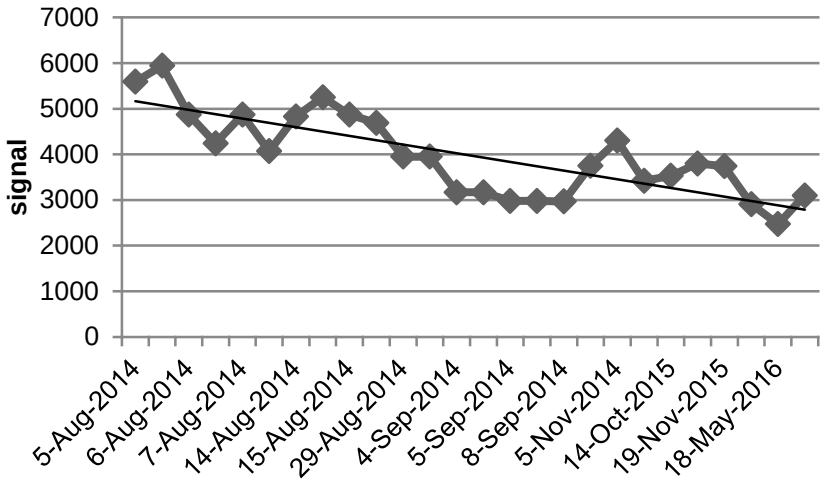
Adaption of titer ranges: FACS assay

- Positive control antibody (stored in refrigerator)
- Trending demonstrated tthat degradation occured within two years
  => Lower signals -> Lower titers of TPC

High PC signal – response over time



Low PC signal – response over time



- Validated TPC range: 640 – 1280, Median titer: 1180
- If using validated TPC range, 16 out of 25 titer plates would have failed because TPC was below 640.
- NC signal trending (not showed here) demonstrating that signal was unchanged, hence demonstrating the stability of the assay itself.

=> In this case TPC range might be adjusted, because the assay itself was stable.

- Further storage of positve control antibody in Freezer

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Discussion 1
Relevance for gene therapy projects

Titration assays are becoming of increased importance in the evaluation of the ADA response for instance for compounds for which pre-existing antibodies against the therapeutic agent can be detected in up to 100% of the pre-dose samples (*e.g*. gene therapy compounds).

This makes the screening and confirmatory steps (almost) optional: the humoral immunogenicity assessment relies on the variation of the titers within an individual rather than on the positive or negative status of the individual samples.

Therefore, sample analysis titer controls are of paramount importance to:

- ensure the relevance of the titer variations observed between two time points collected from the same individual.
- determine if a titer variation is relevant or not (i.e boosted by the compound administration or within the normal assay variability range)

-> Information on the drug label

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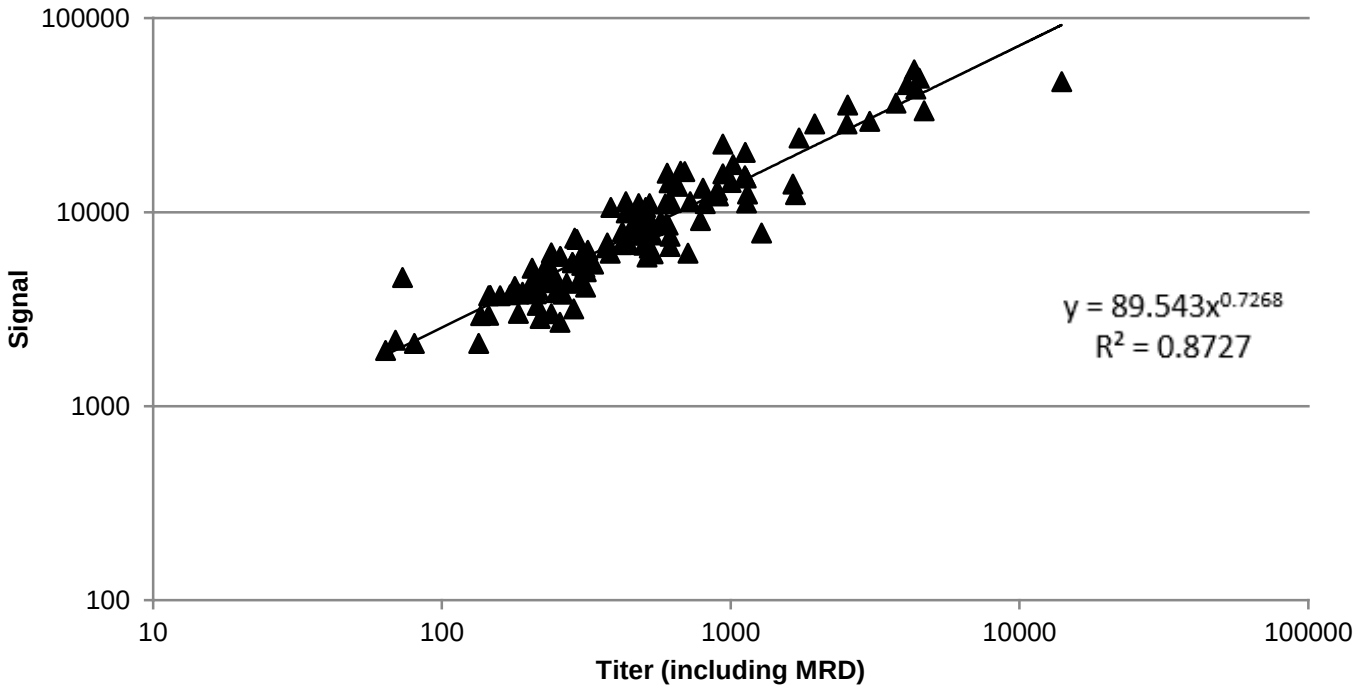
Discussion 2: Correlation between signals and titer

Assay with broad working range => correlation between titer values and assay signal



⇒ Assessment of magnitude of ADA response with assay signals

⇒ Sample titration would become unnecessary

⇒ Prerequisite: drug tolerant and precise assay

Starcevic Manning et al., (2017) Assay signal as an alternative to titer for assessment of magnitude of an antidrug antibody response

Julien Couturier
Bernd Potthoff
Franck Picard

Thank you

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Backup slides

Establishment of the tier range: example 2

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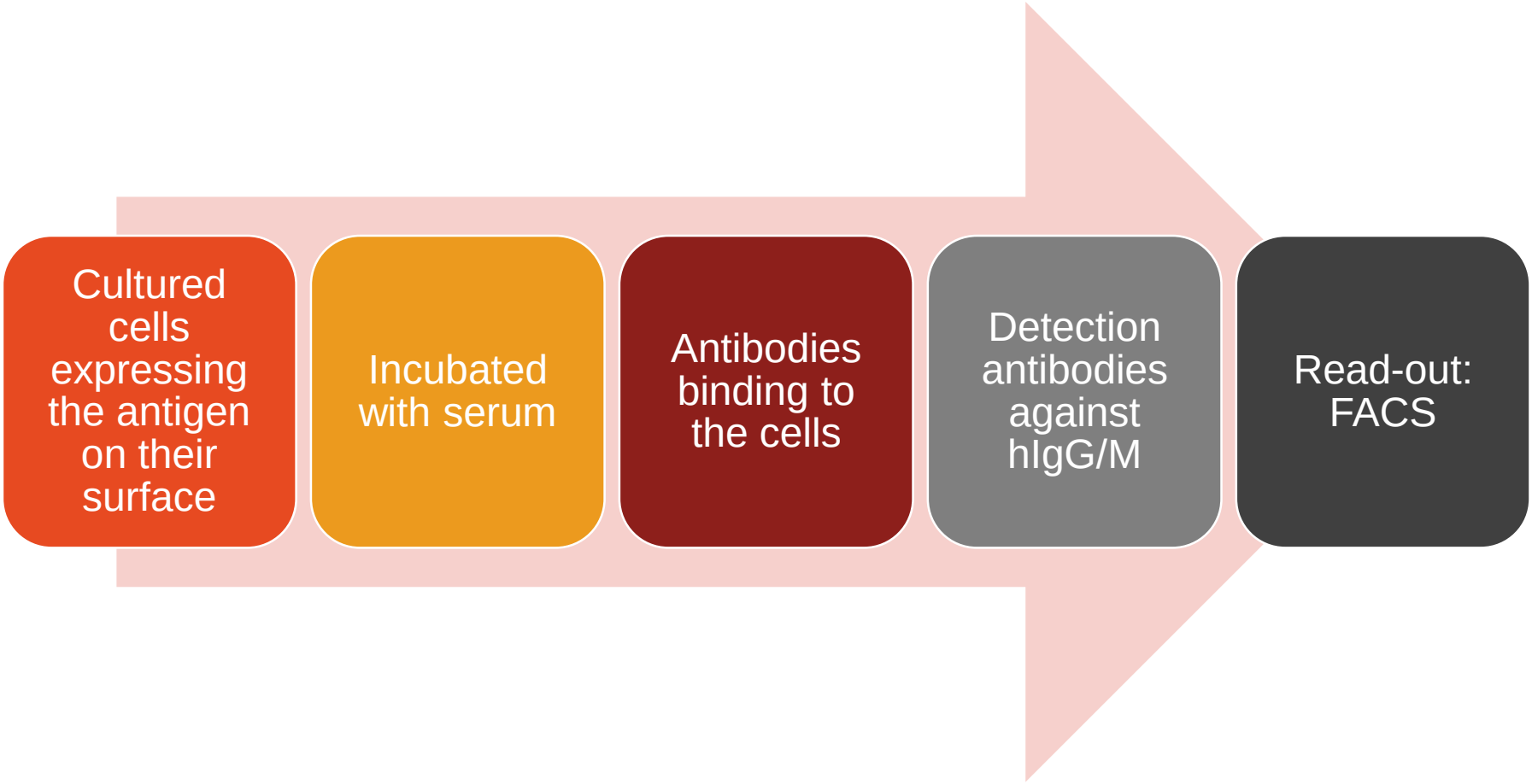
Example 2: Cell-based ADA assay



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The various acceptance ranges for the titer PC

$n * \sqrt{MSR}$ to $n / \sqrt{MSR}$

Range: 714 – 325

$n * MSR$ to n / MSR

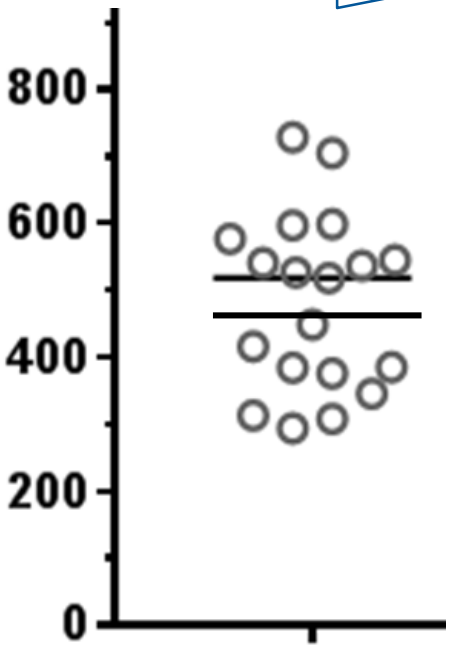
Range: 1059 - 219

$n \pm 3.09SD$

Range: 886 – 76

$M * 2$ to $M / 2$

Range: 1036 – 259



Titer values of the PC from validation / FIH (19 titer curves)

Mean: n= 481

Median: M = 518

SD = 131

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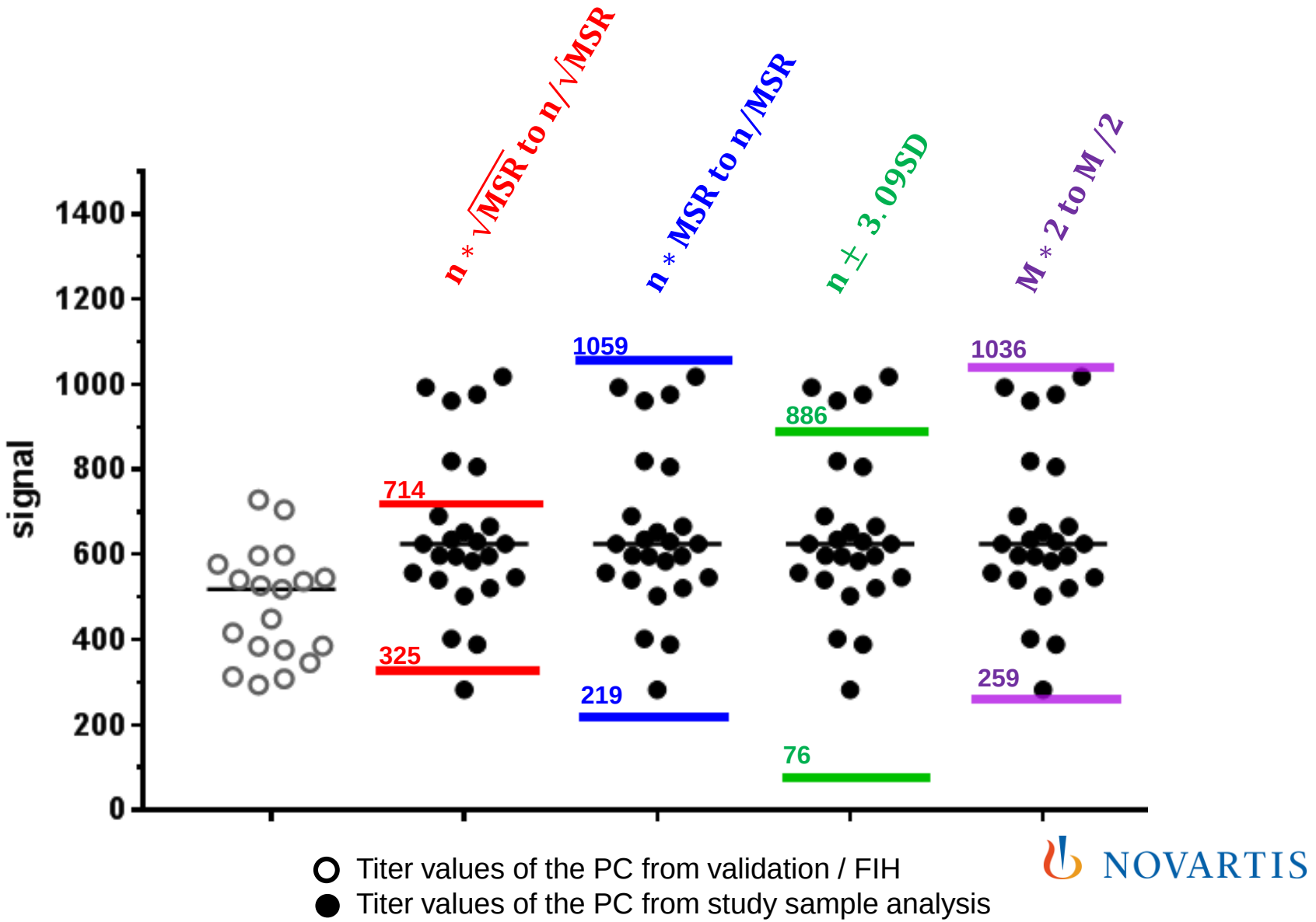
Graphical representation of the various acceptance ranges



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Example 1, enlarged graphs

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Establishment of PC titer ranges

Titer was defined as the reciprocal dilution calculated with the intercept method

- intercept of the linear regression of the two titration points which produce assay signals directly above and below the TCP, with the TCP
- TCP setup

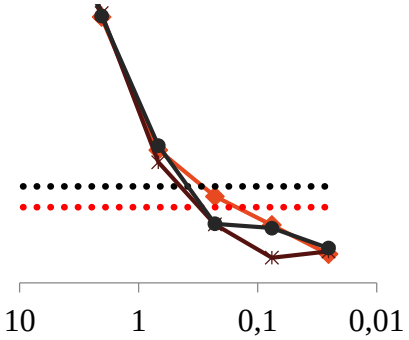
Minimum Significant Ratio (MSR) used to express precision of titer values

$$MSR = 10^{2*\sqrt{2}*SD}$$

- log transformed intra-assay and inter-assay titer
- NB. base 10 was used in the log transformation of titer results

Titer Positive Control acceptance ranges were calculated:

- with titer data from validation
- with different methods to investigate the impact on titer range calculation



n: mean of the titer values

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$n * \sqrt{MSR}$ to $n/\sqrt{MSR}$

$n * MSR$ to n/MSR

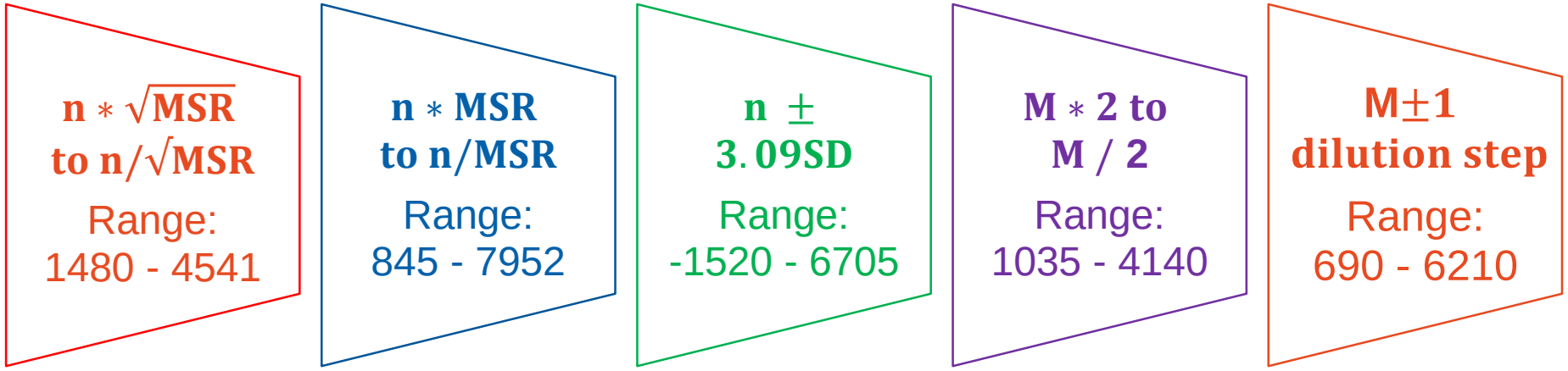
$n \pm 3.09SD$

$M * 2$ to $M/2$

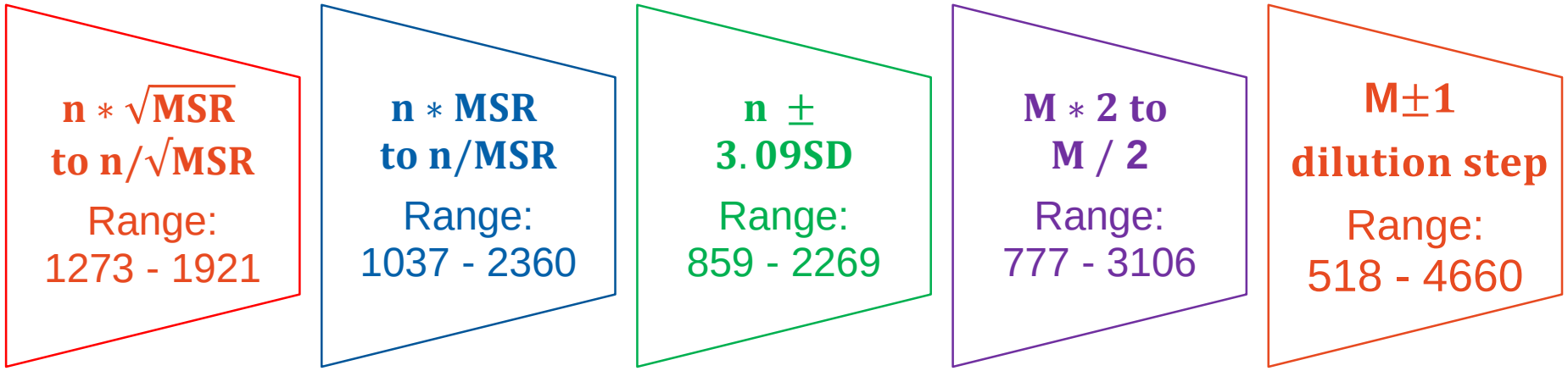
$M \pm 1$ dilution step

The various acceptance ranges for the titer PC

TCP = SCP



TCP = mean NC+3.09pSD



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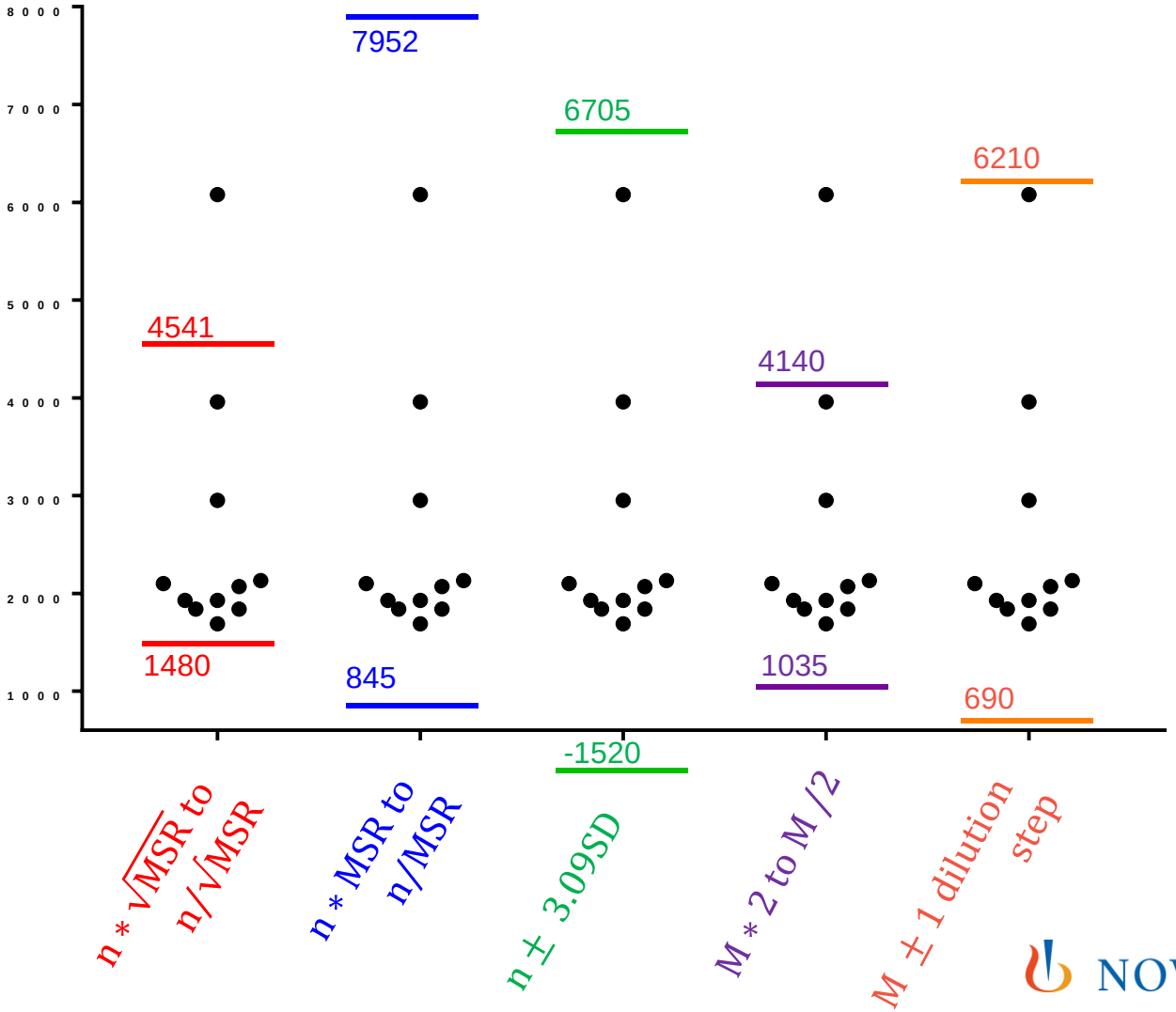

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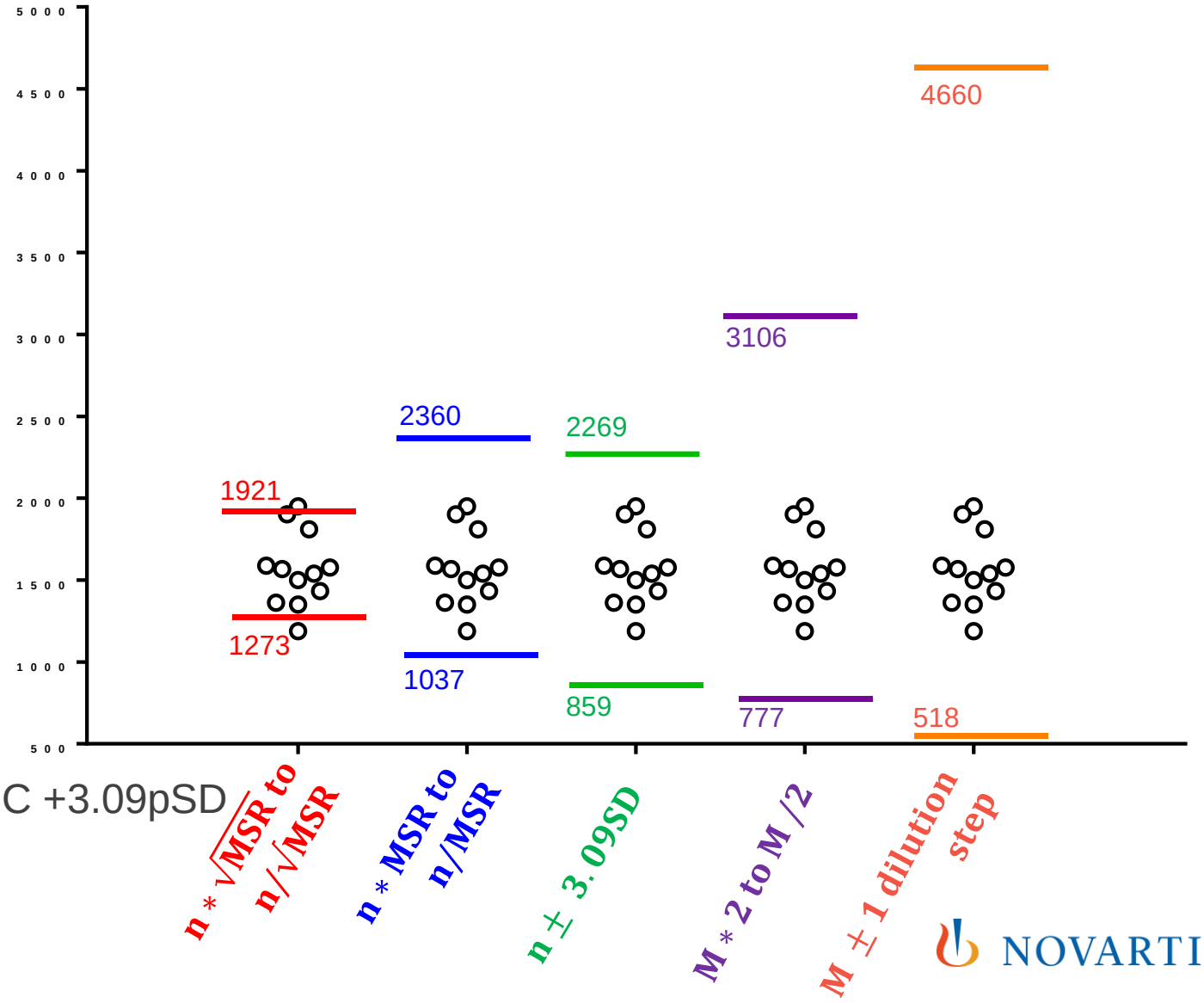

TCP = mean NC +3.09pSD
M = 1553
n =1564
MSR = 1.51

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