Discussion of possible interpretations of the Low-Quality Control Sample determination in immunogenicity assays

8th EBF Young Scientist Symposium

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Concept of Immunogenicity – immune response

Immunogenicity is the ability of a particular substance (antigen/epitope) to provoke an immune response

Desired immune response

- Provoke an immune response against the pathogen
- Upon infection, neutralizing antibodies (NAb) will be produced

Vaccine

Undesired immune response

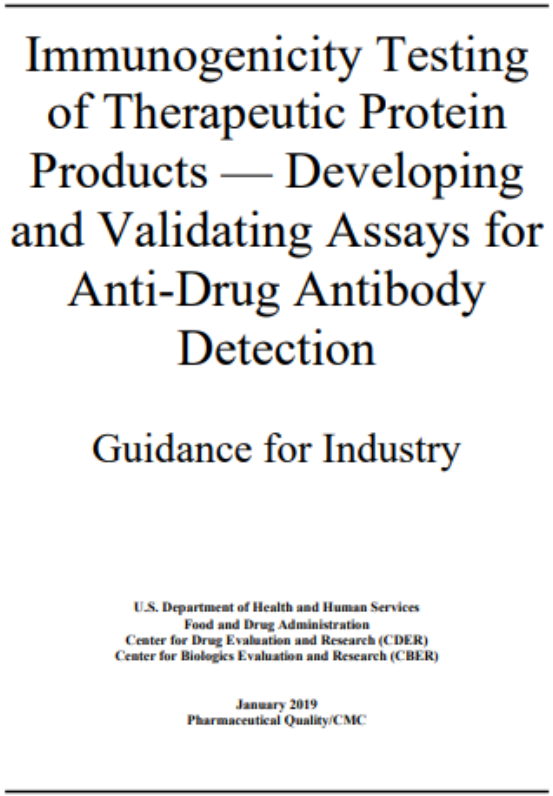
Therapeutic protein

– Has potential to induce an immune-mediated response
– Often measured in terms of “anti-drug antibodies” or ADA
– Hypersensitivity (anaphylaxis)
– Precipitated large therapeutic ADA complex can cause tissue damage and organ failure
– Impacts the drug PK, PD responses, clinical efficacy and patient safety

Guidelines for Immunogenicity testing

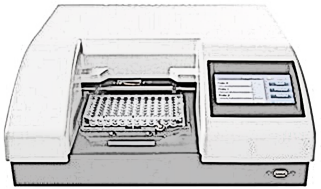
18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

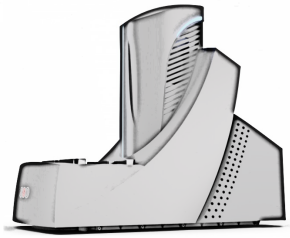


EMA Immunogenicity Guideline (2017) and FDA Guidance for Industry (2019)

Three tier approach to identify and characterize ADAs



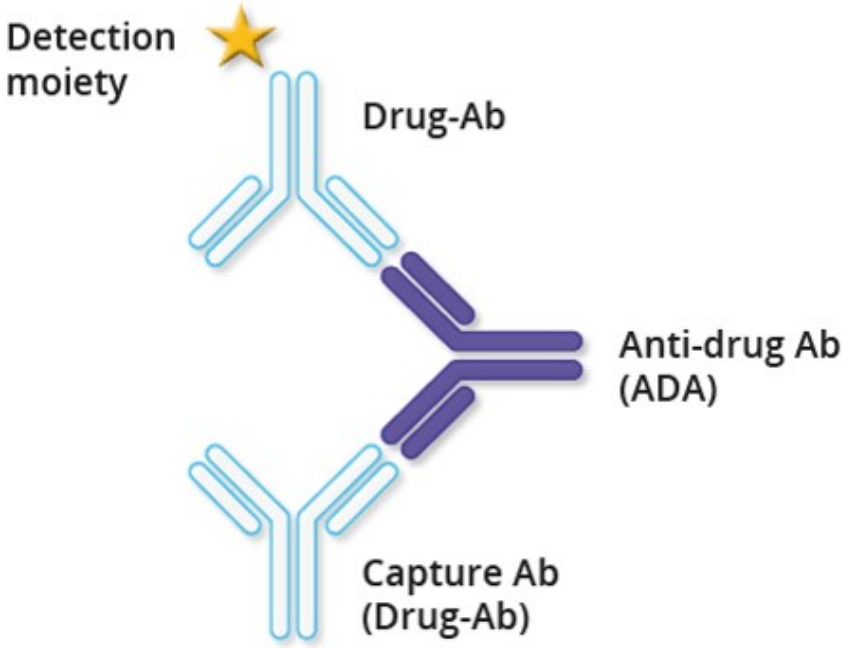
ELISA



MSD-ECL

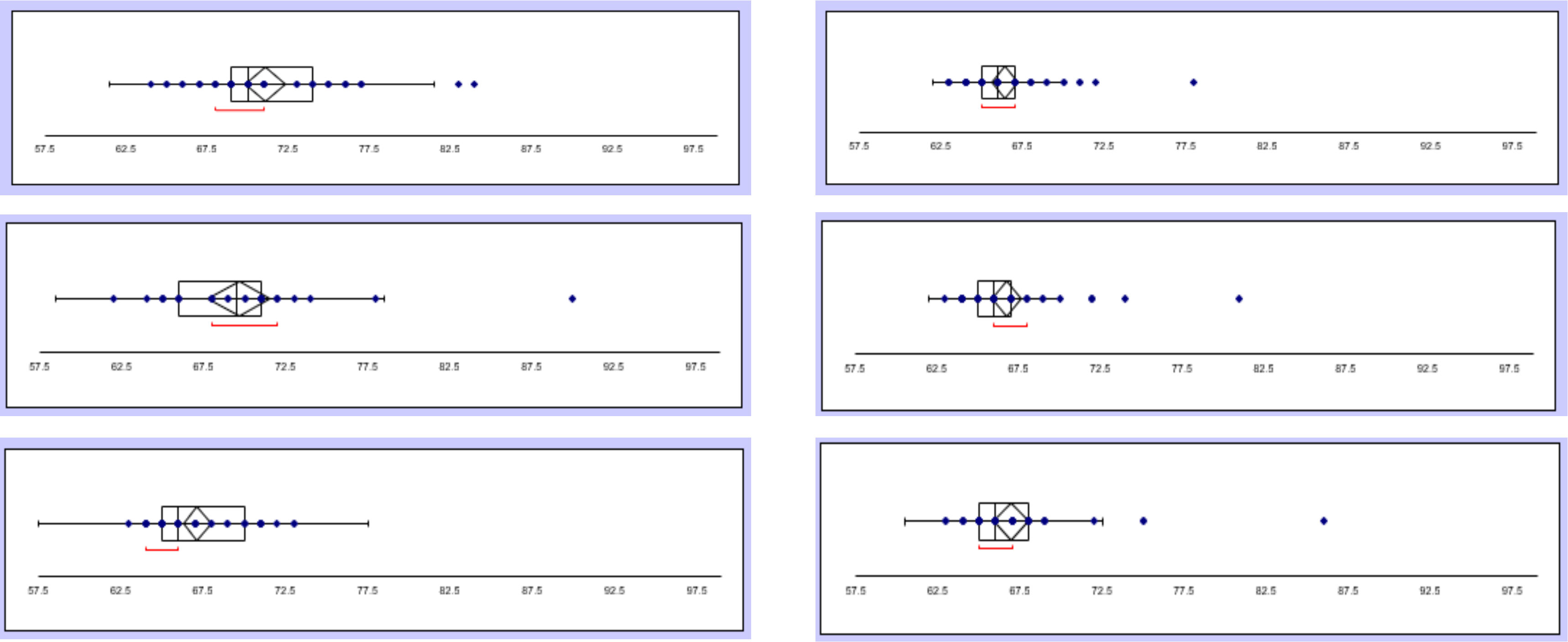
Control Samples:

- Neg-QC score -
- Low-QC score +
- Med-QC score +
- High-QC score +



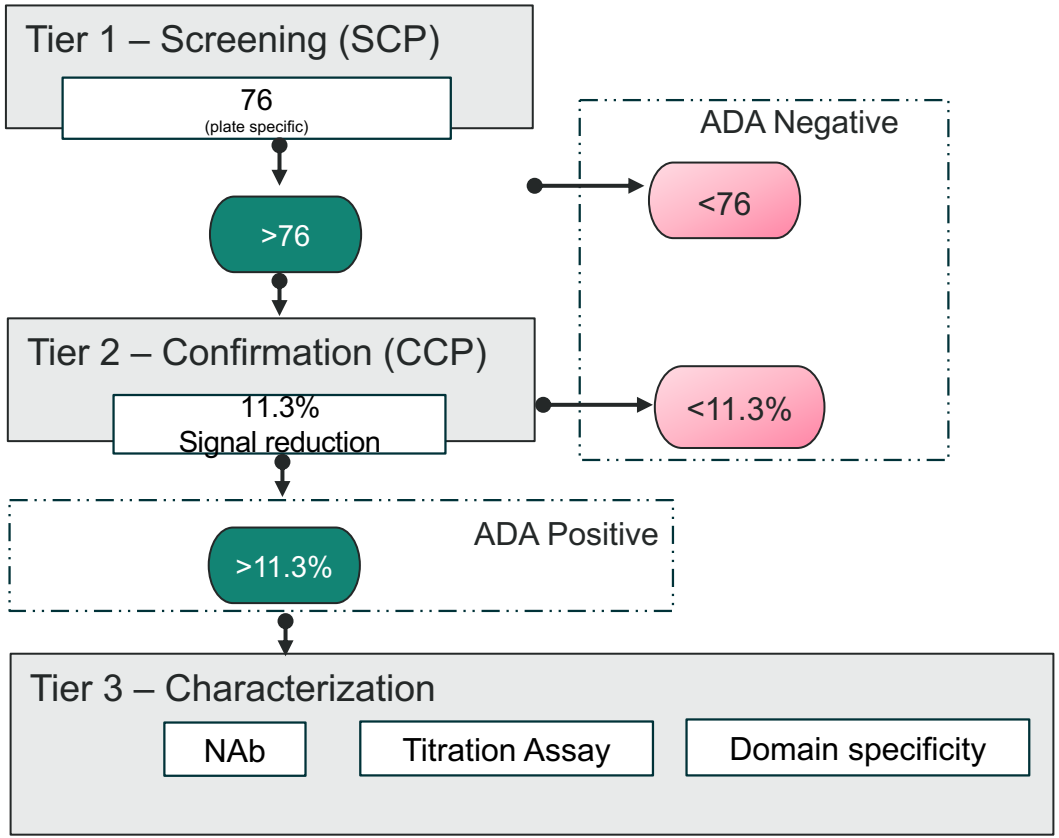
Statistical methods of cut-point determination - Example

"The cut point should be determined statistically with an appropriate number of treatment-naïve samples, generally around 50, from the subject population."



Determination of cut-points

SCP: screening cut-point
CCP: confirmatory cut-point

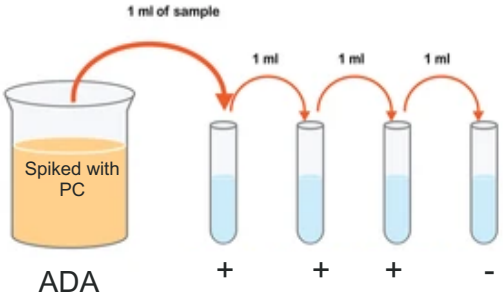


Determination of the Low-QC sample – Challenge!

"For the low-positive QC sample, we recommend that a concentration be selected that, upon statistical analysis, would lead to the rejection of an assay run 1% of the time."

Our Interpretation:

- To find the lowest detectable concentration → Sensitivity run



- 4 runs analyzed on at least 2 different days by at least 2 Lab Analysts
- a linear fit between the two points at either side of the cut-point is used to interpolate the sensitivity concentration
- Assessment of the Low-QC concentration at 99% confidence interval ("rejection of an assay run 1% of the time")

Low-positive control sample and sensitivity – Example

"For the low-positive QC sample, we recommend that a concentration be selected that, upon statistical analysis, would lead to the rejection of an assay run 1% of the time."

Dilution Series	Undil.	2	4	8	16	32	64	128	256	512
Conc. ADA	500	250	125	62.5	31.3	15.6	7.81	3.91	1.95	0.977
SCP Value	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10
71	297 (+)	182 (+)	125 (+)	93 (+)	80 (+)	75 (+)	71 (+)	68 (-)	67 (-)	67 (-)
71	290 (+)	179 (+)	124 (+)	91 (+)	82 (+)	75 (+)	69 (-)	70 (-)	65 (-)	65 (-)
70	297 (+)	182 (+)	123 (+)	95 (+)	84 (+)	73 (+)	70 (+)	68 (-)	67 (-)	66 (-)
71	318 (+)	188 (+)	130 (+)	96 (+)	82 (+)	75 (+)	70 (-)	67 (-)	65 (-)	66 (-)
71	296 (+)	183 (+)	128 (+)	95 (+)	83 (+)	75 (+)	72 (+)	70 (-)	66 (-)	66 (-)
69	308 (+)	190 (+)	128 (+)	94 (+)	81 (+)	75 (+)	69 (+)	68 (-)	67 (-)	68 (-)
70	276 (+)	170 (+)	119 (+)	89 (+)	78 (+)	72 (+)	69 (-)	66 (-)	66 (-)	63 (-)
68	274 (+)	170 (+)	118 (+)	89 (+)	77 (+)	72 (+)	68 (+)	67 (-)	65 (-)	65 (-)
70	275 (+)	171 (+)	115 (+)	90 (+)	81 (+)	77 (+)	71 (+)	68 (-)	67 (-)	65 (-)
70	276 (+)	170 (+)	117 (+)	89 (+)	79 (+)	75 (+)	70 (+)	67 (-)	66 (-)	65 (-)
70	283 (+)	175 (+)	121 (+)	94 (+)	80 (+)	74 (+)	70 (+)	69 (-)	69 (-)	68 (-)
70	269 (+)	170 (+)	118 (+)	93 (+)	81 (+)	75 (+)	71 (+)	67 (-)	68 (-)	67 (-)

Dilution Series	Undil.	2	4	8	16	32	64	128	256	512
Conc. ADA	500	250	125	62.5	31.3	15.6	7.81	3.91	1.95	0.977
CCP Value	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10
11.3	74.4 (y)	60.4 (y)	46.4 (y)	29.0 (y)	15.0 (y)	13.3 (y)	7.0 (n)	1.5 (n)	1.5 (n)	1.5 (n)
11.3	73.8 (y)	62.0 (y)	46.0 (y)	31.9 (y)	20.7 (y)	14.7 (y)	8.7 (n)	7.1 (n)	1.5 (n)	1.5 (n)
11.3	74.1 (y)	61.0 (y)	43.1 (y)	29.5 (y)	17.9 (y)	6.8 (n)	5.7 (n)	0.0 (n)	0.0 (n)	0.0 (n)
11.3	75.2 (y)	63.3 (y)	46.2 (y)	33.3 (y)	17.1 (y)	12.0 (y)	7.1 (n)	1.5 (n)	3.1 (n)	1.5 (n)
11.3	74.3 (y)	62.3 (y)	49.2 (y)	31.6 (y)	21.7 (y)	12.0 (y)	8.3 (n)	8.6 (n)	1.5 (n)	4.5 (n)
11.3	74.7 (y)	62.1 (y)	44.5 (y)	28.7 (y)	19.8 (y)	10.7 (n)	5.8 (n)	1.5 (n)	3.0 (n)	5.9 (n)
11.3	72.1 (y)	60.0 (y)	44.5 (y)	25.8 (y)	14.1 (y)	11.1 (n)	5.8 (n)	1.5 (n)	4.5 (n)	-1.6 (n)
11.3	73.4 (y)	59.4 (y)	45.8 (y)	29.2 (y)	16.9 (y)	12.5 (y)	10.3 (n)	7.5 (n)	6.2 (n)	6.2 (n)
11.3	72.7 (y)	59.1 (y)	43.5 (y)	30.0 (y)	21.0 (y)	16.9 (y)	9.9 (n)	8.8 (n)	7.5 (n)	4.6 (n)
11.3	73.2 (y)	60.6 (y)	47.0 (y)	32.6 (y)	22.8 (y)	14.7 (y)	10.0 (n)	7.5 (n)	7.6 (n)	6.2 (n)
11.3	72.4 (y)	58.9 (y)	43.0 (y)	30.9 (y)	16.3 (y)	10.8 (n)	4.3 (n)	4.3 (n)	5.8 (n)	5.9 (n)
11.3	71.4 (y)	57.1 (y)	43.2 (y)	29.0 (y)	18.5 (y)	12.0 (y)	7.0 (n)	4.5 (n)	1.5 (n)	4.5 (n)

	sLow-QC
Mean	8.02
SD	1.41
n	12
t0.99	2.72
calculation sLow-QC (ng/mL)	11.9
	cLow-QC
Mean	14.2
SD	3.55
n	12
t0.99	2.72
calculation cLow-QC (ng/mL)	23.9
difference sLow and cLow (%)	-67.2

(y) : confirmed positive
(n) : confirmed negative
sLow: screening Low-QC
cLow: confirmatory Low-QC

Rejected runs due to the calculated Low-QC concentration

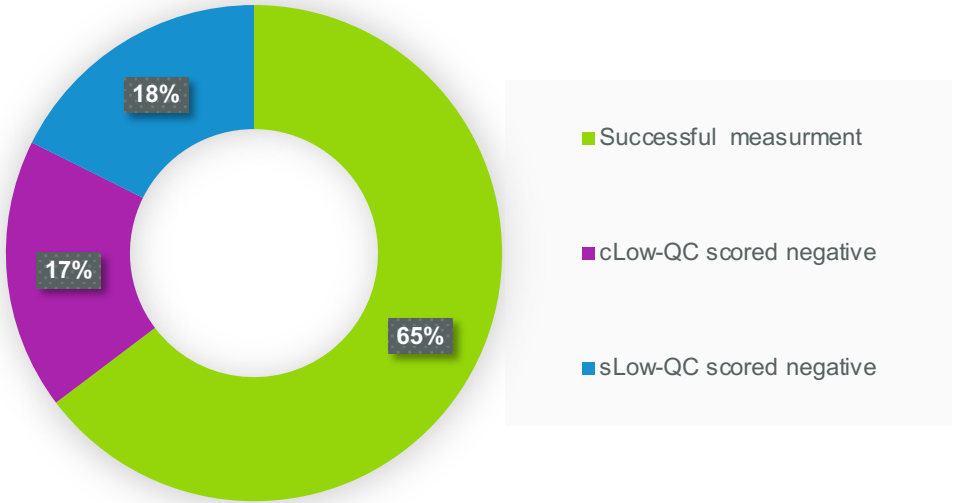
sLow-QC

Responses-drug (ng/mL)						12 ng/mL
x1	x2	x3	Mean	CV (%)	n (pos)	SCP Value
79 (+)	77 (-)	N/A	78 (+)	1.8	1	78
71 (+)	73 (+)	N/A	72 (+)	2.0	2	68
76 (+)	78 (+)	N/A	77 (+)	1.8	2	76
85 (-)	77 (-)	N/A	81 (-)	7.0	0	87

cLow-QC

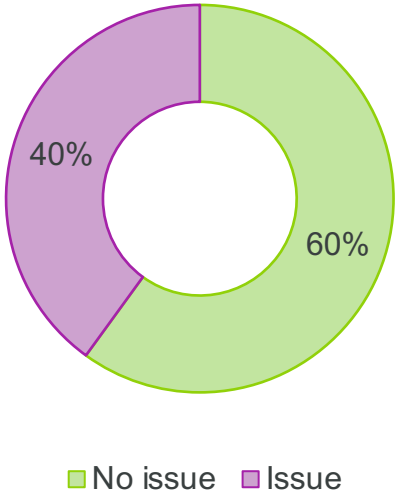
Responses-drug (ng/mL)						24 ng/mL
x1	x2	x3	Mean	CV (%)	n (pos)	SCP Value
81 (+)	83 (+)	N/A	82 (+)	1.7	2	78
76 (+)	80 (+)	N/A	78 (+)	3.6	2	68
82 (+)	82 (+)	N/A	82 (+)	0.0	2	76
81 (-)	84 (-)	N/A	83 (-)	2.6	0	87

4 rejected runs - 16 samples



Possible solutions for Low-QC determination

ADA studies 2021



Possible solutions:

- Arbitrarily increase the Low-QC concentration
- Increase the Low-QC with a percentage of the original concentration (e.g. 20%)
- Re-evaluation of the sensitivity data or log transformation of the data
- Other??

Conclusion

- According to the 2019 FDA guideline for immunogenicity assays → Low-QC sample that, based on statistical analysis, would lead to the rejection of an assay run 1% of the time

- There is a need for specific recommendation for Low-QC determination

› Bioanalysis. 2014 May;6(10):1409-13. doi: 10.4155/bio.14.95.

EBF recommendation for stability testing of anti-drug antibodies; lessons learned from anti-vaccine antibody stability studies

Susanne Pihl [1], Lydia Michaut, Jenny Hendriks, Ralf Loebbert, Janka Ryding, Martin Nemansky, Laurent Vermet, Arjen Companjen

Affiliations + expand

PMID: 24958124 DOI: 10.4155/bio.14.95

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› Bioanalysis. 2019 Oct;11(19):1787-1798. doi: 10.4155/bio-2019-0248. Epub 2019 Oct 28.

EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays

Susanne Pihl [1], Barry Wa van der Strate [2], Michaela Golob [3], Janka Ryding [4], Laurent Vermet [5], Birgit Jaitner [6], Joanne Goodman [7], Philip Timmerman [8]

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PMID: 31657235 DOI: 10.4155/bio-2019-0248

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Thank you for your attention!

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Questions?