Regulatory Requirements Translated Into Day-to-Day Practice

Jonas Blaes, PhD

DMPK-BA, AbbVie Germany
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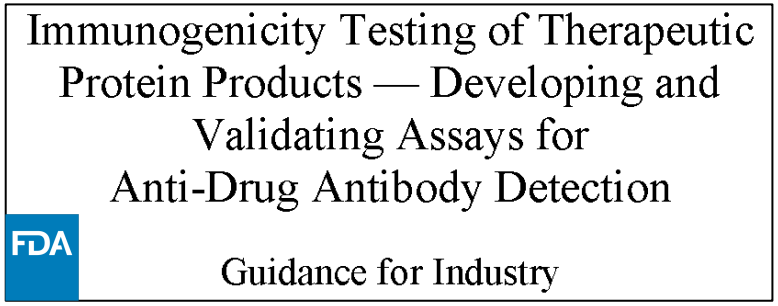
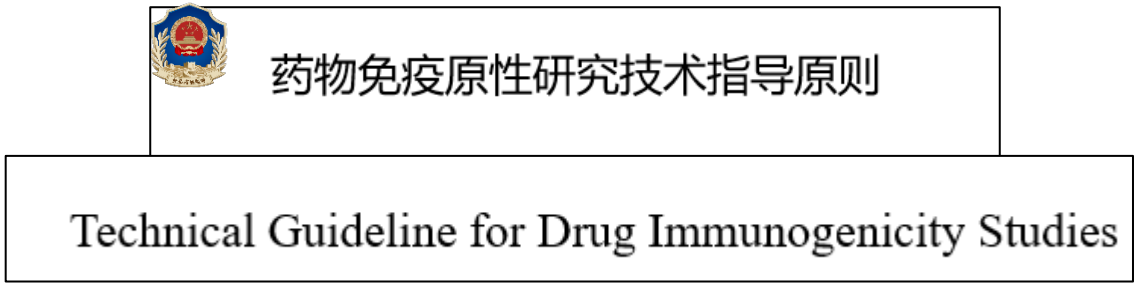
Regulatory Requirements …





Presentation Overview

Drug Tolerance
- *guideline perspective*
- *example 1: assessment during MDV*
- *example 2: application of drug tolerance?*

Multi-Tiered Analysis
- *guideline perspective*
- *repeat analysis vs. true reassay*
- *example 3: definition*
- *example 4: dealing with true reassays*

Cross Validation
- *guideline perspective*
- *example 5: PK-oriented approach*
- *example 6: different studies, different labs, different matrices*

Back Up:
- *qualification of assay plates*
- *certificates of analysis*

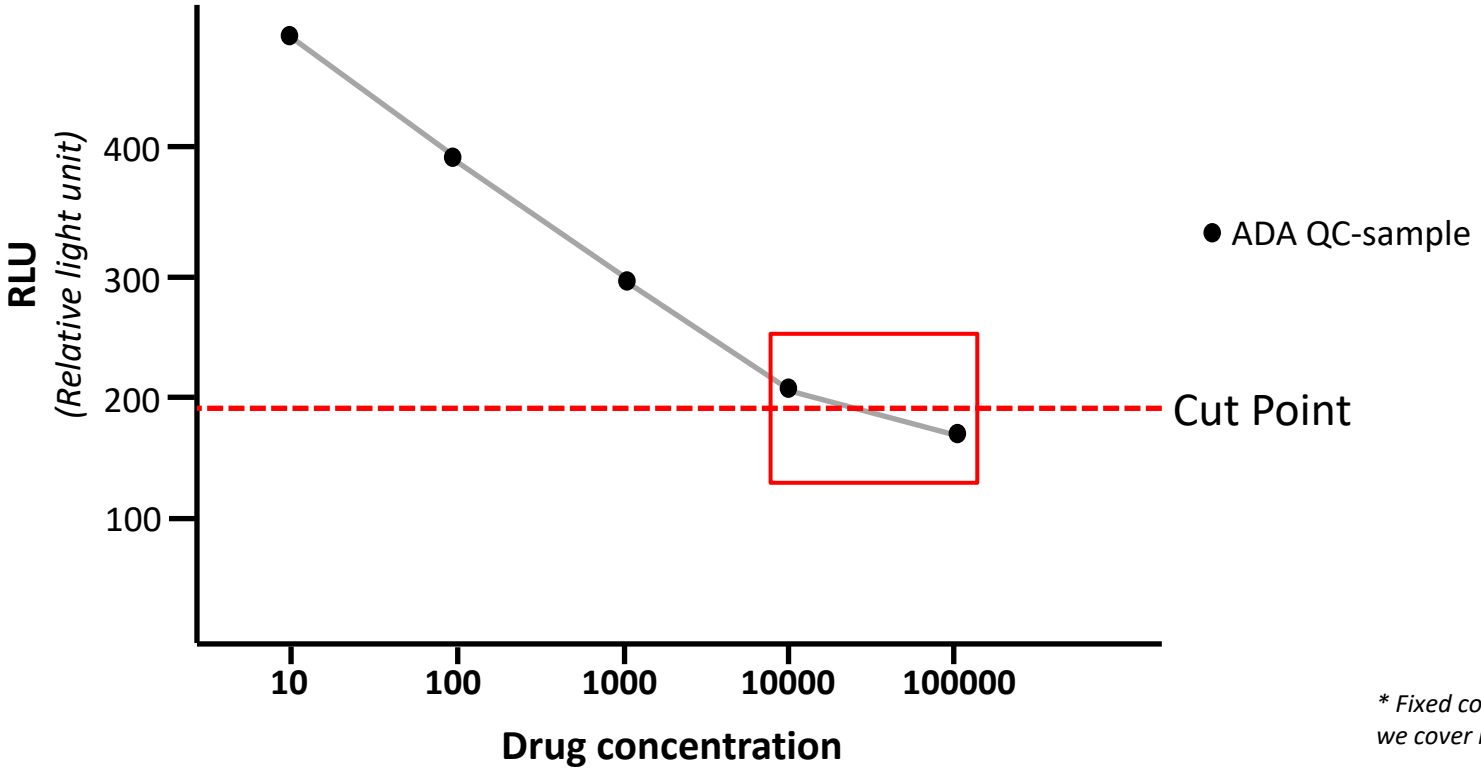
Drug Tolerance of ADA Assays

Guideline	Recommendation
FDA 2019	*The Sponsor may examine drug tolerance by deliberately adding different known amounts of positive control antibody into ADA-negative control samples in the absence or presence of different quantities of the therapeutic protein product to determine whether the therapeutic protein product interferes with ADA detection. [….]* *Interference from the therapeutic protein product can be minimized by collecting subject samples at trough drug levels.*
EMA 2017	*[…], the Applicant has to demonstrate that the tolerance of the assay to the therapeutic exceeds the levels of the therapeutic protein in the samples for ADA testing.*
NMPA Draft 2020	*Generally, blank matrix pool is used to dilute the tested drug to the appropriate series of concentrations, and the series of diluted samples are mixed with the positive control antibody samples at different concentrations (including at least low level concentration of positive control antibody or 100ng/mL of positive control antibody) for detection.* *[…] the maximum drug concentration that can be tolerated by the positive control antibody*

Assessment of Drug Tolerance

example 1: experimental design during MDV

- adding increasing concentrations of drug to different concentrations of polyclonal ADA control antibodies spiked into human serum
- drug is added e.g. at 10 - 1000000 ng/mL and polyclonal ADA is used in concentrations of the HQC, MQC, LQC and 100 ng/mL.
- Interpolation of drug/ADA ratio which still yields a result at/above the assay cut point



** Fixed concentration of 100 ng/mL to ensure we cover required minimal sensitivity.*

Application of Drug Tolerance?

hypothetical scenario:

- the drug tolerance of an ADA assay permits a detection of 100 ng/mL ADA in the presence of 30 µg/mL drug. What do you do with individual patient samples in which the PK concentration exceeds 30 µg/mL?

Is to ‚not analyze for ADA' an option?

Validation perspective:

- the drug tolerance of the ADA assay does not enable detection of ADA

Scientific Perspective:

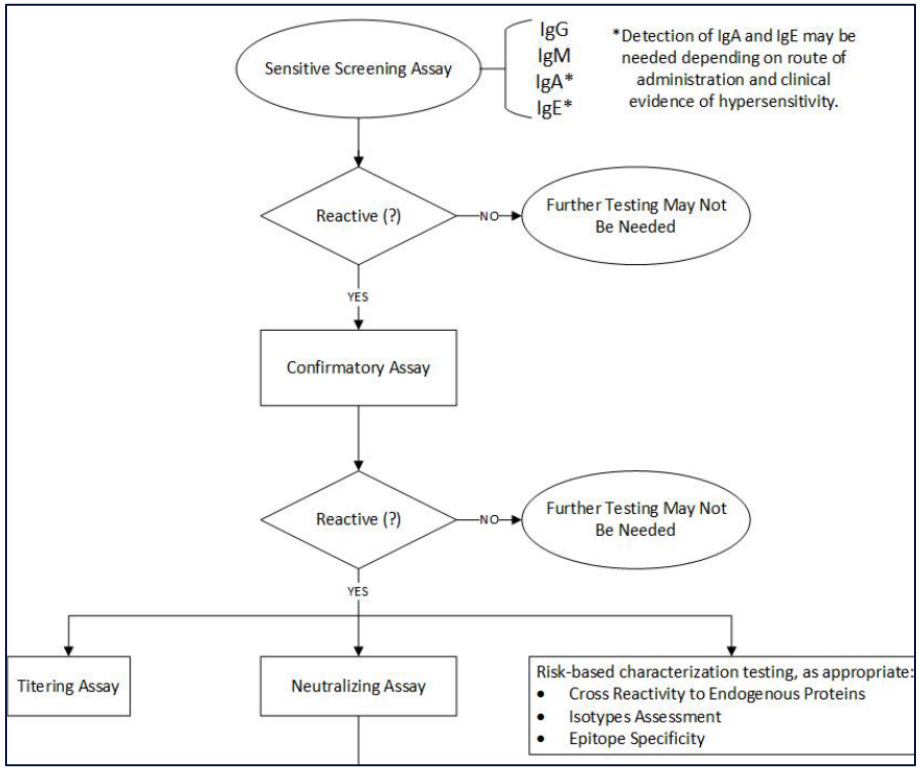
- drug tolerance is typically assessed with surrogate ADAs derived from animals. Won't individual patient-generated ADA behave differently? How can you be sure if you do not test?
- Even PK result could be dependent on remaining ADA (ADA-tolerance of PK-assay!)

example 2: assessment of drug tolerance with different ADAs

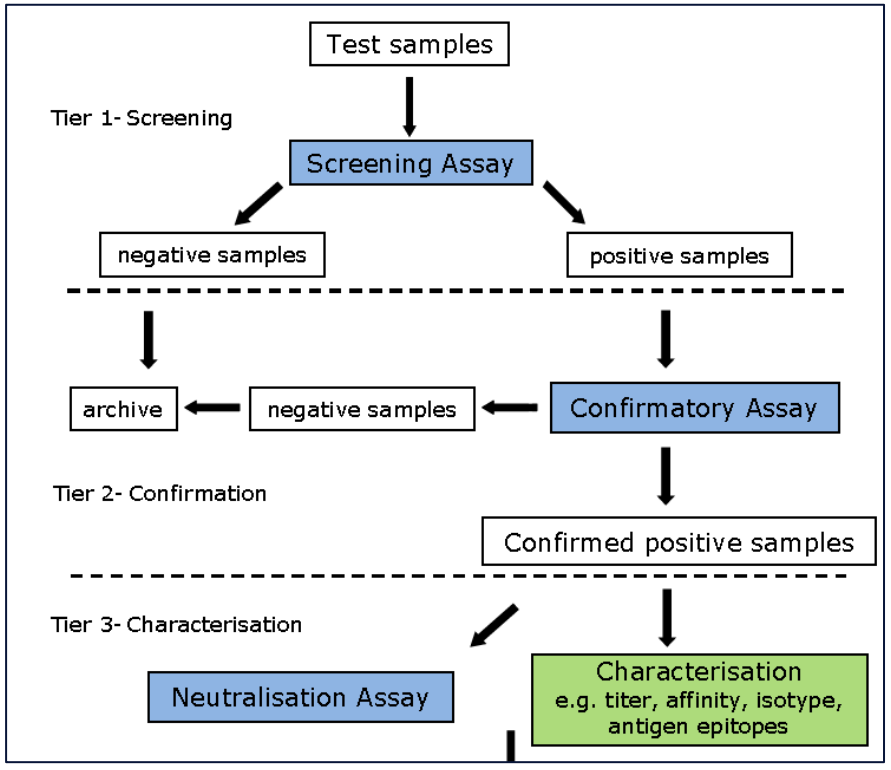
- Drug tolerance of an assay depends on surrogate control material

	Surrogate ADA 1	Surrogate ADA 2
Drug tolerance	100 ng/mL detected positive in the presence of 26004 ng/mL drug (**260** fold drug conc. tolerated)	100 ng/mL detected positive in the presence of 122770 ng/mL drug (**1227** fold drug conc. tolerated)

Multi-Tiered Analysis



Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. Guidance for Industry. FDA. 2019



Guideline on Immunogenicity of therapeutic proteins. EMA. 2017

Multi-Tiered Analysis: ‘Reassays’

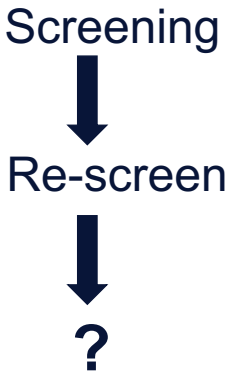
Guideline	Recommendation
FDA 2019	*not defined/mentioned*
EMA 2017	*not defined/mentioned*
NMPA Draft 2020	*not defined/mentioned*

Multi-Tiered Analysis: ‘Reassays’

Reanalyzing samples ‘by nature of design‘

[example 4: reassay by mistake]

- Screening was done twice by accident

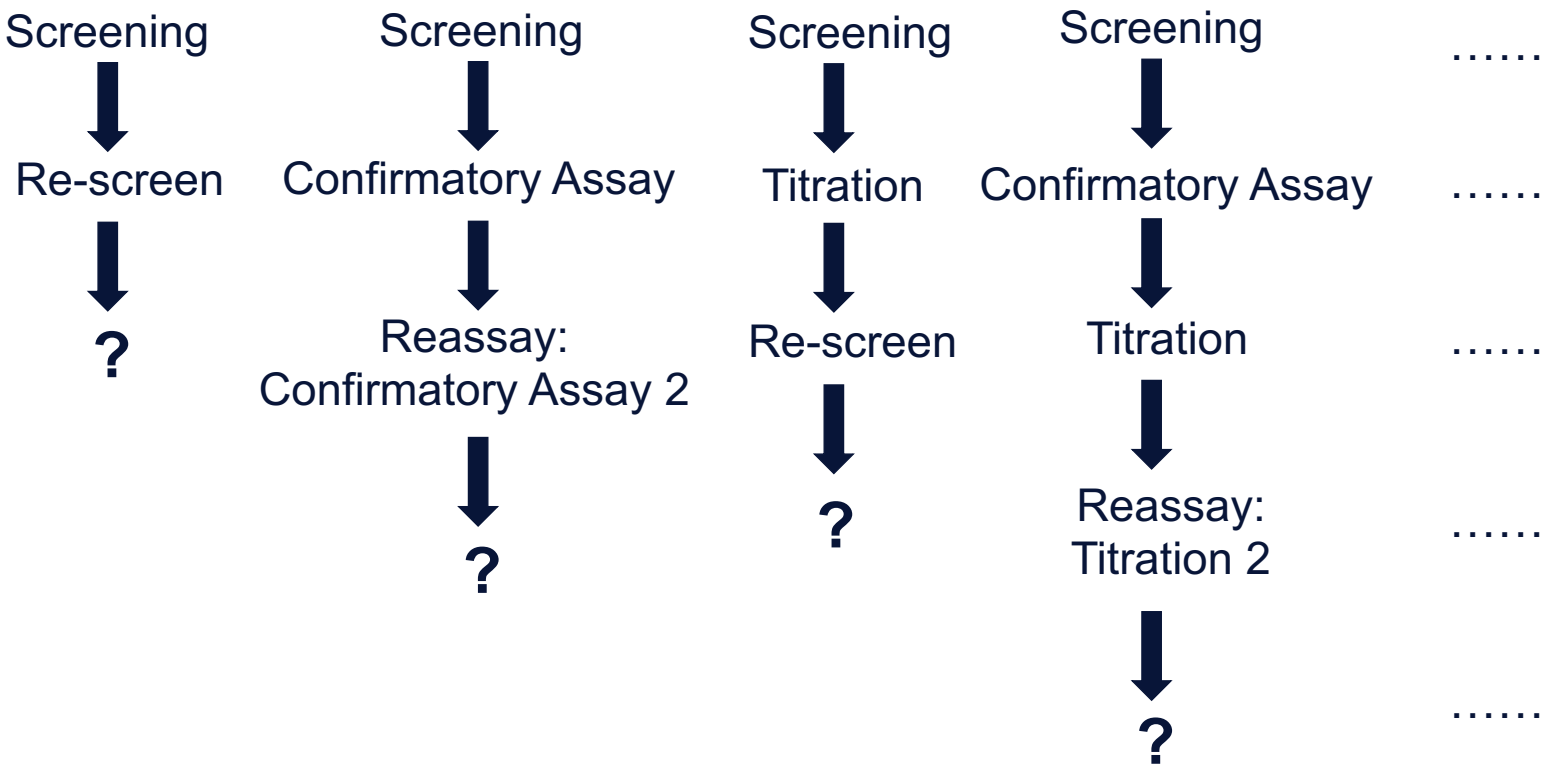


[example 3: our definition]

all steps required to determine a final analytical result (screening, titration, confirmation) are not considered as true reassays and are not reported as such.

Multi-Tiered Analysis: ‘Reassays’

many additional possible scenarios



Cross-Validation

Guideline	Recommendation
FDA 2019	*Reproducibility (also called cross-validation) is needed when more than one laboratory will be used to assess samples.* *Reproducibility is an important consideration if an assay will be run by two or more independent laboratories during a study, and a sponsor should establish the comparability of the data produced by each laboratory. Comparable assay performance, including sensitivity, drug tolerance, and precision, should be established between laboratories.*
EMA 2017	*Regardless of the approach used for immunogenicity assessment of a **biosimilar** product versus the reference product, it is recommended that the assays are cross-validated using both antigens, antibody positive controls and preferably clinical samples to demonstrate similar performance*
NMPA Draft 2020	*If samples are tested by two or more independent laboratories during a study, reproducibility is an important consideration and the comparability of data produced by the different laboratories should be established. Comparable assay performance, including sensitivity, drug tolerance, and precision, should be established between laboratories.*

Cross-Validation

example 5: PK-orientated approach for cross-validation

- Typically following an assay transfer from lab A → lab B (same reagents, comparable sensitivity, drug-tolerance etc.)
- Preparation of 30 spiked serum/plasma samples (+/- ADA & +/- drug & blank samples)
- Analysis in both labs using multi-tiered approach
- Comparison of results. Acceptance criterion = ADA-status (+/-)
- 90% of the results need to match

	Confirmatory Assay Results		Comparison / acceptance	Titer Assay Results	
Sample Id	Lab 1	Lab 2		Lab 1	Lab 2
01	negative	negative	pass	n.a.	n.a.
02	negative	positive	does not pass	n.a.	10
03	positive	positive	Pass	151000	164000
04	negative	negative	Pass	n.a.	n.a.
05	positive	positive	Pass	2400	2200
…	…..	…	….	….	…

Cross-Validation

*[…] an important consideration if an assay will be run by two or more independent laboratories during **a** study.*

What about multiple studies?

example 6: plasma vs. serum ADA assays

- early studies were analyzed by lab A using plasma, later studies by lab B using serum

→ Data to support comparability of the new vs the previous ADA assay methods (across data sets)

But:

*- Overall, the assays should be validated using the **same matrix** as the samples to be analyzed (EMA)*

*- the sponsor should define the matrix and dilution factor that will be used for preparation of subject samples **before** performing validation studies (FDA)*

*- cut-point should be determined statistically with an appropriate number of treatment-naïve samples, generally around 50, from the **subject population** (FDA)*

Back-Up

Qualification of Assay Plates

Guideline	Recommendation
FDA 2019	*If plate homogeneity of response is not demonstrated, alternative plate layouts should be used during cut-point determination.*
EMA 2017	*not mentioned*
NMPA Draft 2020	*not mentioned*

example 7: plate homogeneity testing

- tested during assay development

	1	2	3	4	5	6	7	8	9	10	11	12
A	138	135	142	137	135	143	128	125	122	119	113	115
B	134	137	133	133	135	131	128	120	119	112	116	117
C	138	141	137	136	130	133	131	122	122	121	117	117
D	143	137	132	133	130	127	126	116	115	116	109	116
E	135	138	138	140	136	134	127	121	115	117	107	110
F	140	136	135	132	134	132	126	120	116	108	112	111
G	133	138	130	132	129	130	125	118	118	114	112	110
H	134	135	129	135	128	132	124	124	120	115	118	112

Certificates of Testing/Analysis (CoT/CoA)

Guideline	Recommendation
FDA 2019	*- not mentioned.* *'Qualification and stability of critical reagents is important for ensuring consistent assay performance'*
EMA 2017	*For all controls the characterization data showing their properties and functionality for the intended use should be provided as part of the MAA submission*
NMPA Draft 2020	*not mentioned*

example 8: request for additional data on positive control material

- the information presented regarding the positive control antibody was considered as insufficient.

→ Data on qualification were provided as CoA. Control material generation was outlined in detail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