Generic ADA Assay

How to speed up early phase and preclinical immunogenicity testing.

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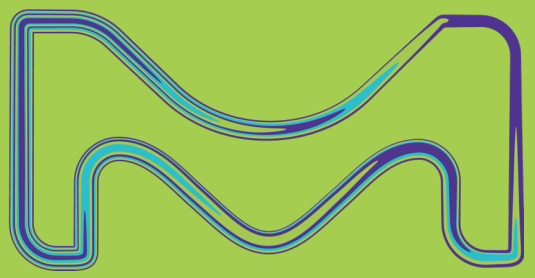
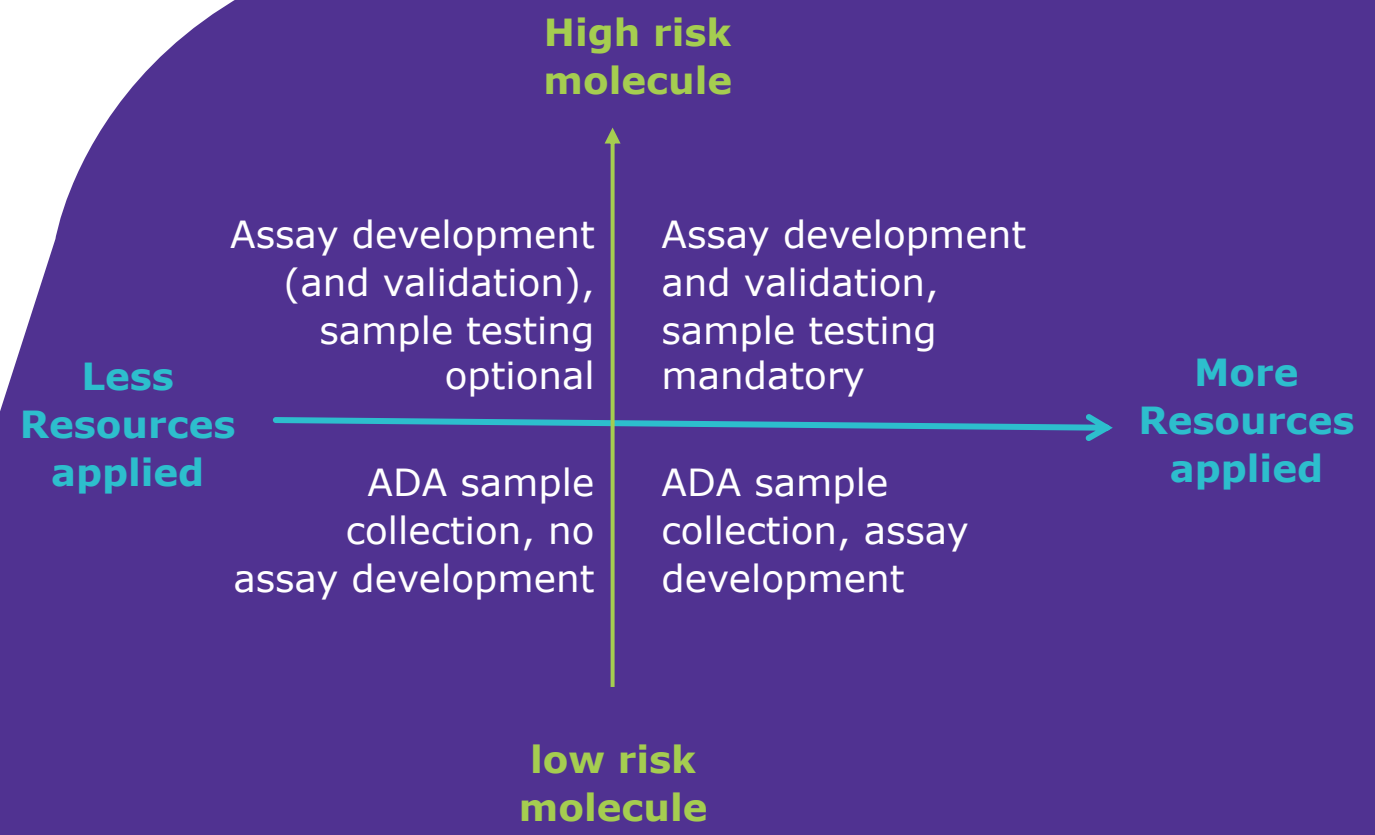
16th EBF Open Symposium



MERCK

Stratecic Preclinical ADA testing approach



Immunogenicity risk assessment

Business risk analysis

Anna Laurén, Joanne Goodman, Jonas Blaes et all**. A strategic approach to nonclinical immunogenicity assessment: a recommendation from the European Bioanalysis Forum. Bioanalysis**. 2021 Apr;13(7):537-549. doi: 10.4155/bio-2021-0028.

Some figures from 2022 non-clinical environment (efficacy, PK, DRF)

Total number of ADA samples tested: 126

- ~1% of total analyzed samples are ADA samples
- ~8% of studies required ADA testing

Total number of ADA methods developed: 5

- 1 qualified method never used
- 3 new qualified methods used during the year
- 1 method developed for further validation next year

Average number of sample tested per qualified method: 31,5

- Average number of exp required for development + qualification: 13
- Average number of analytical runs required for sample testing: 1

Conclusion

Only in limited studies specific ADA methods are required

The effort needed to cover these activities is way greater then the entirety of the sample testing

Moreover… a labour intensive case study

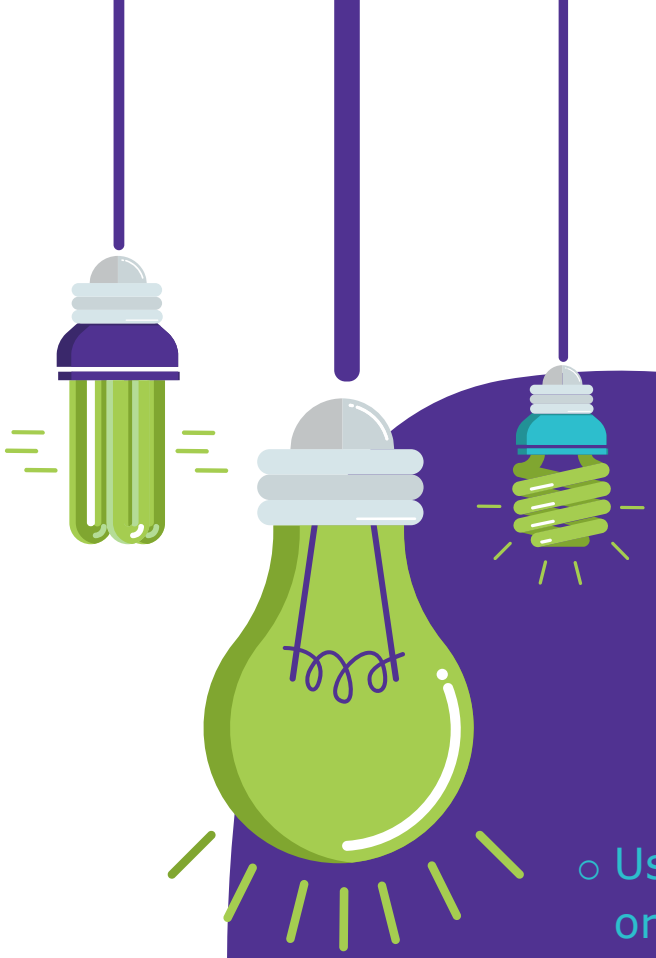
Efficacy mouse study with three mouse surrogate test items

48 samples belonging to three treatment groups (3 test items)

ADA analysis meant:

- 6 labellings (biotin and sulfo-tag for 3 test items) were performed
- 14 runs for method development (for optimizing each test item condition) were done
- 9 runs for «qualification» were performed
- 3 different assay cut-points were established
- 1 analytical method was finalized (containing options and variations for each test item)
- Samples testing: 3 analytical runs, one per test item, were done

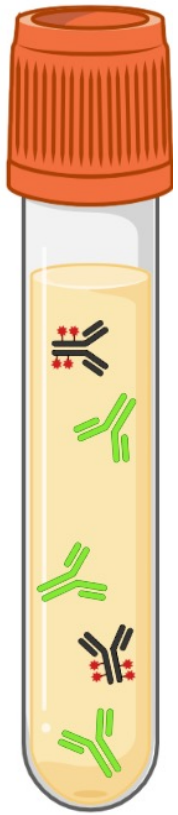
Total time spent on this activity: 1,5 month



Idea:
Generic ADA Assay

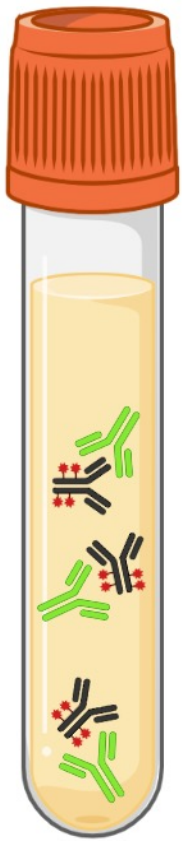
- Usage of common reagents → no need for specific reagents purchase or labelling waiting time
- Skip method development → only need for checking the «applicability» to the new drug/matrix
- Promptly able to perform immunogenicity sample testing as required → shorter time-to-result

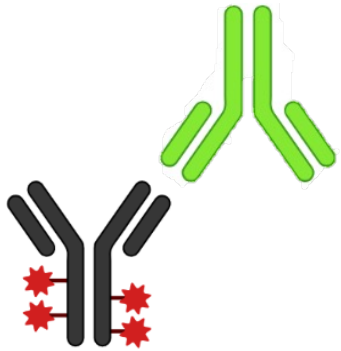
Generic approach
Principle of the assay



Mouse sample with ADA and drug

Add excess drug to bind all ADA





ADA – Positive Control

Mouse Anti-Human IgG (H+L specific)

DRUG – human IgG

Biorender

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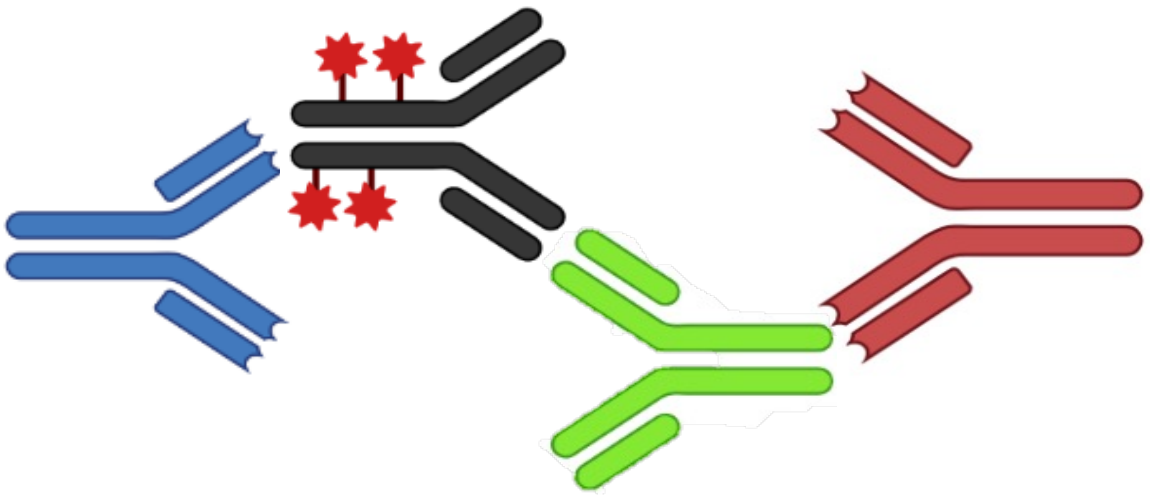
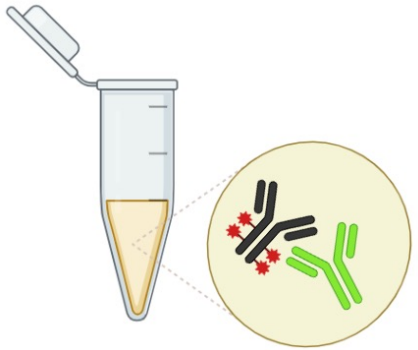
Generic approach
Assay Format



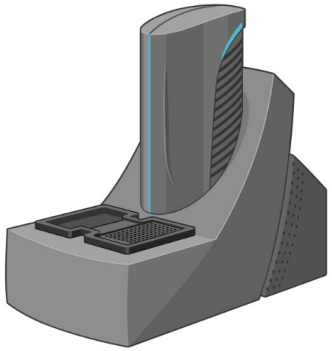
1. Capture
biotinylated anti-human IgG fc

2. Drug-additioned sample



3. Detection

SULFO-TAG anti-mouse IgG



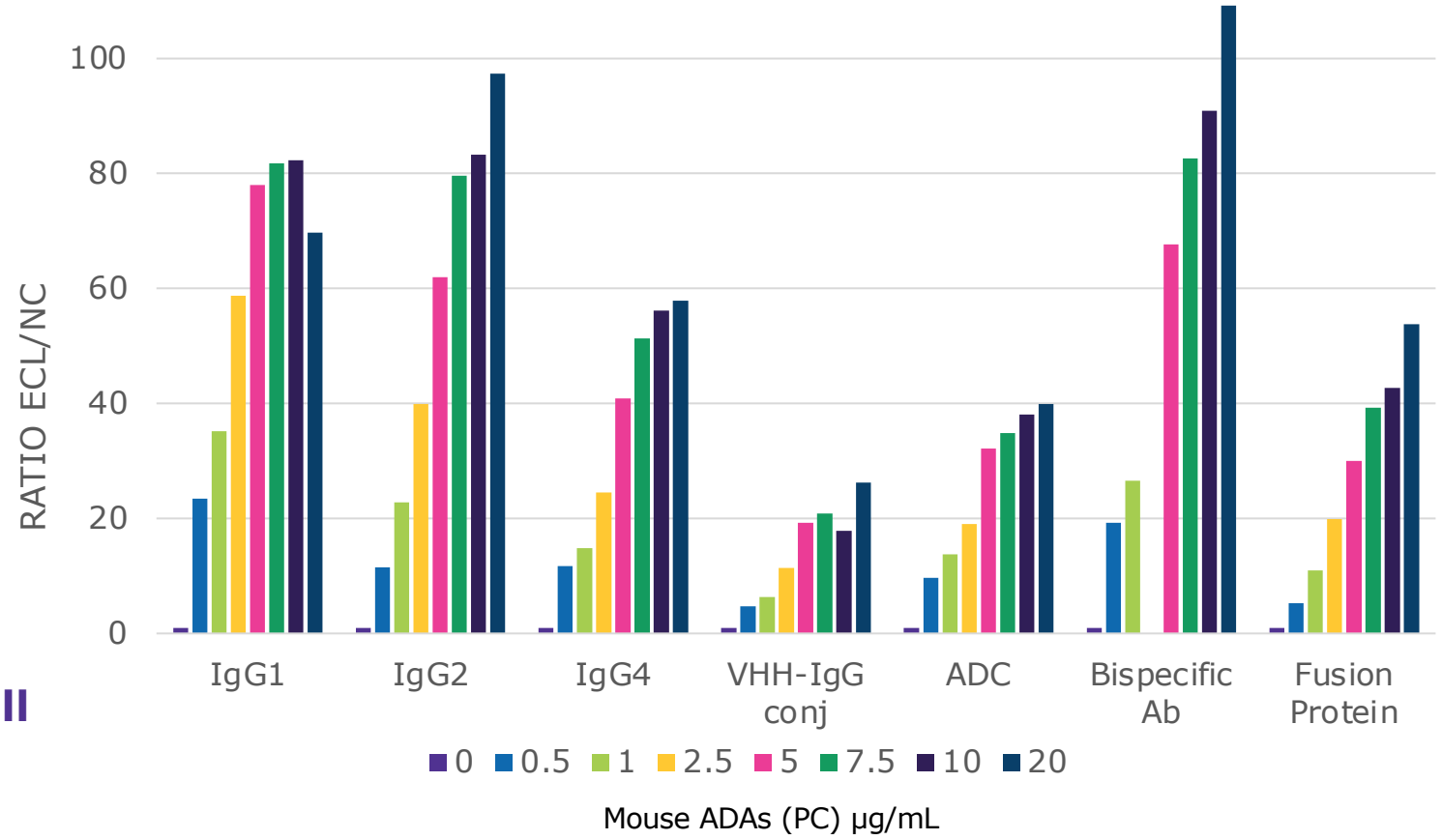
4. Plate reading
MesoScale Discovery

Biorender

Generic ADA assay
Test on Different Drug Modalities

Different drug modalities were tested to assess method performances

- IgG1
- IgG2
- IgG4
- VHH-IgG conjugated
- Antibody Drug Conjugate (ADC)
- Bispecific antibody SEED
- Fc Fusion Protein

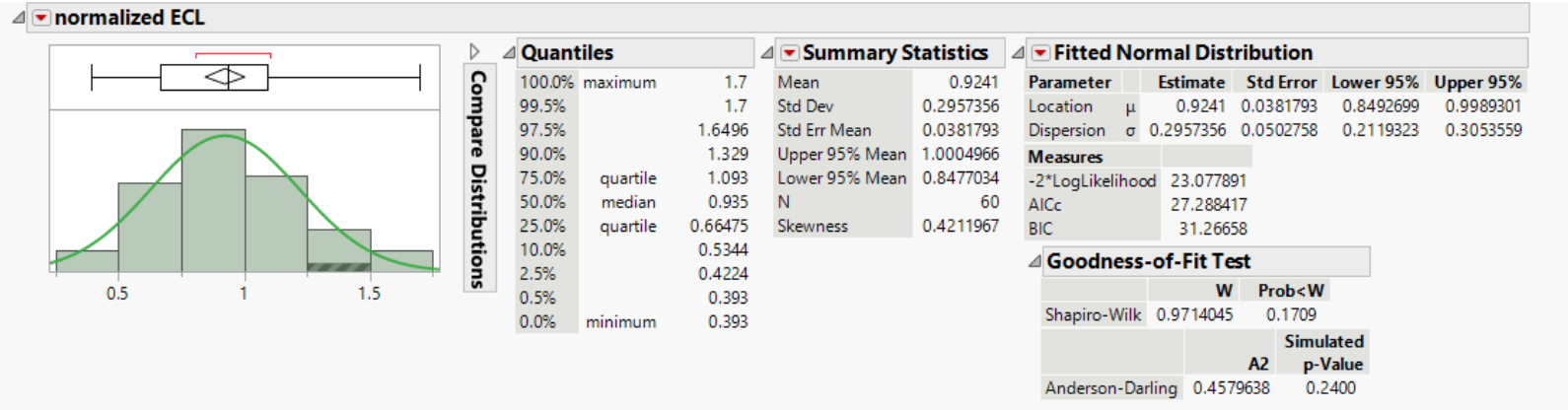


Different entities of responses with different molecules

Overall good performances with all the drug modalities tested

Method Qualification

- Three runs of 20 individuals (total 60 data points), FPR 1%



CP =mean+$t_{0,99}$ SD = 1,63

- Selection of LPC: 3 concentration levels tested within CP runs: middle LPC selected at 250 ng/mL (as tested positive in all determinations)
- **Drug Tolerance**:
  - **for HPC > 1.5 mg/mL drug** (max concentration tested)
  - **For LPC > 1.5 mg/mL drug** (max concentration tested)

Sensitivity: 250 ng/mL

Example of new drug «applicability», the short way: 2 days

Day 1

Assay feasibility

First testing with the Drug: different PC concentrations, drug tolerance, NC

Focus areas

- Checking the best drug concentration to apply
- Choice of the LPC and HPC concentration
- Definition of parameters to troubleshoot

Day 2

Sample Testing

15 individual naive samples + PCs + study samples + NC

Focus areas

- Dynamic CP calculated in run
- Direct sample testing: S/N comparison before and after treatment samples

Applicable to early discovery studies

Example of new drug «applicability», the long way: 1 week

Day 1

Assay feasibility

First testing with the Drug: different PC concentrations, drug tolerance, NC

Focus areas

- Checking the best drug concentration to apply
- Choice of the LPC and HPC concentration
- Definition of parameters to troubleshoot

Day 2-3

Screening CP and PC precision

4 analytical runs: 2 operators, 25 individual samples + NC, LPC and HPC

Focus areas

- Definition of Screening CP
- Choice of LPC for sample testing

Day 4

Drug Tolerance

If needed, evaluation of drug tolerance according to study expected levels

Focus areas

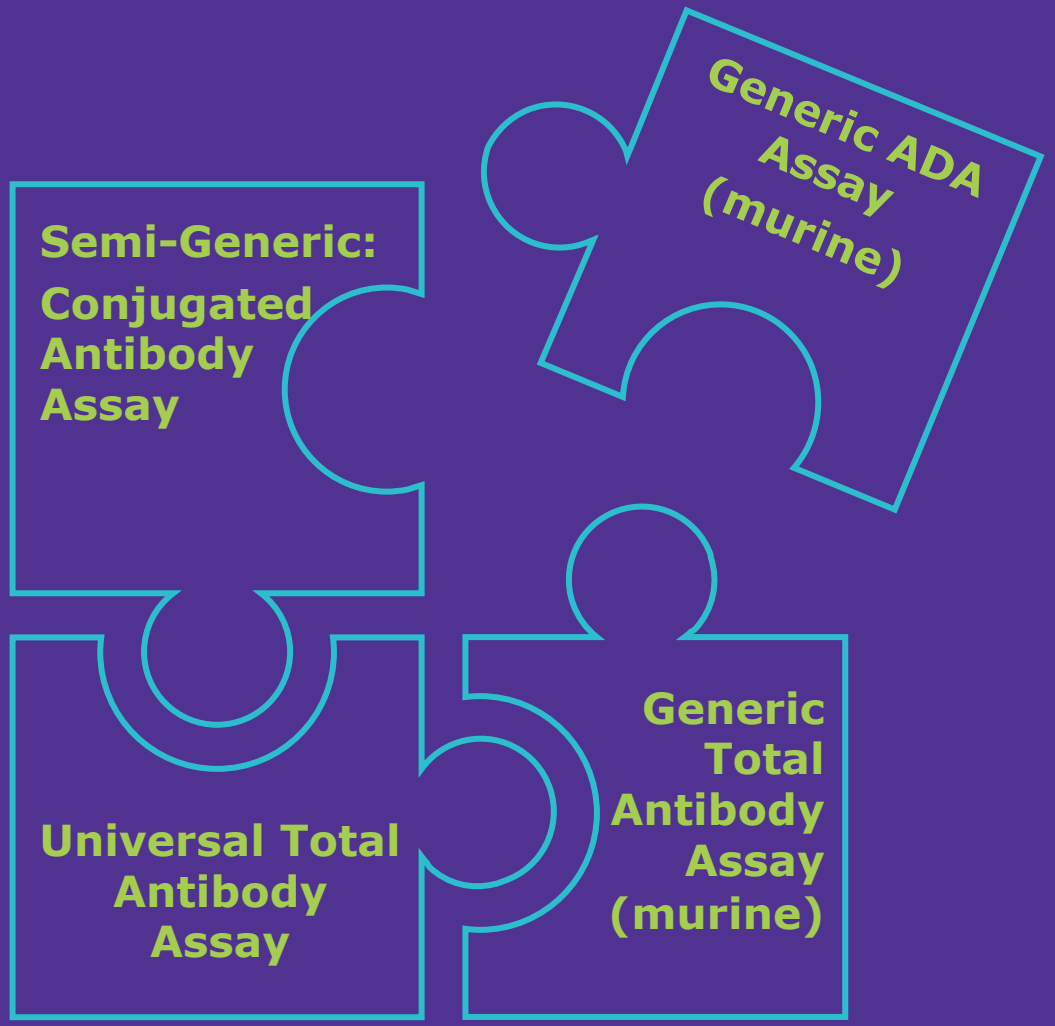
- Definition of drug tolerance level for LPC and HPC

Day 5

- Sample Testing

Applicable to more advanced studies

Solving the non-clinical puzzle



- For human IgG based molecules complete testing package up to GLP tox
- No method development required: only applicability to be confirmed for new test item/matrix

Pros & cons

1 No assay development needed

2 Fixed format: no need for labelling or specific reagents purchase from time to time

3 Quick turnaround (2-5 days from start to result generation)

1 IgG backbone needed (drugs with different structure will not be assessed)

2 Species-specific method (only suitable for murine studies)

3 IgG-specific method (will not capture IgM or other immunoglobulins)

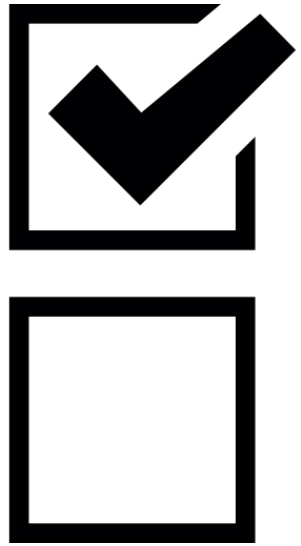
Thinking outside the Tick box

Measurement of ADA in nonclinical studies could be evaluated when there is:

1. evidence of altered PD activity
2. unexpected changes in exposure in the absence of a PD marker
3. evidence of immune-mediated reactions

Immunogenicity assessment should not be a tick box!

A strong scientific rationale should be driving immunogenicity evaluations.



future directions

- Application of the same method to all rodent matrices.
- Exploration of this generic approach for cynomolgus monkey matrix (evaluation of a commercial kit).
- Evaluation of applicability in minipig studies
- Improvement of sensitivity/throughput by applying different technologies.

Thanks

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LBA Laboratory Team