



## On the road to simplified and fit-for-purpose immunogenicity assays to support pre-clinical studies

*Development of ADA assays in cynomolgus monkey and mouse serum as part of a fusion protein project*

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# Introduction

- + Clinical ADA assessment:
  - + Immune responses may pose problems for both patient safety and drug efficacy
  - + Serious potential adverse effects:
    - + anaphylaxis
    - + cytokine release syndrome
    - + cross-reactivity with endogenous counterpart
- + Preclinical ADA assessment:
  - + Higher incidence of ADAs is expected in animals
  - + Interpretation of PK and TK data

***Why should be developing and validating them in the same way?***

- + New FDA guidelines published in January but focusing on clinical immunogenicity assessment
- + Shankar has been the reference for the last decade but alternative methods have since been published (Devanarayan, 2017)

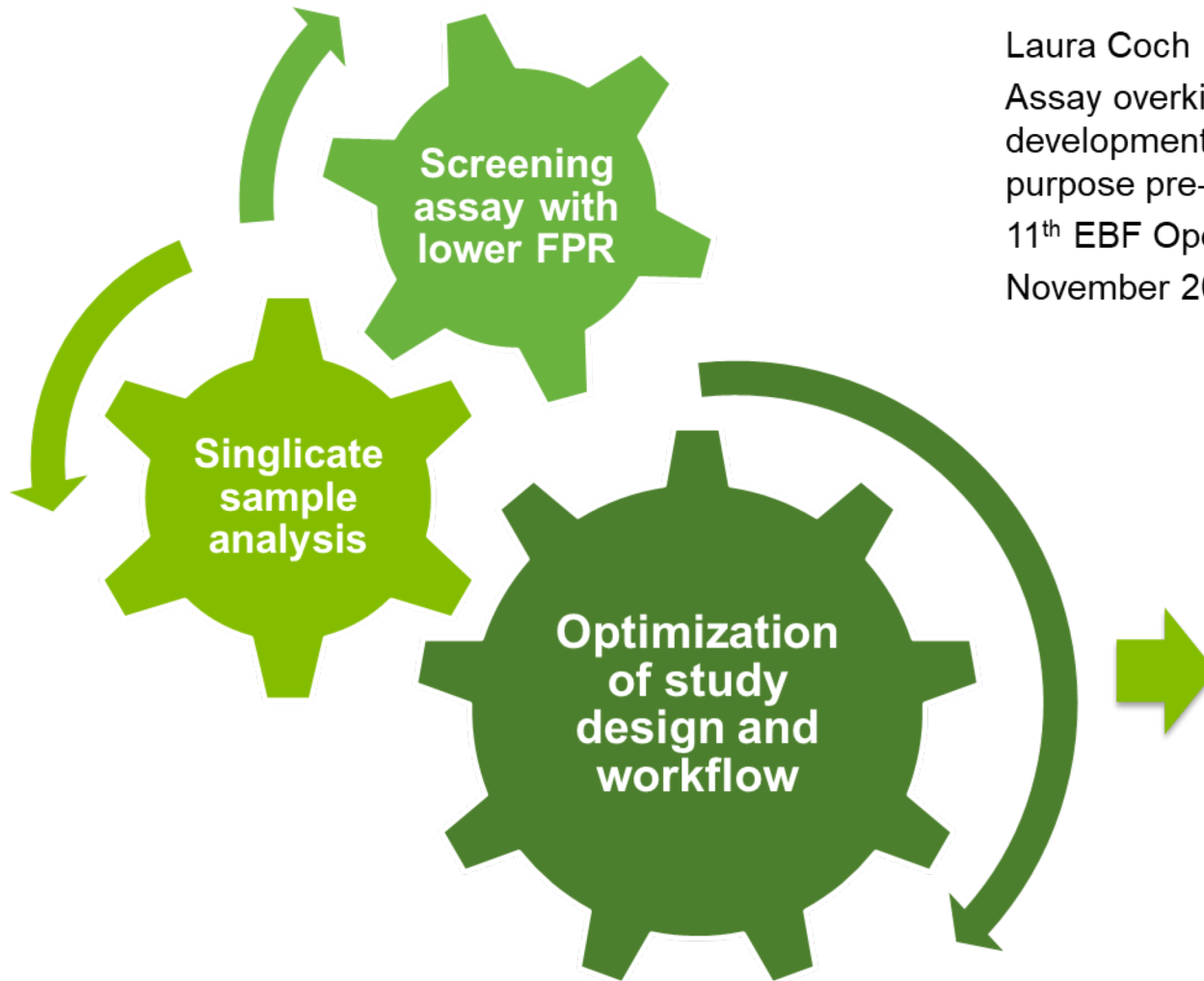
# ADA results of 4-week toxicology study

Treatment Group	Animal number	Time points				
		PT	Day 4	Day 11	Day 18	Day 25
Control group	1	109	101	110	97	100
	2	391	268	274	272	350
	3	122	147	137	123	167
	4	108	128	122	119	156
	5	104	127	106	129	125
	6	111	112	103	109	115
	7	108	114	108	115	125
	8	184	110	108	97	99
	9	113	125	104	116	119
	10	117	124	104	109	109
Low dose group	11	96	127	293	195	240
	12	94	95	619	1506	1475
	13	111	105	294	1009	521
	14	101	93	304	1414	608
	15	178	107	619	788	395
	16	112	224	842	934	609
	17	109	122	252	291	178
	18	162	119	427	347	395
	19	124	111	404	727	413
	20	110	96	261	471	218
High dose group	21	104	118	269	736	240
	22	100	98	169	292	162
	23	181	106	174	900	545
	24	99	116	260	1300	1010
	25	118	102	144	705	473
	26	104	98	121	142	509
	27	100	100	567	940	599
	28	140	103	157	255	237
	29	115	99	121	244	261
	30	113	99	132	305	270

	Mean response
NC	110
CPC	240
LPC	330
HPC	20000

- Consistent signal between plates (4 plates)
- LPC > arbitrary CP (CPC)
- High signal for all time points in 1 control animal: pre-existing ADA?
- Drug induced ADA in all dosed animals

# Previous suggestions



Laura Coch

Assay overkill: Practical solutions for development and validation of fit-for-purpose pre-clinical immunogenicity assays

11<sup>th</sup> EBF Open Symposium

November 2018

- Fit for purpose assays
- Efficient assay development and validation
- Reduction of time and resources

# Assay format and strategy



## Monkey

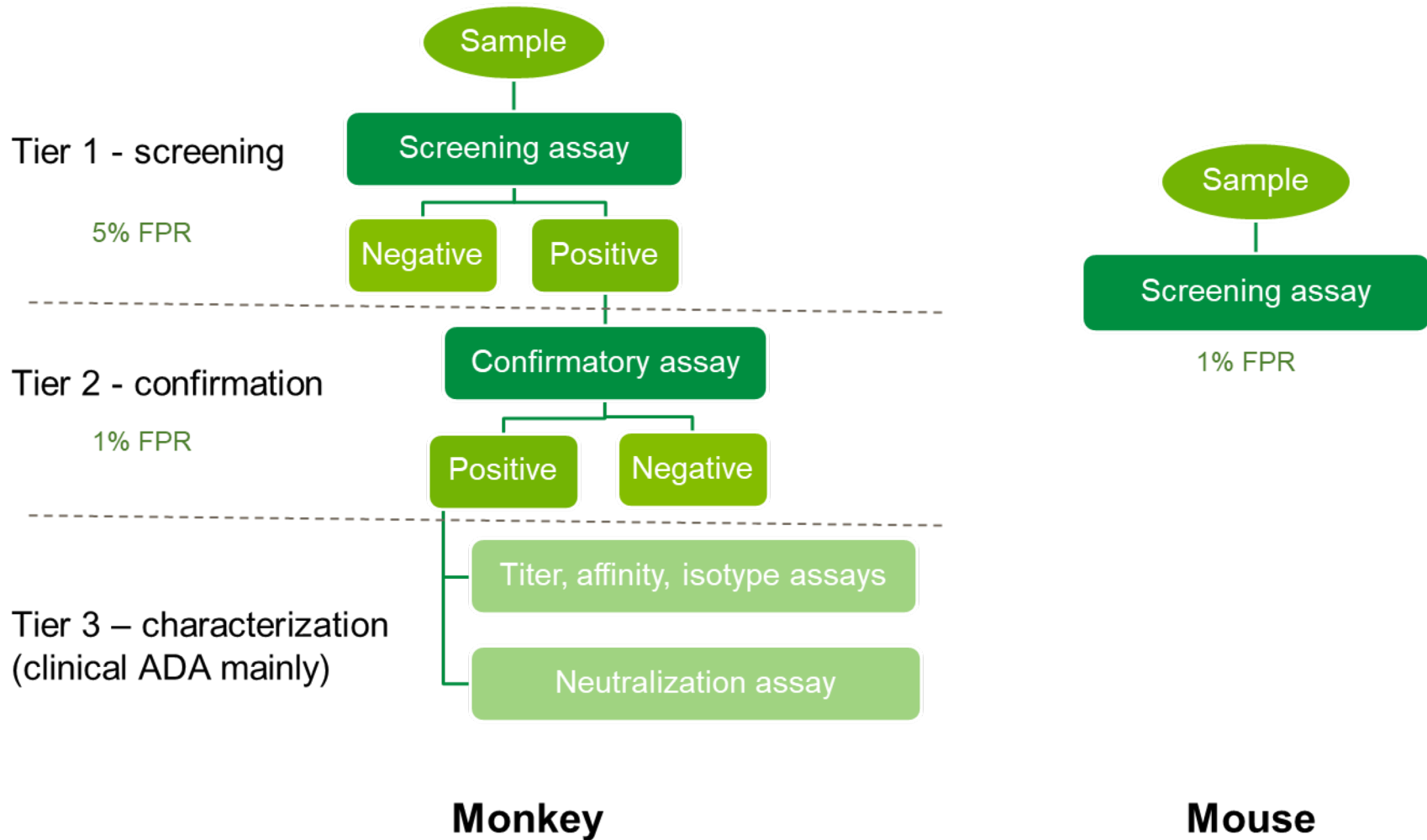
- Bridging assay
- MSD platform
- Acid dissociation
  
- Positive control A
- Two-tier approach



## Mouse

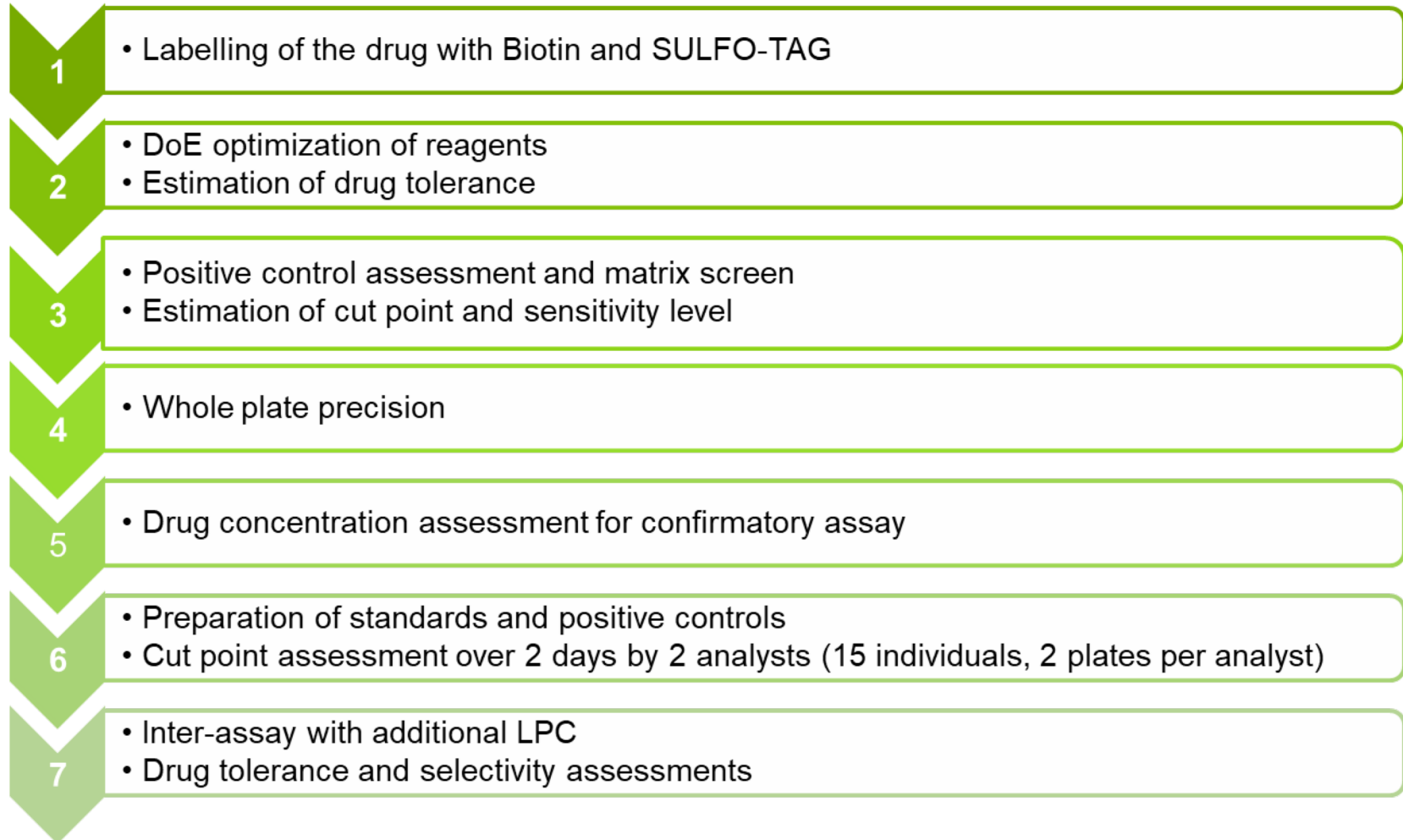
- Bridging assay
- MSD platform
- Acid dissociation
  
- Positive control B
- One-tier approach

# Screening assay with 1% False Positive Rate

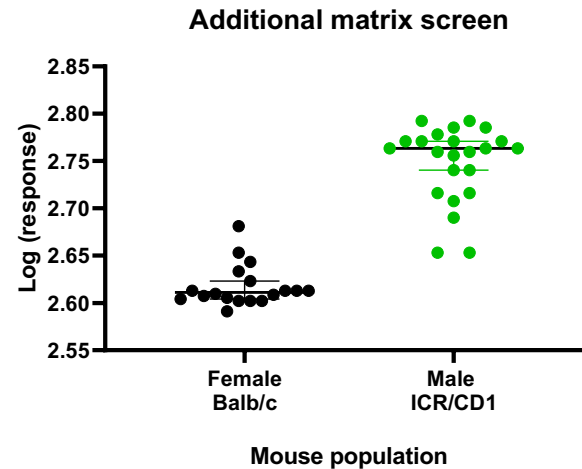
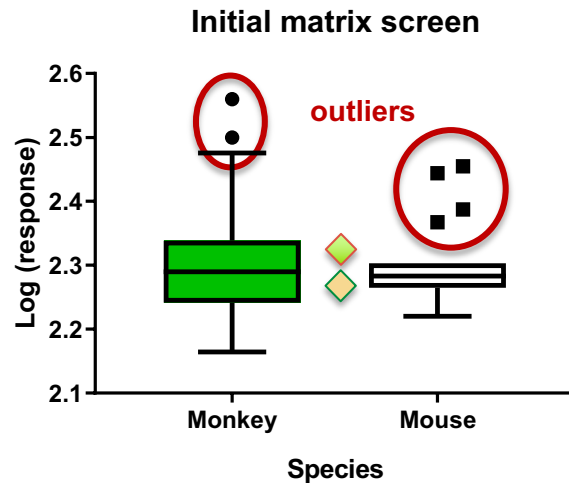


# Assay development for Cynomolgus plasma

+ Monkey chosen to develop the assay due to serum volume



# Matrix screen



- + 20 individuals screened for each species
- + Separate pool used for mouse due to low sample volume
- + Cut point to be reassessed in validation with same population as toxicology study

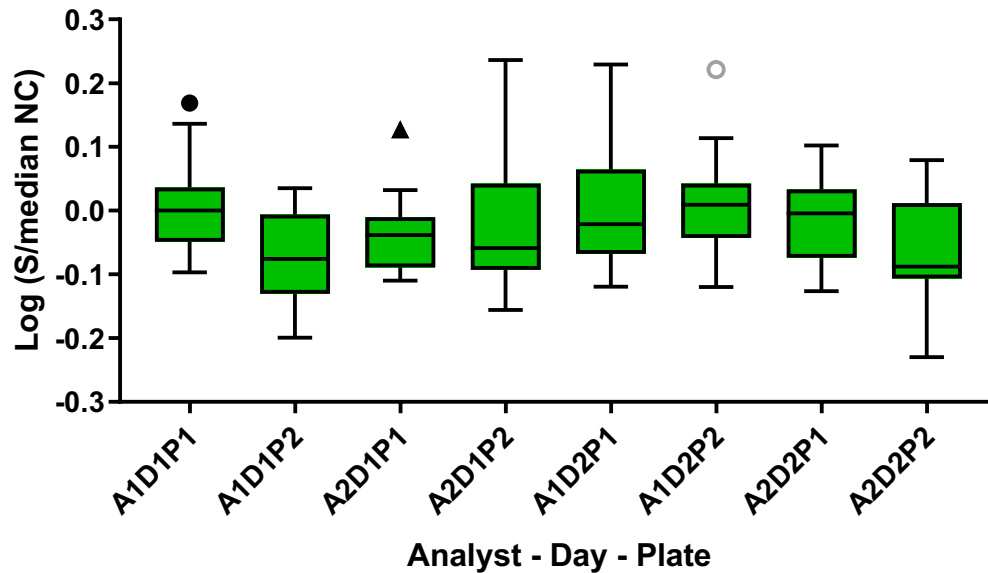


# Outlier identification

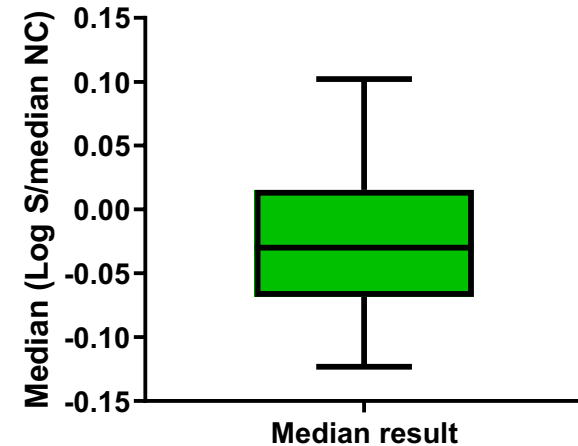
## + Devanarayan's approach

- + Normalization by the NC median
- + Log transformation
- + Exclusion of analytical and biological outliers

Analytical outlier identification

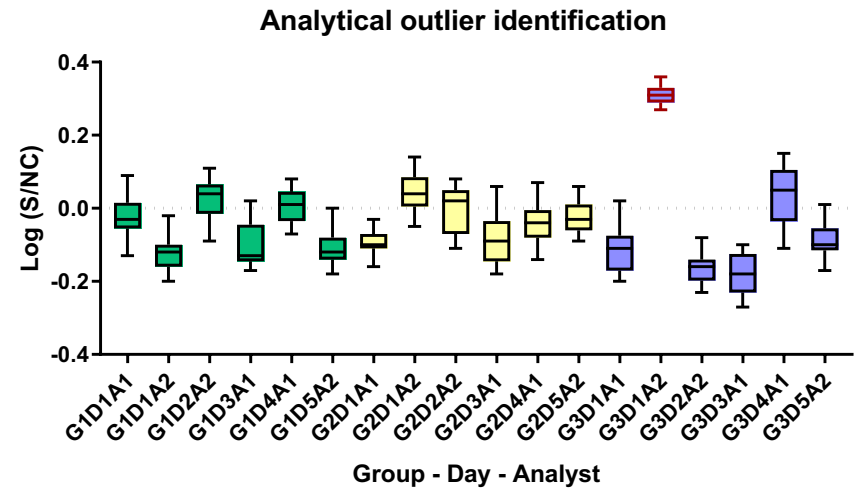
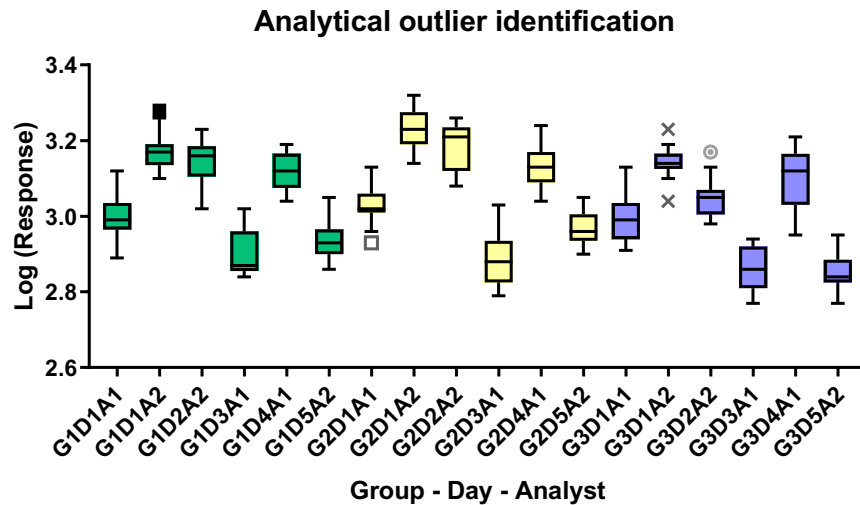


Biological outlier identification



# Normalisation with blank

- + Normalization with the blank previously used in a clinical study



- + Only duplicate wells used during the cut point assessment leading to the exclusion of one dataset (Group 3 Day 1 Analyst 2)

# Cut point assessment plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	Std 6	Std 6	Ind 4	Ind 4	Ind 12	Ind 12	Ind 2	Ind 2	Ind 10	Ind 10
B	NC	NC	Std 7	Std 7	Ind 5	Ind 5	Ind 13	Ind 13	Ind 3	Ind 3	Ind 11	Ind 11
C	LPC	LPC	Std 8	Std 8	Ind 6	Ind 6	Ind 14	Ind 14	Ind 4	Ind 4	Ind 12	Ind 12
D	Std 1	Std 1	Std 9	Std 9	Ind 7	Ind 7	Ind 15	Ind 15	Ind 5	Ind 5	Ind 13	Ind 13
E	Std 2	Std 2	Std 10	Std 10	Ind 8	Ind 8	Blank	Blank	Ind 6	Ind 6	Ind 14	Ind 14
F	Std 3	Std 3	Ind 1	Ind 1	Ind 9	Ind 9	Blank	Blank	Ind 7	Ind 7	Ind 15	Ind 15
G	Std 4	Std 4	Ind 2	Ind 2	Ind 10	Ind 10	LPC	LPC	Ind 8	Ind 8	NC	NC
H	Std 5	Std 5	Ind 3	Ind 3	Ind 11	Ind 11	Ind 1	Ind 1	Ind 9	Ind 9	NC	NC

No drug

Drug

- + Cut point assessment over 2 days by 2 analysts (15 individuals, 2 plates per analyst)
- + Order of individuals changed between plates 1 and 2

# Results of cut point assessment

## + Monkey study:

- + Evidence of statistically significant differences between the means for the analysts and days
- + For analyst 1, the correction factor (CF) was 45 and for analyst 2 it was 1
- + Sensitivity with 95% CL: 157 ng/mL (analyst 1) and not calculable for analyst 2
- + Cut point for confirmatory assay: 74% inhibition after
- + LPC prepared at 160 ng/mL and above cut point in all inter-assays (analyst 1)

## + Mouse study:

- + Evidence of statistically significant differences between the means for the analysts and days
- + For analyst 1, the correction factor (CF) was 72 and for analyst 2 it was 1
- + Sensitivity with 95% CL: 28 ng/mL (analyst 1) and 33 ng/mL (analyst 2)
- + Sensitivity with 99% CL: 523 ng/mL (analyst 1) and 77 ng/mL (analyst 2)
- + LPC prepared at 35.0 ng/mL and above cut point in all inter-assays (analyst 1)

# Following steps

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## + New cut point assessment in validation using:

- + Same strain as toxicology study
- + Assessment over 3 days
- + Devanarayan's method

## + Continuous improvement

## + Giving choice to customers

- + Shankar or Devanarayan approach
- + Screening assay only or screening followed by confirmatory
- + Singlicate or duplicate sample analysis

# Acknowledgments

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- + Laura Coch
- + James Lawrence
- + Paolo Repeto
- + Jennifer Thomas
- + Dean Brown and the BBC immunoassay team in Huntingdon



Together, we make the world a  
safer and healthier place to live.

