Troubleshooting Analyte Carryover on the Gyrolab

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Assay Background

Assay 1

Aim → Design of Experiments (DoE) Method Development → Final Assay Conditions

Develop a Gyrolab PK assay with the following parameters:

LLOQ: 100-300 ng/mL

HLOQ: 50,000 ng/mL

Screening Run:

- Assay buffer diluent
- Capture concentration
- Detection concentration
- MRD
- CD Type

Optimisation Run:

- Capture concentration
- Detection concentration

Range: 300 to 30,000 ng/mL in human serum

Capture: neutralising anti-id at 600 nM in Rexxip A

Detect: mouse anti-human IgG at 25 nM in Rexxip F

MRD: 1/10 in Rexxip A

Bioaffy CD: 200

GSK

Problem: erratic positive bias across the range

Quality Control Conc. (ng/mL)	Run 01	Run 02	Run 03	Run 04
300	-13.00	2.33	4.67	7.33
	-3.00	13.00	35.00	10.33
	-4.00	-3.67	-7.00	28.67
	3.67	3.33	15.67	21.00
	-8.67	12.00	-12.33	9.67
	-3.67	29.00	15.67	23.33
900	5.44	7.00	-1.11	22.22
	15.56	12.22	2.22	14.44
	4.78	10.44	-9.33	13.33
	12.22	15.56	6.11	11.11
	3.78	18.89	-11.56	26.67
	13.33	21.11	-6.78	16.67
3000	14.33	4.33	-1.33	9.67
	17.00	11.67	10.33	15.00
	10.33	4.67	4.33	12.67
	13.67	9.00	9.33	31.00
	16.00	14.67	10.00	17.33
	27.33	21.67	17.00	16.00
24000	9.17	2.50	4.17	5.00
	20.83	10.83	13.75	17.92
	6.25	5.42	7.50	4.58
	22.08	14.17	18.33	13.33
	-3.33	7.08	12.50	16.67
	3.75	12.92	26.25	25.83
30000	24.00	5.00	1.00	*-26.33*
	20.00	14.00	8.33	14.67
	-1.00	0.00	-3.67	10.00
	10.67	10.33	11.67	17.33
	4.33	7.67	2.00	0.00
	9.00	-3.33	22.67	8.33

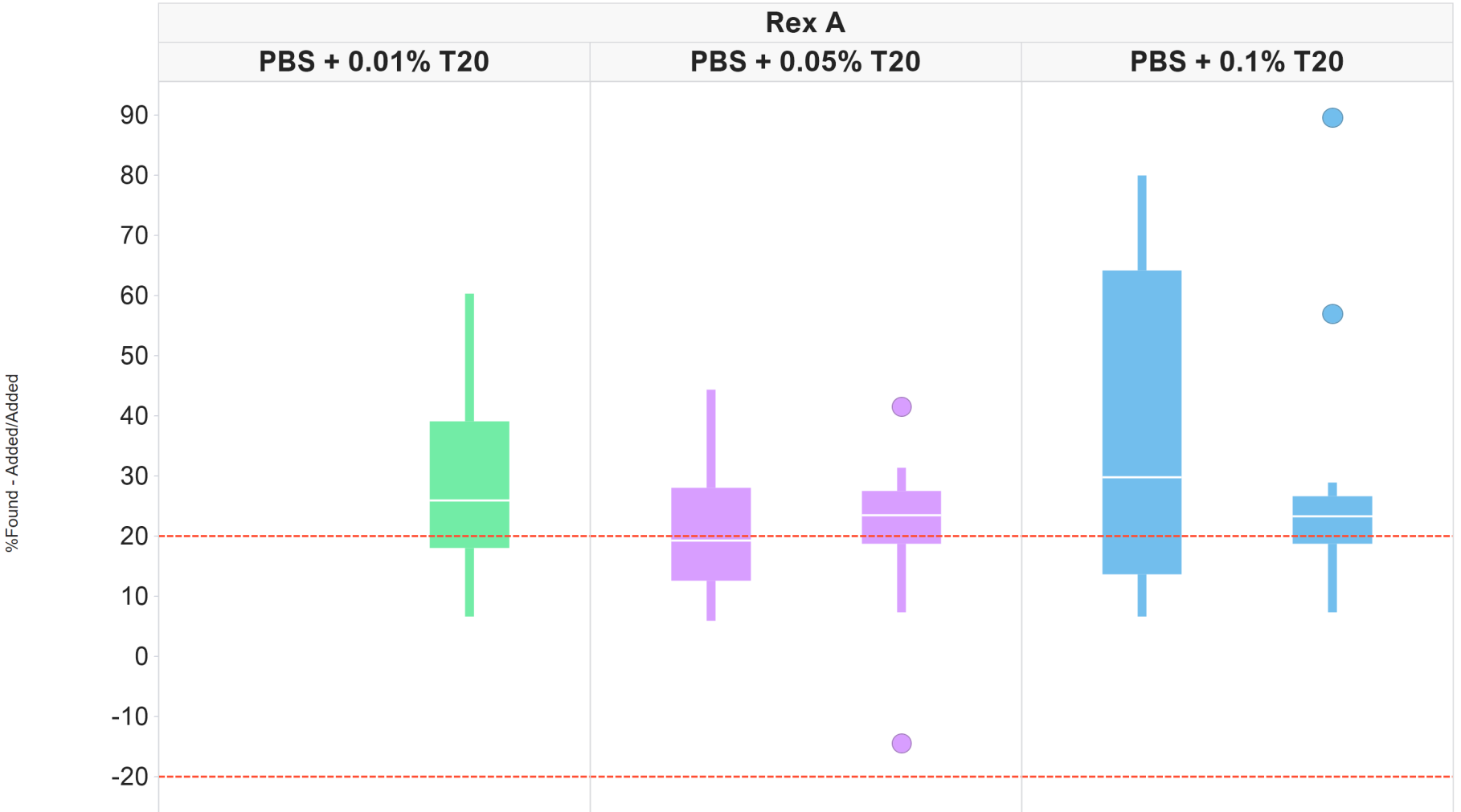
Note: 17/18 QC failures attributed to positive bias

Carryover assessment

Testing format

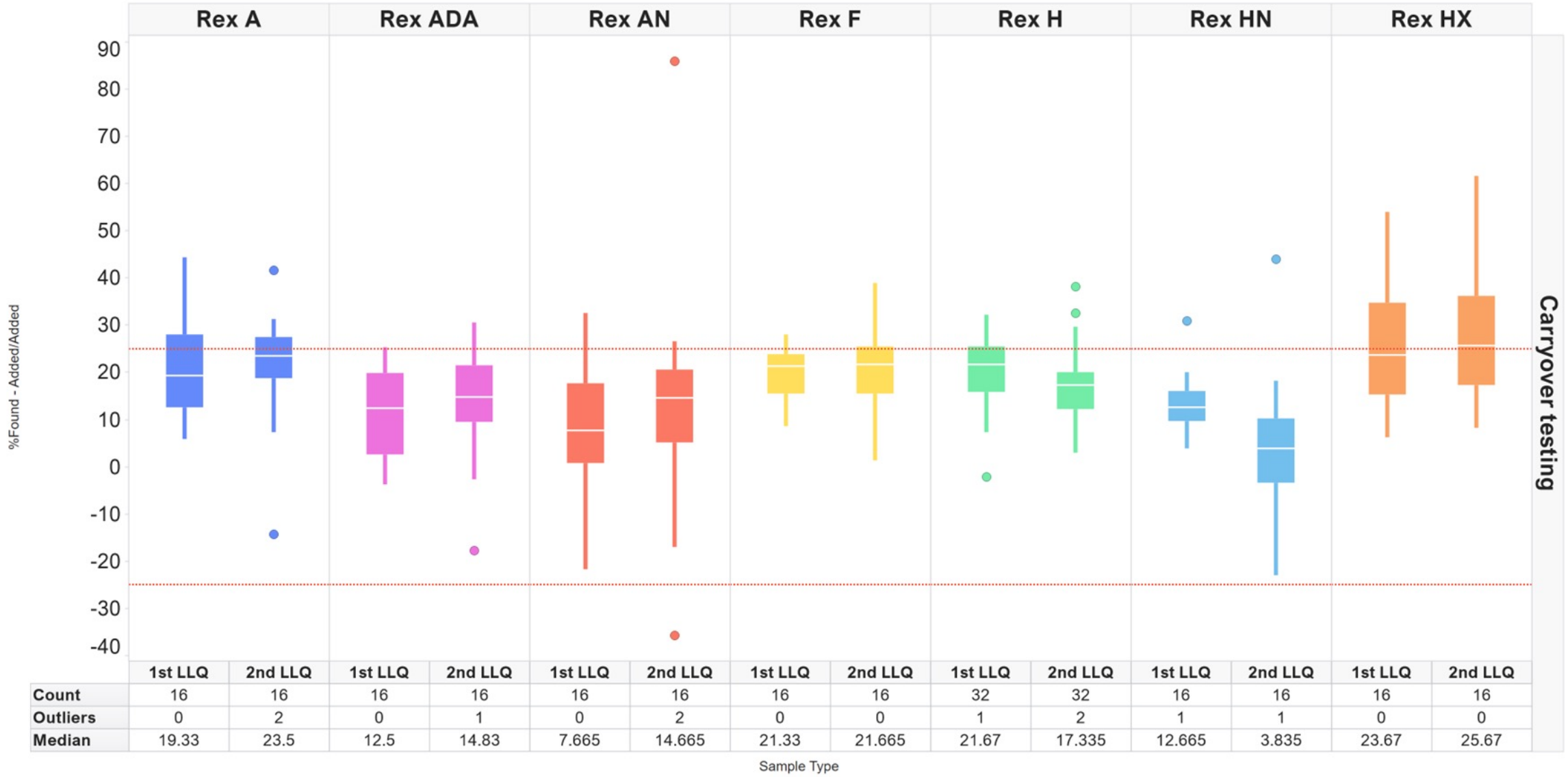
- Performed by assessing the change in response/ back-calculated concentration of a blank or LLOQ sample after a high concentration (or HLOQ) sample has been taken up by the instrument.
- Format used to perform Carryover assessment:
  - LLOQ Sample (Row A)
  - Calibration line (Row B)
  - HLOQ Sample (Row C)
  - LLOQ Sample (Row D)

Troubleshooting Carryover: On-plate wash buffer



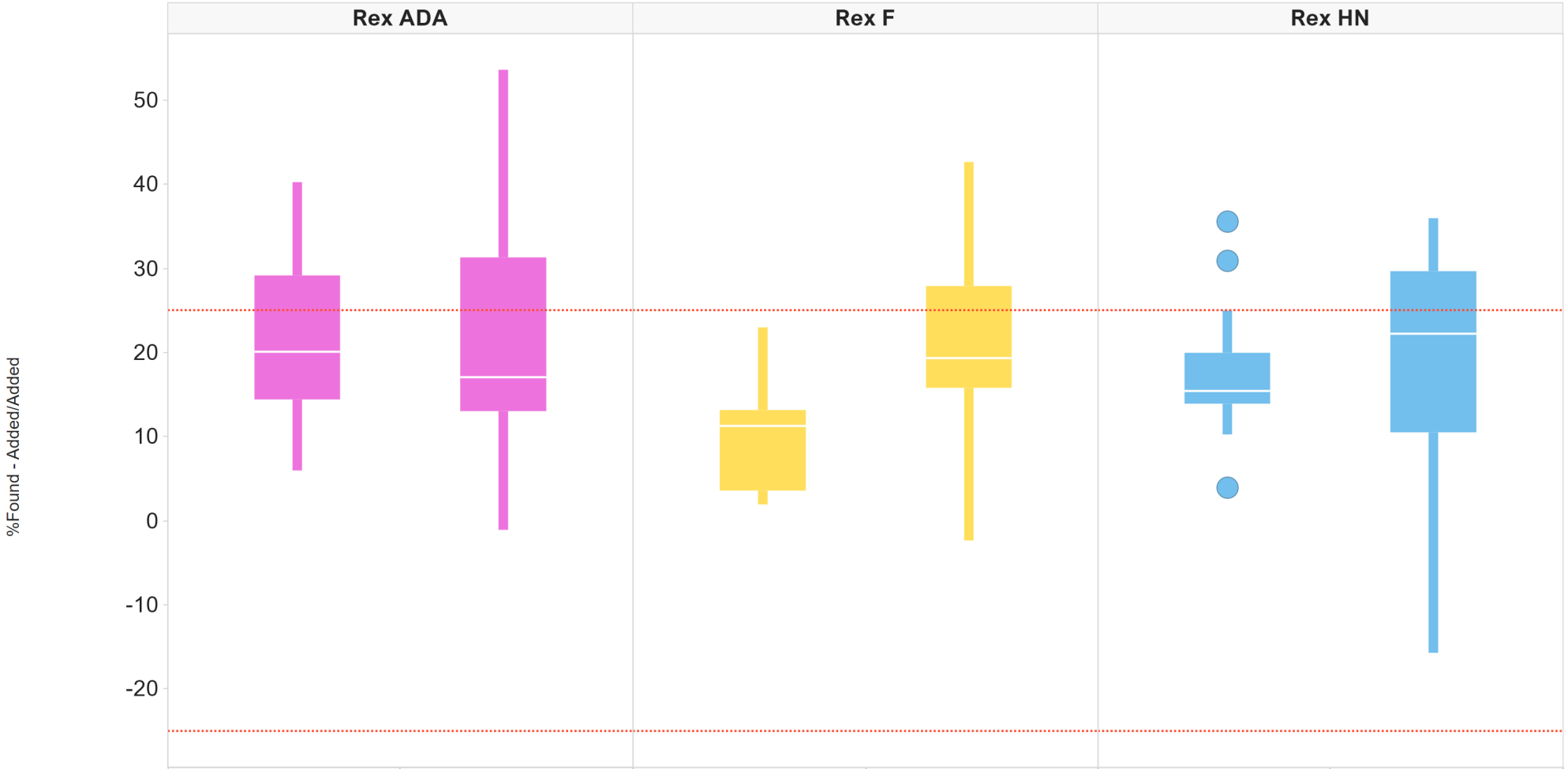
	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ
Count		32	16	16	16	14
Outliers		0	0	2	0	2
Median		26	19.33	23.5	29.835	23.33

Troubleshooting Carryover: Assay Buffer



	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ
Count	16	16	16	16	16	16	16	16	32	32	16	16	16	16
Outliers	0	2	0	1	0	2	0	0	1	2	1	1	0	0
Median	19.33	23.5	12.5	14.83	7.665	14.665	21.33	21.665	21.67	17.335	12.665	3.835	23.67	25.67

Troubleshooting Carryover: Assay Buffer & Capture Diluent Matching



	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ	1st LLQ	2nd LLQ
Count	16	16	16	16	16	16
Median	20.165	17.165	11.33	19.335	15.5	22.33
Outliers	0	0	0	0	3	0

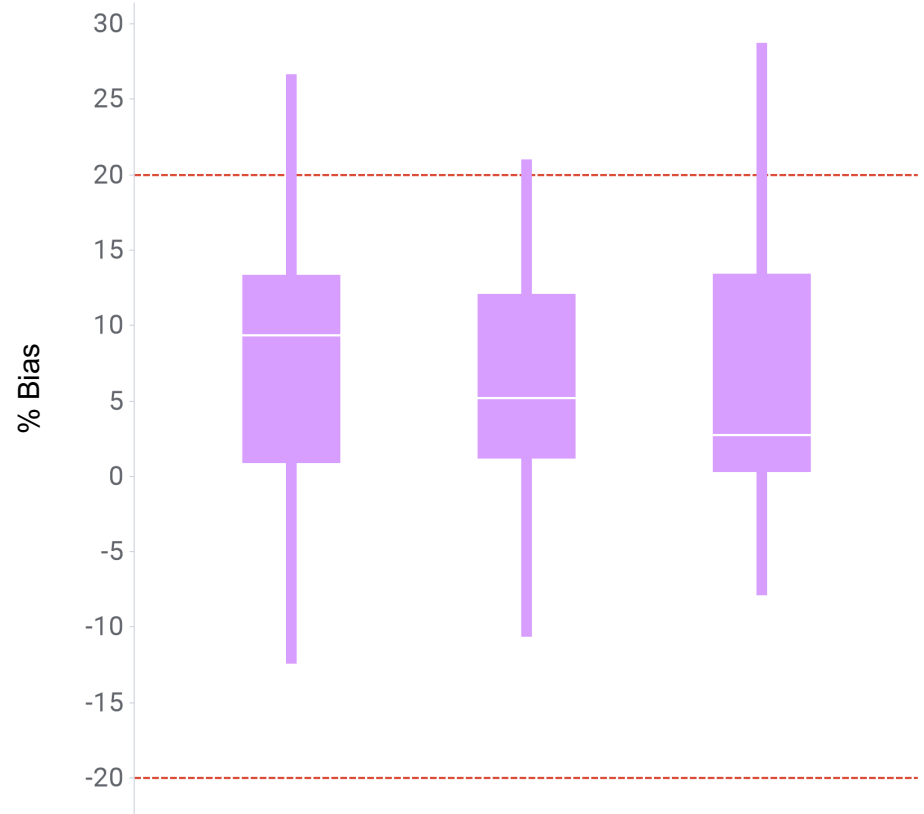
Troubleshooting Summary

Assay 2

Troubleshooting	Final Assay Conditions	Validation
• Adjusted Tween-20 concentrations within on-plate wash buffer had **no impact** • Assay buffer **impacted** observed carryover • Matching assay buffer with capture diluent had **no impact**	**Range:** 300 to 30,000 ng/mL in human serum **Capture:** neutralising anti-id at 600 nM in Rexxip A **Detect:** mouse anti-human IgG at 25 nM in Rexxip F **MRD:** **1/10 in Rexxip HN** **Bioaffy CD:** 200	**Assay 2 was successfully validated, however robustness issues were still observed during sample analysis.** Further assay troubleshooting was conducted.

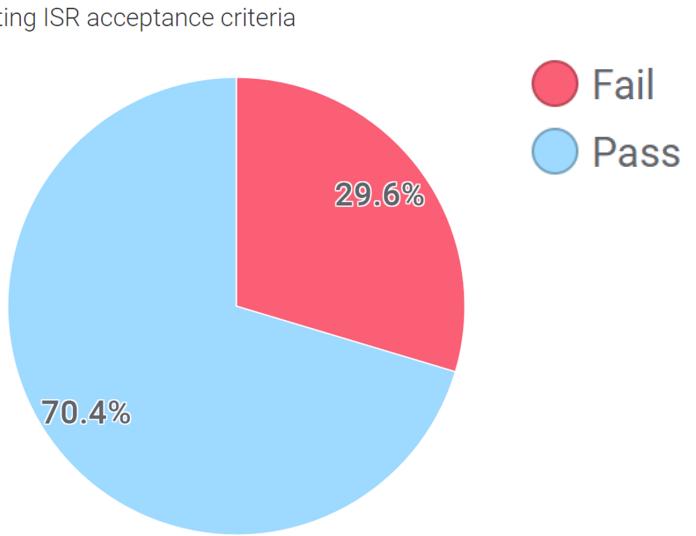
QC trending and ISR

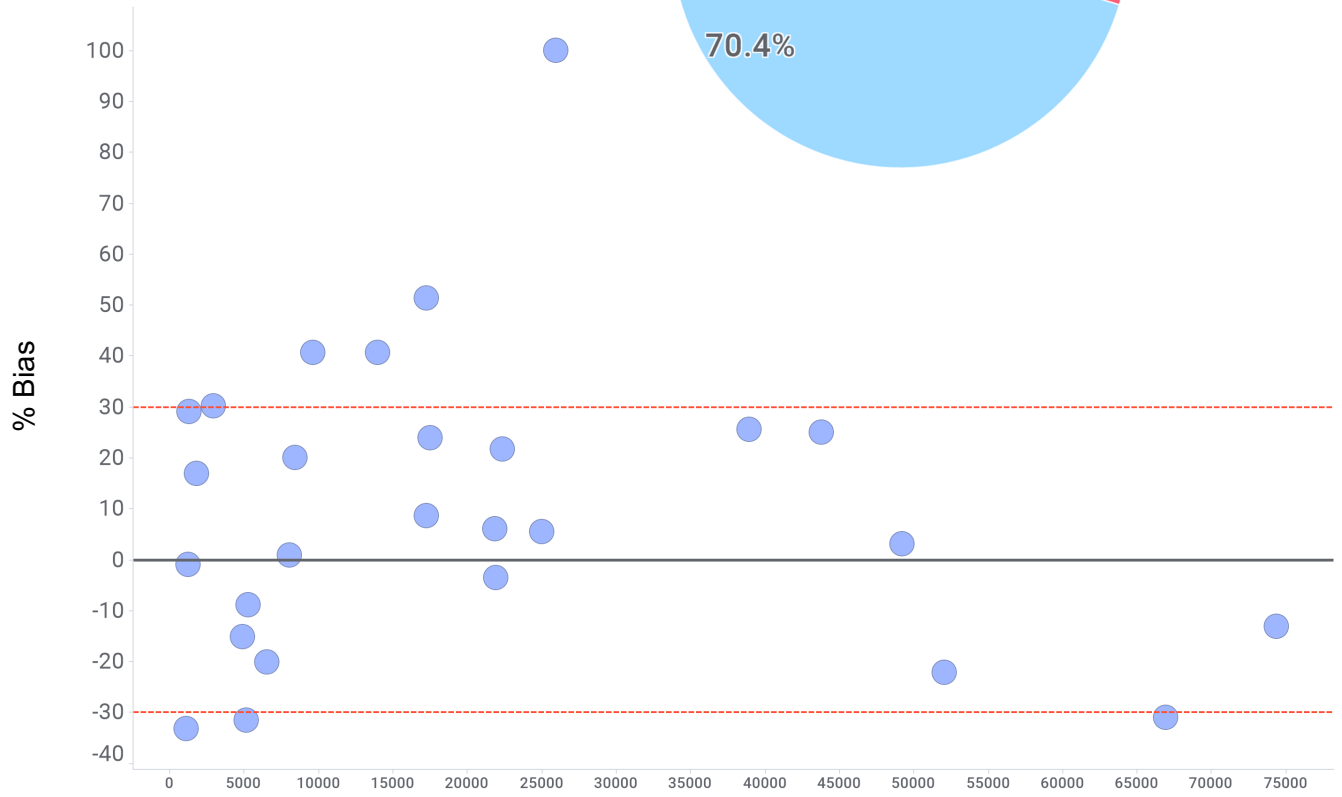
Assay 2



	900	3000	24000
Count	20	20	36
Median	9.385	5.17	2.71
Max	26.67	21.00	28.75
Min	-12.44	-10.67	-7.92

Nominal Concentration (ng/mL)





Concentration (ng/mL)

Accuracy & Precision

Assay 3

Range: 500 to 50,000 ng/mL in human serum

Capture: neutralising anti-id at 600 nM in Rexxip A

Detect: mouse anti-human IgG at 25 nM in Rexxip F

MRD: 1/100 in Rexxip HN

Bioaffy CD: 200

Assay 3 was successfully validated

	Nominal Concentration (ng/mL)				
	500	**1500**	**5000**	**40000**	**50000**
Mean (ng/mL)	467	1380	4750	37300	44300
SD	71.2	141	499	3310	5120
%CV	15.25	10.22	10.51	8.87	11.56
%Bias	-6.6	-8	-5	-6.75	-11.4

Troubleshooting Summary

Assay 1 → Assay 3

Assay 1

- Problem: Carryover
- Adjusted Tween-20 concentrations within on-plate wash buffer had **no impact**
- Assay buffer **impacted** observed carryover
- Matching assay buffer with capture diluent had **no impact**

Assay 2

- Robustness issues observed in the form of **borderline ISR** and **poor precision** across study level QC's
- Assay range **shifted**, to increase the LLOQ – **improved A&P**
- **MRD increased** to reduce matrix effects

Assay 3

- Successfully validated

Will be used to support future work

Adapted Method Development Workflow

Key learnings

- Carryover can manifest itself in several ways, understanding the impact of reagent and analyte physio-chemistry are essential in troubleshooting
- We now explicitly test for carryover prior to DoE method development and alter the parameter input into the DoE model accordingly
- Following this, we perform additional robustness testing, and selectivity assessments to confirm optimised conditions are suitable for method validation.

Gyrolab maintenance:

- Bi-weekly Deep clean
- Daily Wash Station Clean
- Desorb between runs

Acknowledgements

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