



## Measuring assay robustness across the life cycle of a bioanalytical method

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EBF 7<sup>th</sup> Open Symposium  
20<sup>th</sup> November 2014

### What is (assay) robustness?



Vauxhall/Opal Chevette (ca. 1985)

– Manufactured to predefined acceptance criteria (e.g. 4 wheels, an engine, lights)



Validation /  
Qualification

– Periodic performance check (MOT)



ISR

– Day to day reliability and robustness



- An ever increasing list of assay validation requirements **will not necessarily** increase assay robustness or make an assay “better”
- An assay can be extremely robust (or conversely un-robust) regardless of whether it is validated to BMV guidance documents or scientifically validated

## Understanding Assay Robustness



Understanding assay robustness is important (to GSK) because...

- Large diverse portfolio of assets (and assays)
- Economic drivers related to run failures
- A desire to transfer reliable and robust assays to CRO partners as part of our outsourcing strategy

Possible “markers” for a reliable assay:

1. Validation or qualification of an assay against predefined criteria
2. Conduct of ISR to demonstrate that an assay is reproducible
3. Assay pass/failure rates; to show reliability of the assay during routine use
4. Assay transfer between laboratories; the ability for additional laboratories to set up and validate (or qualify) an existing assay
5. Cross-validation between laboratories – to show that equivalent study data can be generated at different laboratories

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## How to spot a “lemon”



- Work-list pass failure rates give a “feel” for bioanalytical lab success
  - < 10% failure rate “seems reasonable”
  - not a statistical approach – “gut feel”
- This does not (generally) give info related to the **robustness of individual assays** (when portfolio of assays is large)
- GSK has adopted a Key Performance Indicator to help identify “less robust” assays



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## Key Performance Indicators (KPIs)



- A KPI is intended to show "direction of travel"
  - o To allow remedial action to affect the outcome
  - o Lag measure: the end goal
  - o Lead measure: a performance indicator

### Lag Measure:

Lose 10kg by summer vacation



### Lead Measure:

Monitor & control daily calorific intake



Lead measures are not always easy to identify or measure

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## Assay Robustness KPI



Assay Name	accepted study worklists (1Q14)	rejected study worklists (1Q14)	accepted validation worklists (12 months)	rejected validation worklists (12 months)	accepted study worklists (12 months)	rejected study worklists (12 months)
GSK1	2	0	0	0	2	0
GSK2	3	0	3	1	31	6
GSK3	3	0	0	0	24	4
GSK4	2	1	0	0	10	2
GSK5	8	4	1	0	12	7
GSK6	2	0	2	0	17	4
GSK7	7	4	0	0	20	9
GSK8	3	0	0	0	3	0
GSK9	3	0	2	0	8	0
GSK10	2	0	0	0	2	0
GSK11	2	2	1	0	6	2
GSK12	14	0	1	0	73	1
GSK13	2	0	0	0	4	0
GSK14	1	0	0	0	3	1
GSK15	1	0	0	0	7	1
GSK16	2	0	1	0	10	1
GSK17	1	0	0	0	1	0
GSK18	2	0	0	0	2	0
GSK19	15	1	1	0	15	1
GSK20	1	0	0	0	1	0
GSK21	2	4	7	1	2	4
GSK22	4	0	13	2	4	0
GSK23	4	1	6	4	4	1
GSK24	1	0	22	5	18	3
GSK103	16	0	0	0	35	0
GSK104	2	0	0	0	2	0
GSK105	2	2	1	0	8	7
GSK106	1	0	0	0	2	0
GSK107	1	0	0	0	1	0
GSK108	1	0	1	0	1	0
GSK109	1	0	0	0	1	0
count (active assays)	109	31				
# of runs	415	46				
% failed runs		10				
active assays with 0 or 1 failed study runs		102				
active assays with 2 or more failed study runs		7				

### Lag Measure:

Successful assay transfer to CRO  
Any issues are resolved and not assay related

### Lead Measure:

Monitor the occurrence of assays with  $\geq 2$  run failures each quarter (3 month period)

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## Assay Robustness KPI

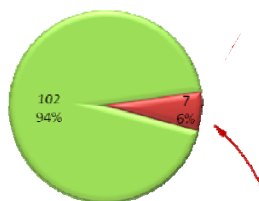


### Lead Measure:

Monitor the occurrence of assays with  $\geq 2$  run failures each quarter (3 month period)



### Active Assays During 1Q14



- active assays with 0 or 1 failed study runs
- active assays with 2 or more failed study runs

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## Assay Robustness KPI

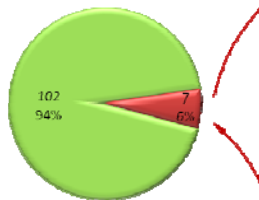


### Lead Measure:

Monitor the occurrence of assays with  $\geq 2$  run failures each quarter (3 month period)

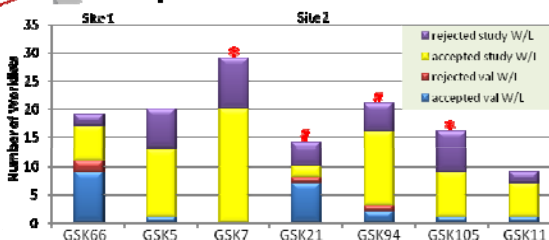


### Active Assays During 1Q14



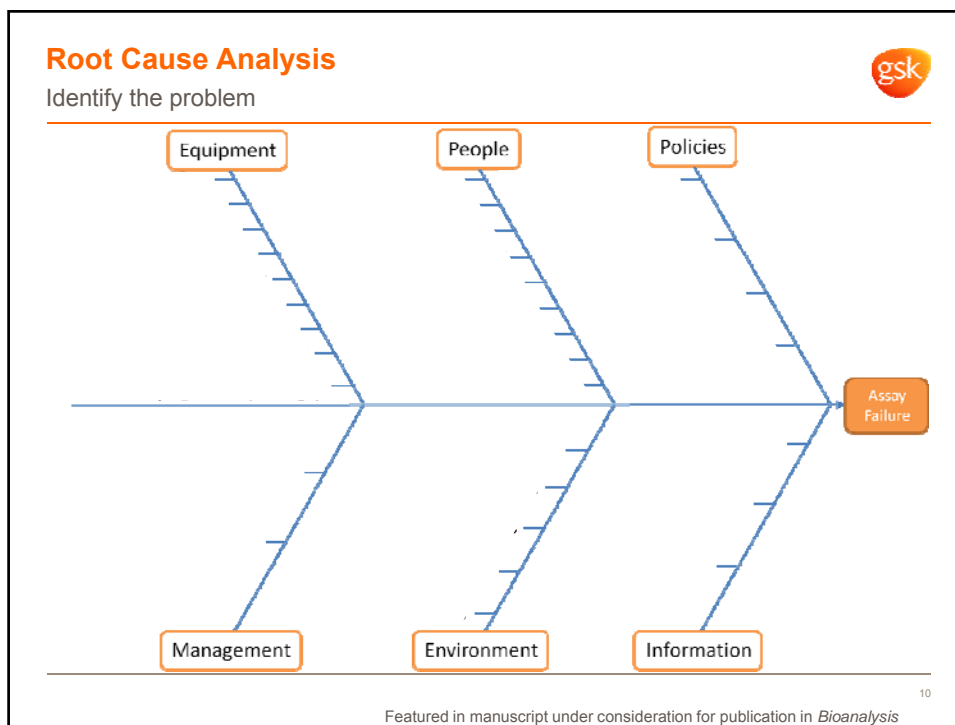
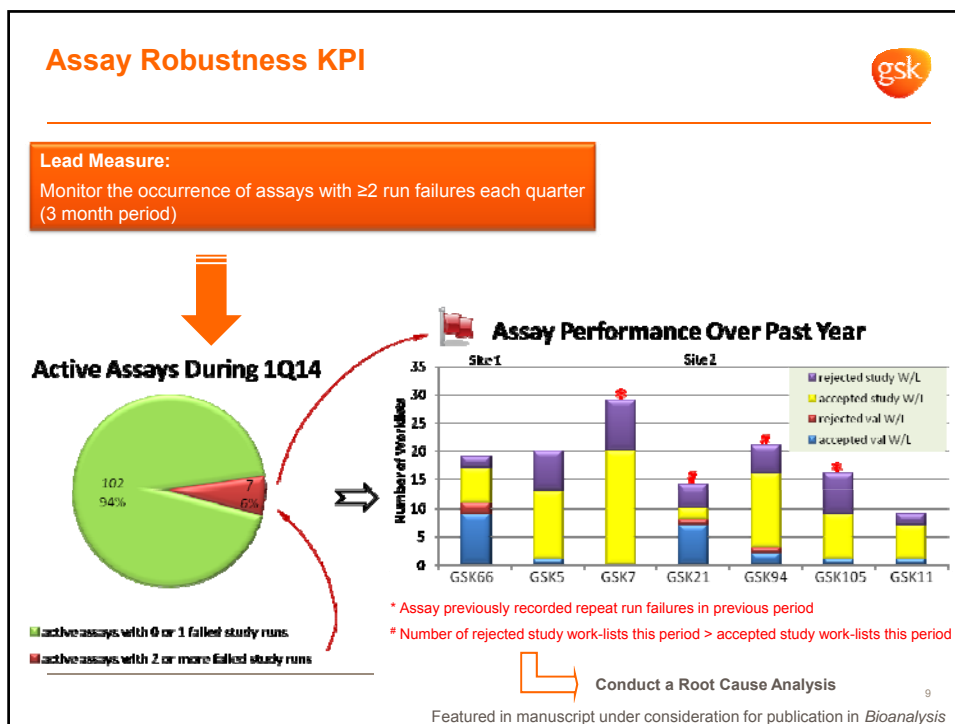
- active assays with 0 or 1 failed study runs
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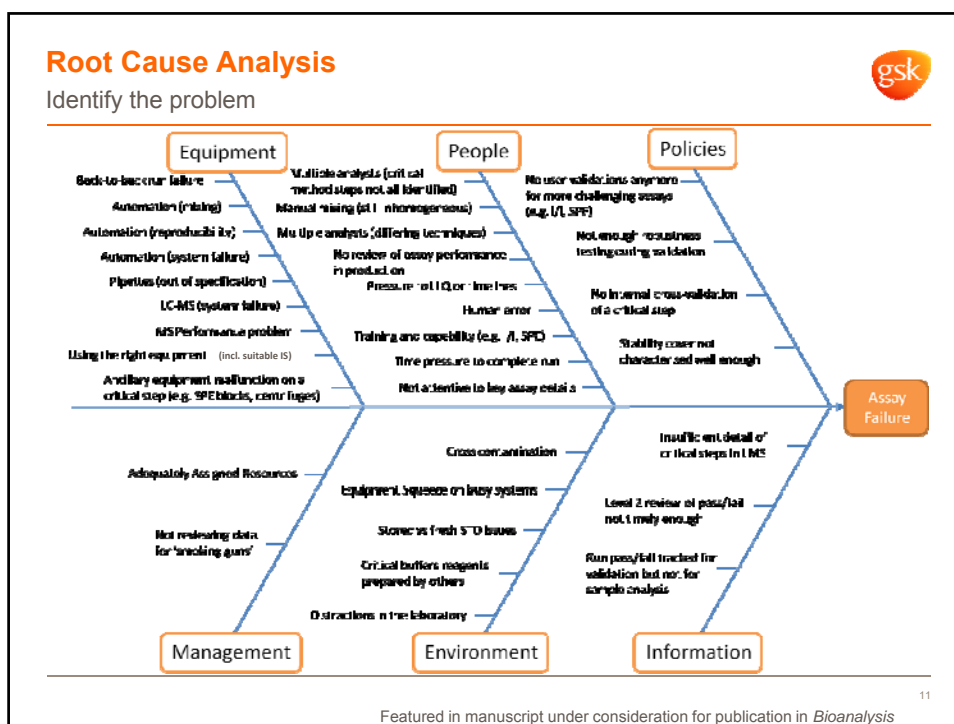
### Assay Performance Over Past Year



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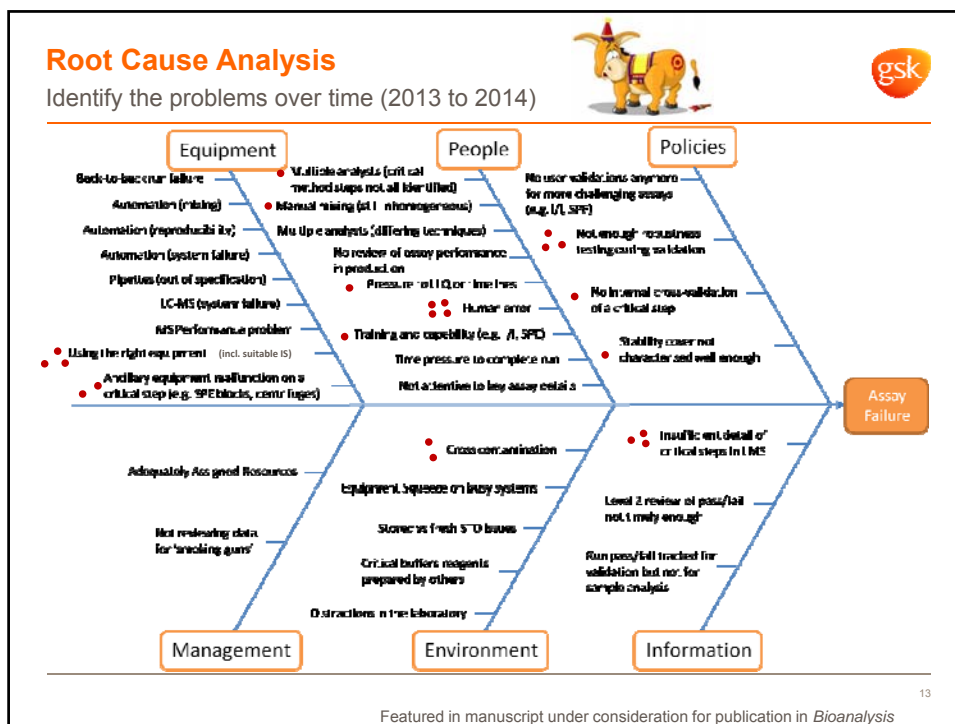
### Root Cause Analysis

Fix the problem

Assay	Platform	Site	Issue	Root Cause Identified
"GSK7"	LC-MS/MS	2	Red flag assay previous period	Previously identified as lacking robustness. Assay is currently being re-developed and re-validated during a recruitment break in ongoing study. Enhancements include automation of extraction steps, simplification of protein digestion process and use of a more suitable IS (to address <i>people, policies &amp; equipment</i> deficiencies).
"GSK21"	LC-MS/MS	2	Rejected work-list this period > accepted work-lists	Incorrect stock solutions (wrong concentration) used for affected runs ( <i>people</i> ) – Error was fed back to the analyst concerned.
"GSK94"	LC-MS/MS	2	Rejected work-list this period > accepted work-lists	Training/capability deficiency ( <i>people</i> ) – inexperienced analyst (on secondment) prepared stock solutions and performed affected runs. Issues resolved once fresh stocks and standards prepared by another team member. TF to follow up with the secondee to identify any training gaps.
"GSK105"	LC-MS/MS	2	Red flag assay previous period	Plans for revalidation after current study is complete - Likely causes identified as contamination ( <i>environment</i> ) during clean-up and suitability of internal standard ( <i>equipment</i> ). Enhancements include automation of extraction steps, simplification of protein digestion process and use of a more suitable IS

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## Assay Transfer & Cross Validation

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## Cross Validation Process



2013 (as presented at OS 2013)

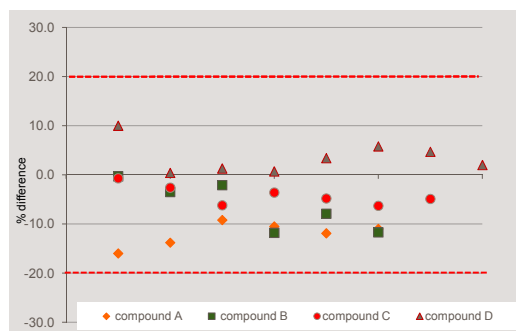
7-8 test samples

Analysed in replicate (n=6) by originator & comparator labs

For each test sample:

$$\% \text{ difference} = \frac{(\text{lab 1 mean} - \text{lab 2 mean})}{\text{overall mean}} \times 100\%$$

**Acceptance criteria =  $\leq \pm 20\%$  for all test samples ( $\pm 30\%$  for LBAs)**



**Example:**  
4 small molecule  
(chromatographic) assay  
cross validations

All human biological samples used were sourced ethically and their research use was in accord with the terms of the informed consents  
Graph featured in manuscript under consideration for publication in *Bioanalysis*

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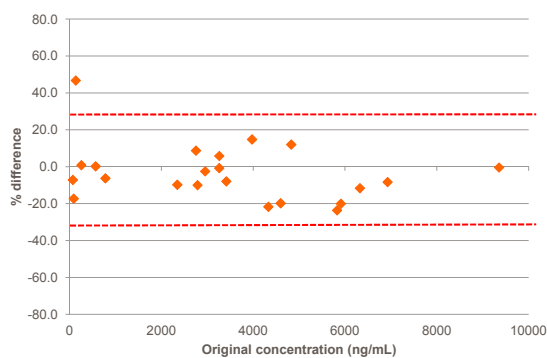
## Cross Validation Process



Revised 1Q14

- Use incurred study samples (by first intent)
- Approx. 20 sample pools
- Test (in singlet) at originator and comparator labs
- Acceptance criteria:

**$\geq 3\%$  rds within  $\pm 20\%$  of the mean ( $\pm 30\%$  for LBAs)**



**Example:**  
Ligand Binding Assay cross  
validation

All human biological samples used were sourced ethically and their research use was in accord with the terms of the informed consents  
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## Assay Transfer & Cross Validation



Our "Lag Measure"

**Lag Measure:**  
 Successful assay transfer to CRO  
 Any issues are resolved and not assay related

"pass following investigation" examples:

- o differences in standard/QC spiking schemes between labs
- o errors in standard preparation at the comparator lab
- o QC test samples being close to acceptance limits upon preparation by the originator lab.



■ pending ■ pass ■ pass following investigation ■ fail

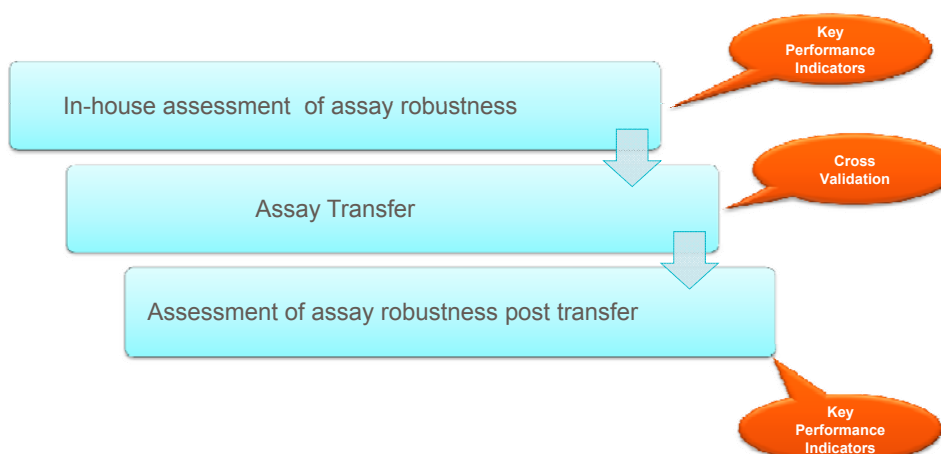
failed cross validation:

- o low pg/mL, multi-analyte assay transfer between two CROs in different continents
- o Resolved in 2014 using pooled incurred samples



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## Building Confidence in Assay Robustness



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## Assay Robustness post Transfer



2Q14 Assay Robustness KPI from a CRO Partner



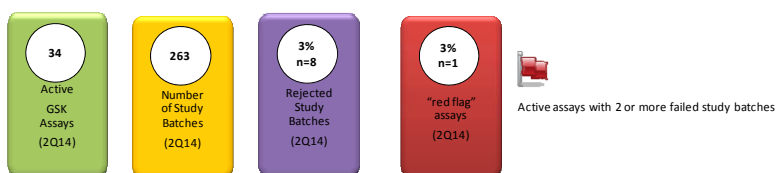
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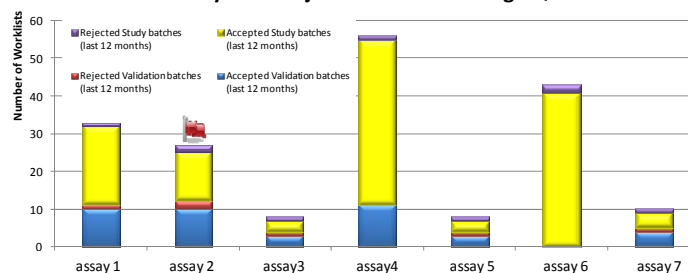
## Assay Robustness post Transfer



2Q14 Assay Robustness KPI from a CRO Partner

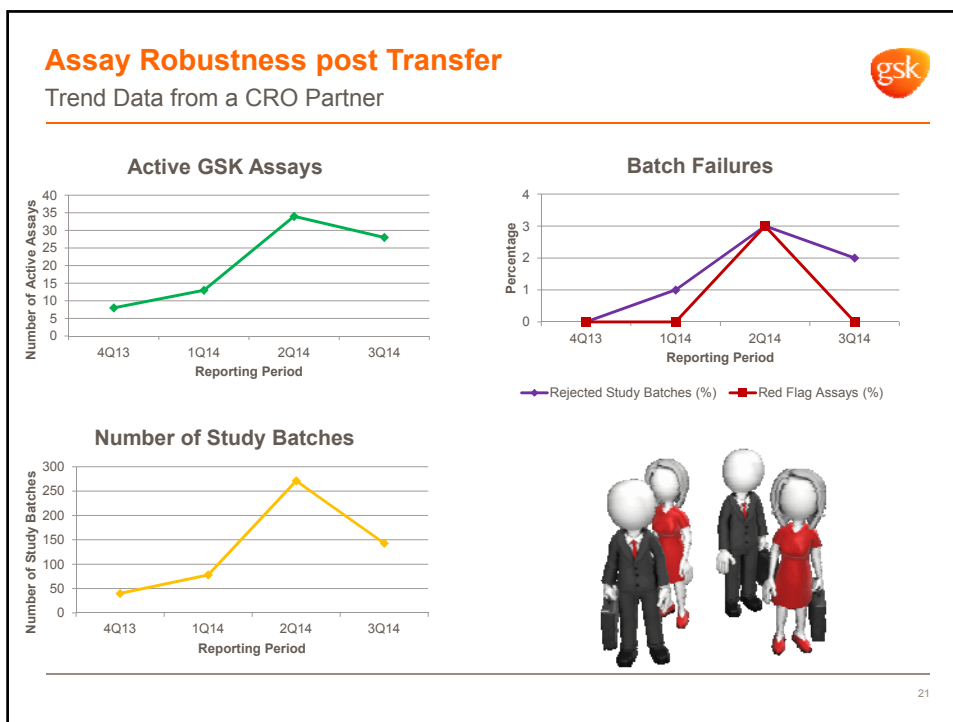


**Assay performance (12 months)  
for assays with rejected batches during 2Q14**



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### Conclusions

The measurement of assay robustness should **not** be considered as yet another regulatory layer, but more as a means for the bioanalyst **to better understand an assay** when used for its intended purpose, i.e. the routine analysis of study samples.

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## Acknowledgements



- Our CRO partners for their continued support
- John Dunn
- Scott Summerfield
- Lorrie Day
- Bonnie Orr
- Glenn Tabolt
- Alex Georgiou
- Robbie Calloway
- Teresa Fuller
- Adam Hughes
- Teresa Heslop

