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Challenges and Solutions in the application of IS variability criteria

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Summary

- Guidelines and Background
- Development of acceptance criteria
- Development of simple automated interface for the bioanalyst
- Give examples of application to several situations
- Discuss IS response investigation experimental design

Guidelines

- Objective criteria need to be established *a priori* if study samples or analytical runs are to be rejected or repeated, based on IS response variability – (3rd AAPS/FDA Bioanalytical workshop report AAPS j. 9(1) E30-E42 2007)

- Reasons for reanalysis: IS response significantly different from the response for calibration standard and QC samples, if such criteria have been pre-defined in a SOP – (EMA Guideline on BMV 2011)

- Internal standard should be monitored for drift. An SOP should be developed *a priori to address issues related to variability of the IS response (FDA draft guidance 2013)*

Background

- Highly variable IS does not always indicate poor assay reliability
- Simple mathematical criteria (e.g. Mean ±50%) – blanket approach
- Perform trend analysis, use IS variation in 'Known' samples to define acceptability of IS response in 'Unknown' samples
- GCC recommendation: Criteria based on the fluctuation of known samples should be applied to the unknowns - Bioanalysis (2011) 3(12)
- Combined approach: IS variance + blanket limit criteria

- Objective: to apply a combined approach suitable to all methods and IS types
- Develop a very simple automated interface for the user.

Development of acceptance criteria

- Look for population differences (most important)
- Look for individual atypical IS responses

- Combined approach used: IS variance + blanket limit criteria
- A long iterative process which is still on-going!

- Two basic calculations
- 1. IS response in Unknowns equivalent to Knowns?
- 2. Define individual atypical IS responses

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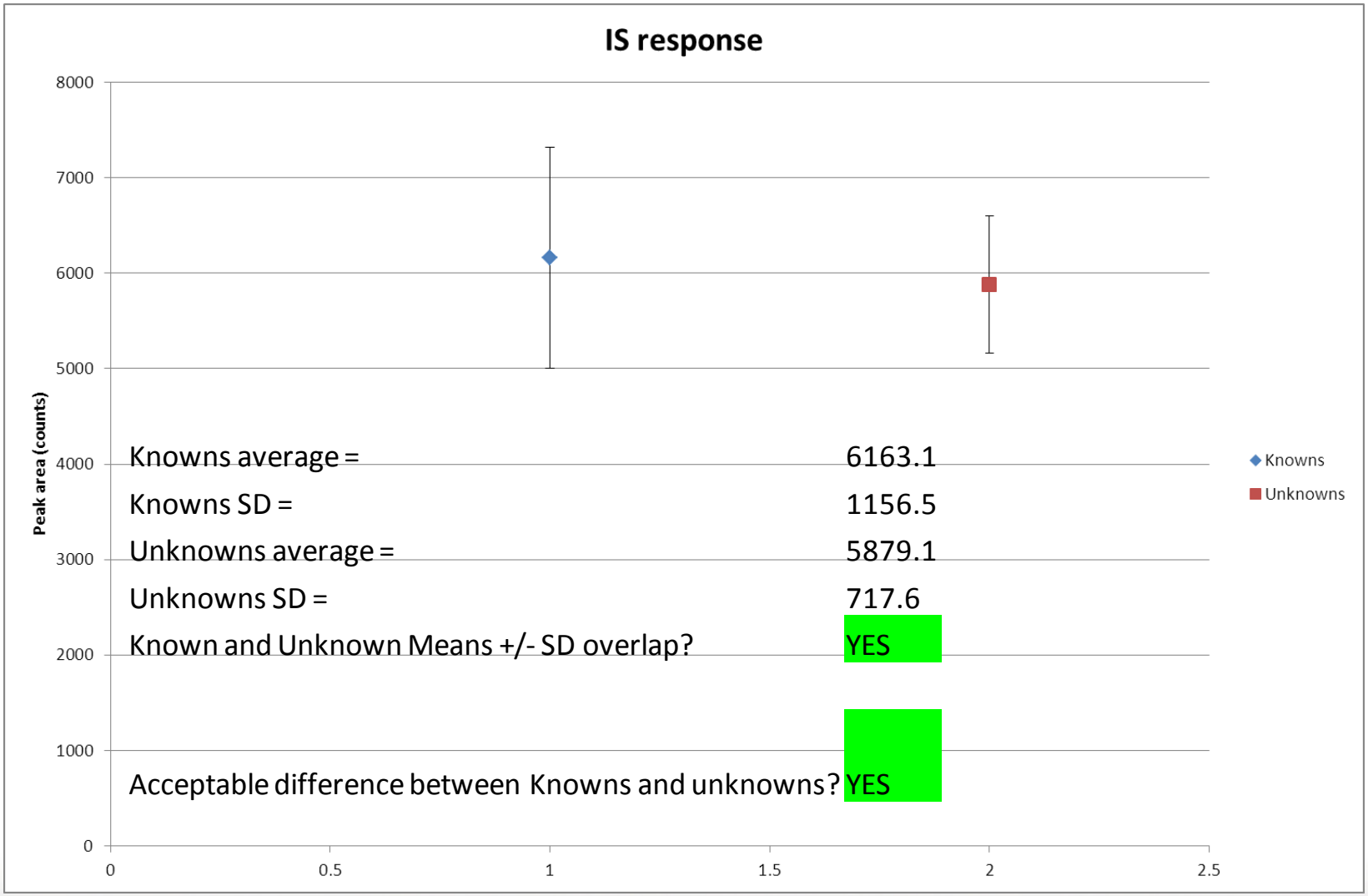
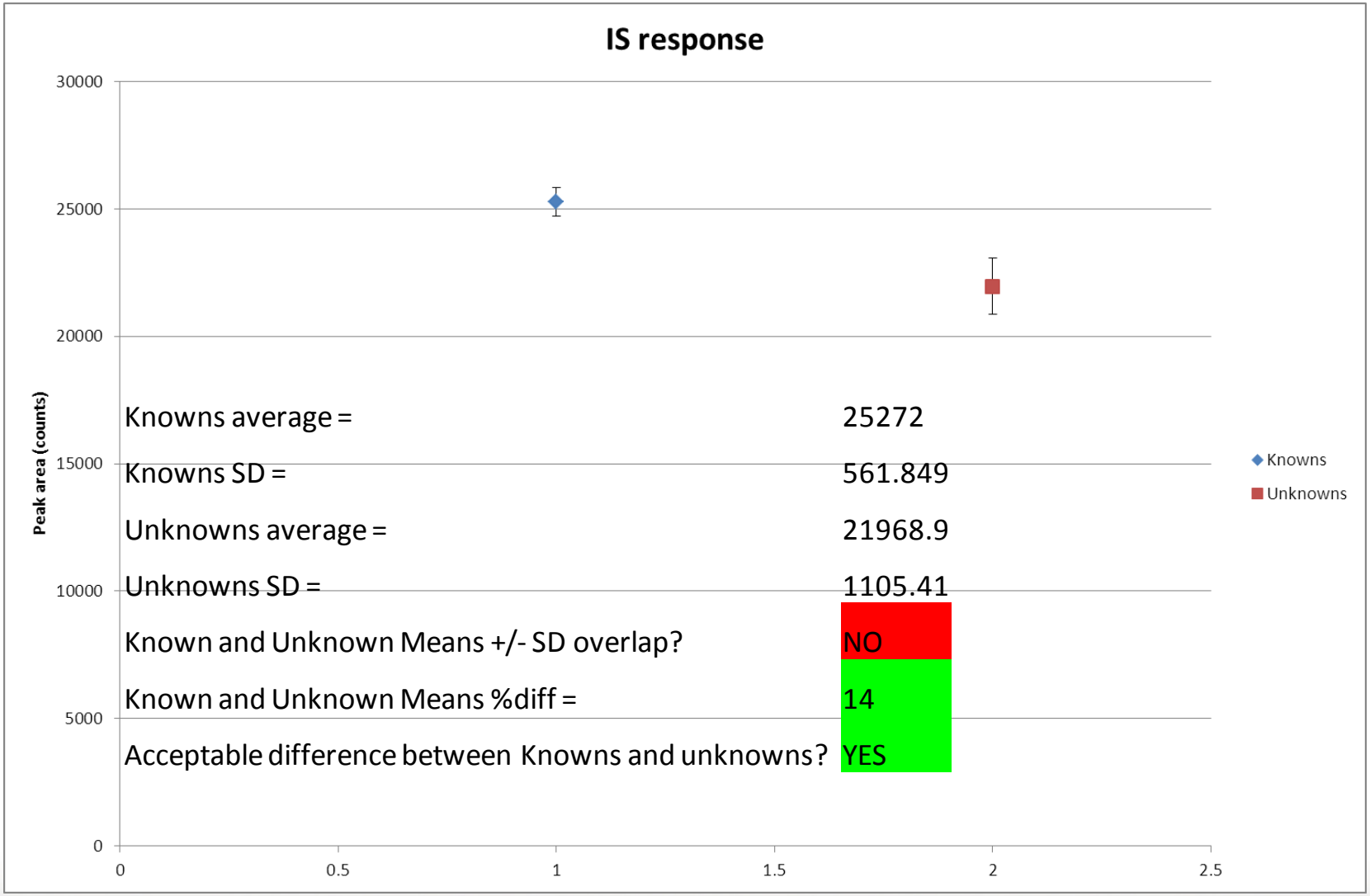
IS response in Unknowns equivalent to Knowns?

- Calculate mean IS response and associated SD in acceptable Knowns and Unkowns
- Check if the means ±SDs cross-over
- Cross-over observed populations equivalent
- Cross-over not observed is the difference in the means ≤15%
- IS macro.xlsx

- If population differences are noted they should be investigated


IS response
Peak area (counts)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
0.5
1
1.5
2
2.5
Knowns
Unknowns
Knowns average = 6163.1
Knowns SD = 1156.5
Unknowns average = 5879.1
Unknowns SD = 717.6
Known and Unknown Means +/- SD overlap? YES
Acceptable difference between Knowns and unknowns? YES


IS response
Peak area (counts)
30000
25000
20000
15000
10000
5000
0
0
0.5
1
1.5
2
2.5
Knowns
Unknowns
Knowns average = 25272
Knowns SD = 561.849
Unknowns average = 21968.9
Unknowns SD = 1105.41
Known and Unknown Means +/- SD overlap? NO
Known and Unknown Means %diff = 14
Acceptable difference between Knowns and unknowns? YES

Individual atypical IS responses: High IS variability run

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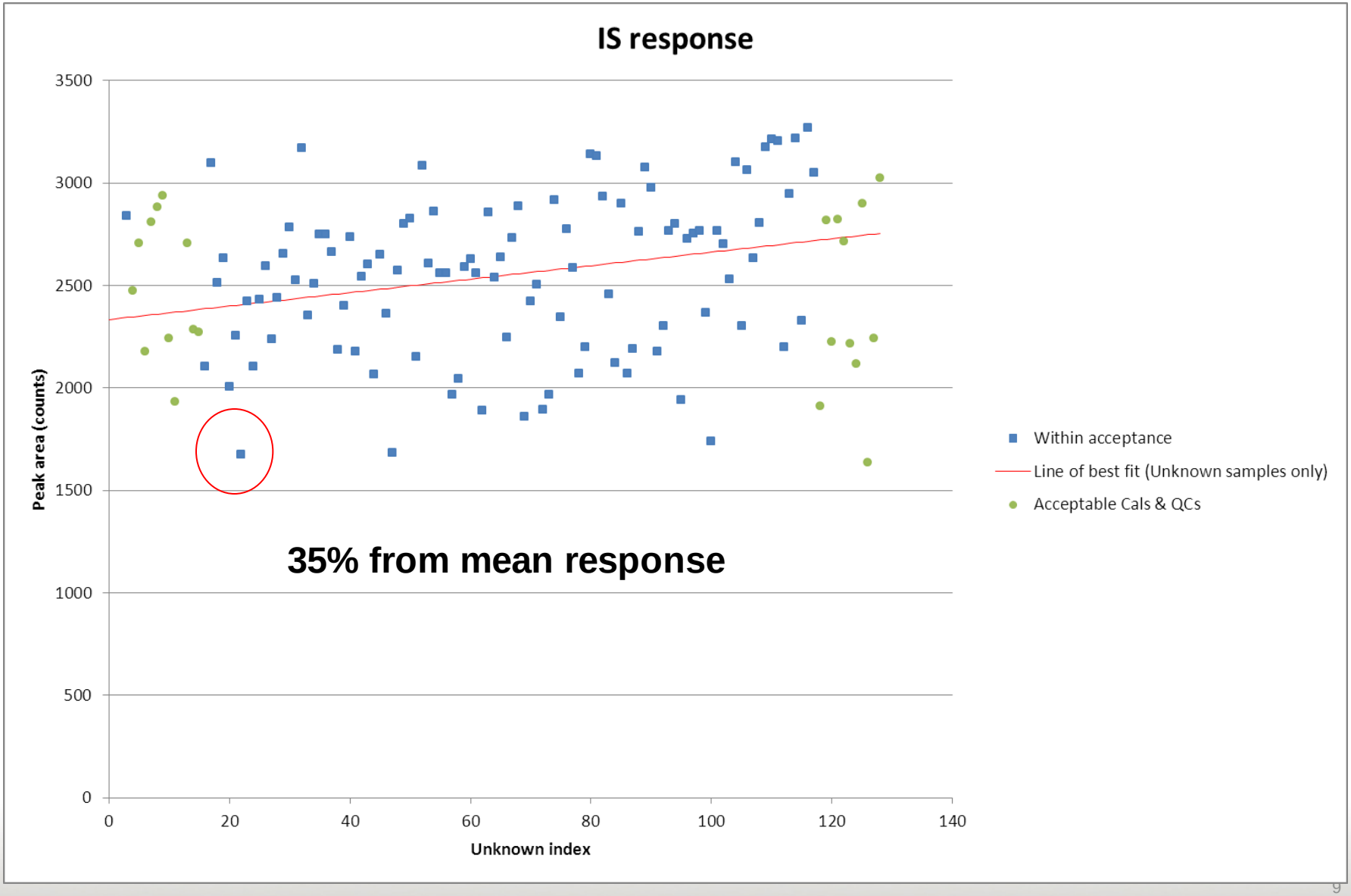

Low IS variability run

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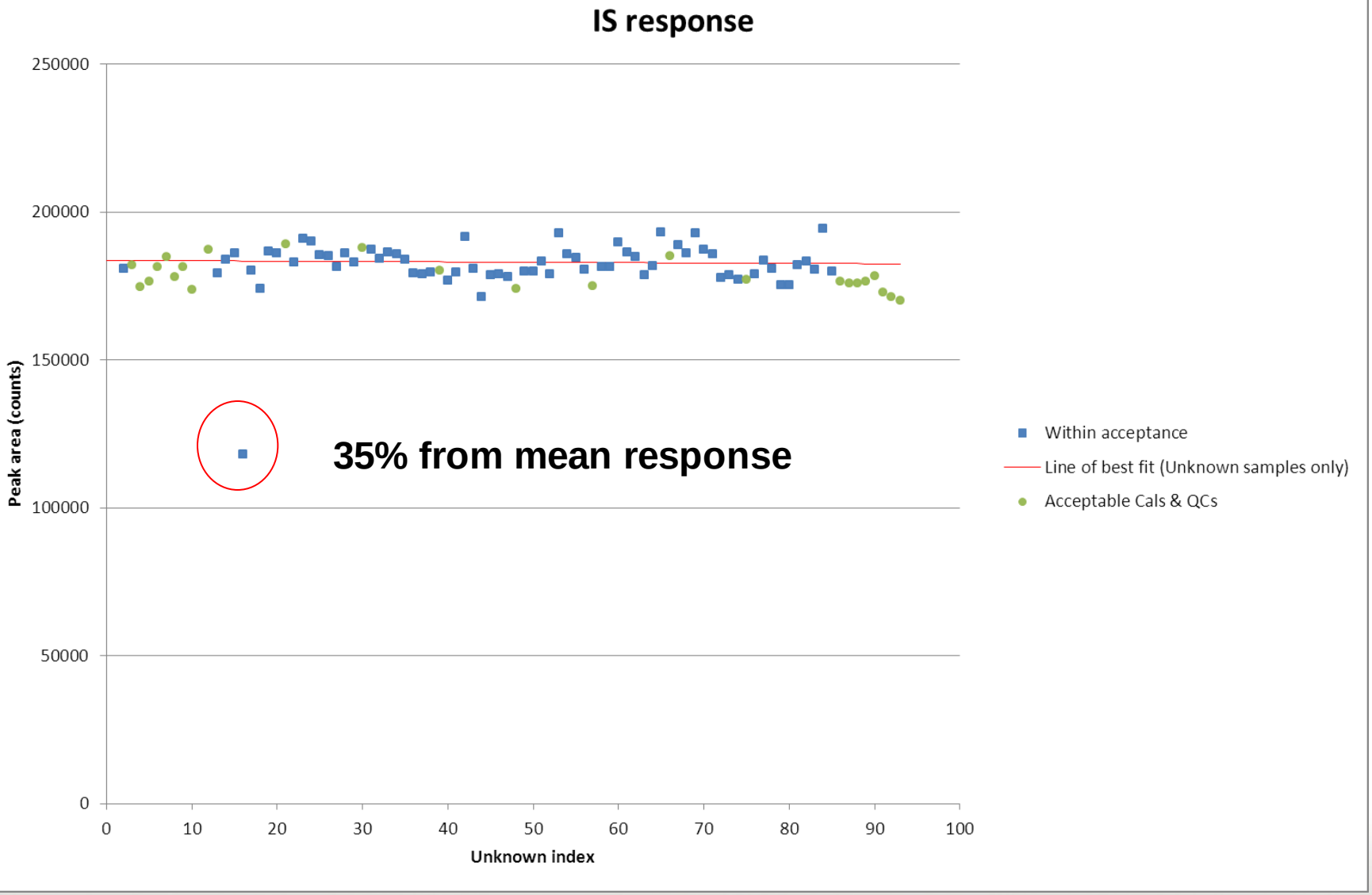

Define individual atypical IS responses

- Combination of blanket limit values and IS variance in acceptable Known samples.

Criteria 1	Criteria 2	Criteria 3
<50% or >185% of the line of best fit	Line of best fit ± XX%	Line of best fit ± XX standard deviation of the acceptable known samples, from the line of best fit

- Regression of unknowns (line of best fit) used rather than a mean
- Criteria 2 and 3 are adjustable depending on methodology e.g. SIL or Analogue IS (Typically: 30% and 3.5 x SD)
- Individual IS responses will be considered to be atypical if they are outside two or more of the above criteria
- Where possible sample analysis will be repeated in singlicate if IS response is considered to be atypical.

Mean vs. Line of best fit

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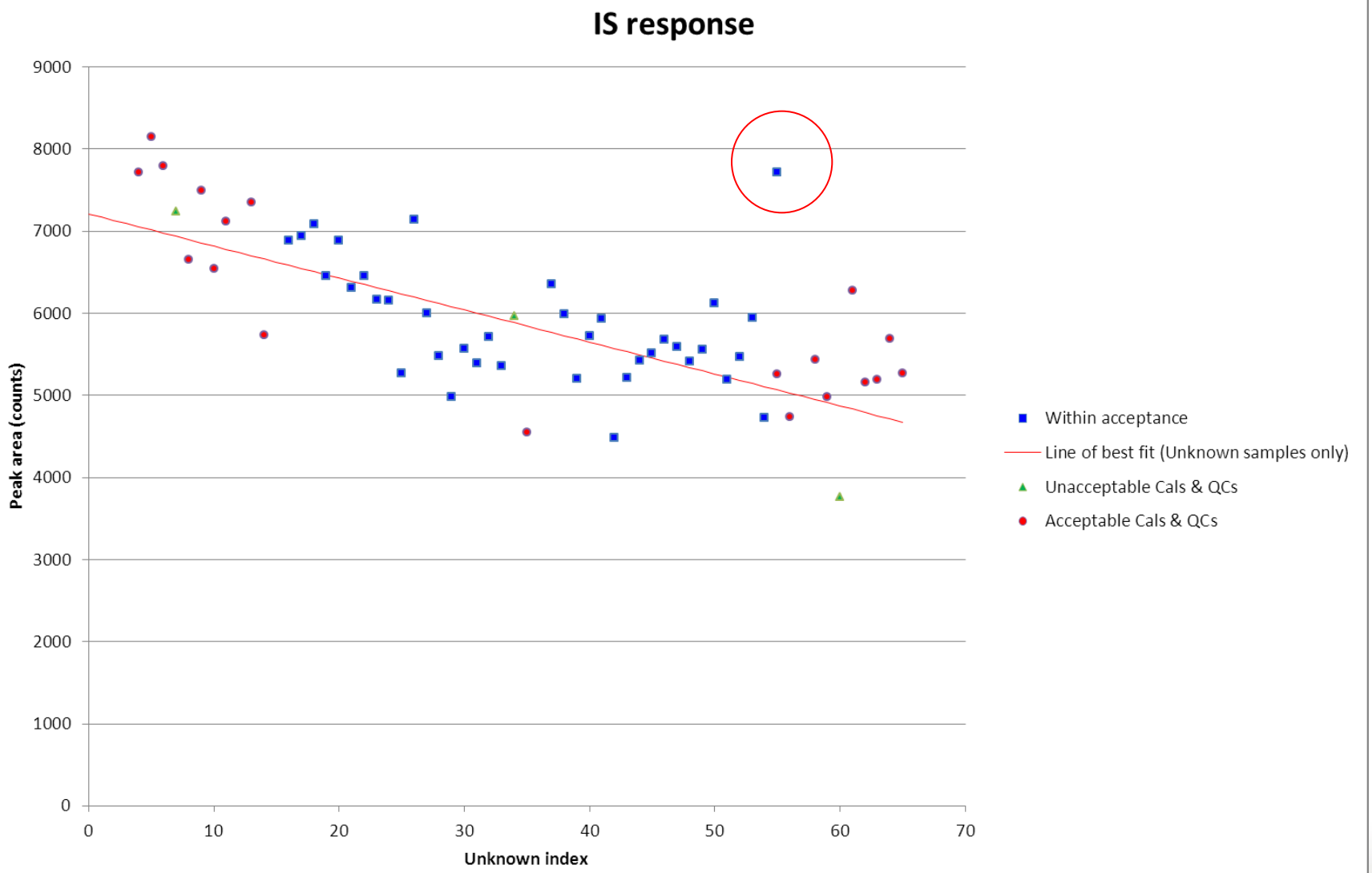

Development of IS variability macro

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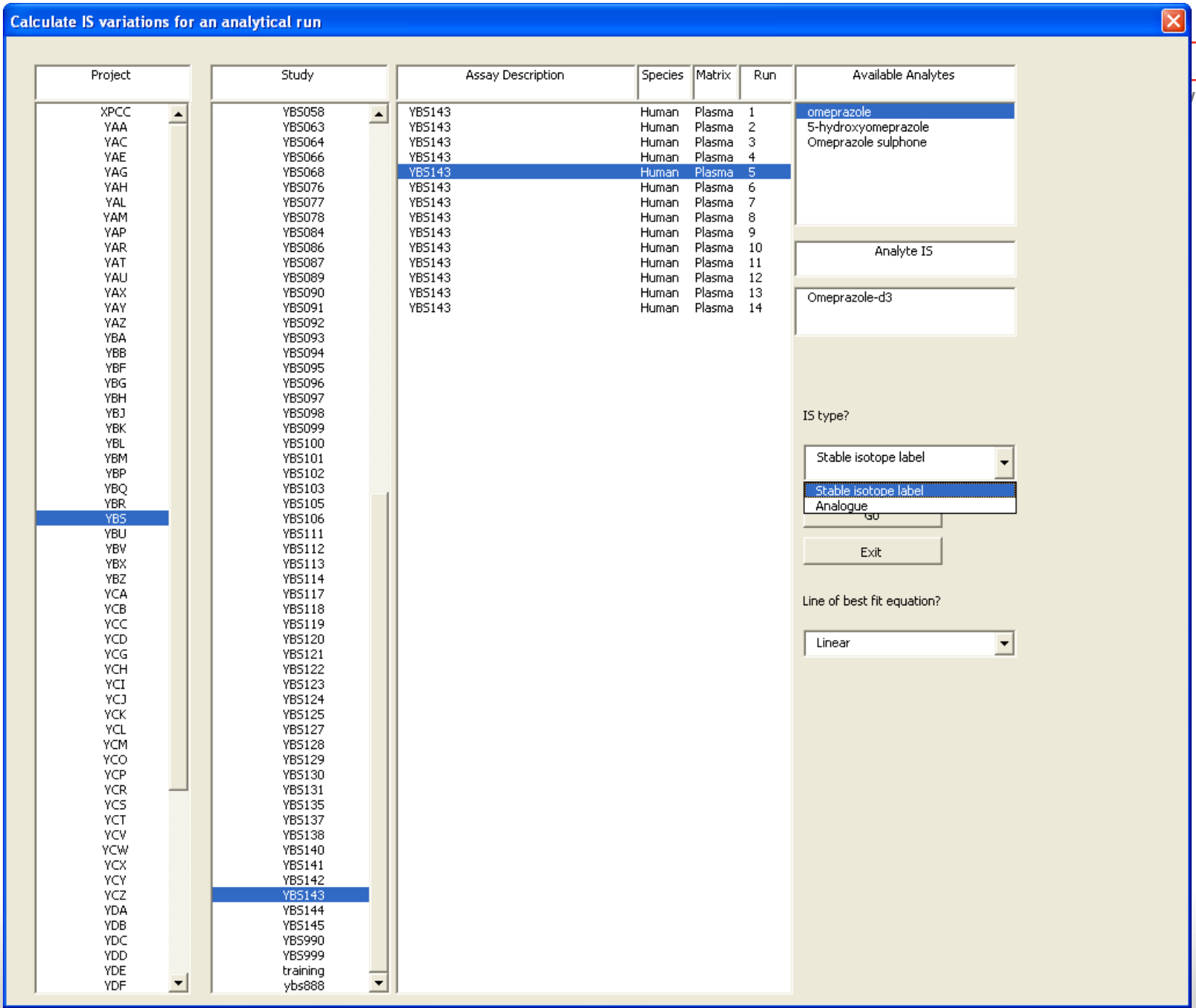
- Macro developed to interface with LIMs system
- Macro validated to negate any subsequent checking
- Simple to use both in processing runs and any interpretation
- Excel based output which is automatically saved electronically in the study records
- Several iterations mainly due to changes in limit values and additional features
- Identifies samples with IS response which is lower than the lowest IS response for an acceptable LLOQ sample (3rd AAPS/FDA Bioanalytical workshop report)
- Allows different criteria for SIL and Analogue IS (Criteria tighter for analogue as less robust to IS variability
- Mis-extraction / mis-injections automatically excluded from stats based on a limit value for peak area.
- Option of using alternative regressions (designed in but not used in practice)


Calculate IS variations for an analytical run
Project
XPCC
YAA
YAC
YAE
YAG
YAH
YAL
YAM
YAP
YAR
YAT
YAU
YAX
YAY
YAZ
YBA
YBB
YBF
YBG
YBH
YBJ
YBK
YBL
YBM
YBP
YBQ
YBR
YBS
YBU
YBV
YBX
YBZ
YCA
YCB
YCC
YCD
YCG
YCH
YCI
YCJ
YCK
YCL
YCM
YCO
YCP
YCR
YCS
YCT
YCV
YCW
YCX
YCY
YCZ
YDA
YDB
YDC
YDD
YDE
YDF
Study
YBS058
YBS063
YBS064
YBS066
YBS068
YBS076
YBS077
YBS078
YBS084
YBS086
YBS087
YBS089
YBS090
YBS091
YBS092
YBS093
YBS094
YBS095
YBS096
YBS097
YBS098
YBS099
YBS100
YBS101
YBS102
YBS103
YBS105
YBS106
YBS111
YBS112
YBS113
YBS114
YBS117
YBS118
YBS119
YBS120
YBS121
YBS122
YBS123
YBS124
YBS125
YBS127
YBS128
YBS129
YBS130
YBS131
YBS135
YBS137
YBS138
YBS140
YBS141
YBS142
YBS143
YBS144
YBS145
YBS990
YBS999
training
ybs888
Assay Description
Species
Matrix
Run
YBS143 Human Plasma 1
YBS143 Human Plasma 2
YBS143 Human Plasma 3
YBS143 Human Plasma 4
YBS143 Human Plasma 5
YBS143 Human Plasma 6
YBS143 Human Plasma 7
YBS143 Human Plasma 8
YBS143 Human Plasma 9
YBS143 Human Plasma 10
YBS143 Human Plasma 11
YBS143 Human Plasma 12
YBS143 Human Plasma 13
YBS143 Human Plasma 14
Available Analytes
omeprazole
5-hydroxyomeprazole
Omeprazole sulphone
Analyte IS
Omeprazole-d3
IS type?
Stable isotope label
Stable isotope label
Analogue
Go
Exit
Line of best fit equation?
Linear

IS response and LLOQ

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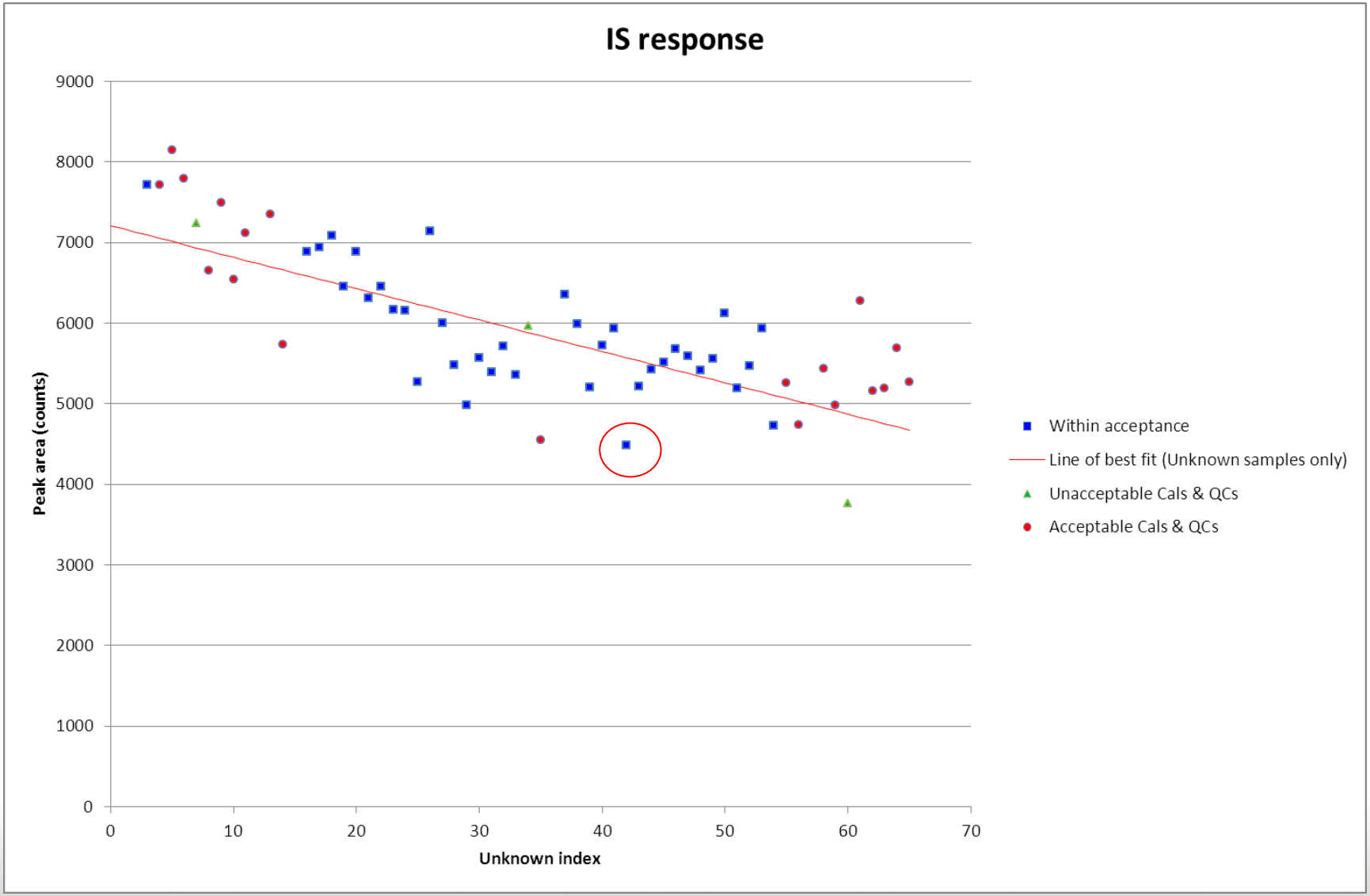

IS response and LLOQ

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Cal A	58	5431.4	STANDARD		
Cal B	59	4979.4	STANDARD		
Cal C	60	3766	STANDARD	X	
Cal D	61	6279.6	STANDARD		
Cal E	62	5156.1	STANDARD		
Cal F	63	5192.9	STANDARD		
Cal G	64	5685.7	STANDARD		
Cal H	65	5268.9	STANDARD		
Sample name	Sample run index	IS area	Sample type	Excluded from stats	<LLOQ IS Area
YBG056 Blank + IS	3	7718.9	STABILITY		
YBG056 422760000054 S012 A Plasma-1 Pre-dose / Day 1	16	6891.3	UNKNOWN		
YBG056 422760000053 S012 A Plasma-1 Day 1 15m	17	6939.6	UNKNOWN		
YBG056 422760000052 S012 A Plasma-1 Day 1 30m	18	7088.6	UNKNOWN		
YBG056 422760000051 S012 A Plasma-1 Day 1 45m	19	6458.9	UNKNOWN		
YBG056 422760000050 S012 A Plasma-1 Day 1 1h	20	6884.8	UNKNOWN		
YBG056 422760000049 S012 A Plasma-1 Day 1 1h 30m	21	6312.8	UNKNOWN		
YBG056 422760000048 S012 A Plasma-1 Day 1 2h	22	6453.7	UNKNOWN		
YBG056 422760000047 S012 A Plasma-1 Day 1 3h	23	6171.9	UNKNOWN		
YBG056 422760000046 S012 A Plasma-1 Day 1 4h	24	6154.6	UNKNOWN		
YBG056 422760000045 S012 A Plasma-1 Day 1 5h	25	5270.3	UNKNOWN		X
YBG056 422760000044 S012 A Plasma-1 Day 1 6h	26	7145.8	UNKNOWN		
YBG056 422760000043 S012 A Plasma-1 Day 1 12h	27	5998.5	UNKNOWN		
YBG056 422760000042 S012 A Plasma-1 Day 1 16h	28	5476.8	UNKNOWN		
YBG056 422760000041 S012 A Plasma-1 Day 1 24h	29	4983.2	UNKNOWN		X
YBG056 422760000040 S012 A Plasma-1 Day 2 36h	30	5569.5	UNKNOWN		
YBG056 422760000039 S012 A Plasma-1 Day 3 48h	31	5391.1	UNKNOWN		X

Runs with small numbers of Unknowns

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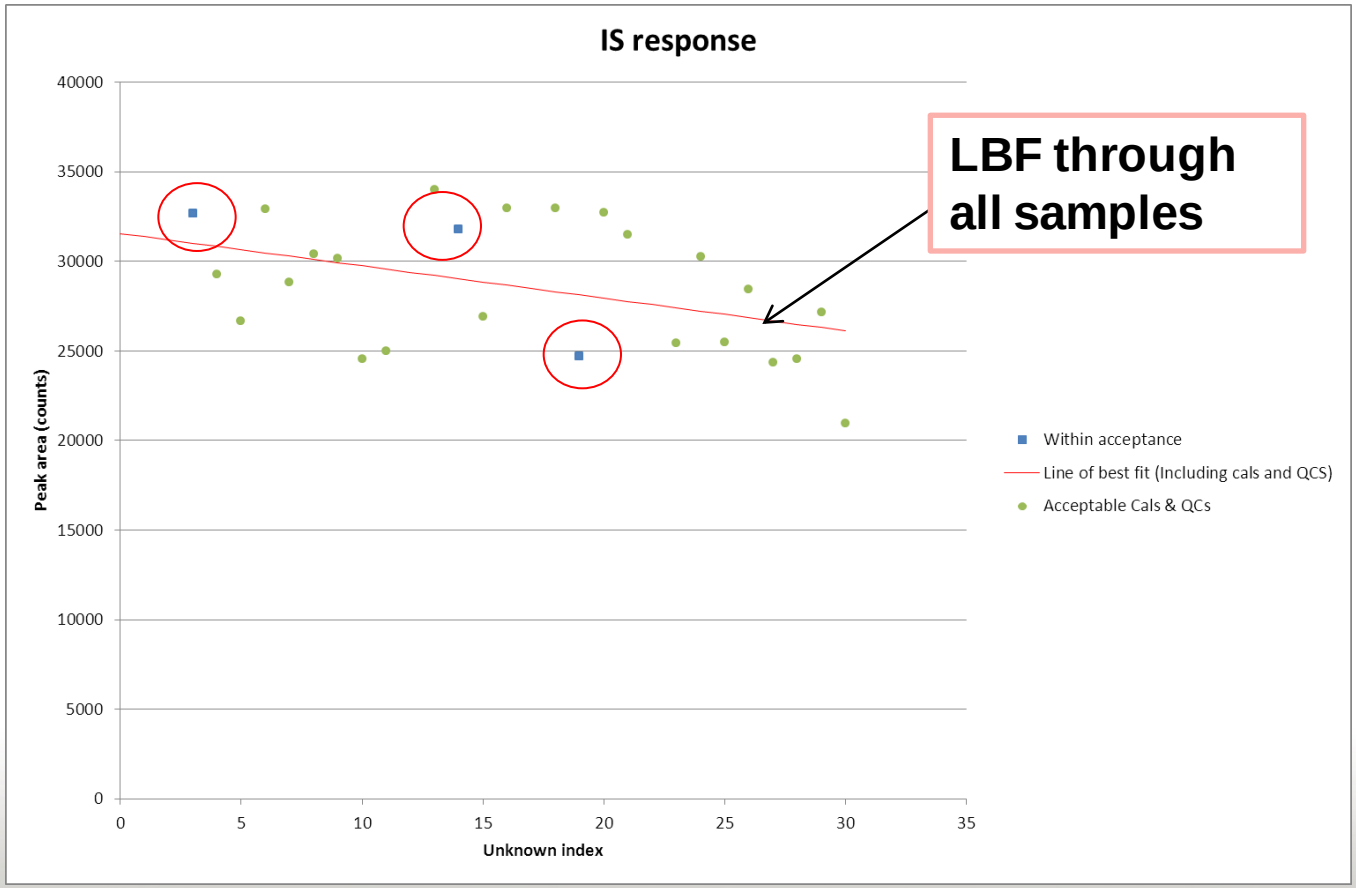
- Knowns-Unknowns equivalence test is not performed
- Unknowns SD is not meaningful due to lack of data
- Line of best fit drawn through all samples (Unknowns and Knowns)
- Individual atypical IS responses defined using same criteria



Application to a Biomarker:

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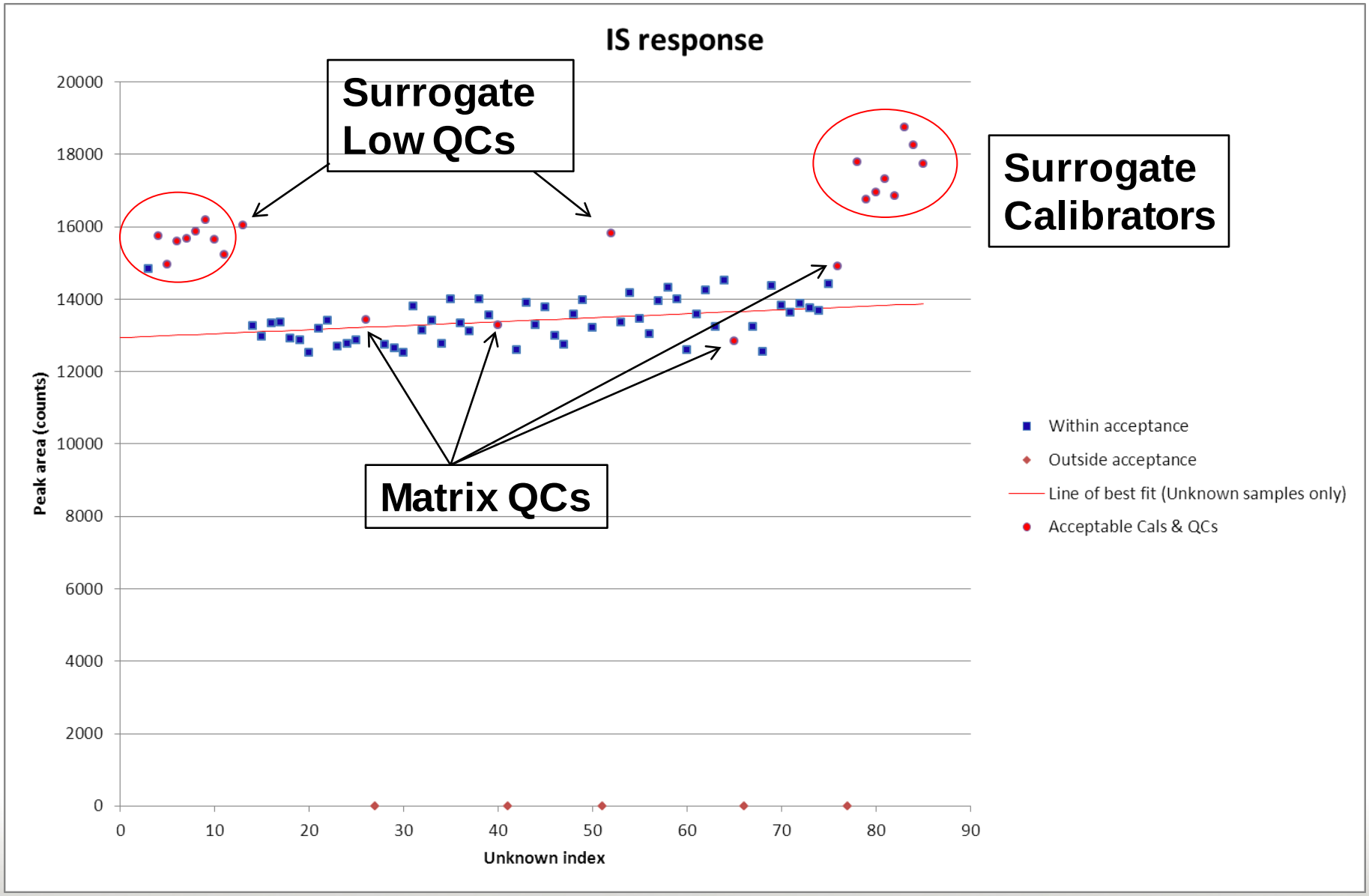

Application to a Biomarker:

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Low QC	13	16051.9	QC
High QC	26	13422.1	QC
Mid QC	40	13275.4	QC
Low QC	52	15830.8	QC
Mid QC	65	12834.6	QC
High QC	76	14909.5	QC
Cal 0.5 ug/mL	4	15736.2	STANDARD
Cal 1 ug/mL	5	14965.1	STANDARD
Cal 5 ug/mL	6	15601.6	STANDARD
Cal 10 ug/mL	7	15680.7	STANDARD
Cal 20 ug/mL	8	15873.6	STANDARD
Cal 30 ug/mL	9	16187	STANDARD
Cal 40 ug/mL	10	15657.2	STANDARD
Cal 50 ug/mL	11	15226.4	STANDARD
Cal 0.5 ug/mL	78	17788	STANDARD
Cal 1 ug/mL	79	16747.7	STANDARD
Cal 5 ug/mL	80	16946	STANDARD
Cal 10 ug/mL	81	17325.8	STANDARD
Cal 20 ug/mL	82	16848	STANDARD
Cal 30 ug/mL	83	18758.6	STANDARD
Cal 40 ug/mL	84	18247.8	STANDARD
Cal 50 ug/mL	85	17742.5	STANDARD

Knowns average =	15984.4
Knowns SD =	1547.6
Unknowns average =	13416.8
Unknowns SD =	582.9
Known and Unknown Means +/- SD overlap?	NO
Known and Unknown Means %diff =	17.5
Acceptable difference between Knowns and unknowns?	NO
LBF R squared value =	0.1312
LBF regression =	Linear

Application to a Biomarker:

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Low QC	13	16051.9	QC
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Cal 10 ug/mL	81	17325.8	STANDARD
Cal 20 ug/mL	82	16848	STANDARD
Cal 30 ug/mL	83	18758.6	STANDARD
Cal 40 ug/mL	84	18247.8	STANDARD
Cal 50 ug/mL	85	17742.5	STANDARD

		Plasma QC	
Knowns average =	15984.4	average =	13610.4
Knowns SD =	1547.6		901.334
Unknowns average =	13416.8		
Unknowns SD =	582.9		
Known and Unknown Means +/- SD overlap?	NO		yes
Known and Unknown Means %diff =	17.5		1.4
Acceptable difference between Knowns and unknowns?	NO		
LBF R squared value = 0.1312			
LBF regression = Linear			

Investigation into population differences

- First confirm issue is method specific and not specific to another factor e.g. Analyst or equipment error, control matrix
- If method specific demonstrate quantification not impacted by IS response in affected population
- Typical experimental design
- Dilute pooled affected incurred samples with control matrix used for known samples
- Compare results undiluted and diluted (6 reps each)
- Precision ≤15%, mean bias ≤20%
- Confirm presence of an IS response population difference in undiluted samples
- Quantification unaffected? – note in Analytical Method
- Quantification affected? – revisit methodology to remove / negate population difference

Investigation into population differences (2)

- Dilution type experiment requires incurred samples with appropriate concentrations
- Alternative - utilise standard addition or spiked pre-dose / control samples
- Any experiment requires use of incurred samples
- Relative importance of IS investigation and use of incurred samples
- Do IS investigation as early as possible – many factors - case by case basis

Summary

- Development of one set of useful criteria is challenging and will always be a compromise in some way
- Implementation of IS criteria - easy if criteria are restrictive and too simple
- Implementation of useful IS criteria requires some IT input to simplify the user interface

Acknowledgements

- YBS Colleagues for both scientific and IT input
- Dr Martyn Ward, University of York

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