Dealing with Internal Standard Variability – Towards a Recommendation

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on behalf of the EBF TT-07

EBF 6th Open Symposium
21st November 2013
Barcelona

Introduction

- Topic Team (TT-07) objective to provide a pragmatic and fit for purpose recommendation on how to identify and deal with internal standard variability
- Two scenarios identified:
  - Individual irregularities (“sporadic flyers”)
  - Systematic variability
- EMA & FDA (draft) BMV Guidelines
  - Specified but not prescriptive

Overview

- Setting the scene – individual irregularities & systematic IS variability
- Current Landscape
- Putting things into context
- “Rule of thumb” vs. mathematics
- Towards a recommendation
- “But this doesn’t work for my assay?”
- Next steps

Questions to Consider…

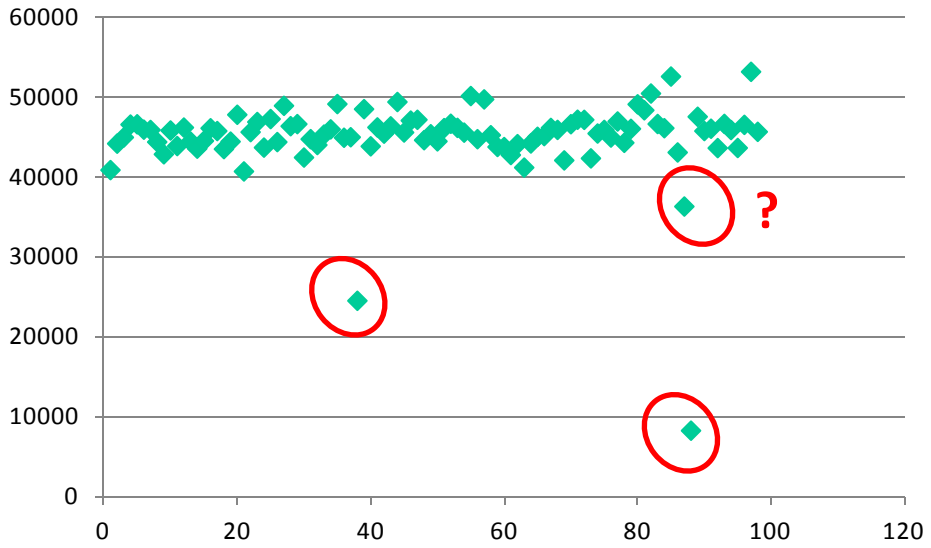
Why have an internal standard?

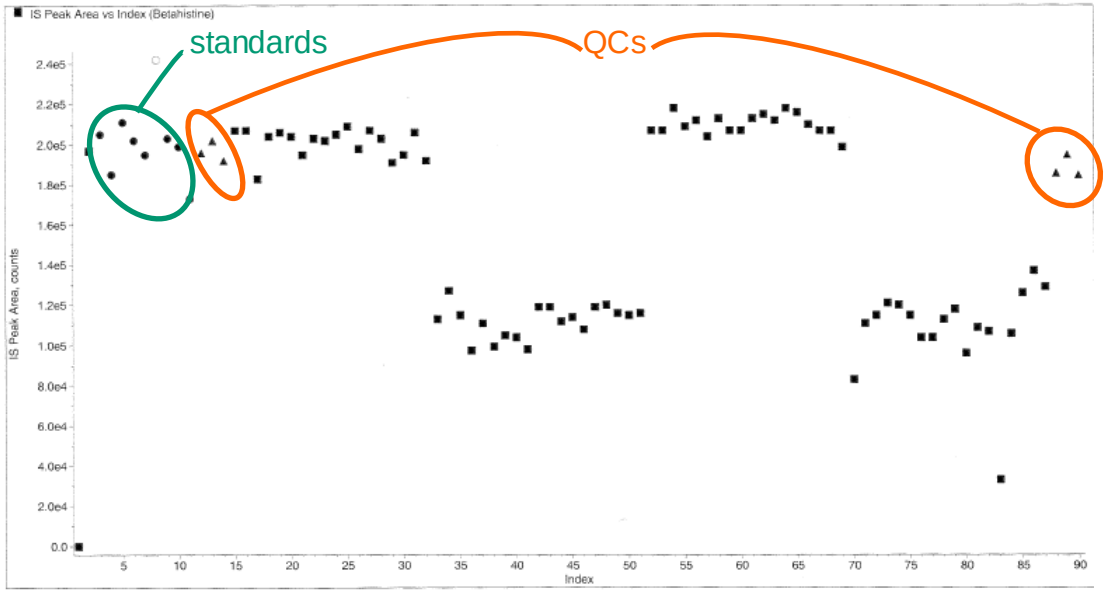
Why strive for a Stable Label Internal Standard?

Should I let the IS "do its job"?

Individual Irregularities & Systematic Variability

Individual irregularities
("sporadic flyers")

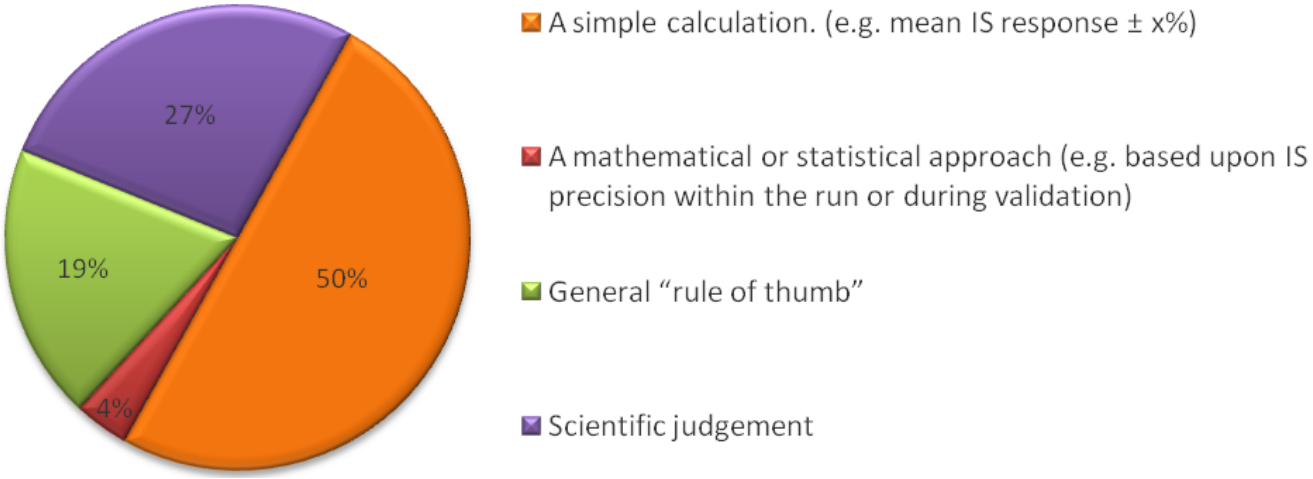




Systematic IS variability

Current Landscape – Individual Irregularities

Current practice…



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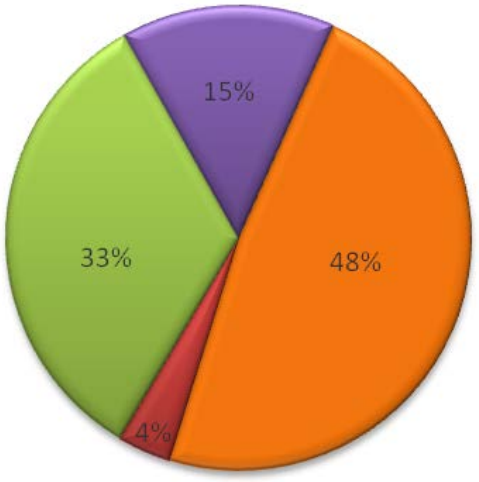
Perceived best practice going forward…



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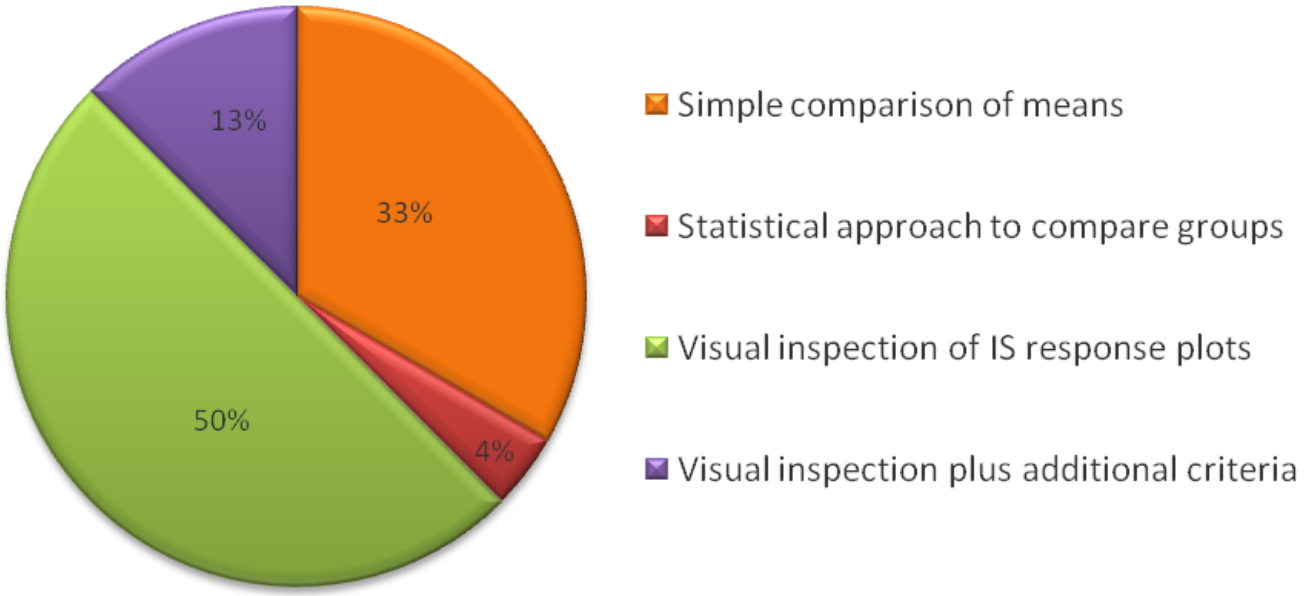
Current Landscape – Systematic Variability



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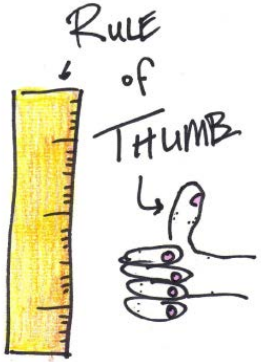
Rule of Thumb vs. Mathematical Approach

- "Rule of Thumb"…

  Inspection of chromatograms and IS response plot followed by a simple set of rules;

  e.g. *"if analyte & IS peaks are both bigger than LLOQ analyte response, then the injection is good!"*

  - Quick & simple
  - No need for calculators or (validated) spreadsheets
  - Relies on a reference point which may not be representative of the whole run



- Mathematical Approaches…

  e.g. *"take mean IS response and apply an acceptance window (±x%) for unknown samples"*

  The value of "x" varies significantly across labs…

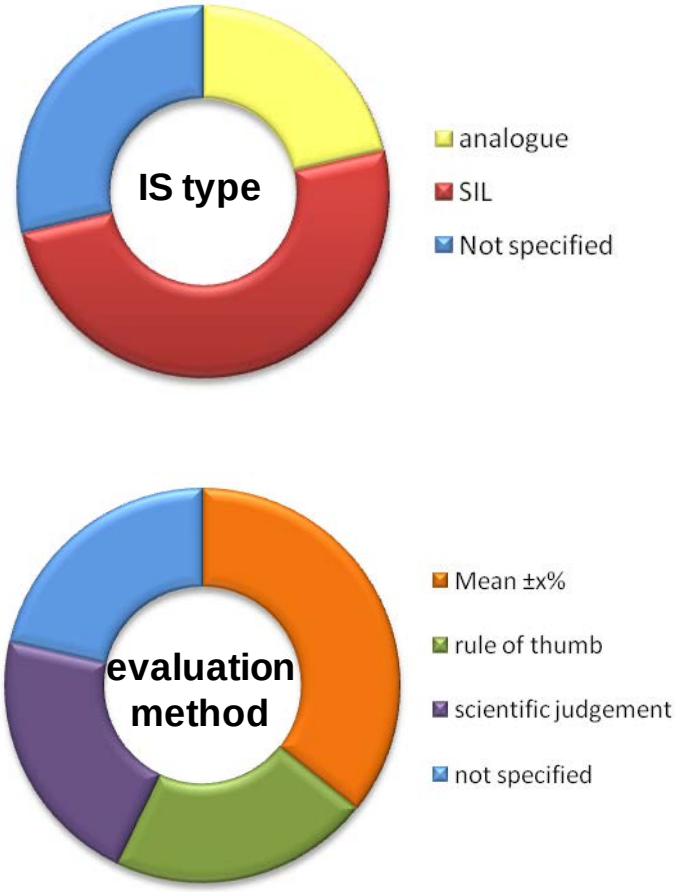
  - seems to be an arbitrary number (e.g. 50-200%, 50-180%, 20-180%)
  - mean of IS response across the whole run **or** just STDs/QCs
  - same window regardless of IS response variability

  May not be as "simple" as it first appears - requiring some kind of spreadsheet…

  - **Checking of data transfer**
  - **Spreadsheet validation**

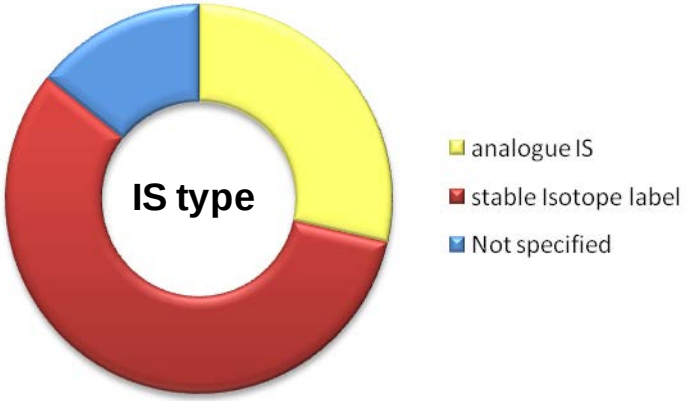
Individual Irregularities – putting it into context

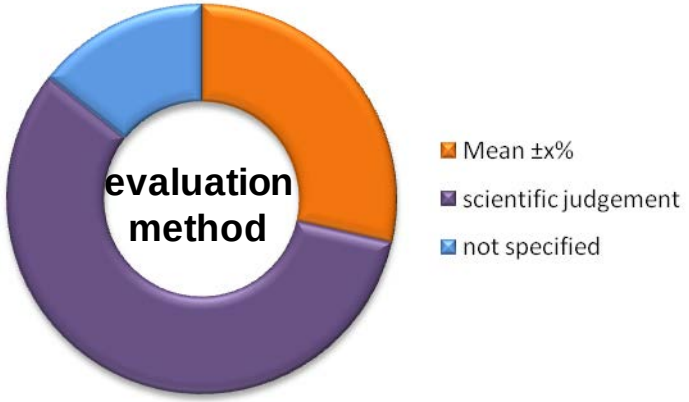
	Number of repeats due to "irregular" IS response (sporadic flyers)	Total number of unknown samples tested (approx)	%age of samples affected by "irregular" (sporadic) IS response
	0	10,000	0.0
	70	913	7.7
	51	9320	0.5
	26	14630	0.2
	96	33843	0.3
	500	40000	1.3
	50	50,000	0.1
	30	4000	0.8
	1	501	0.2
	0	6109	0.0
	0	100	0.0
	49	11163	0.4
	0	2347	0.0
	36	50486	0.1
Total =	**909**	**233412**	**0.4**



Systematic Variability – putting it into context

	Number of runs where systematic IS variability has been observed	Total number of analytical runs (approx)	%age of runs affected by systematic IS variability
	2	250	0.8
	0	20	0.0
	0	200	0.0
	5	5000	0.1
	0	18	0.0
	4	187	2.1
	1	424	0.2
Total =	**12**	**6099**	**0.2**





Pragmatic & fit for purpose

- How big does the hammer need to be?

Individual Irregularities: < 0.4% of samples analysed

Systematic Variability: < 0.2% of analytical runs

Feedback from EBF members

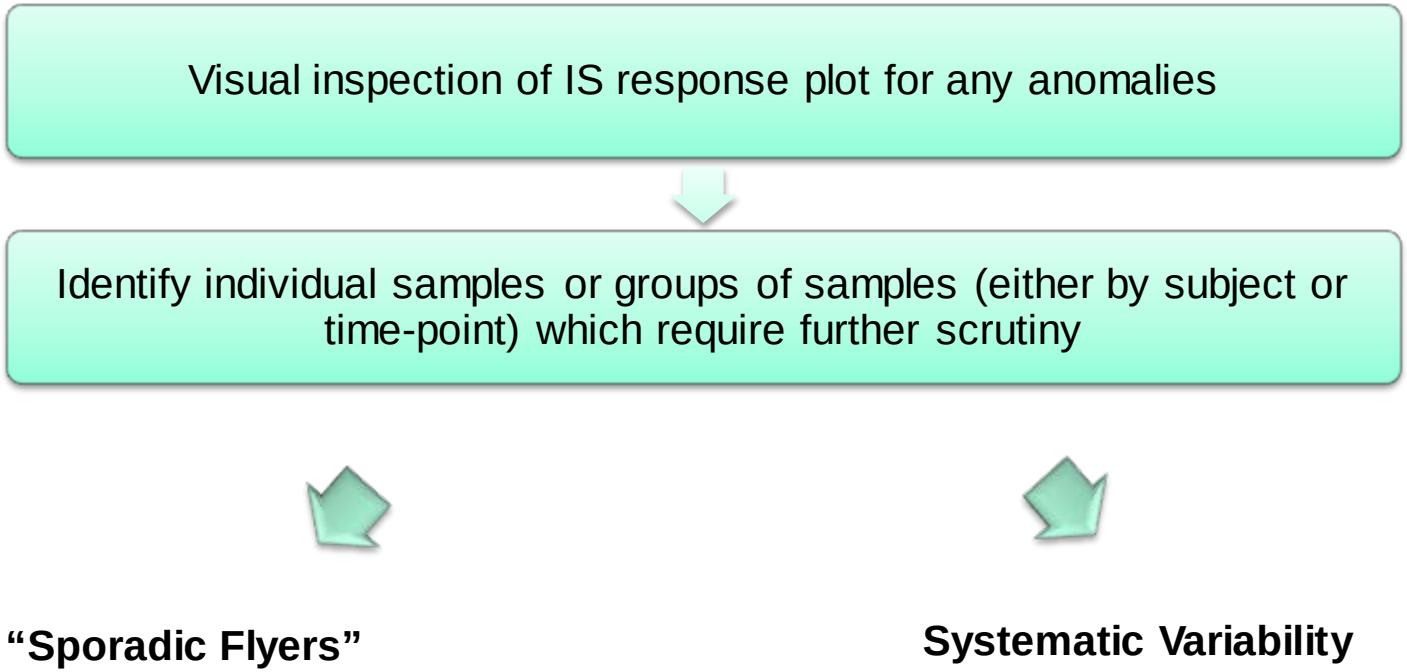
- Member companies in favor of a simple to implement "set of rules"
- More concern over systematic variability than individual irregularities ("sporadic flyers")
- General acceptance that a "rule of thumb" approach would be appropriate for most instances
- Accepted limitations of applying reference point(s) required for a "rule of thumb"

Towards a Recommendation

- Apply a “rule of thumb” approach (where applicable)
- Where this is clearly inappropriate (poor IS precision or analogue IS)… adopt a mathematical approach reflective of IS run variability rather than an arbitrary acceptance window

EBF Recommendation...

Within a run, take the lowest and highest IS response in accepted known samples (STDs & QCs) as the reference points (Ref_{low}, Ref_{high})



EBF Recommendation (continued)...

"Sporadic Flyers"

Assign as an analytical repeat if IS response is > 2x Ref_{low} or <10% of Ref_{low}

Assign as an analytical repeat if IS response is <50% of Ref_{low} **and** the analyte response is < lowest LLQ standard response

Repeat result for individual samples supersedes original (rejected result)

Systematic Variability

Investigate further if IS response across the group of samples is:

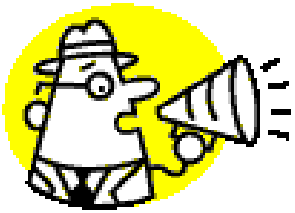
>2x Ref_{high} or <50% of Ref_{low}

Further investigation of result suitability for affected unknowns could include…

- Prepare test QCs using pre-dose matrix (if available)
- Dilution of selected unknown samples using control matrix (used for preparing STDs & QCs)

Results from this investigation will either confirm that original sample results (with questionable IS response) are acceptable, or whether all affected samples require reanalysis following dilution (or using alternative methodology)

“But this doesn’t work for my assay…”



- Consider alternative reference points or % windows
- Consider a mean ± 3 SD window (based upon known samples in the run)
- Examples may include;
  - Assays with analogue IS
  - Assays with poor IS precision
- Should be able to identify these assays during method development & validation

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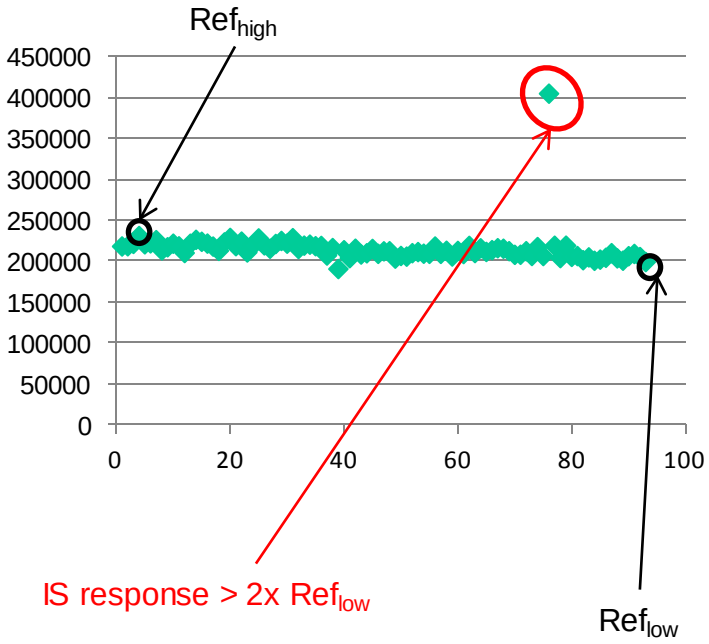
Recommendation in Practice…..

➢ Example 1

Sporadic flyers (1)

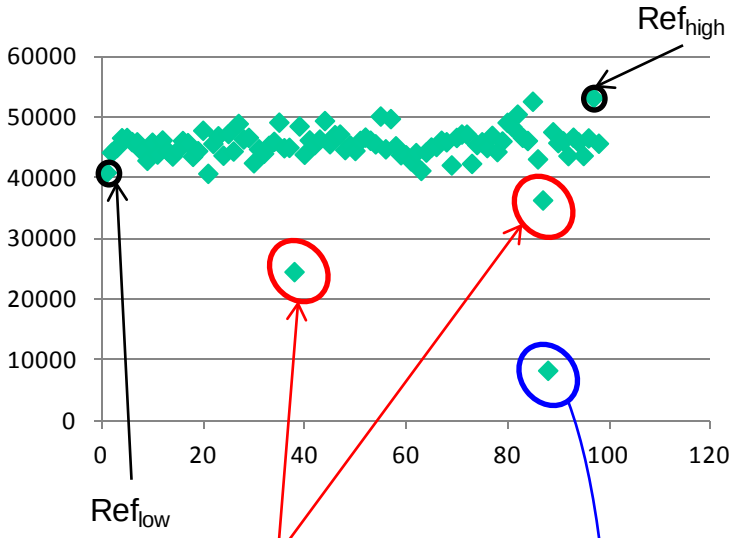


IS response > 2x Ref$_{low}$

Likelihood of double spiked IS

➢ Example 2

Sporadic flyers (2)



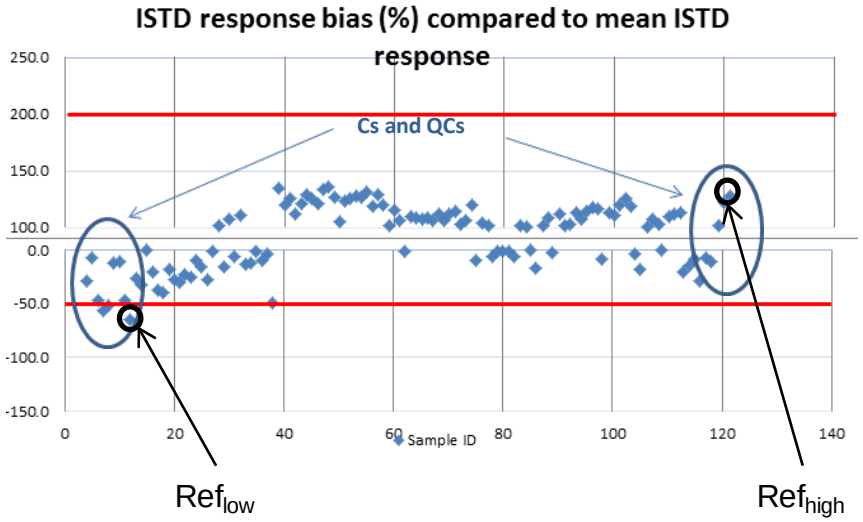
IS response > 50% of Ref$_{low}$
Assume the IS is "doing its job"

- IS response < 50% of Ref$_{low}$
- Reject if analyte response < LLQ STD analyte response
- Accept if analyte response > LLQ STD analyte response (assumes SIL IS)

Recommendation in Practice…..

➢ Example 3

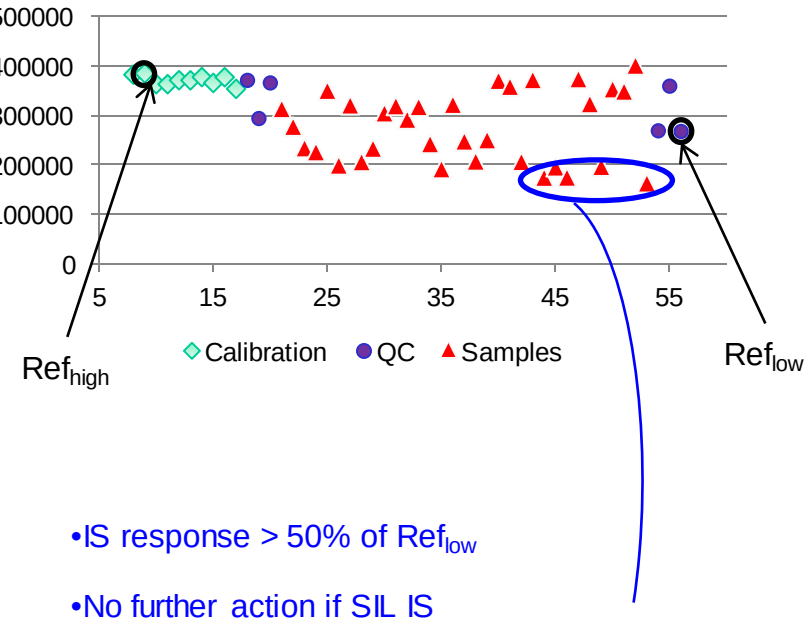
IS response drift across the analytical run



IS drift is covered by known samples at start and end of the run

➢ Example 4

IS response differs between STDs, QCs and samples

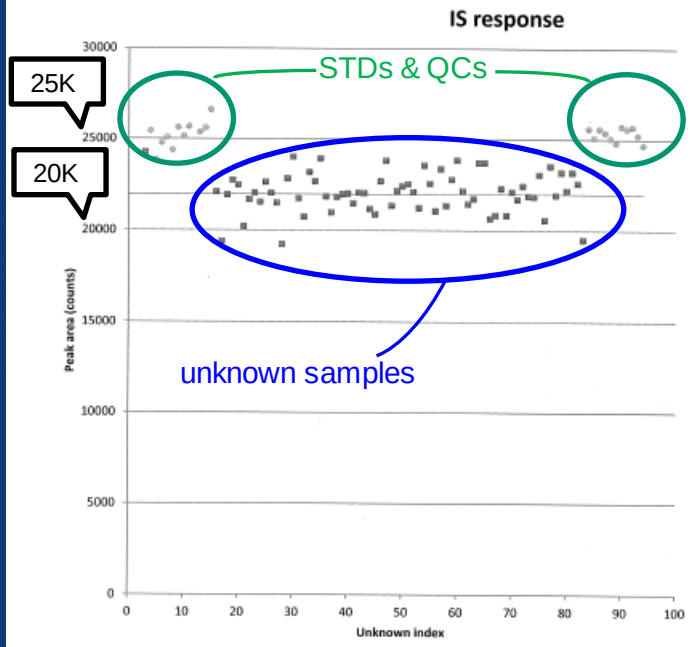


- IS response > 50% of Ref$_{low}$
- No further action if SIL IS
- May wish to investigate further if analogue IS

Recommendation in Practice…..

➢ Example 5

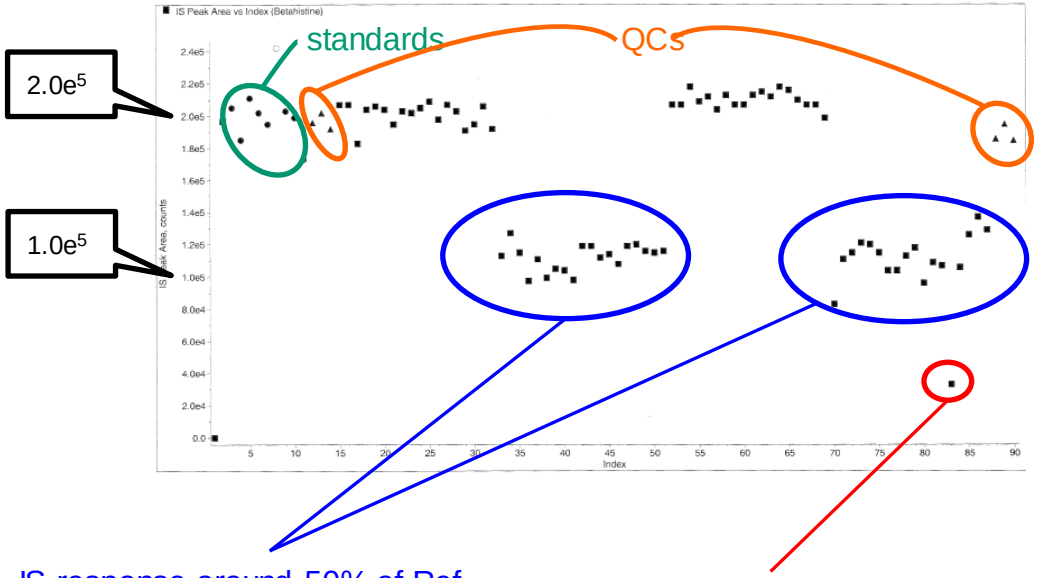
Different IS response between known & all unknown samples



- IS response > 50% of Ref_{low}
- No further action if SIL IS
- May wish to investigate further if analogue IS

➢ Example 6

Systematic variability for some unknown samples



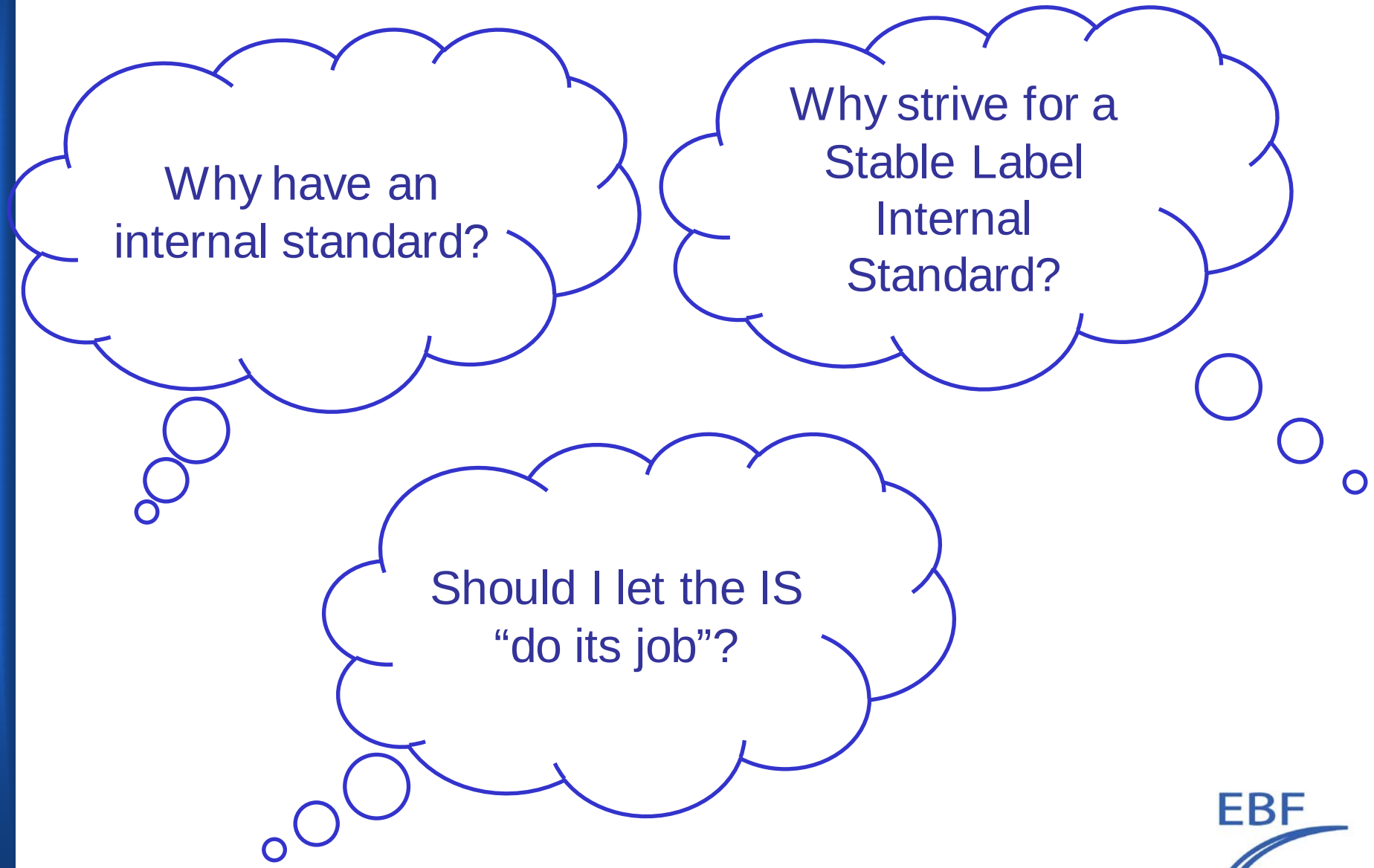
- IS response around 50% of Ref_{low}
- Further investigation likely

- Sporadic flyer
- Reject if analyte response <LLQ STD analyte response

Summary

- EBF TT-07 proposes a pragmatic, simple to implement and fit for purpose approach for dealing with IS variability
- Recommendation is:
  - based up feedback from EBF member companies
  - Prevalence of “the issue”
  - Impact on study data

Questions to Re-consider…



Next Steps

- Feedback please…
- Propose to submit a recommendation paper (on behalf of EBF TT-07) to *Bioanalysis* soon after Barcelona meeting

Acknowledgements

TT-07 members:

Fabrice Salavert	Actelion
Jeff Long	Shire
Tom Verhaeghe	Janssen
Neil Adcock	Quotient Bioresearch
Stuart McDougall	Covance
Magnus Knutsson	Ferring
Berthold Lausecker	CRS
Jim Hillier	Gen-Probe
Timothy Sangster	Charles River
Fernando Romero	Novartis (replaced 2Q13 by Walid Elbast)
Peter van Amsterdam	EBF Steering Committee Sponsor

The EBF community for survey responses and provision of data

Global Bioanalysis Consortium (GBC) – Team S3

Thank You!