Matrix effects in practice: sense and nonsense of internal standard response acceptance criteria

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Content

- Introduction
- What do guidances say?
- IS criteria currently used by J&J
- Some real life examples
- Fine tuning of current criteria
- Conclusion

Introduction

- The internal standard (IS) is used to compensate for any variability during sample preparation, injection and detection.
- IS is selected to mimic the analyte as closely as possible.
- If IS closely tracks the analyte, why monitor IS response?
  - Inconsistent IS response points to issues with the assay
  - Impact on reliability of the results?
  - IS will not compensate for human error

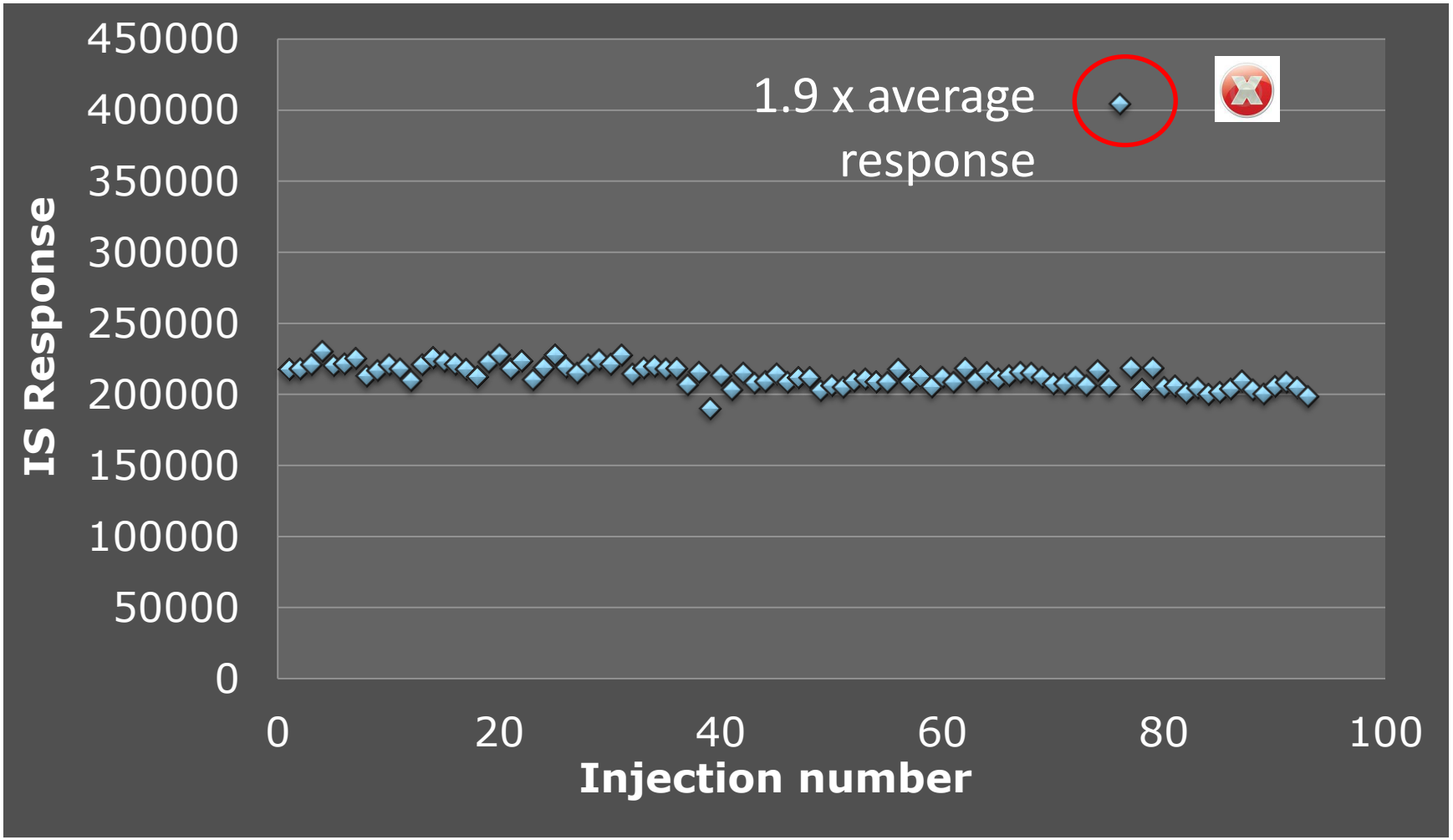
What do guidances say?

- Workshop/Conference Report on the 3rd AAPS/FDA Bioanalytical Workshop *(C.T. Viswanathan et al. The AAPS Journal 2007; 9 (1) Article 4 (http://www.aapsj.org))*
  - Mentioned under "Validation topics with no consensus" is Acceptance criteria for internal standards:
  - …in case of low IS response it is important to determine that the assay continues to have the ability to accurately quantify the LLOQ…
  - …If study samples or analytical runs are rejected or repeated based on IS response variability, objective criteria are necessary and need to be established a priori…
- Although there was no consensus on the topic, it is good practice to have some criteria in place to exclude subjectivity in accepting/rejecting results

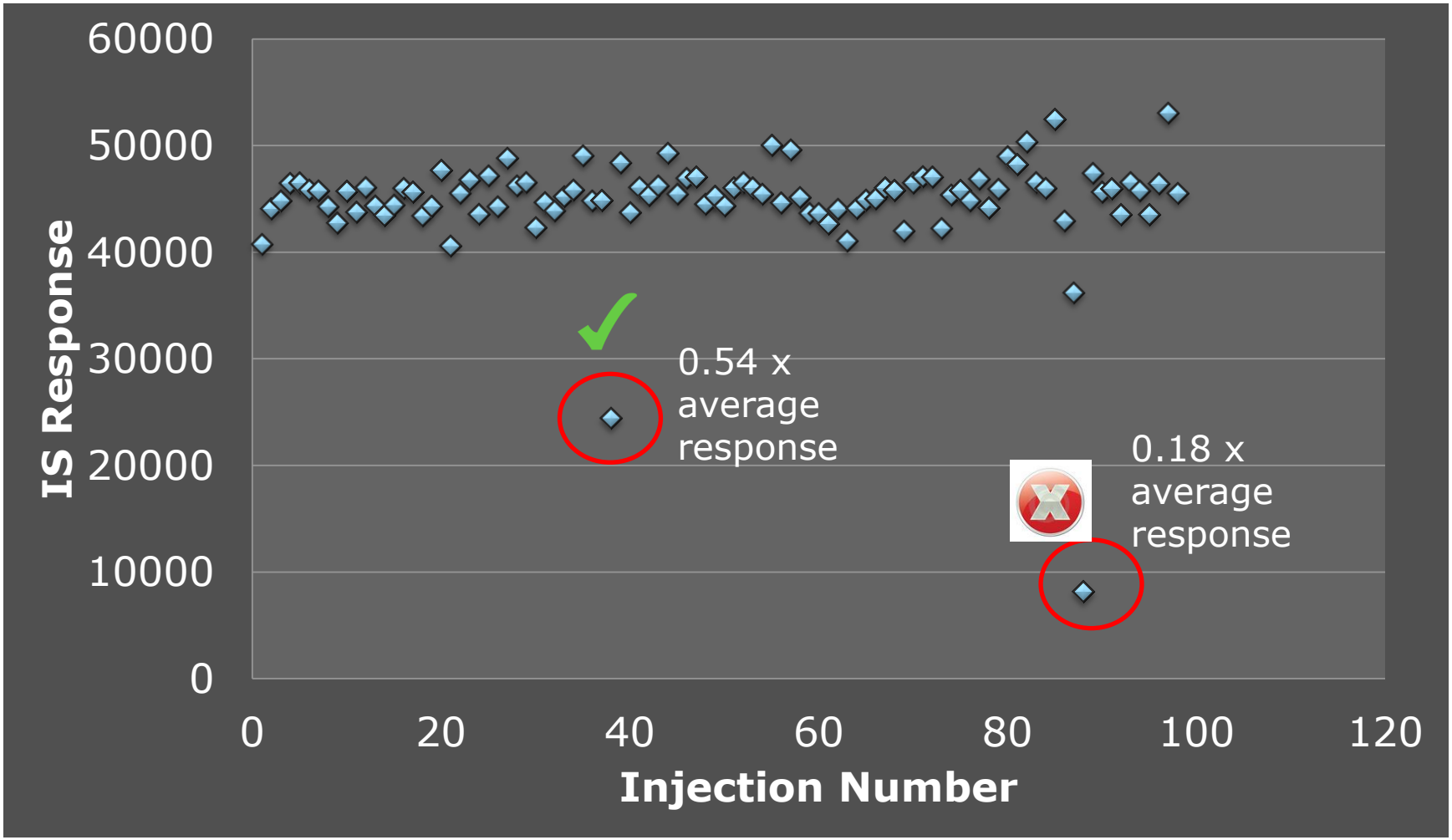
IS acceptance criteria currently used by Janssen Research and Development

- Criteria straightforward and easy to use; no complicated calculations
- Criteria should enable to filter out gross errors (e.g. spiking errors)
  - Calculate average IS response over the entire batch
  - Lower limit: if IS response < 25% (STIL) or 50% (analog) of average then reject; ability to accurately quantify the LLOQ
  - Upper limit: if IS response > 175% of average then reject
  - Do not reject calibration standards or QCs based on this criterion; BCV will tell you if IS compensates for response variations in these samples

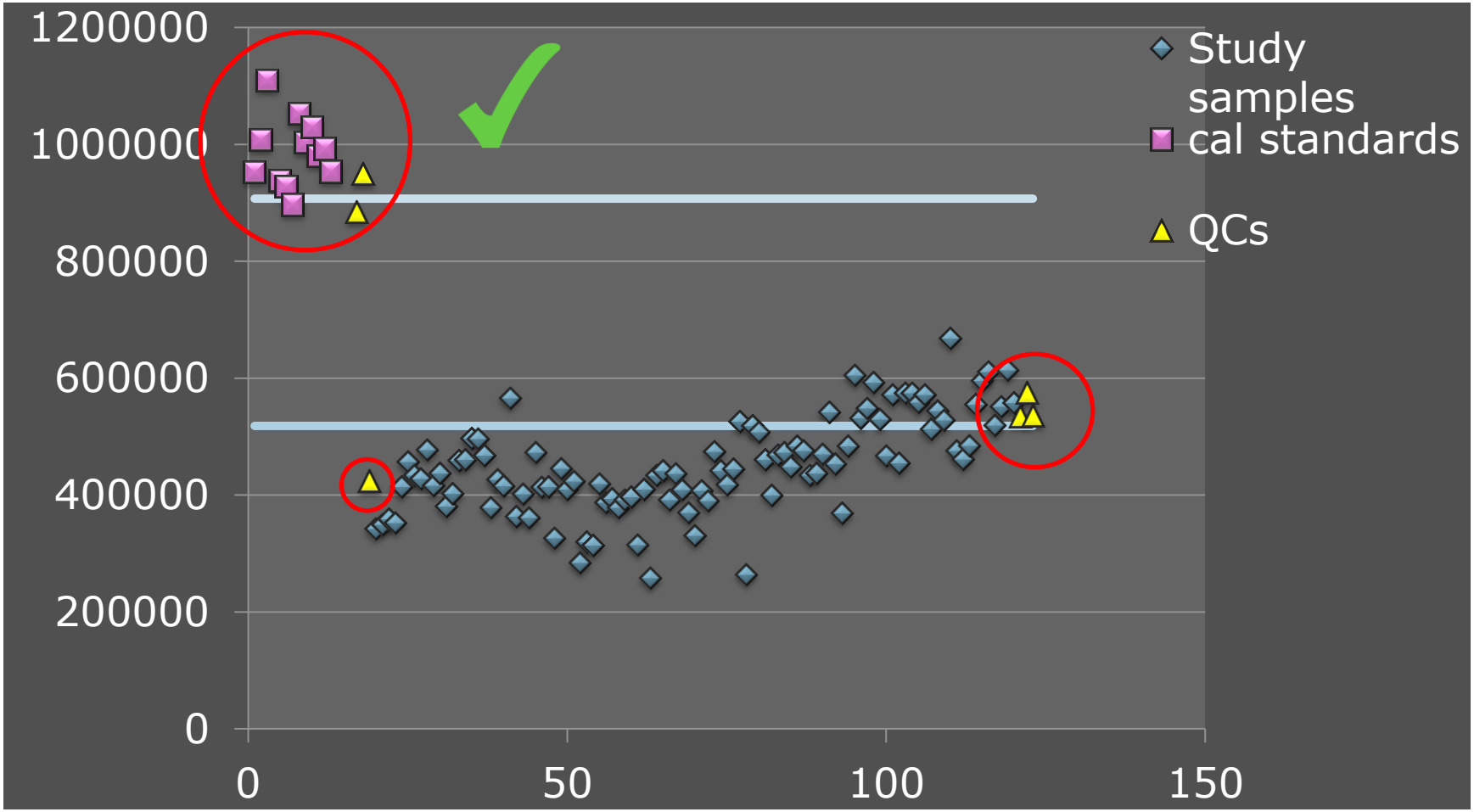
Example 1


450000
400000
350000
300000
250000
200000
150000
100000
50000
0
IS Response
1.9 x average response
0
20
40
60
80
100
Injection number

Example 2


60000
50000
40000
30000
20000
10000
0
IS Response
0
20
40
60
80
100
120
Injection Number
0.54 x average response
0.18 x average response

Example 3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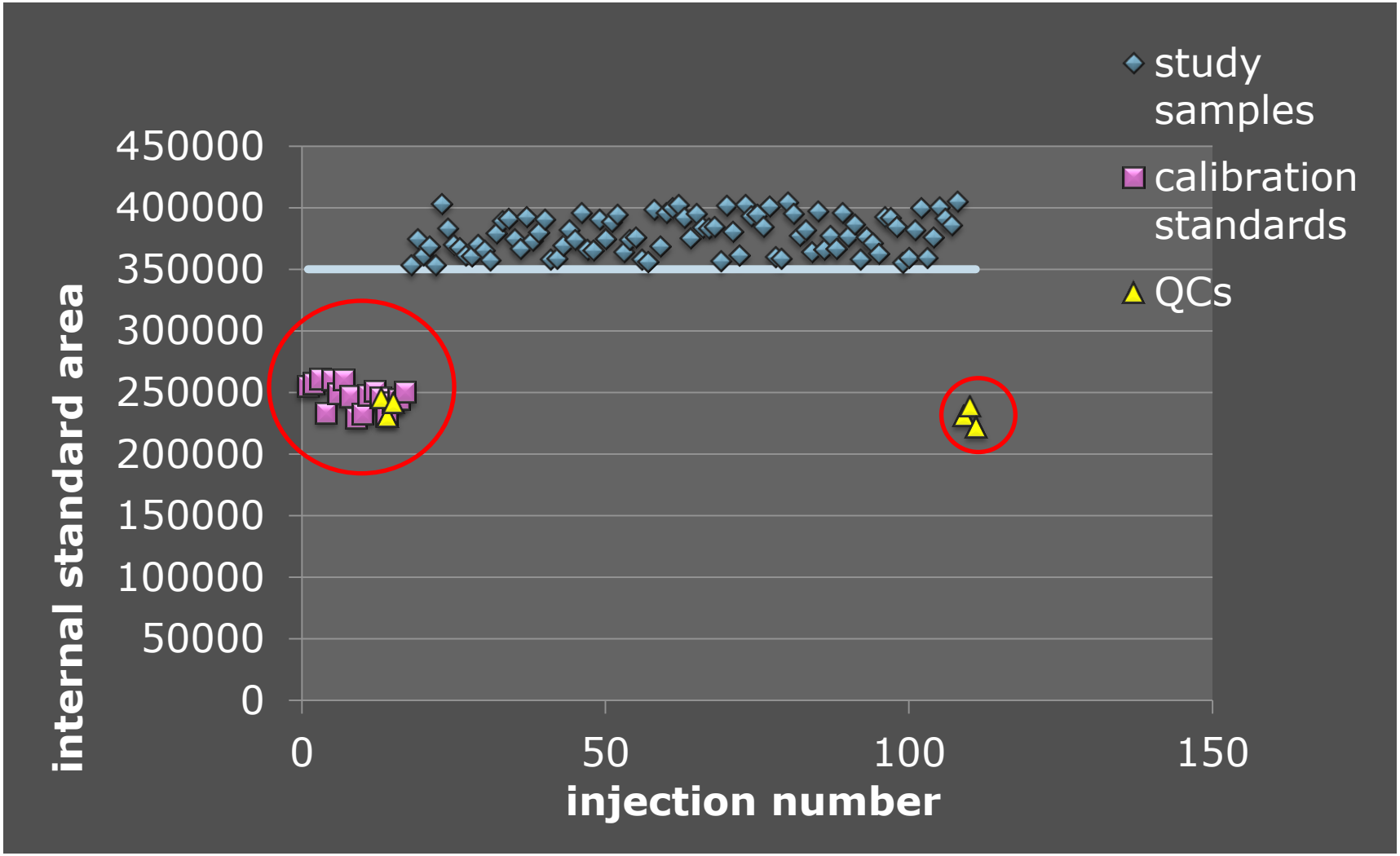
1000000
800000
600000
400000
200000
0
0
50
100
150
Study samples
cal standards
QCs

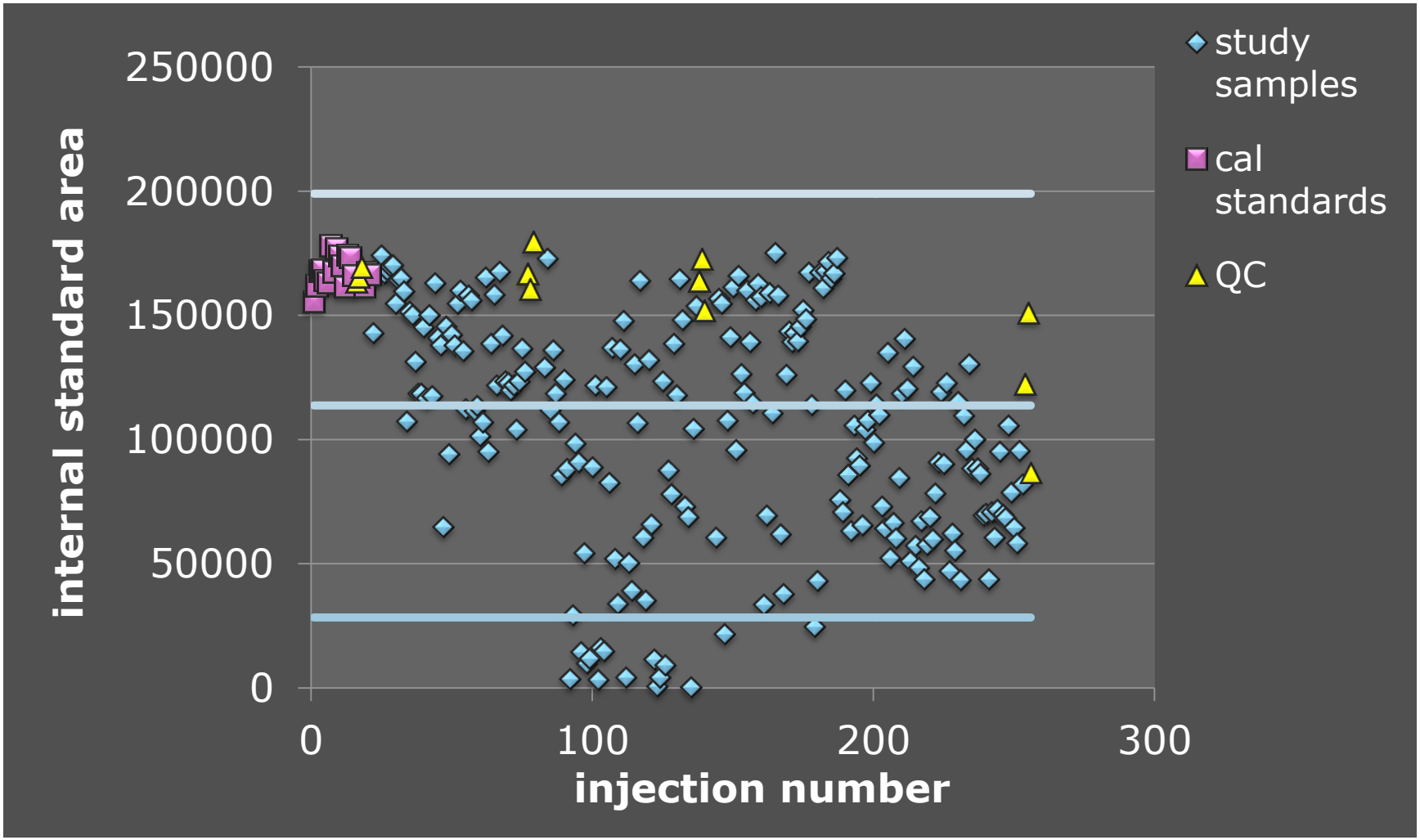
Are these criteria ideal?

- No, need some fine tuning
- Even when acceptance criteria are met, IS response differences can question reliability of the results
- Additional criteria needed to flag batches where further investigation into root cause is required
- Outcome of root cause investigation could be to
  - accept batch
  - re-inject (instrument problem)
  - re-analyse
- If problem cannot be solved method may require re-development

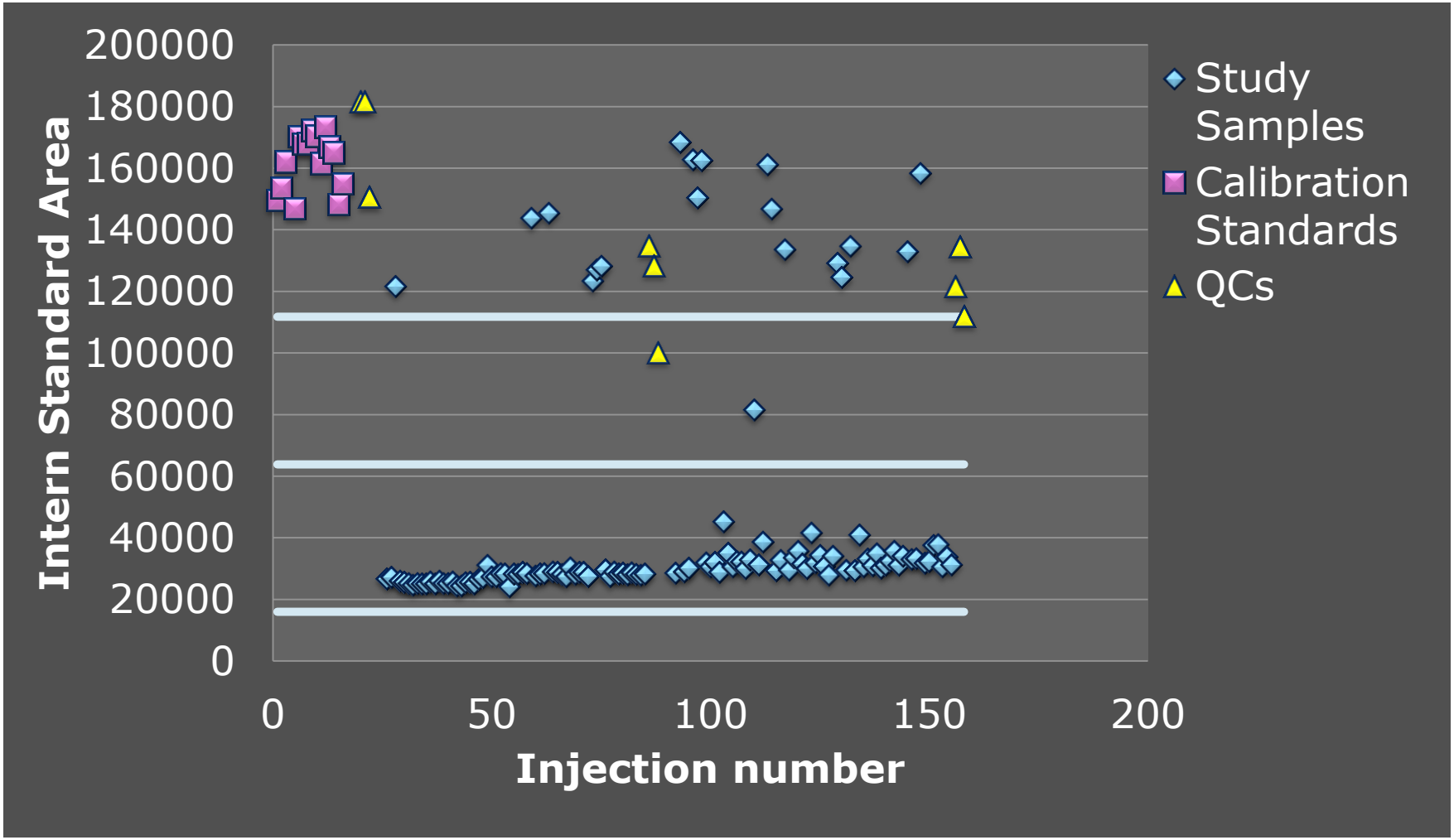
Example 4


study samples
calibration standards
QCs
internal standard area
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
50
100
150
injection number

Example 5


250000
200000
150000
100000
50000
0
internal standard area
0
100
200
300
injection number
study samples
cal standards
QC

Example 6


Intern Standard Area
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
50
100
150
200
Injection number
Study Samples
Calibration Standards
QCs

Fine tuning of criteria

- Criteria should pick up
  - Difference in IS response standards-QCs-study samples
  - Difference in IS response between subjects
  - Large variability in IS response overall
  - Gradient in IS response throughout the run
- Add criteria to initiate further investigation (no hard criterion to reject the batch):
  - 1. overall %CV < 30%
  - 2. average response study samples within 70 – 130% average response standards & QCs
- If one of the above is not met: investigate
- If both of the above are met, apply lower & Upper IS response criterion to accept/reject study samples

Conclusion

- Having a priori IS response criteria adds value
- Criteria currently used at Janssen R&D are being fine tuned
  - Start by applying first set of criteria to flag unexpected IS behavior requiring further investigation
  - If first criteria pass, apply second set of criteria to reject individual samples based on lower and upper limits
- Proposed criteria should be easy to apply