



Confirmatory Re-analysis of Study Samples – An Industry Perspective

Mario L. Rocci Jr., Ph.D.
Prevalere Life Sciences, Inc.
Whitesboro, NY



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Background

- Crystal City III Conference Consensus Report (The AAPS Journal, 2007)
- Viswanathan, et. al. have suggested that an evaluation of the reproducibility in the analysis of incurred samples be performed:
 - On each species used for GLP toxicology assessments
 - An “appropriate evaluation” of incurred sample reproducibility from clinical studies





Industry Perspectives

- Few laboratories (~8% based on one survey in 2005) perform repeat assays using incurred samples
- General lack of consensus and guidance as to how this analysis is to be accomplished.
- Some question whether the re-analysis of incurred samples is value-added due to the time and costs associated with it, as well as the likelihood that differences will actually be detected.
- Others feel it is a worthwhile exercise and have at least some data to back their contention.
- Approaches directed toward the confirmatory re-analysis of incurred samples must employ scientific and statistically-based methodology. It must also be practical.
- Was required for Canadian submissions – requirement was removed in 2003 – “We didn’t know what to do with the data.”



Practical/Scientific Questions Surrounding this Issue

- From what types of studies should samples be reanalyzed?
- How many samples should be re-analyzed to assure ourselves that the assay is reproducible?
- In what manner should the data be analyzed to arrive at valid conclusions?
- What actions, if any, should be taken, once the analysis is completed, and the results have been evaluated?

In What Types of Studies Should Samples be Re-analyzed?

- Pre-clinical GLP Toxicology Studies – Incurred samples from one study conducted in each species
- Guideline for Clinical Studies (highly dependent on what is known about the macromolecule):
 - “Normal Volunteer” Studies (when conducted) – one study
 - “Patient Population Studies – evaluate on a case-by-case basis
 - What disease are we treating – is “patient” matrix likely to be substantially different from “normal” matrix? (e.g., Cancer, Renal, Liver Disease)
 - Has the method been partially validated in “patient” matrix?
 - Apart from inherent differences between “patient” and “normal” matrix could disease specific instability issues (Ex/ increase in endogenous protease levels) or other disease related factors (pseudo-receptors, endogenous inhibitors etc.) affect the integrity of incurred sample analysis?
 - Generic Bioequivalence Trials – one study from each submission



How Many Samples Should be Re-Analyzed to Assure Ourselves that the Assay is Reproducible?

- Differences in Incurred Sample Reproducibility
 - Systematic
 - Random
- Systematic Differences – would like to detect a 25% difference between assay results with >80% power and a 5% probability of a Type I error (falsely claiming a difference when none exists)
 - Assuming a CV of 25% (acceptance criteria at the LLOQ and ULOQ), as few as 10 samples would need to be re-analyzed
- Random Differences – more samples would be required than is necessary to detect a systematic difference
- Limited Model Testing – 20 samples sufficient in most cases to detect either a significant systematic or a random difference





In What Manner Could Data be Analyzed to Arrive at Valid Conclusions?

- This approach is analogous to the steps outlined by Eastwood et al, 2006, for evaluating the reproducibility of compound potency results between two results (see: <http://www.ncgc.nih.gov/guidance/section2.html#analysis-potency>)
- Results from this approach can be summarized in graphical format using a Bland-Altman plot.
- Detailed explanation of the calculations necessary for the criterion in the previous slide will be published by Rocci Jr., et. al., in The AAPS Journal in 2007.



In What Manner Should the Data be Analyzed to Arrive at Valid Conclusions?

- Appropriate statistical methods should be used to determine the level of reproducibility of incurred samples and should include:
 - Accuracy Criterion – calculation of the mean ratio of the sample results and its 95% confidence limits (Ratio Limits) – useful in detecting systematic differences
 - Mean Ratio – average fold change in the sample results between the runs
 - Precision Criterion - the characterization of the “67% limits of agreement” between the results which will prove useful in detecting random differences
 - 67% Limits of Agreement – range within which the ratio of sample results is expected to fall approximately 2/3 of the time.

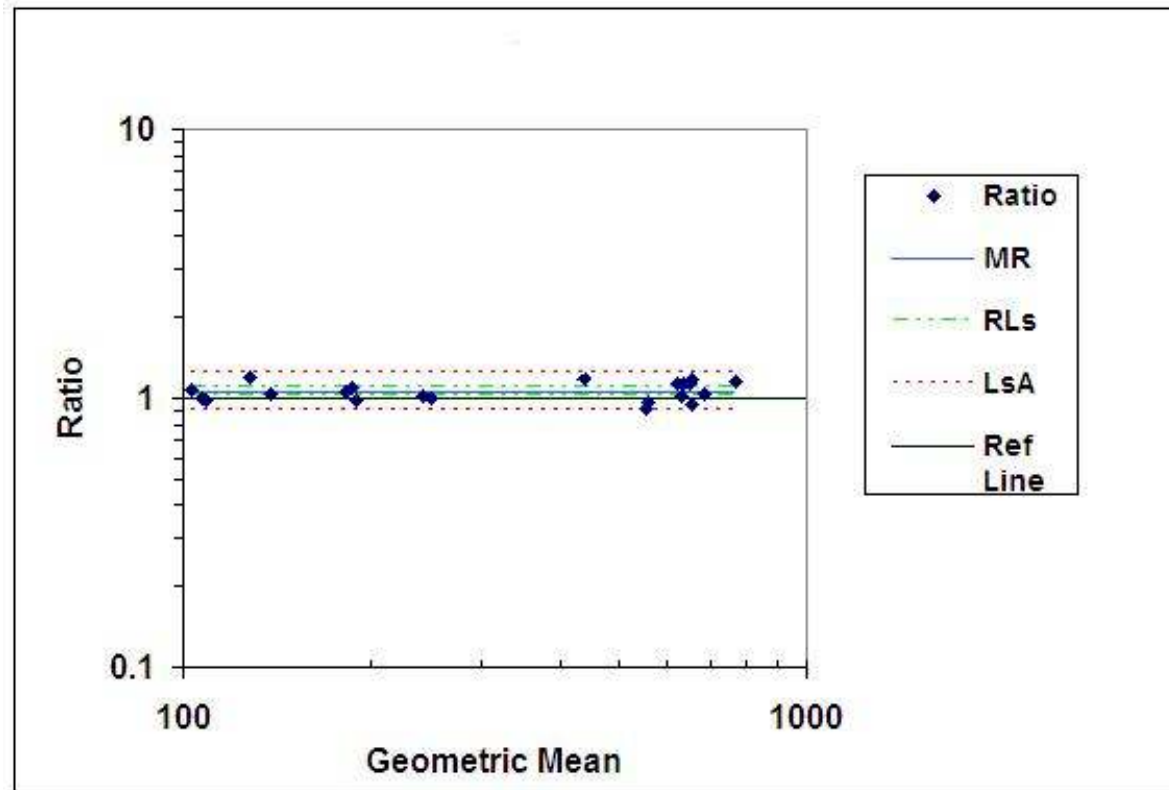
Example One

| <u>Subject</u> | <u>Original Result (ng/mL)</u> | <u>Repeat Result (ng/mL)</u> | <u>Percent Difference*</u> |
|----------------|------------------------------------|----------------------------------|--------------------------------|
| 4001 | 478 | 406 | -16.3 |
| 4002 | 107 | 107 | 0.0 |
| 4031 | 826 | 718 | -14.0 |
| 4031 | 108 | 109 | 0.9 |
| 4003 | 248 | 250 | 0.8 |
| 4004 | 696 | 674 | -3.2 |
| 4004 | 141 | 135 | -4.3 |
| 4005 | 194 | 179 | -8.0 |
| 4006 | 548 | 564 | 2.9 |
| 4007 | 676 | 598 | -12.2 |
| 4009 | 636 | 676 | 6.1 |
| 4032 | 635 | 624 | -1.7 |
| 4032 | 244 | 240 | -1.7 |
| 4009 | 527 | 579 | 9.4 |
| 4009 | 139 | 117 | -17.2 |
| 4010 | 712 | 601 | -16.9 |
| 4010 | 107 | 99.3 | -7.5 |
| 4033 | 664 | 583 | -13.0 |
| 4033 | 187 | 176 | -6.1 |
| 4033 | 690 | 610 | -12.3 |
| 4033 | 187 | 190 | 1.6 |

*((Repeat Result -Original Result)/Average) expressed as a %



Example One – Bland-Altman Plot



N=21 samples

MR = 1.06

RLs = 1.02 to 1.09 (Range for acceptance= 0.83-1.20)

LsA = 0.98 to 1.14 (Range for acceptance= 0.83-1.20)



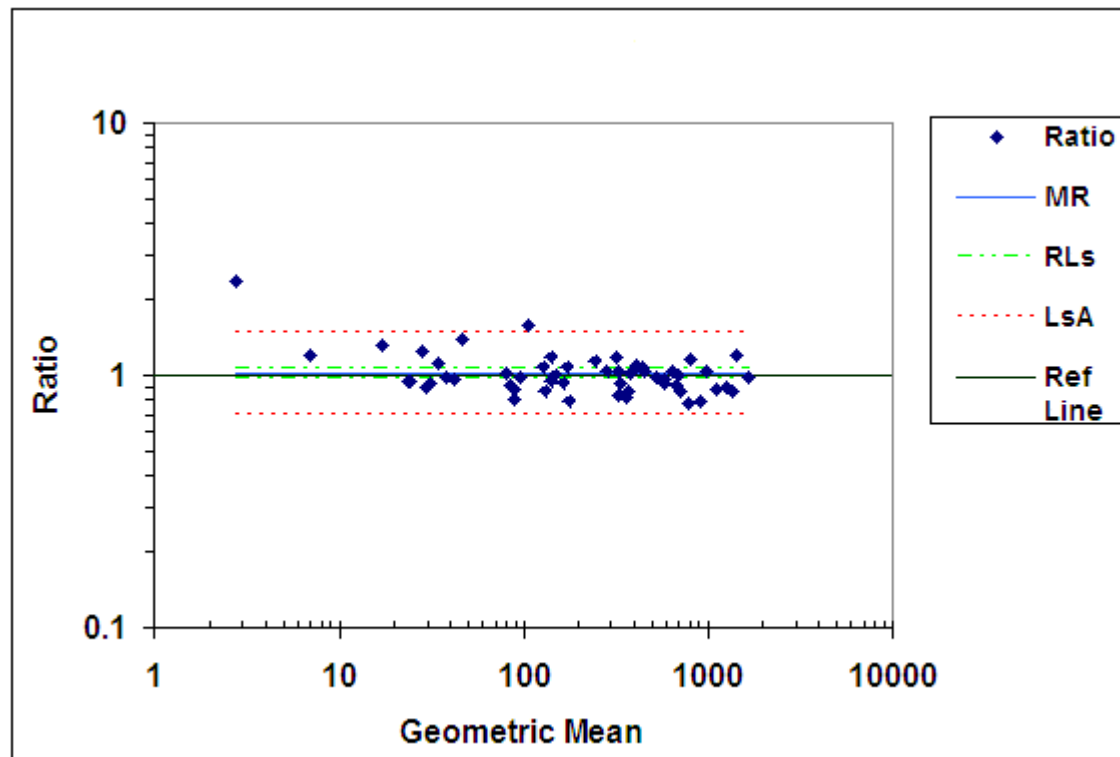


Example Two – Percent Differences

| | | | | | | | |
|-------|-------|------|-------|-------|-------|-------|-------|
| 5.9 | 5.4 | -2.4 | -7.0 | -33.0 | 9.5 | -9.8 | 1.7 |
| 10.8 | -81.1 | 3.4 | -6.5 | 9.7 | 2.5 | 13.9 | -3.7 |
| -0.9 | -22.1 | -7.4 | 0.7 | 23.0 | 20.5 | 7.8 | 14.6 |
| -17.8 | -26.7 | -2.9 | 1.3 | -3.8 | -45.0 | -2.7 | -14.9 |
| 8.1 | -4.3 | 7.8 | -11.3 | 24.6 | 12.8 | -3.7 | 4.3 |
| -18.2 | -17.2 | -5.9 | 12.8 | 18.7 | 13.7 | -12.6 | 7.5 |
| 10.6 | 14.4 | 22.5 | -16.0 | 24.5 | 2.7 | 5.6 | |



Example Three – Bland-Altman Plot



N=55 samples

MR = 1.01

RLs = 0.96 to 1.06 (Range for acceptance= 0.83-1.20)

LsA = 0.84 to 1.22 (Range for acceptance= 0.83-1.20)





What actions, if any, should be taken, once the analysis is completed, and the results have been evaluated?

- Criteria for accuracy and precision are met and all of the data looks great - on to bigger and better things!
- Criteria for accuracy and precision are met but the differences observed in a few samples are of concern
 - Investigate to assure yourself that assay or sample-related factors are not to blame
- Criteria for accuracy and precision are not met
 - in-depth evaluation of causative factors is critical



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