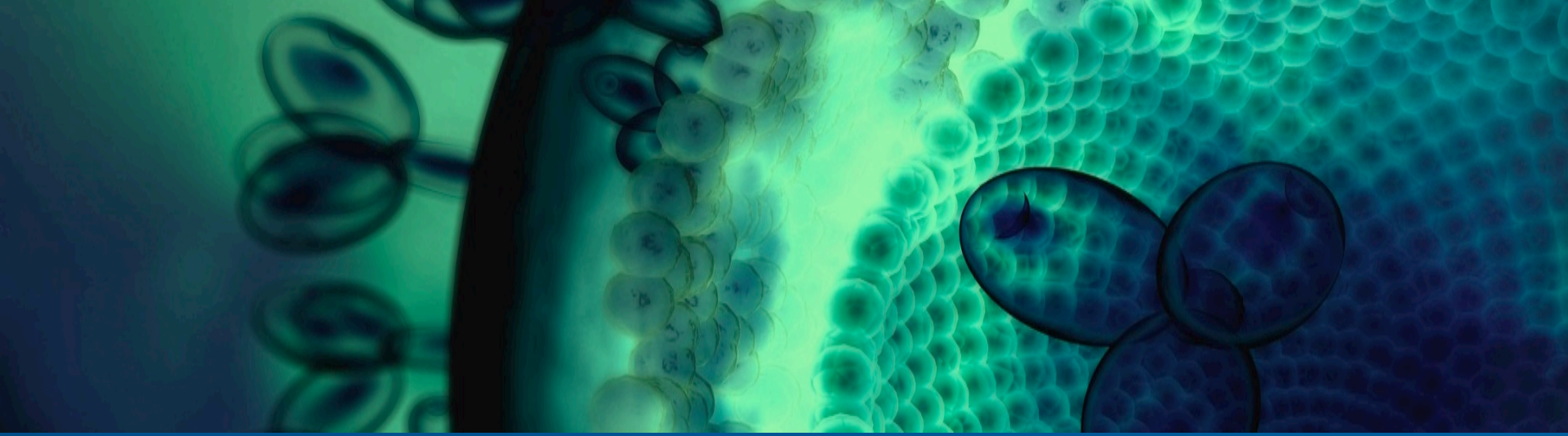


ISR: what have we learned after a decade of experience?

Pictured above: The structure of HIV.

Tom Verhaeghe
EBF Symposium| November 17, 2017

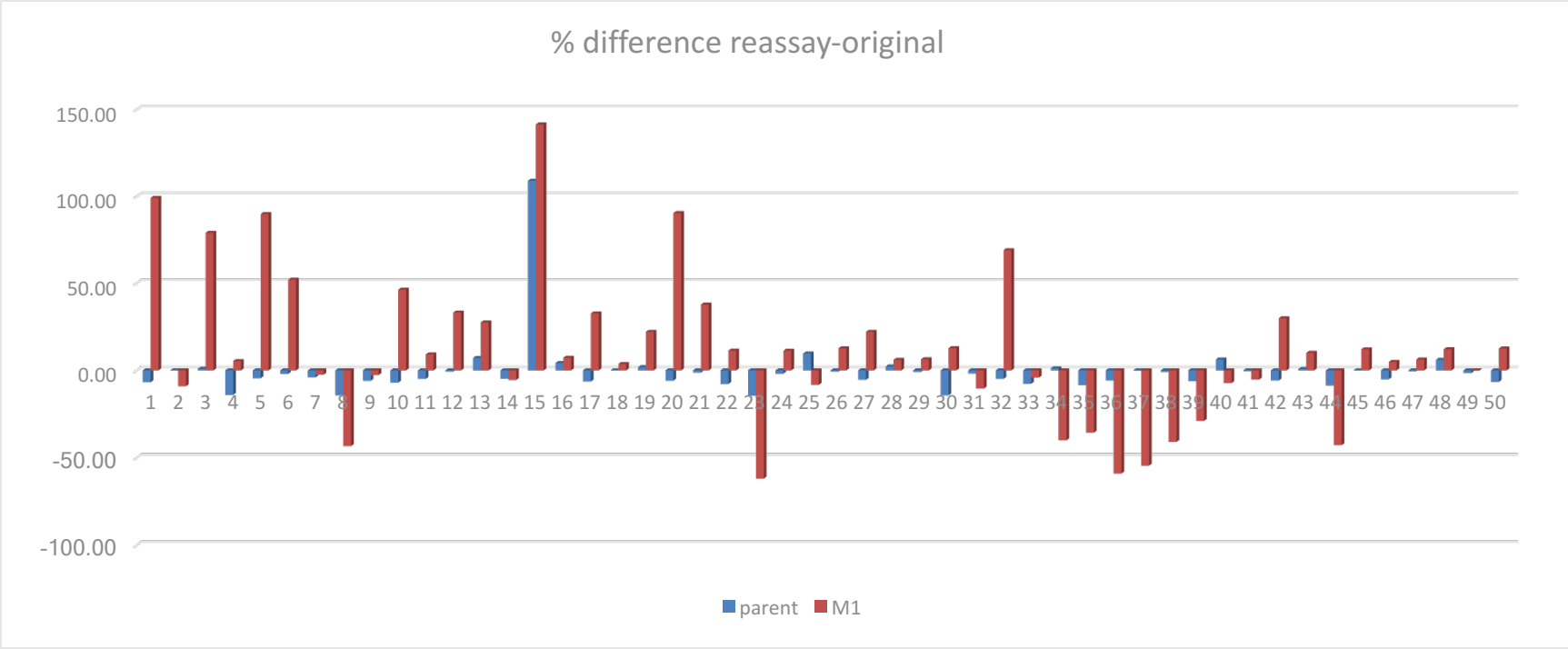
Introduction

- ISR introduced in 2007 as a tool to identify methodological failures
- Collected data from 10 years of experience with Janssen projects in GLP and clinical studies
- 10 examples found of failing ISR, in Janssen lab and at CRO
- Categorized different cases
  - Methodological failure
  - (likely) human error
  - Unidentifiable error
  - Instrument error

Case #1: methodological failure

- In-licenced project
- 2-in-1 assay quantifies parent (R-CO-NH2) & M1 (R-COOH).
- ISR passes for parent but consistently fails for M1 (in clinical and GLP studies).
- M1 results reproducible after single dosing but not after multiple dosing

Case #1: methodological failure



Case #1: methodological failure

- Investigation revealed study samples contain high levels of M1-glucuronide (up to 80x M1 conc. after RD)
- Assay uses evaporation step; M1-glucuronide can partially decompose to M1
- Assay re-developed without evaporation step; issue resolved

Case #2: methodological failure

- GLP study in Tg.rasH2 transgenic mice
- ISR fails for 21/24 samples; all negative bias
- Instability? → time between initial assay and ISR was 99 or 274 days…
- …but validation data indicate 24h@RT, 4xF/T, 315 days at -70 °C stability in CD-1 mouse plasma
- Difference in stability between transgenic mouse plasma and CD-1 mouse plasma ?

Case #2: methodological failure

- Additional experiments revealed severe instability in all plasma sources tested
- No significant difference between fresh plasma from Tg.rasH2 or CD-1 mice
- Apparent difference between fresh and old plasma; more pronounced at higher temperatures
- Stability data from validation not confirmed in old plasma tested in new experiment
- Failing ISR might be attributed to instability of analyte upon repeated thawing or during storage between initial and repeat analysis
- ISR results were rejected
- Initial results were retained but flagged as possibly an underestimation of true value

Case #3: human error

- Clinical study, 2134 samples, rolling ISR
- ISR passed but results from 2 runs could not be confirmed
- The 2 runs with identical sample numbers and plate design were processed in parallel; 96-well plates from the 2 runs were interchanged during processing
- Issue resolution: reject 2 original runs; re-analyse samples from both runs

Case #4: human error

- 13 week GLP dog study
- 1400 samples, many dilutions (2-, 5-, 10-, 20- and 50-fold dilutions)
- 102/140 ISR results fail; %bias from -84% to +490%; majority of failures were diluted samples
- Initial results rejected; all samples reanalysed in duplicate by 2 lab technicians
- 99% of duplicates matched
- High probability of human error in dilution steps

Case #5: likely human error

- GLP study, 168 samples analysed in one run
- 18/24 ISR results fail; all negative bias
- 4 selected samples from ISR repeated in duplicate confirm ISR results
- Results from original run rejected; all samples reanalysed; ISR performed (24/24 pass)
- cause unidentified; most likely pipetting errors during initial analysis (CMS assay)

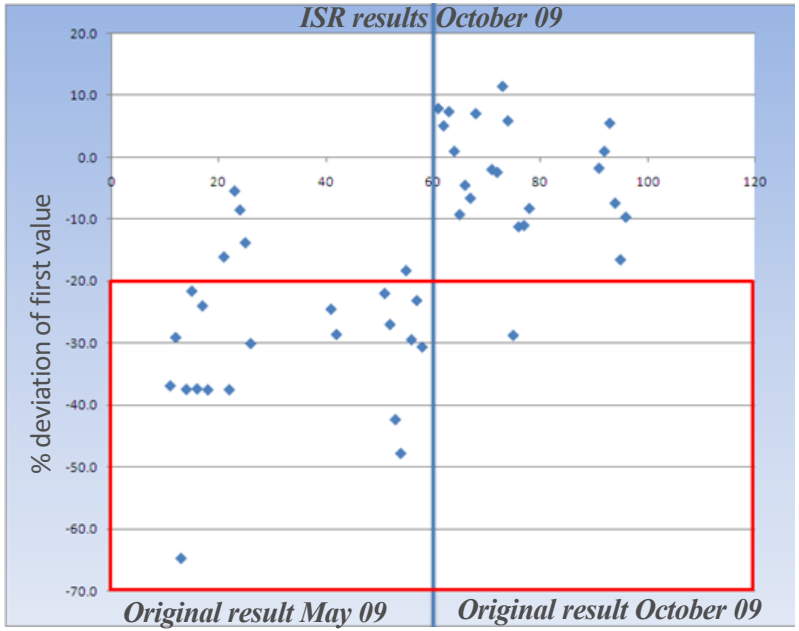
Case #6: likely human error

- Clinical study 1308 samples, 3-in-1 assay
- ISR at end of study fails for all 3 analytes (mostly +bias) in the same samples
- Reanalysis of selection of ISR samples in duplicate confirms ISR results.
- All results rejected, all samples reanalysed with rolling ISR
- ISR passes
- No identifiable cause; likely pipetting errors; samples in brown tubes and processed under yellow light.

Case #7: likely human error

- Phase 3 clinical study
- ISR batch contained 2 sets of samples
- Set 1 ISR 5 months after initial result; fail
- Set 2 ISR 2 days after initial result; pass

Case #7: likely human error



Case #7: likely human error

- Instability during 5 month freezer storage?
- ISR repeated after 5 months on selection of samples from set 2: passed
- No instability; most likely human error during analysis of samples from set 1

Case #8: unidentifiable error

- Study in pediatric subjects; samples analysed in multiple small batches
- ISR passed overall but failed for all samples from 1 subject
- Samples from this subject reanalysed in duplicate; confirmed ISR result
- No assignable cause found

Case #9: unidentifiable error

- Clinical study; 3150 samples analysed with 2-in-1 assay
- 1 cohort with elderly subjects: ISR only for that cohort
- ISR fails for both analytes in same samples (14/28; 11/28)
- In previous studies ISR passed
- Selection of samples from ISR repeated in duplicate, original results confirmed
- Additional samples selected for ISR; fails again for both analytes in same samples (22/63; 20/63).
- All 7 initial runs rejected; implemented rolling ISR; now perform ISR on all cohorts; pass
- No assignable cause, suspected inhomogeneous samples but could not be confirmed

Case #10: instrument calibration error

- Clinical study 252 samples
- 11/28 samples fail ISR
- Samples initially analysed on instrument 1, ISR on instrument 2
- Big difference in calibration line slopes and sensitivity between both instruments
- Mass calibration settings outside of acceptance for instrument 1
- All results rejected; samples reanalysed after fixing calibration issue; ISR passed

Conclusion

- In our experience over past 10 years, only 2/10 of failing ISR cases attributable to methodological failures
- Instead ISR more often serves as quality control check for lab based errors
- Even in cases where overall ISR does not fail it can identify errors in individual batches
- For large studies rolling ISR is preferable; don't wait until end of study!
- Re-analysis in duplicate valuable tool to investigate failed ISR
- Assays with multiple analytes can be helpful for identifying failing ISR cause.

Acknowledgements

- Marc De Meulder
- Vera Hillewaert
- Hans Stieltjes
- Peter Pruim


janssen
PHARMACEUTICAL COMPANIES
OF Johnson&Johnson