Dilution Linearity & Parallelism

Presenter: Robert NELSON,
on behalf of the EBF

Focus Workshop

(*In collaboration with the AAPS and JBF*)

Industry input into ICH M10: Experimental data as the cornerstone for a science driven bioanalytical guideline

The Altis Grand Hotel Lisbon,
Portugal September 24-26, 2017

Problem Statement

- Regulatory guidances provide differing levels of detail and requirements for assessment of dilutional linearity and parallelism

Part 1: Dilutional Linearity

Dilutional Linearity

- Dilutional linearity experiments are performed to demonstrate that high concentrations of the analyte of interest can be accurately measured by diluting into the assay's quantitative range and multiplying the measured concentration by the dilution factor.
  - Particularly relevant to LBA assays where samples of high concentration may require significant dilution to achieve the working range of the assay
  - Hook effect is typically assessed in the same experiment by including samples spiked with very high concentrations of analyte which are tested without dilution beyond MRD
  - GBC L2 Global Harmonization Team Recommendations. Stevenson et al. (2014) AAPS J. 16(1): 83-88

Bioanalytical Guidelines

- Guidelines (both chromatographic and LBA) indicate that dilution of samples should not affect the accuracy and precision
  - But varying levels of detail provided

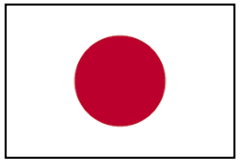
Bioanalytical Guidelines

EMA, 2011	
4.1.7. Dilution integrity Dilution of samples should not affect the accuracy and precision. If applicable, dilution integrity should be demonstrated by spiking the matrix with an analyte concentration above the ULOQ and diluting this sample with blank matrix (at least five determinations per dilution factor). Accuracy and precision should be within the set criteria, i.e. within ±15%. Dilution integrity should cover the dilution applied to the study samples.	7.1.1.9. Dilutional linearity Because the narrow range of the calibration standard curve, it is necessary to demonstrate with QC samples that the analyte of interest, when present in concentrations exceeding the range of quantification (above ULOQ), can be accurately measured by the assay after dilution in blank matrix to bring the analyte concentrations into the validated range for analysis. An additional reason for conducting dilutional experiments is to detect a possible prozone or “hook effect”

Bioanalytical Guidelines

FDA, 2001	FDA, 2013 draft
VI. APPLICATION OF VALIDATED METHOD TO ROUTINE DRUG ANALYSIS The following recommendations should be noted in applying a bioanalytical method to routine drug analysis: • Any required sample dilutions should use like matrix (e.g., human to human) obviating the need to incorporate actual within-study dilution matrix QC samples	C. Validated Method: Use, Data Analysis, and Reporting Concentrations in unknown samples should not be extrapolated below the LLOQ or above the ULOQ of the standard curve. Instead, the standard curve should be extended and revalidated, or samples with higher concentrations should be diluted and reanalyzed.

Bioanalytical Guidelines



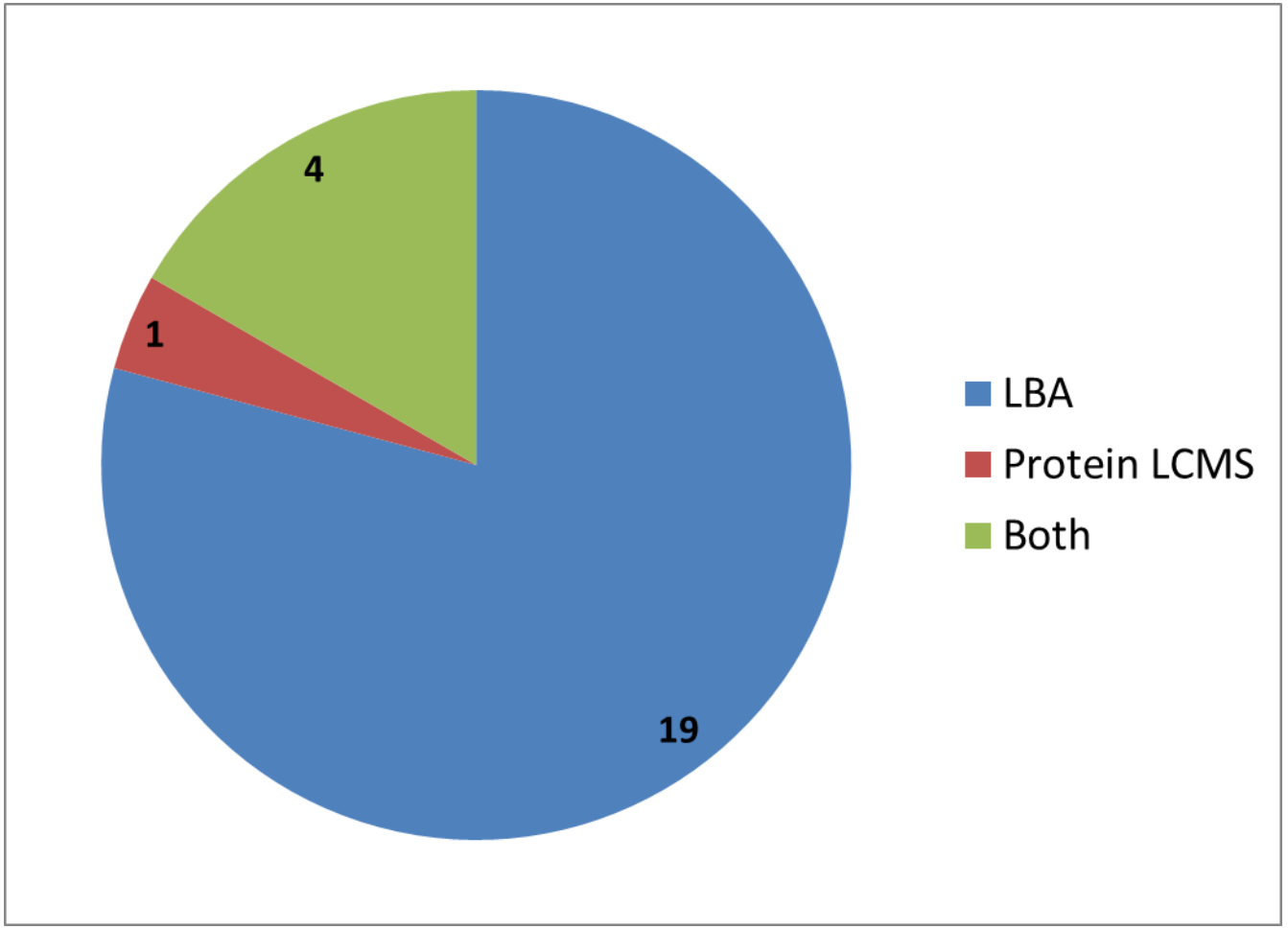
MHLW, 2014	ANVISA*, 2012	CFDA*, 2015
Dilutional linearity is assessed to confirm the following: (i) the method can appropriately analyze samples at concentrations exceeding the ULOQ of a calibration curve without influence of a hook effect or prozone; (ii) measured concentrations are not affected by dilution within the calibration range. Dilutional linearity is evaluated by analyzing a QC sample exceeding the ULOQ of a calibration curve and its serial dilutions at multiple concentrations.	In the case of reanalysis runs with diluted samples, DQC (dilution quality control) samples shall be included	Dilution Reliability • Dilution of samples should not interfere with the accuracy and precision. • Dilution reliability should be demonstrated by spiking the matrix with an analyte concentration above the ULOQ and dilution of this sample with blank matrix (at least five determinations per dilution factor). • Accuracy and precision should be within ±15%. • Dilution reliability should cover the applied dilution of the study samples.

* Non-official translation

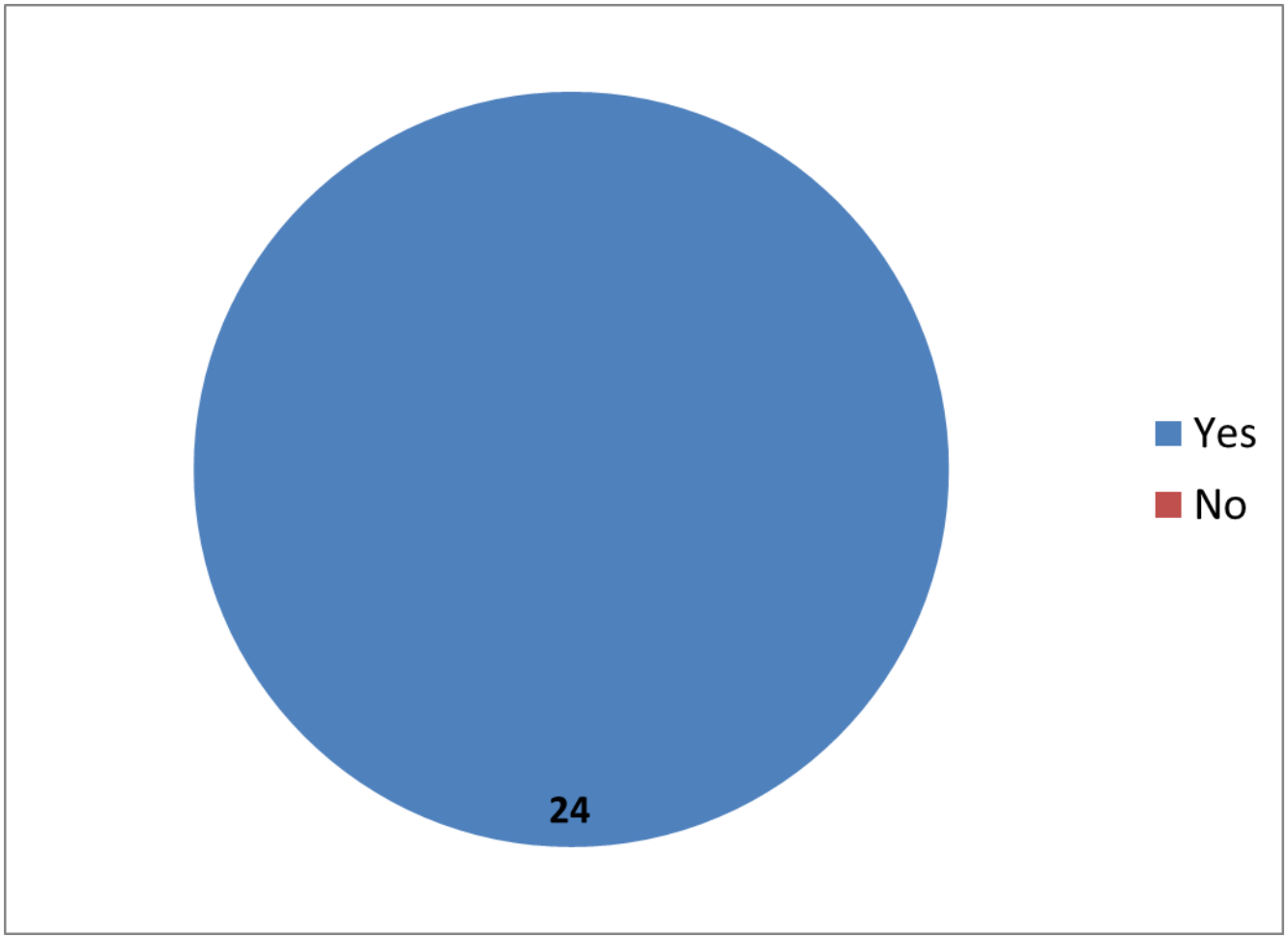
Survey in the EBF Community

- Review of current common practices
  - 24 responses: 15 Pharma, 9 CRO

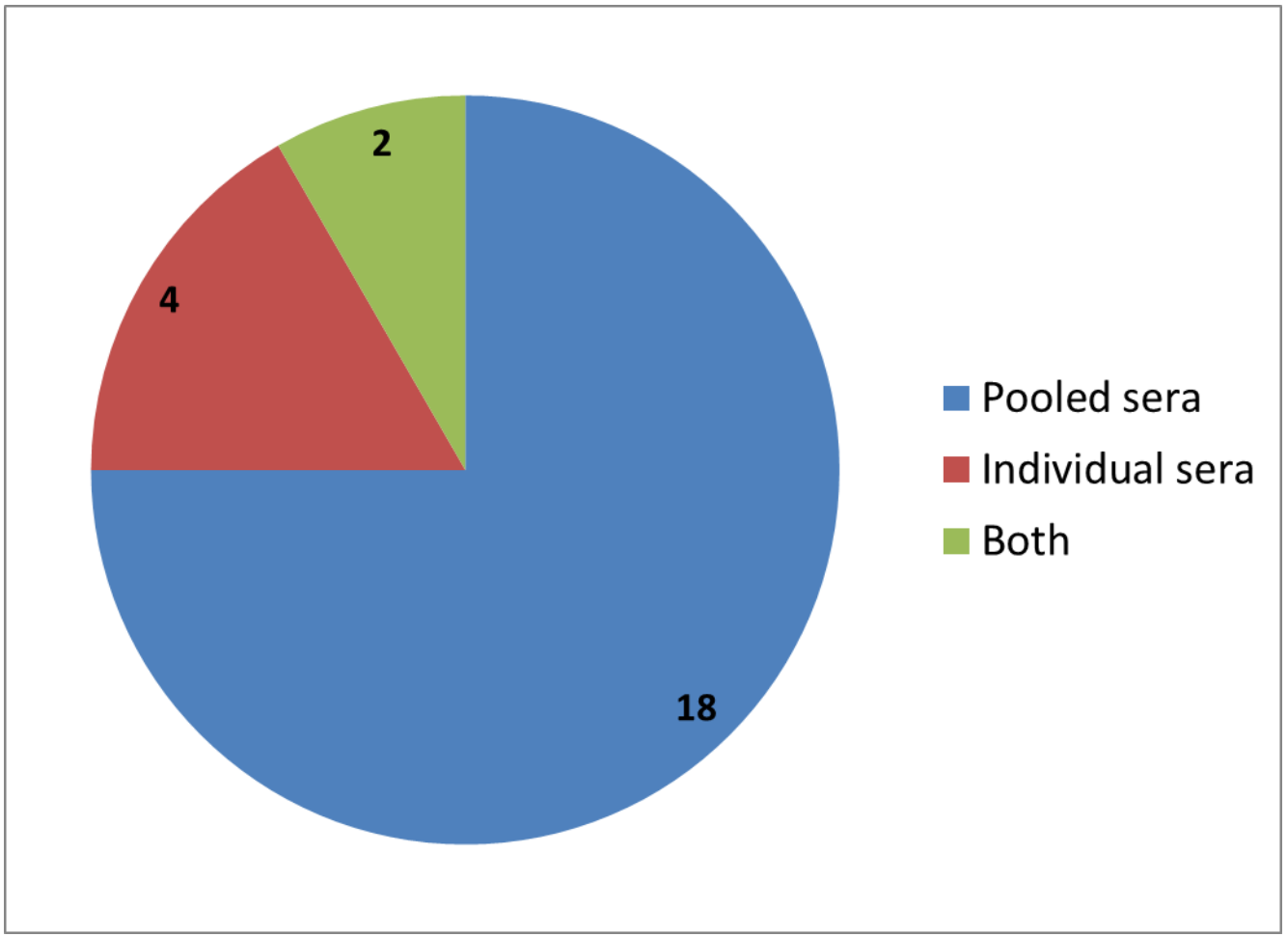
Q1: Do you perform?



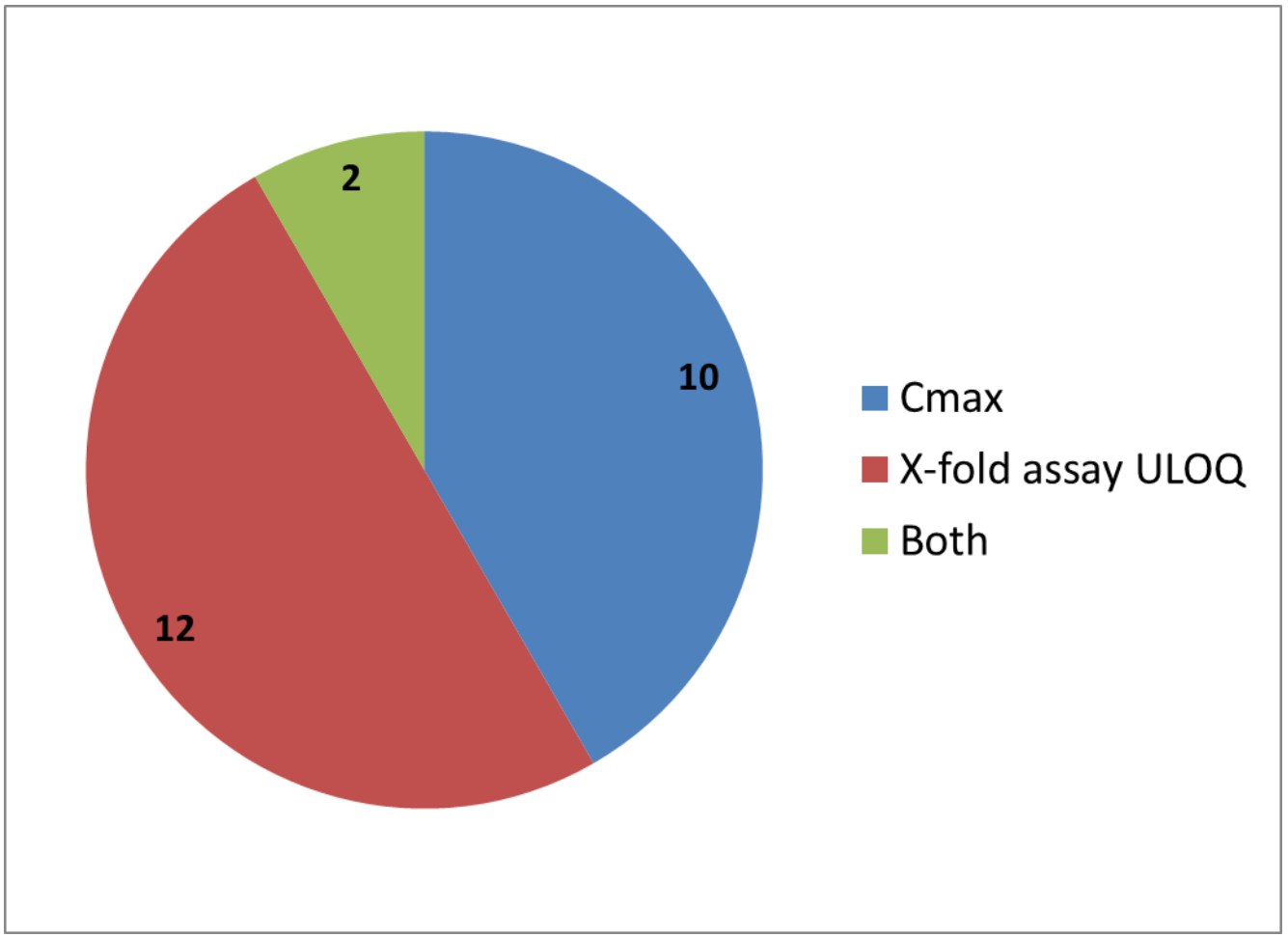
Q2: Do you perform dilution linearity regularly for PK methods?



Q3: How do you perform dilution linearity?

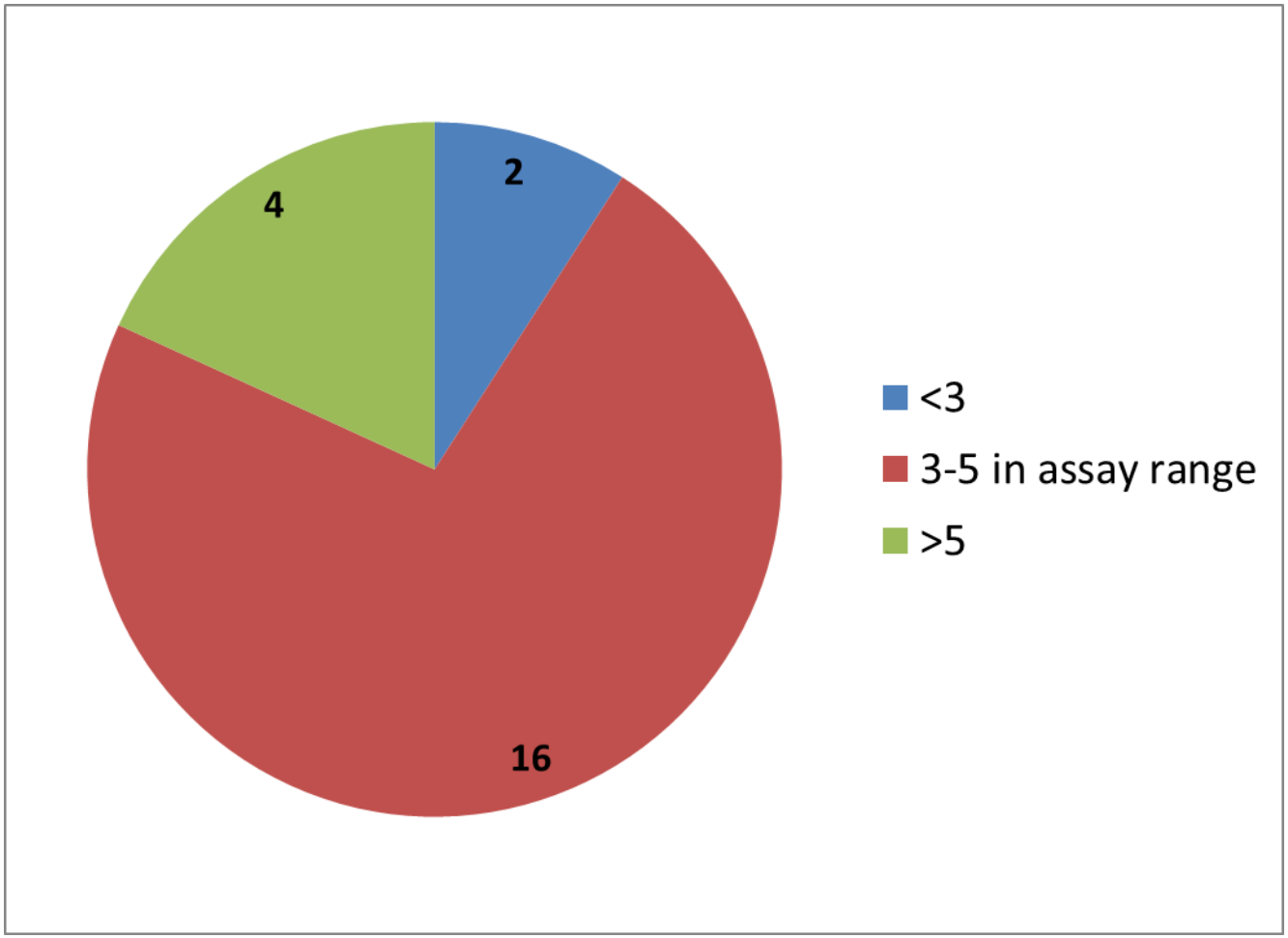


Q4: How do you define the concentration levels?

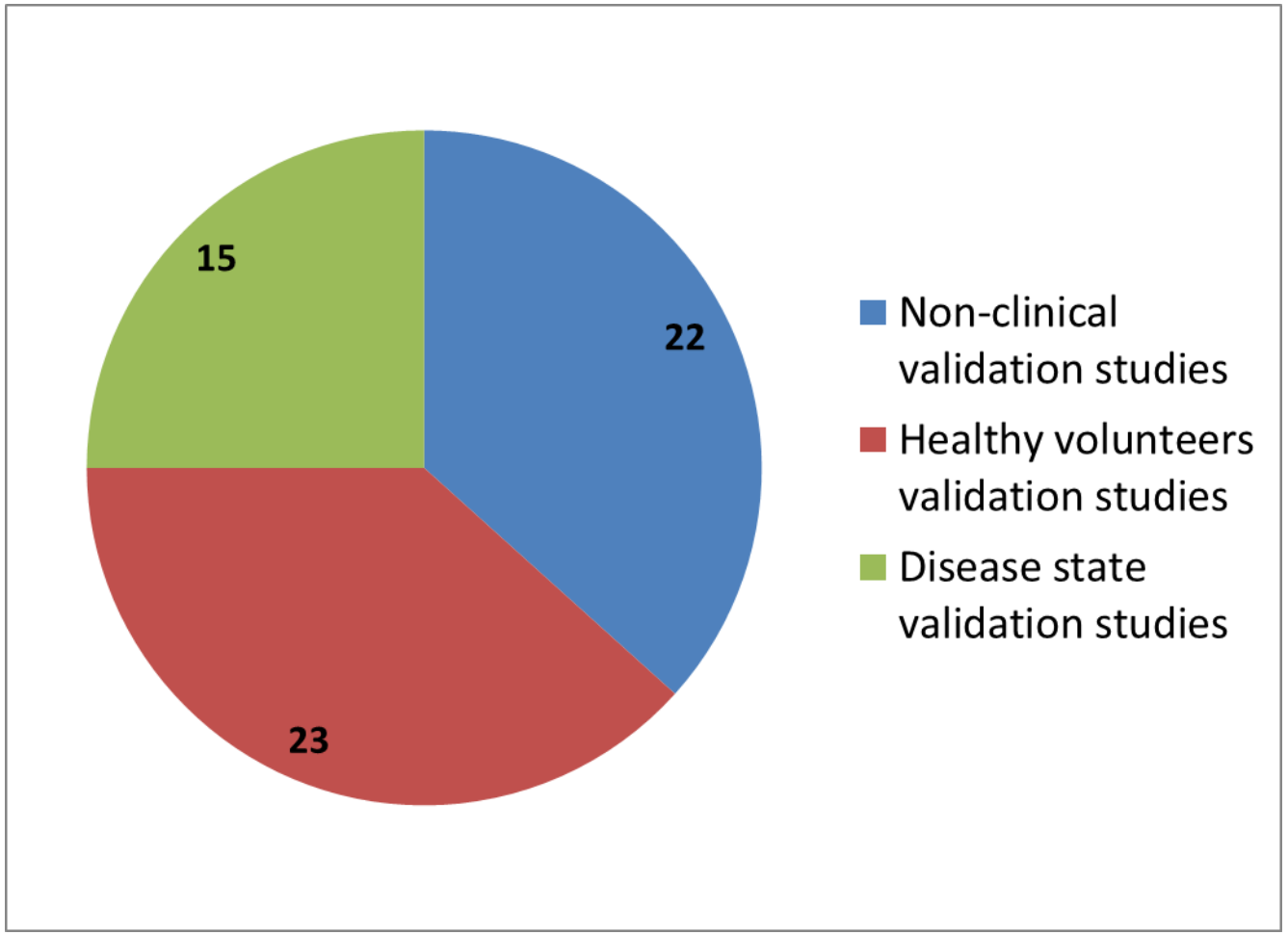


– X-fold often defined as highest possible concentration with 90% or 95% matrix

Q5: How many dilutions do you perform?

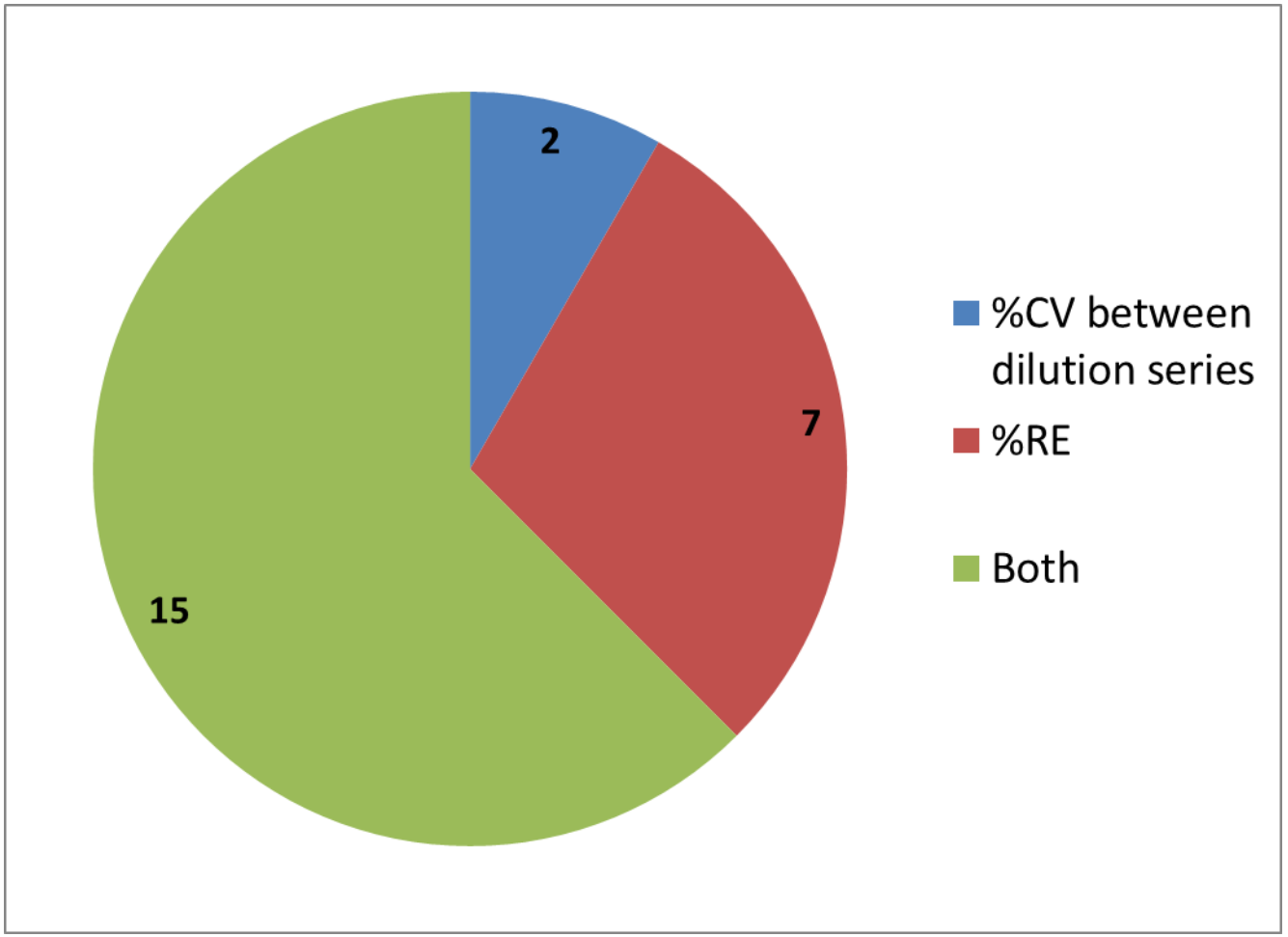


Q6: When do you perform dilution linearity?



– Total out of 24 responses

Q7: How do you evaluate the acceptance of dilution linearity?



Summary of the Survey

- Common approach for dilutional linearity
  - to spike pooled blank matrix
  - to evaluate hook effect with anticipated Cmax or highest possible concentration maintaining 90% or 95% matrix
  - 3-5 dilutions in assay range
  - To apply %RE and %CV acceptance criteria

- Very much in line with GBC L2 white paper

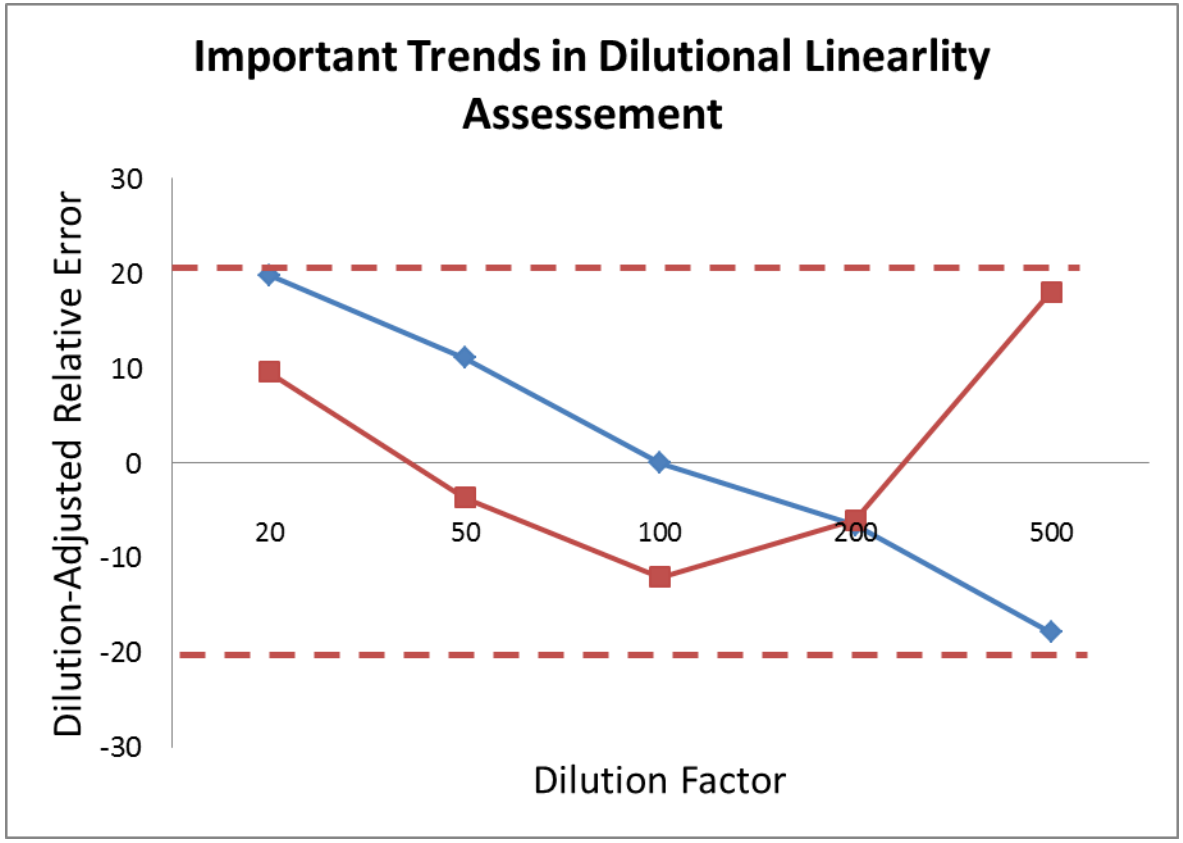
Harmonization of the Guidances

- *Recommendation:*
  - *Where dilution of samples will be required to bring the analyte level into the analytical range, dilutional linearity should be evaluated at multiple dilutions across the quantitative range of the assay*
    - *at least one above the ULOQ (to evaluate hook effect), 3–5 within the quantitative assay range, and one below the LLOQ to fully characterize dilutional linearity.*
    - *Spiked at or above the maximum anticipated concentration (Cmax) levels expected in study samples, or at the highest feasible concentration of analyte where the sample is composed of least 90% matrix.*
  - *Dilutional linearity samples with measured concentrations within the quantitative range of the assay should return values that are within ±20% of theoretical.*
    - *Trends that may have meaningful impact on the study data should be evaluated.*

Harmonization of the Guidances

- *Not Included in Recommendation:*
  - *Precision (%CV) of the cumulative back-calculated concentrations for all in-range samples should be ≤20%.*
    - *Precision criteria should be used with caution, as precision of a dilution series can be misleading*

Evaluation of Trends



- Series 1: CV = 14.6%
- Series 2: CV = 12.2%

Part 2: Parallelism

Parallelism

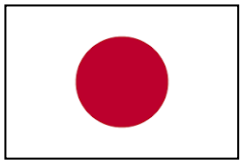
- The concept of parallelism is similar to dilutional linearity except that parallelism assesses incurred study samples.
- Incurred samples (pooled or individual) are tested at multiple dilutions that are expected to yield concentrations that fall above the assay ULOQ (to evaluate prozone or hook effect) as well as within the assay range.

  – GBC L2 Global Harmonization Team Recommendations. Stevenson et al. (2014) AAPS J. 16(1): 83-88

Bioanalytical Guidelines

EMA, 2011	FDA, 2013 draft
7.1.1.10. Parallelism If study samples are available, parallelism between the calibration standard curve and serially diluted study samples should be assessed to detect possible matrix effect or differing affinities for metabolites. A high concentration study sample (preferably close to Cmax) should be diluted to at least three concentrations with blank matrix. The precision between samples in a dilution series should not exceed 30%	B. Bioanalytical Method Development and Validation / 1. Selectivity / b. Matrix Effects Matrix effects should be evaluated. For example: The calibration curve in biological fluids should be compared with calibrators in buffer to detect matrix effects using at least ten sources of blank matrix. Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects.

Bioanalytical Guidelines

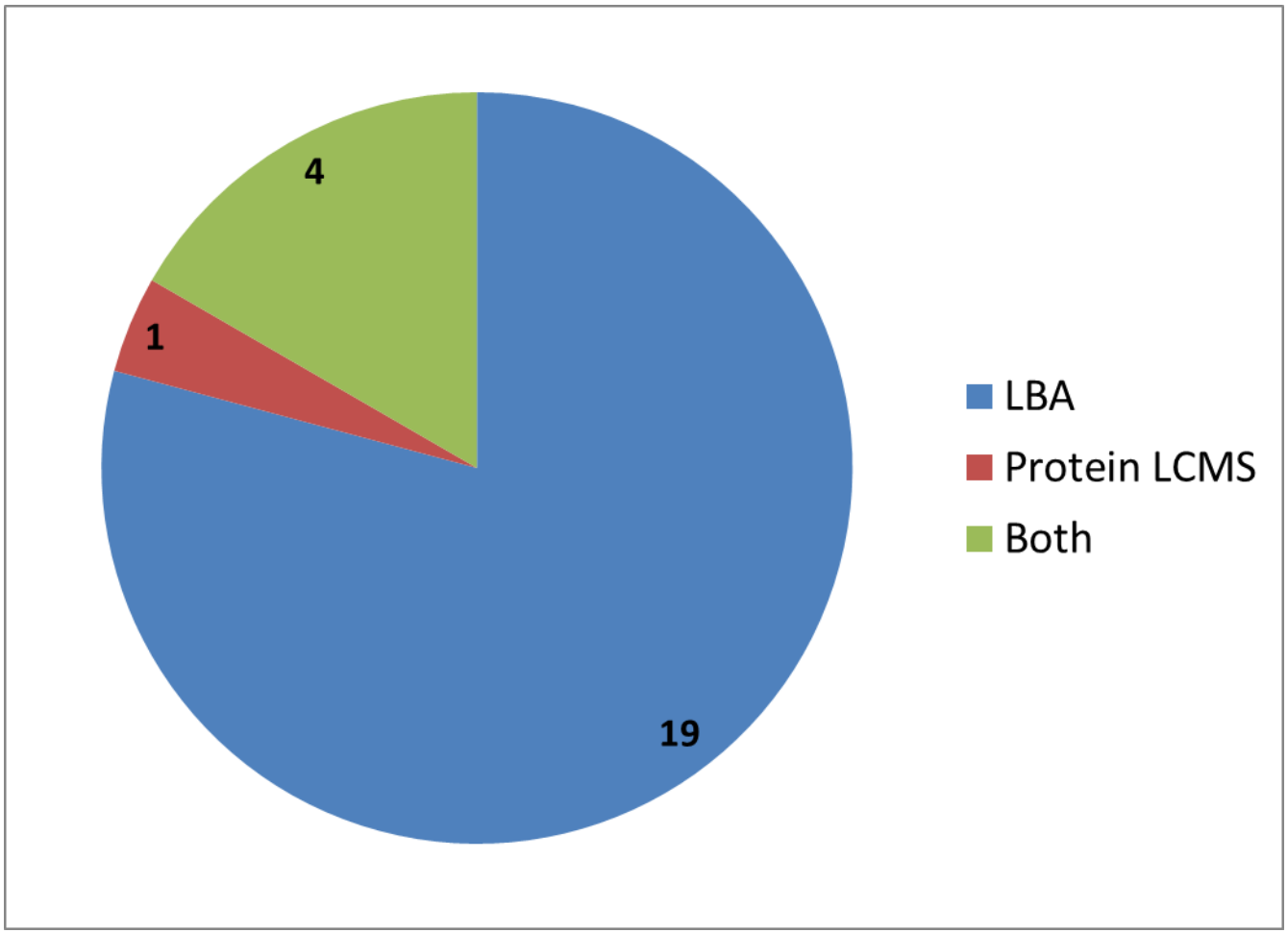


MHLW, 2014

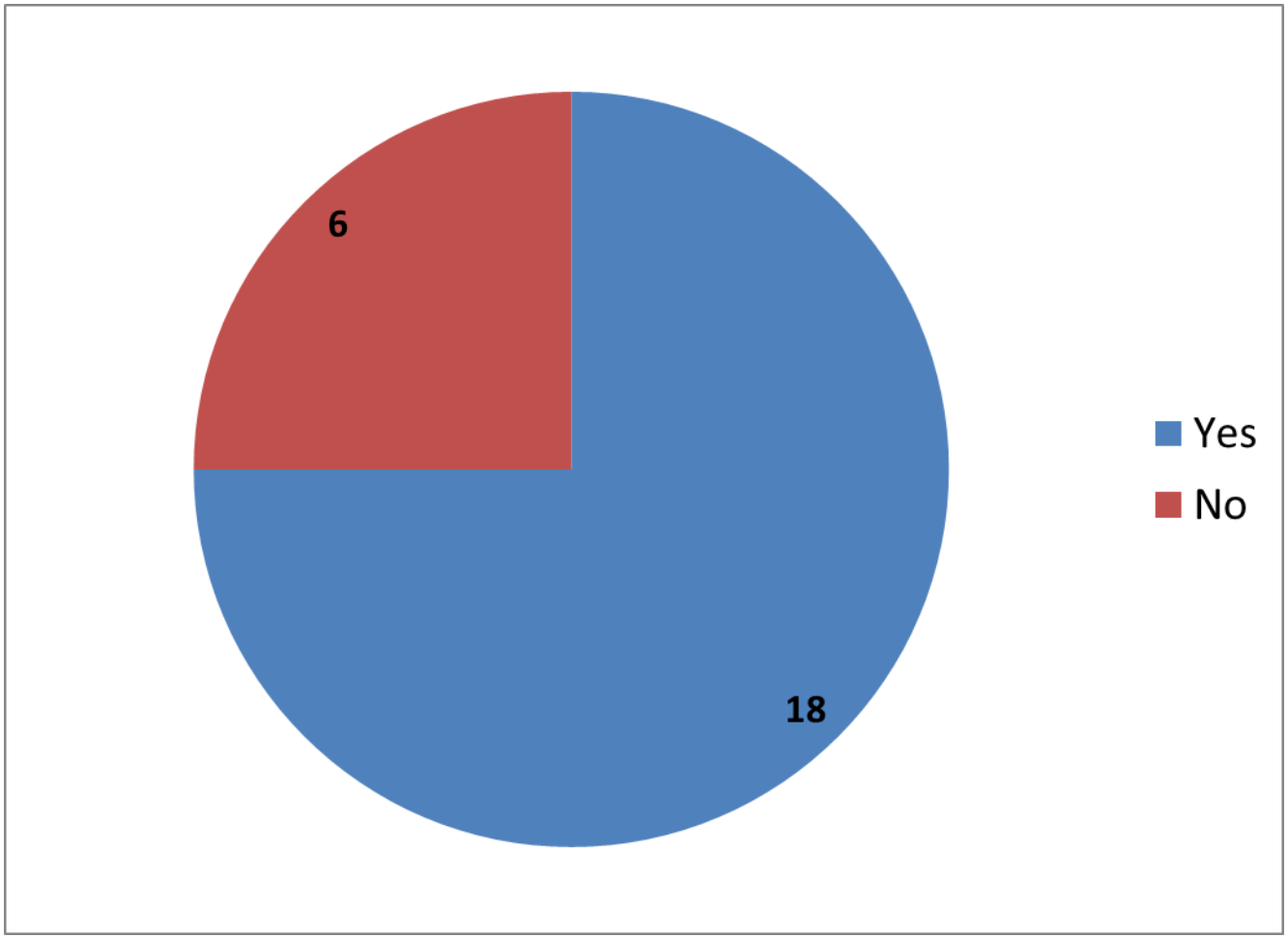
Questions and Answers (Q&A) for the Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development

Q17. Is it not necessary to evaluate parallelism?
A17. Parallelism is defined as an established parallel relationship between a dose-response curve from a study sample dilution series and a curve from a calibration standard series, with no difference among back-calculated concentrations for multiple dilutions of a study sample. As of the issuance of this guideline, domestic and international knowledge has neither accumulated nor discussion yet matured regarding cases in which parallelism was not established, causes for failing to establish parallelism, and the extent of impact the failure might have on pharmaceutical development. Therefore, evaluation of parallelism is not necessarily required for all analytical methods. ***However, if parallelism is an intrinsic issue for an LBA-based bioanalytical method and is likely to cause a problem based on the nature of the analyte or method or data accumulated in the course of pharmaceutical development, scientifically valid evaluation and assessment of the impact on measured concentrations should be considered to the extent possible***.

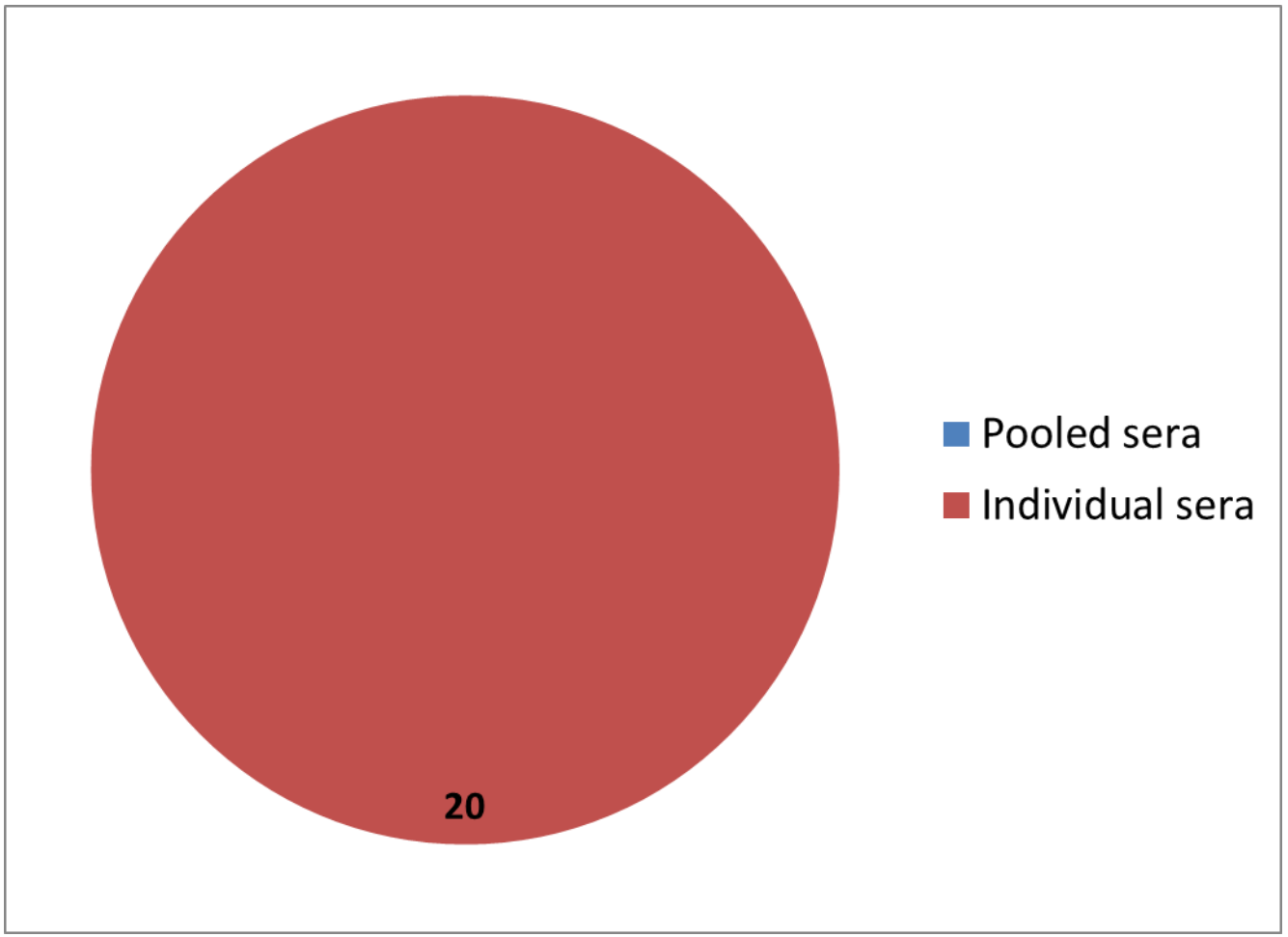
Q1: Do you perform?



Q2: Do you perform parallelism regularly for PK methods?

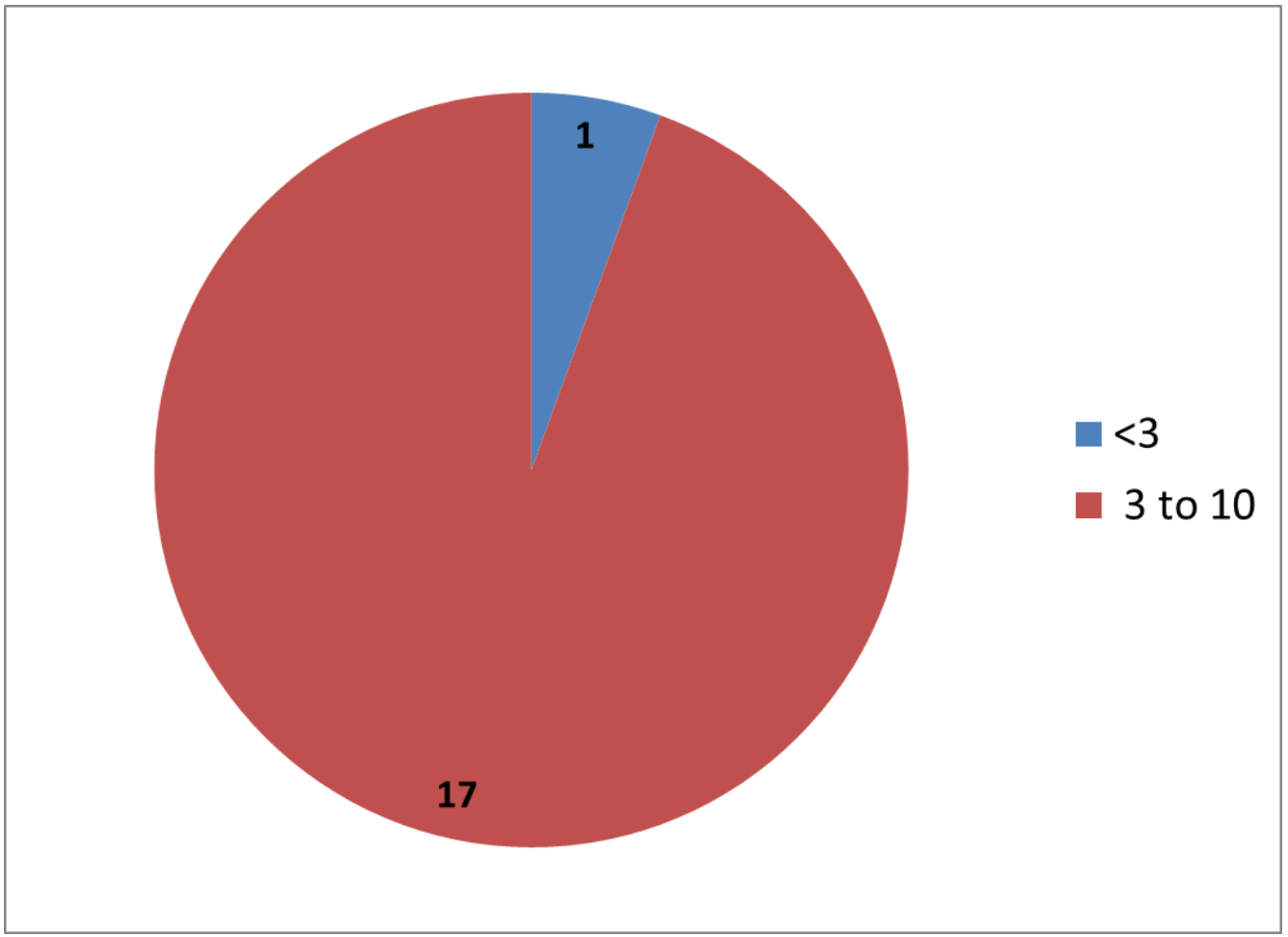


Q3: How do you perform parallelism?

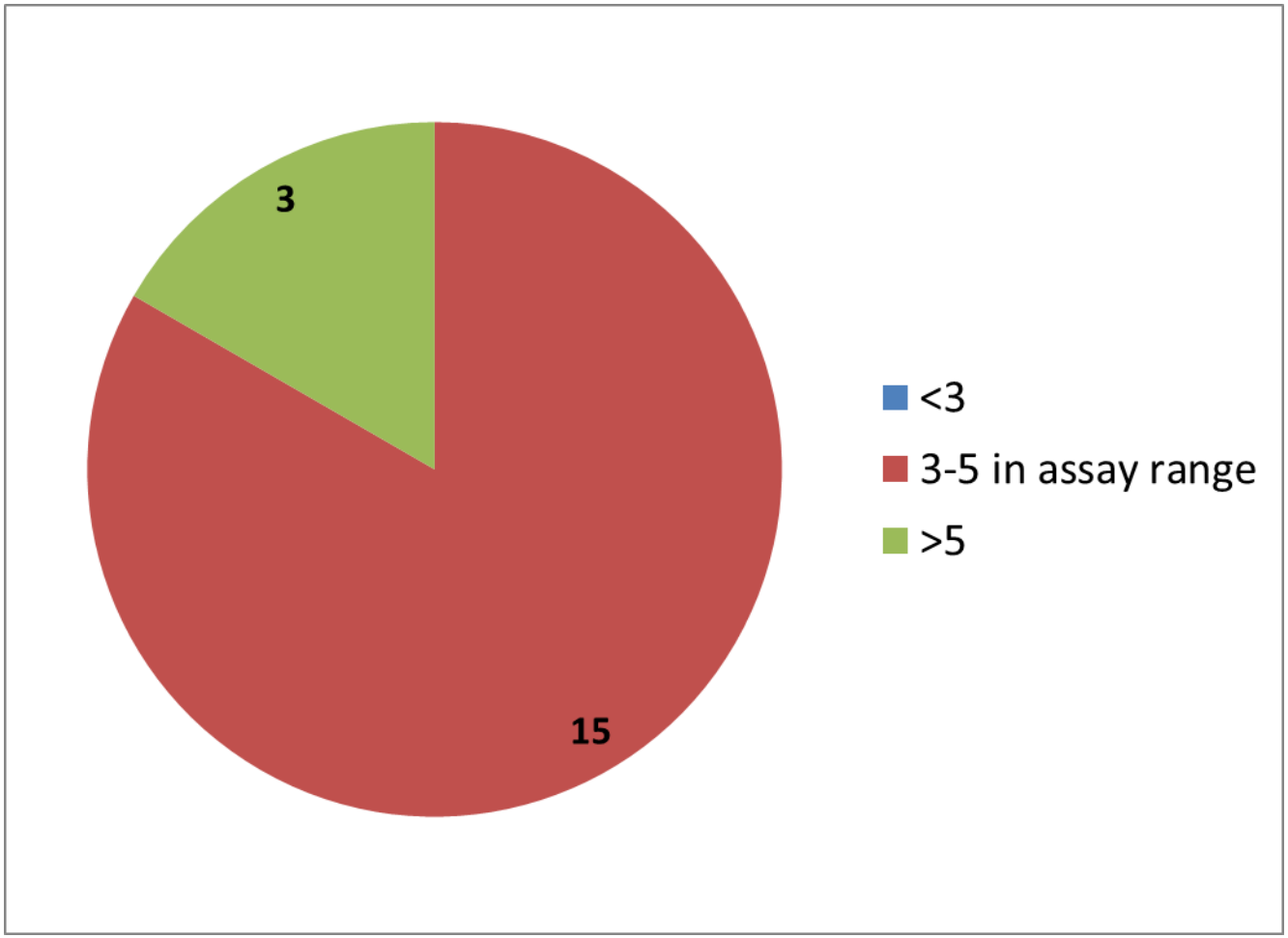


– 2 non-routine responders also answered

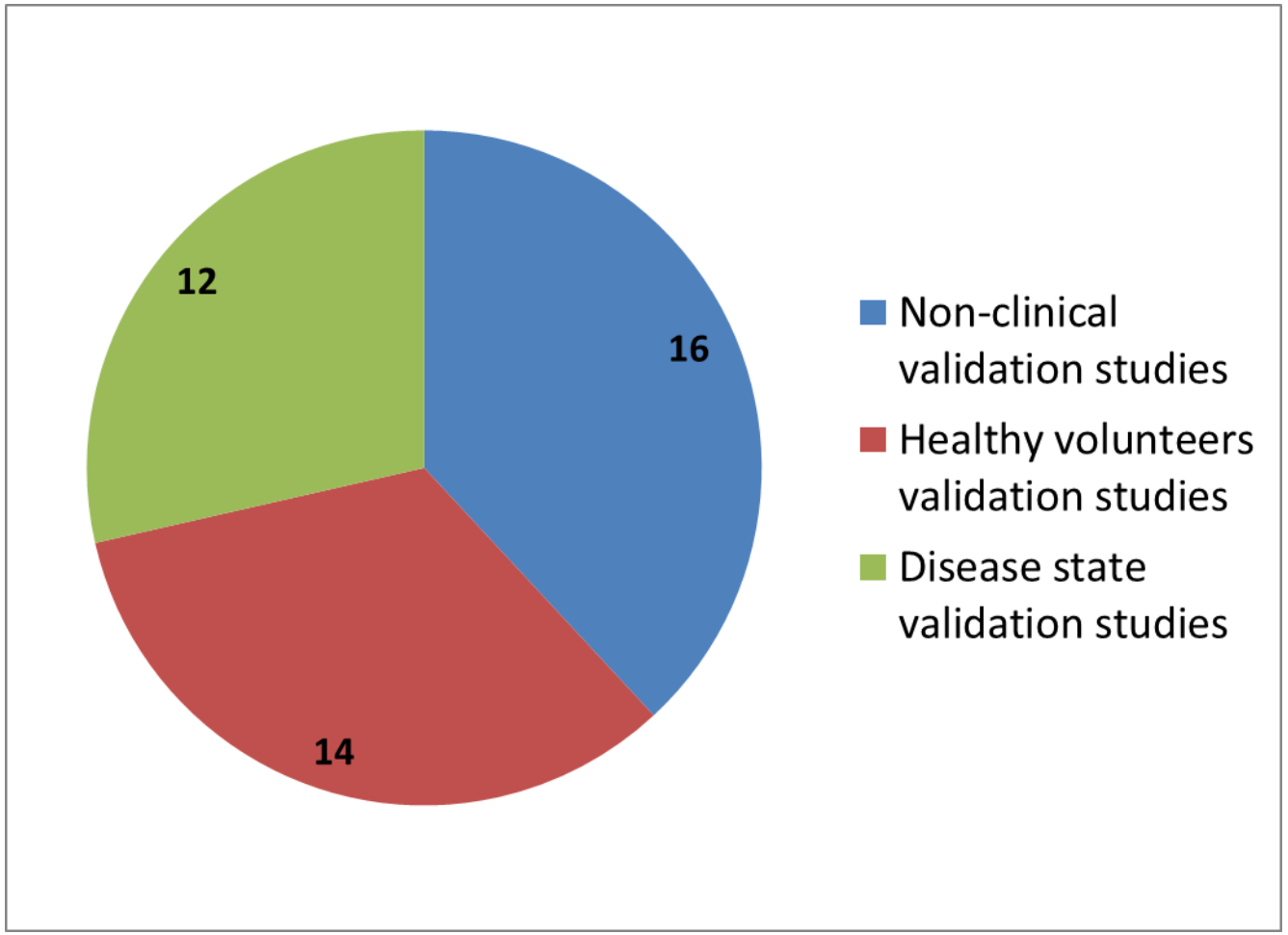
Q4: Number of individual sera?



Q5: How many dilutions do you perform?

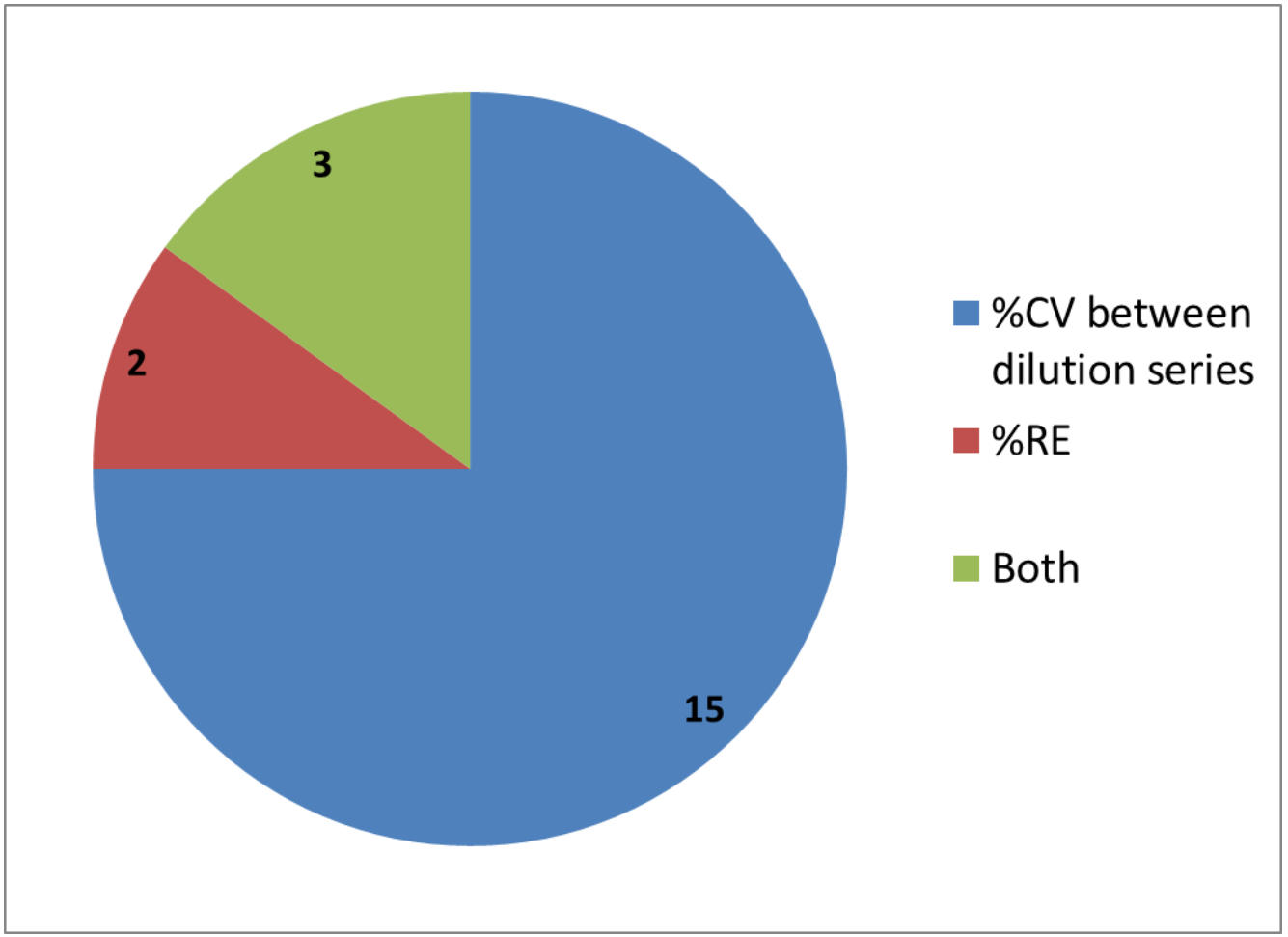


Q6: When do you perform parallelism?



– Total out of 18 responses

Q7: How do you evaluate the acceptance of parallelism?



Summary of the Survey

- Approx. ¾ responders perform parallelism assessment routinely

- Common approach for parallelism
  - to assess 3-10 individual samples
  - 3-5 dilutions in assay range
  - To apply %CV acceptance criteria
    - To a lesser extend evaluate %RE trends

- In line with GBC L2 white paper

Harmonization of the Guidances

- *<u>Recommendation</u>:*
  - *If a parallelism assessment is deemed necessary, incurred samples should be tested at multiple dilutions across the quantitative range of the assay*
    - *Individual samples recommended*
    - *but pooled may be justified in some cases (e.g. low sample volume)*
  - *Samples that fall above the assay ULOQ should be included (to evaluate hook effect), as well as samples within the assay range.*
  - *Trends that may have meaningful impact on the study data should be evaluated.*

Harmonization of the Guidances

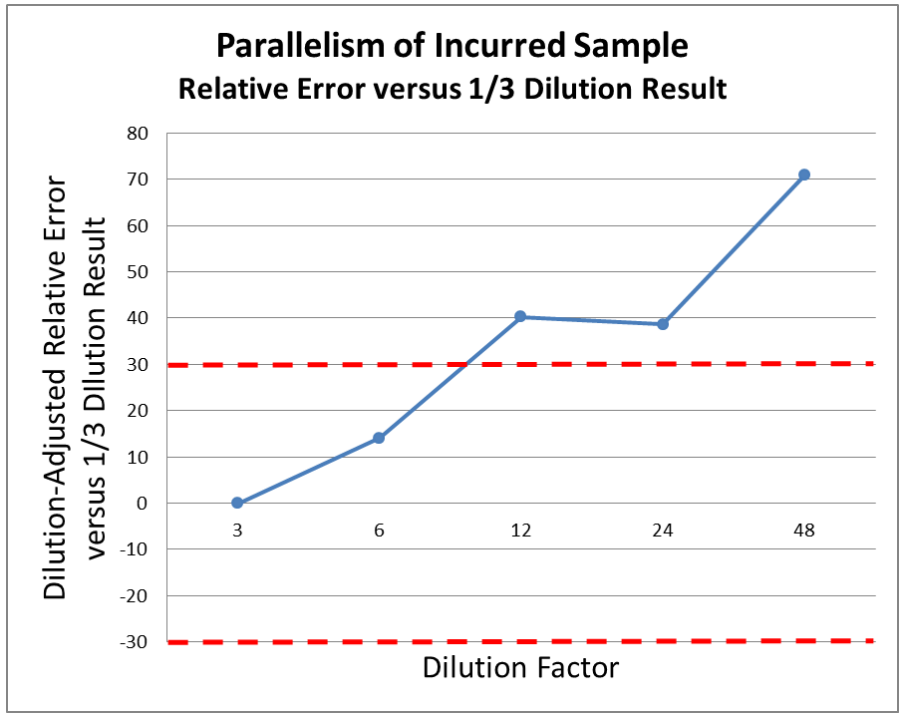
- *Not Included as Recommendation:*
  - *Precision (%CV) of the cumulative back-calculated concentrations for all in-range samples should be ≤30%.*
    - *Precision criteria should be used with caution, as precision of a dilution series can be misleading*

Evaluation of Trends

Dilution Factor (Fd)	Mean Conc. (ng/mL)	Mean Conc. x Fd (ng/mL)	Precision of Series (%)
3	279.6	279.6	
6	159.5	319.0	
12	98.1	392.2	20.5
24	48.4	387.4	
48	29.9	477.7	

CV <30%

BUT…

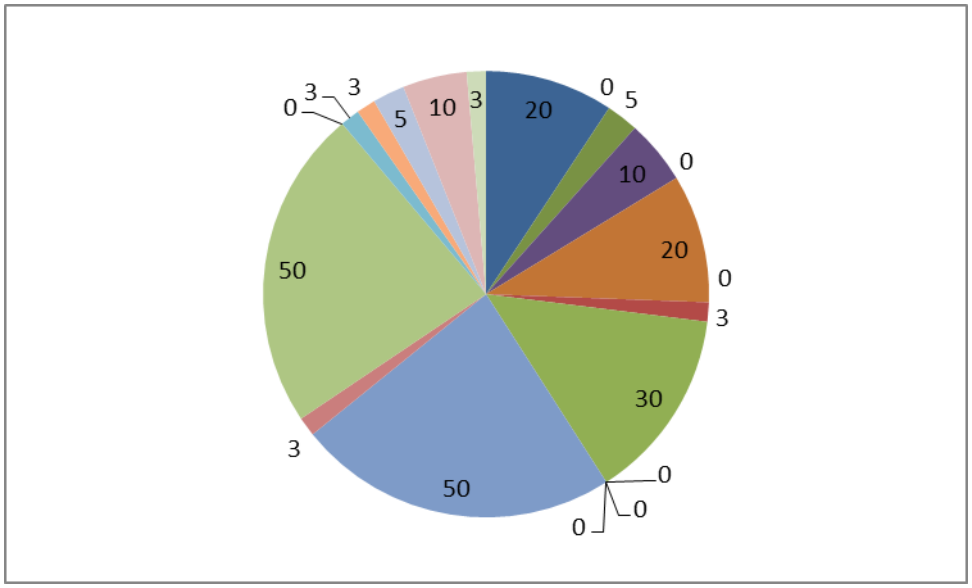


Follow-up to the Survey

- *Gather further data on incidence of parallelism failure*
  - *Type(s) of analyte, reason for failure*
    - *EBF Finger-on-the-Pulse (FotP) performed*
  - ***Workshop delegate input welcome…!***

Parallelism FotP

- *Q: How many parallelism assessments (approx.) have you performed in the past 2 years?*



  - *21 responders*
  - *Total: approx. 215 parallelism assessments*
  - *Note: Clinical and Preclinical assays*

Parallelism FotP

- *Q: What type of molecules have you assessed?*
  - *Antibodies*
  - *Antibody fragments*
  - *Peptides*
  - *Enzymes*
  - *Fusion proteins*
  - *ADC*
  - *Therapeutic proteins*
    - *Hormones*
    - *Chemokines*

- *Q: How many parallelism assessments have failed?*
  - ***5***

Parallelism Failures

- *Q: What type of molecules failed?*
  - *Antibody (2)*
  - *Peptide (1)*
  - *Therapeutic protein (1)*
  - *Not disclosed (1)*

- *Q: Why did parallelism fail?*
  - *ADA interference (2)*
  - *Assay not reached equilibrium (1)*
  - *Metabolism compound (1)*
  - *Cause not identified (1)*

Failures:
3 of 215 = 1.4%

  - *Note: Instances of soluble target interference causing failure of some dilutions*

Parallelism Failures

- *Q: What were the consequences of failure?*
  - *Assay redeveloped to address parallelism, and samples reanalysed (4)*
  - *Samples were analysed in multiple dilutions and reported as semi-quantitative (1)*

Parallelism Recommendation

- *<u>Recommendation</u>: The need to perform a parallelism assessment should not be mandated, but be driven by the characteristics of the drug, its binding partners and the assay reagents' specificity. Scientific rationale should exist that explains why the assessment is warranted.*

Acknowledgements

- EBF community for survey feedback
- Michaela Golob, survey co-author
- Jo Goodman, Marianne Scheel Fjording, Daniela Stoellner for discussion and input

- GBC L2 Harmonization Team
- EBF TT-35
- EBF TT-61

References

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Clare Kingsley. A global view on parallelism. Presented at EBF Open Symposium 2013, Barcelona.

http://bcn201311.europeanbioanalysisforum.eu/slides/

Edwin Janssen on behalf of EBF TT-35: Validation of immunoassays: the importance of parallelism. Presented at EBF Open Symposium 2013, Barcelona.

http://bcn201311.europeanbioanalysisforum.eu/slides/

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