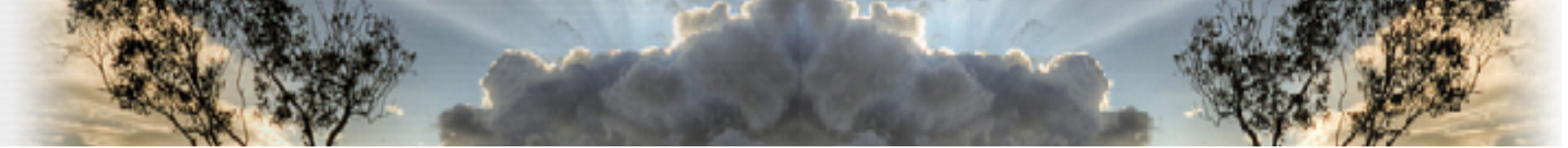


Workshop on current application of qPCR in bioanalysis

(*In collaboration with the JBF*)

EBF qPCR Survey Feedback

Presenter: Stephen Williams, on behalf of the EBF

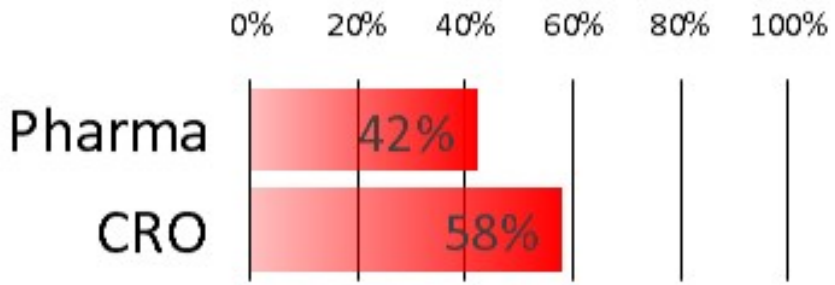
European Bioanalysis Forum (EBF) 11th Open Symposium
Barcelona, 21-23 November 2018

Survey Summary

- Survey run in Sep/Oct 2018 amongst 62 EBF member companies
  - 57 companies responded – thank you!
  - 15 companies (26%) have qPCR bioanalysis activities in Europe

- 19 surveys completed by 15 companies
  - 3 surveys included from companies with North American site conducting analysis
  - 3 companies with 2 or 3 sites conducting analysis for different types of studies
  - Reported percentages are from the 19 surveys

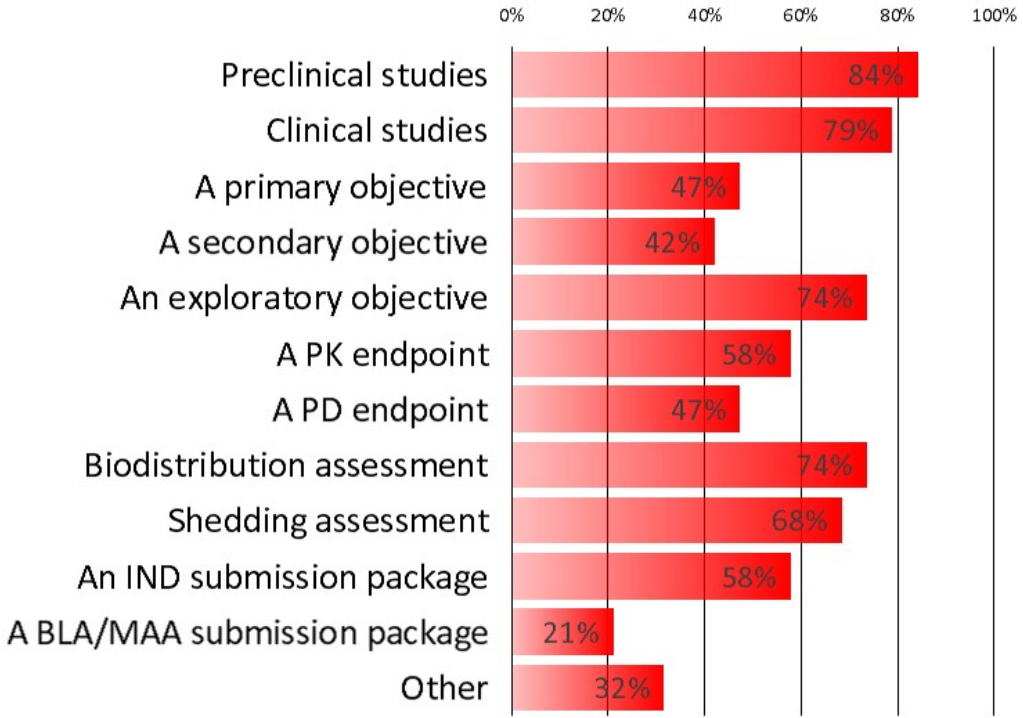
Q1. What is your affiliation?



Q2. Would you like to join the EBF qPCR team?

- Applications are still open…
  - We've reached out to the people who said yes
  - If you are interested in contributing to future efforts in this space, please let us know

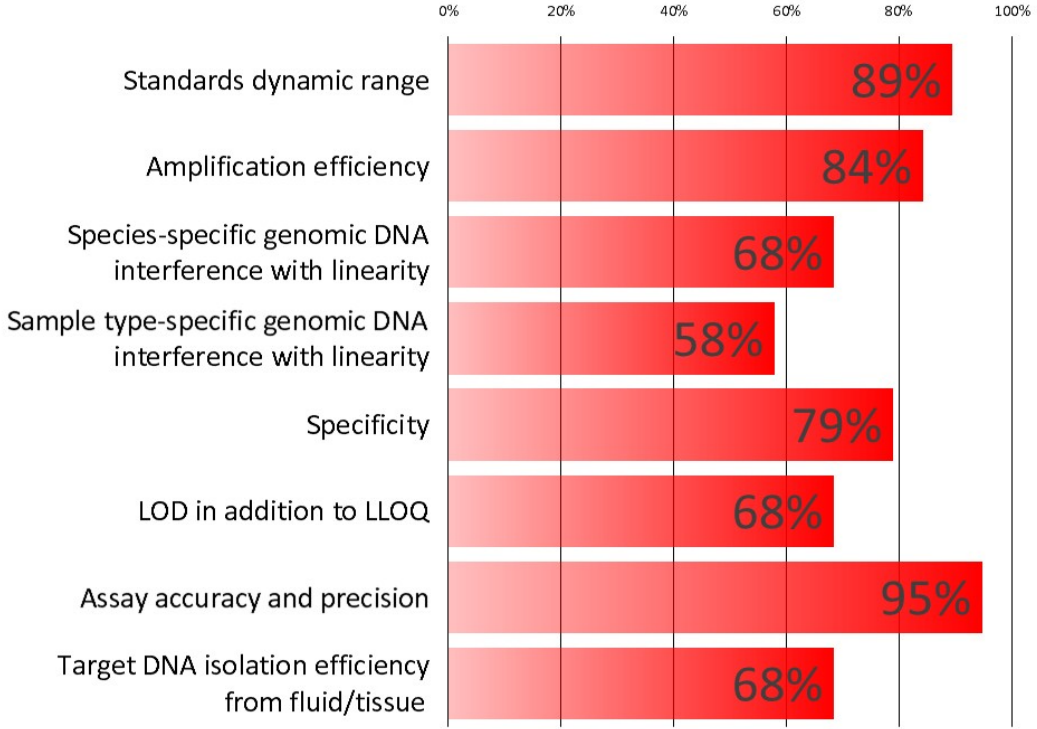
Q3. What studies do you use qPCR to support?



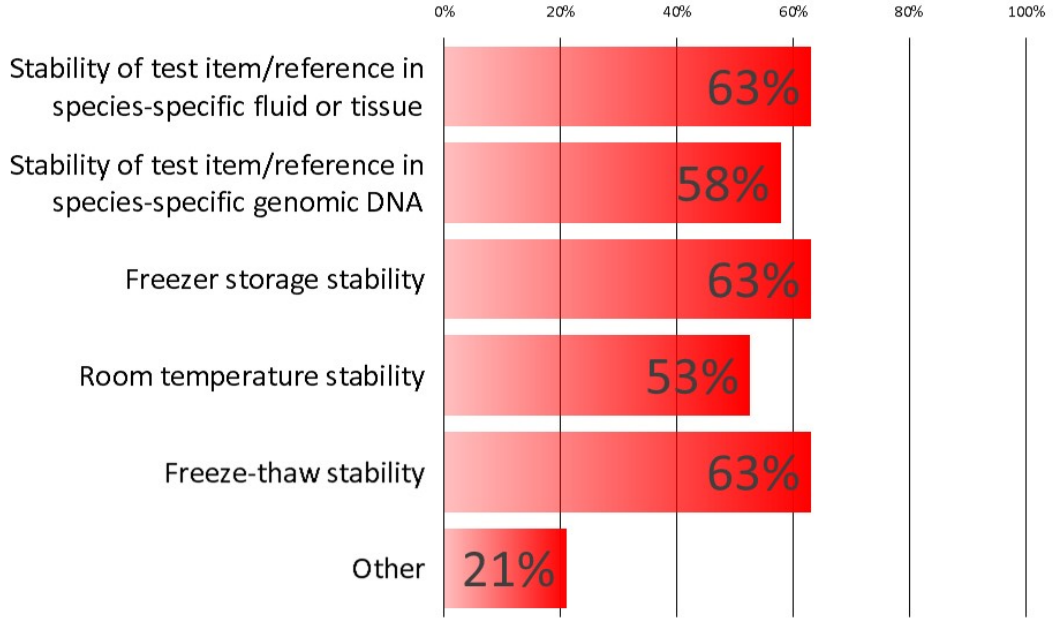
Q3. What studies do you use qPCR to support?

- Other/Comments
  - Discovery
  - Safety assessment
  - Genomic biomarkers for patient selection
  - CMC packages
  - Infectivity assays
  - Replication competence
  - Potency assays
  - Host cell DNA
  - Transgene distribution and expression

Q4a. When performing a full qPCR method validation, what do you assess?



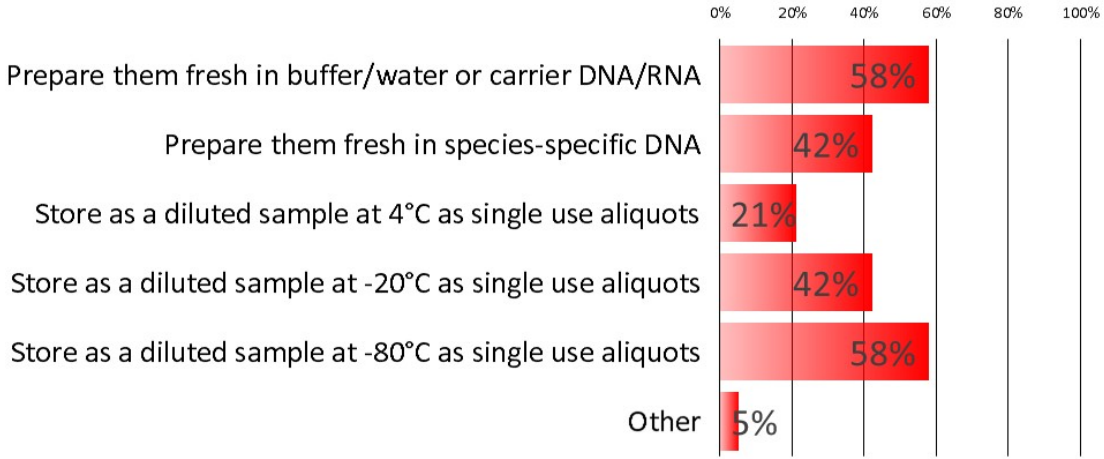
Q4b. When performing a full qPCR method validation, what do you assess?



Q4. When performing a full qPCR method validation, what do you assess?

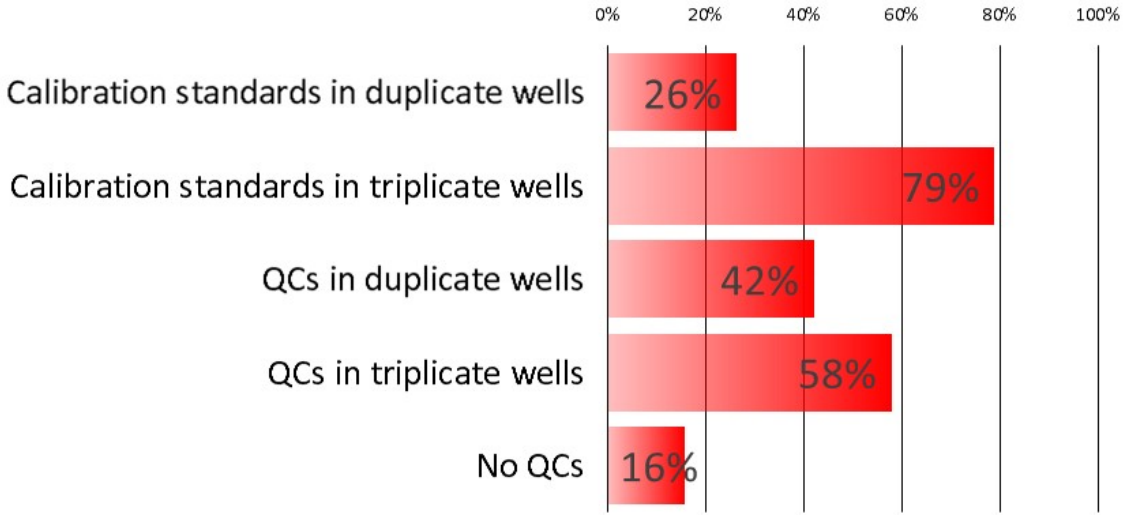
- Other/Comments
  - Benchtop stability of working solutions
  - Amplification efficiency assessed during method development
  - DNA sample purity more important than isolation efficiency
  - All validated assay differ in one way or another, so not all assays are validated with all parameters above – depends on regulations, phase, application, client requirements….

Q5. How do you prepare calibration standards and QCs?

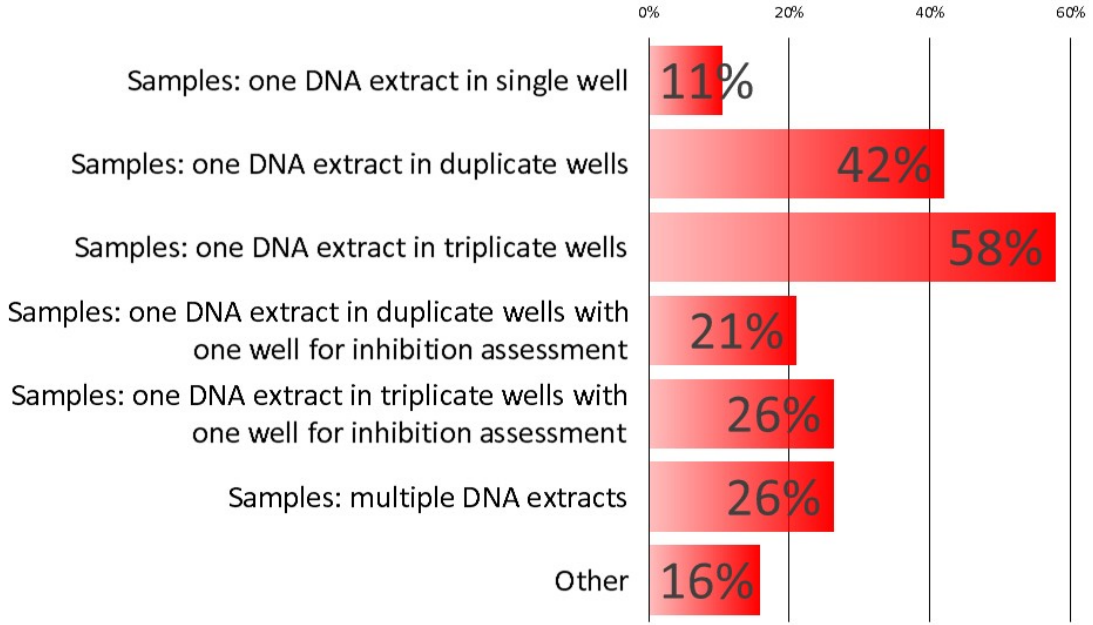


- Other/Comments
  - We have multiple different ways that assays are set up and run
  - Fresh QCs for validation, frozen QCs for sample analysis
  - Use stored samples if stability has been demonstrated

Q6a. How many replicates do you analyse?



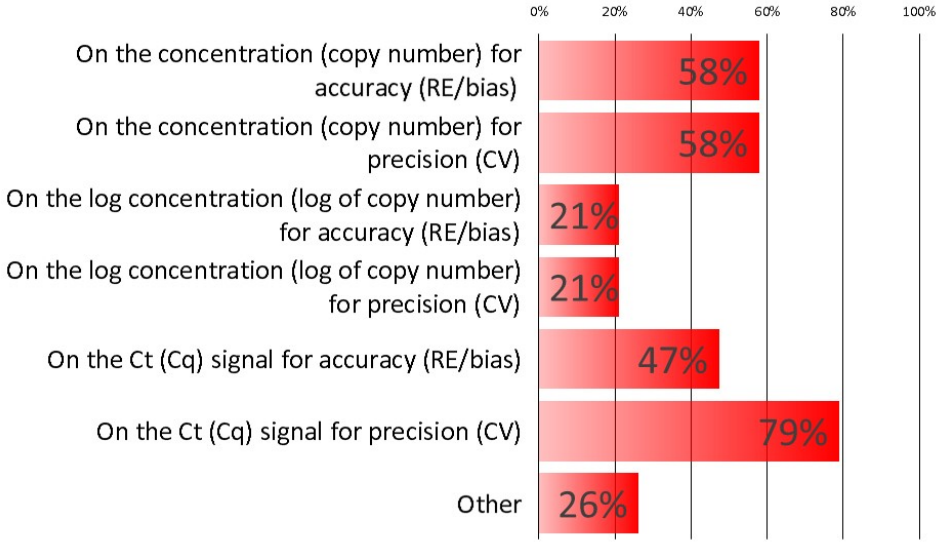
Q6b. How many replicates do you analyse?



Q6. How many replicates do you analyse?

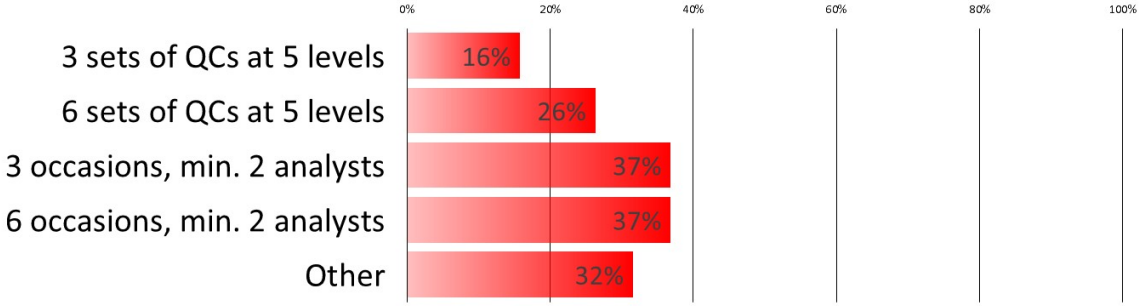
- Other/Comments
  - Samples: multiple DNA extracts pooled to one sample
  - 2 x inhibition, 2 x Test Item
  - Samples: multiple DNA extracts in triplicate wells
  - If assay showed performance, then we would take decision to run less replicates
  - 10% of samples analysed in duplicate
  - Assays for different applications
  - Use of duplicate/triplicate wells assessed during method development

Q7. What do you set acceptance criteria on standards and QCs?



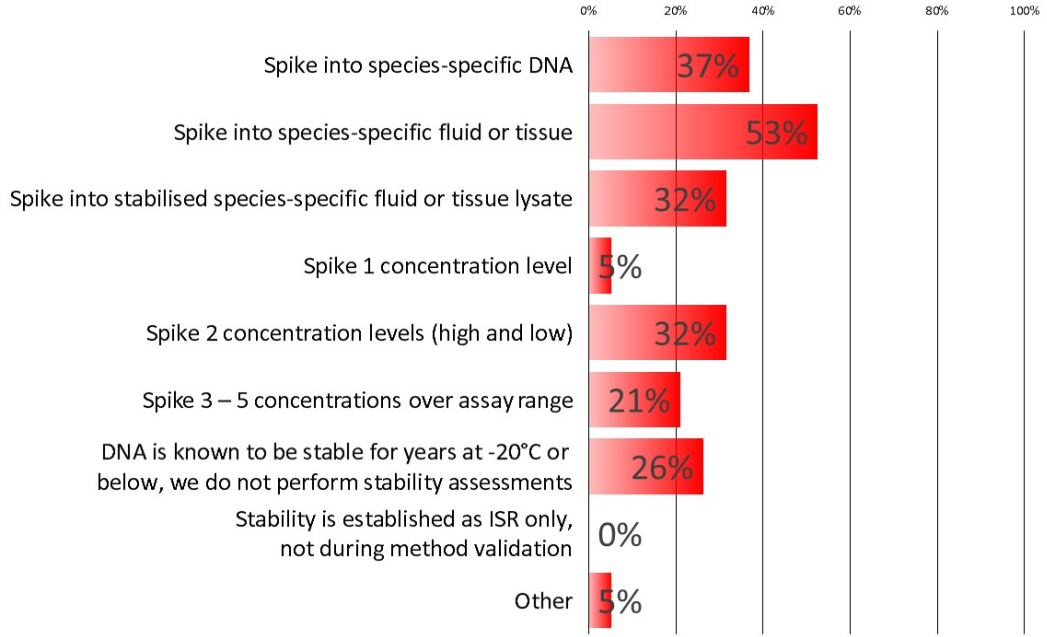
- Other/Comments
  - % Theoretical
  - Amplification efficiency
  - R^2
  - Slope
  - Extraction efficiency
  - Log conc for QCs, Ct for standards

Q8. How many accuracy and precision assessments do you perform?



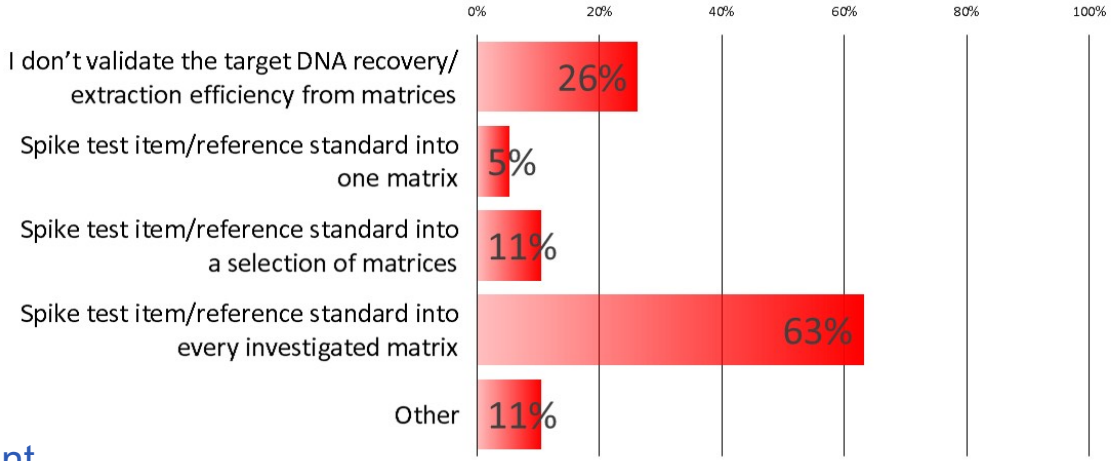
- Other/Comments
  - 5 sets of QCs at 4 levels
  - 5 occasions, min. 2 analysts
  - 5 sets of QCs at up to 5 levels
  - 4 levels; 1 occasion with 6 sets, 6 occasions with 3 sets
  - 3 occasions, 1 analyst
  - 9 replicates of 3-4 levels of QCs
  - At least 30 samples at different levels for accuracy and 3 samples at different levels for precision (Intra/inter/reagent/instrument/operator)

Q9. How do you assess the test item stability in samples?



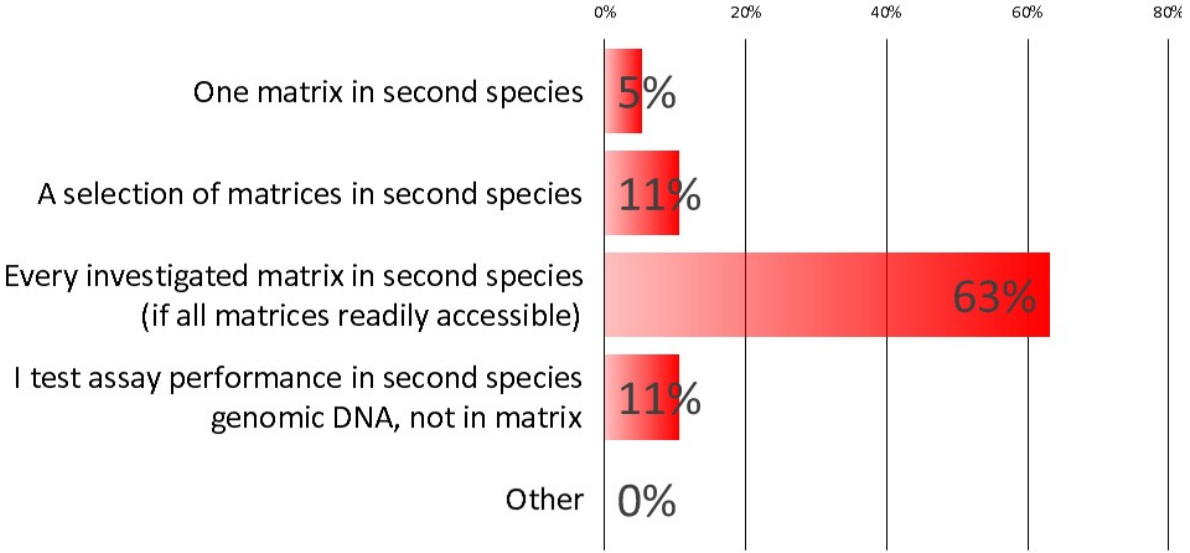
- Other:
  - Stability is not assessed for every QPCR assay
  - Need to assess stability from tissue to DNA extraction before sample DNA is isolated

Q10. How do you validate the target DNA recovery (i.e. extraction efficiency) from fluids or tissues (i.e. matrices)?

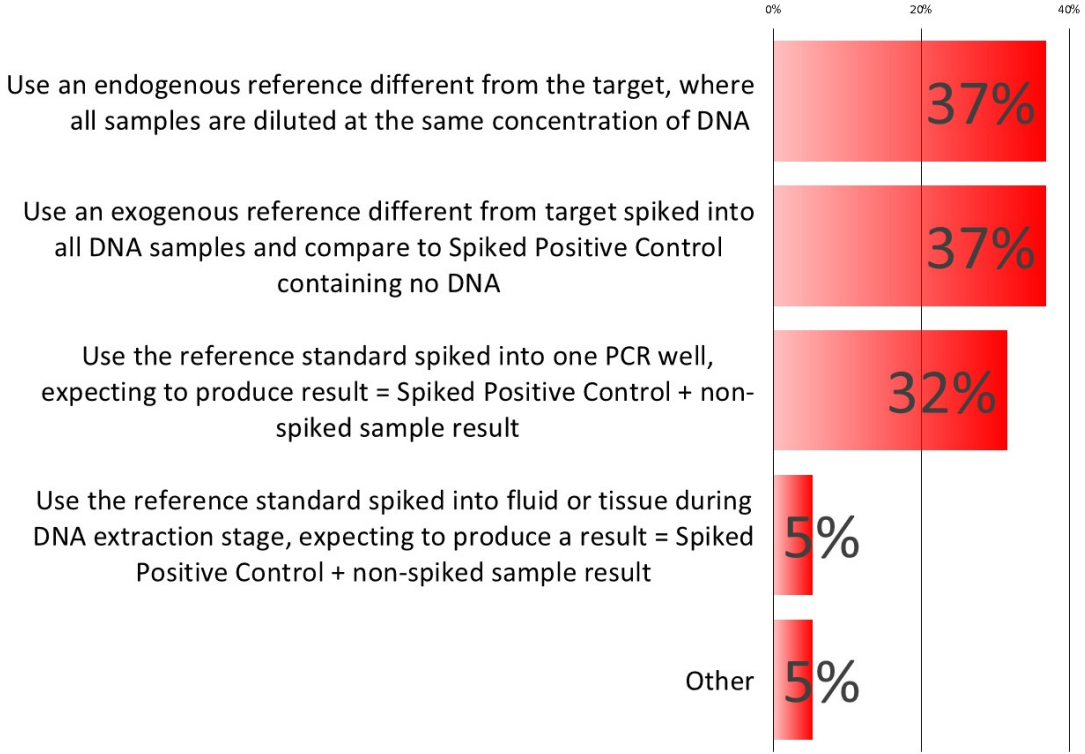


- Other/Comment
  - Total DNA recovery can be estimated by calculating how much tissue is being used, for example, cell counts from whole blood sample so extraction efficiency can be estimated that way from recovered amount of DNA after extraction
  - Extraction reproducibility
  - Not always done for preclinical studies

Q11. When transferring the assay to a new species, for fluid or tissue matrix do you validate:



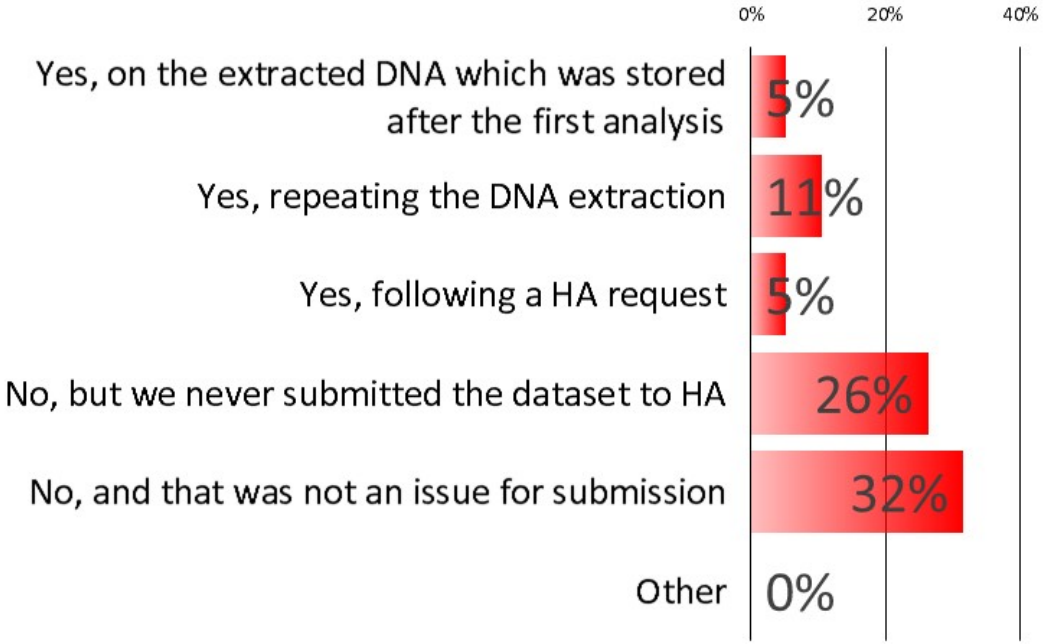
Q12a. During DNA sample analysis, how do you assess inhibition of the PCR reaction?



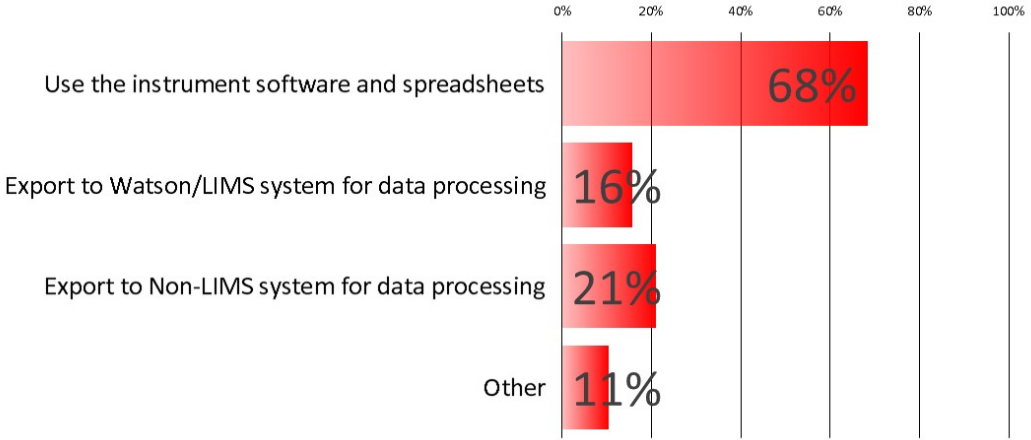
Q12b. During DNA sample analysis, how do you assess inhibition of the PCR reaction?

- Other/Comment
  - Amplification efficiency
  - Standard linearity
  - Do not perform
  - DNA concentrations of endogenous reference and target are different
  - How do we use the copy number from housekeeping gene to normalise the transgene copy?

Q13. Do you perform ISR?



Q14. How do you analyse qPCR data?



- Other/Comment
  - Excel and Qiagen Omicsoft Array Studio
  - How do we use the copy number from house keeping gene to normalize the transgene copy?
  - Our group is mainly focused on BM analyses by qPCR and is switching to digital PCR that may avoid the use of calibration standards. In addition, they perform relative quantification of RT-qPCR data

Areas of commonality

- Surprisingly few, but this may be due to the large number of types of studies supported

  - Extraction efficiency determined in every investigated matrix (is this still true for biodistribution studies with 30 matrices?)
  - Every matrix in second species validated
  - Use of instrument software and spreadsheets for data processing

Areas of divergence

- Use of fresh standards/QCs *vs* frozen
- Use of duplicate/triplicate wells for standards/QCs
- Use of concentration *vs* Ct value for assessing accuracy/precision
- QC occasions/levels/analysts for assessing accuracy/precision
- Use of DNA/matrix/stabilised matrix for assessing stability (or no assessment!)
- Assessment of inhibition

Current state of the industry

- QPCR is used for a wide variety of applications
  - Perhaps no surprise that there are multiple approaches to performing these analyses
  - Probably all valid in the context in which the results are being used, e.g. internal decision making *vs* regulatory submission

Current state of the industry

- Is there a requirement/willingness to harmonise for specific applications, eg preclinical biodistribution studies or clinical shedding studies
  - Tiered/differential approach to different studies types – i.e. one size will not fit all
  - Definition of “validation”
  - For multiple species/matrices do all need to be validated, or just some (with partial/no validation for others)?

Acknowledgements

- EBF community for survey feedback
- Survey preparation team
  - Steve Williams, Milena , Amanda Wilson, Lydia Michaut, Rob Nelson
- Extended team for workshop preparation
  - Paul Bryne, Chris Cox
- JBF collaborators

Workshop discussion points

- 6 teams – 1 topic each
  1. Standards and QCs preparation
  2. How many wells
  3. Use of concentration *vs* Ct value for assessing accuracy/precision
  4. Assessing accuracy/precision
  5. Assessing stability
  6. Assessment of inhibition
- Discuss the advantages/disadvantages of the approaches within the team
  - Is there a single approach that would suit all users (harmonisation)?
  - Can a tiered approach be identified?
- Feed back to the workshop with a 2 minute summary

Workshop discussion points - team 1

- Standards and QCs preparation
  - Preparation medium (TE buffer/water/carrier DNA/species specific DNA)?
  - Prepare fresh for every batch?
  - Prepare single use aliquots and store chilled/frozen?
  - Only use chilled/frozen once stability has been demonstrated?
  - Other…

Workshop discussion points - team 2

- How many wells
  - Duplicate/triplicate wells for standards/QCs/samples
  - Same or different number of replicates for the different standard sample types (e.g. triplicate standards but duplicate samples)?
  - Can you define/reject an outlier replicate value?
  - Assessment of inhibition?
  - Other…

Workshop discussion points - team 3

- Use of concentration *vs* Ct value for assessing accuracy/precision
  - Assessment of instrumental accuracy and precision (Ct signal)
  - Assessment of derived data accuracy and precision (concentration/copy number)
  - Relationship between instrument output and concentration data (non-linear)
  - Other…

Workshop discussion points - team 4

- Assessing accuracy/precision
  - Number of QC levels
  - Duplicate/triplicate wells
  - Number of replicates
  - Number of occasions
  - Number of analysts
  - Acceptance criteria
  - Other…

Workshop discussion points - team 5

- Assessing stability
  - Stability in extracted DNA
  - Stability in matrix
  - Stability in stabilised matrix
  - Number of concentrations/replicates
  - No assessment
  - Other…

Workshop discussion points - team 6

- Assessment of inhibition
  - Use of endogenous reference
  - Use of exogenous reference
  - Use of reference standard
  - FDA guidelines
  - Other…