CHALLENGES IN QPCR ASSAY DEVELOPMENT AND VALIDATION FOR BIODISTRIBUTION AND SHEDDING EVALUATION OF GENE AND CELL THERAPIES

Philippe Ancian, Senior Director, Biomarkers, Charles River

EBF, 21st November 2019, Barcelona

EVERY STEP OF THE WAY

charles river

BIODISTRIBUTION STUDIES FOR GT/CT PRODUCTS

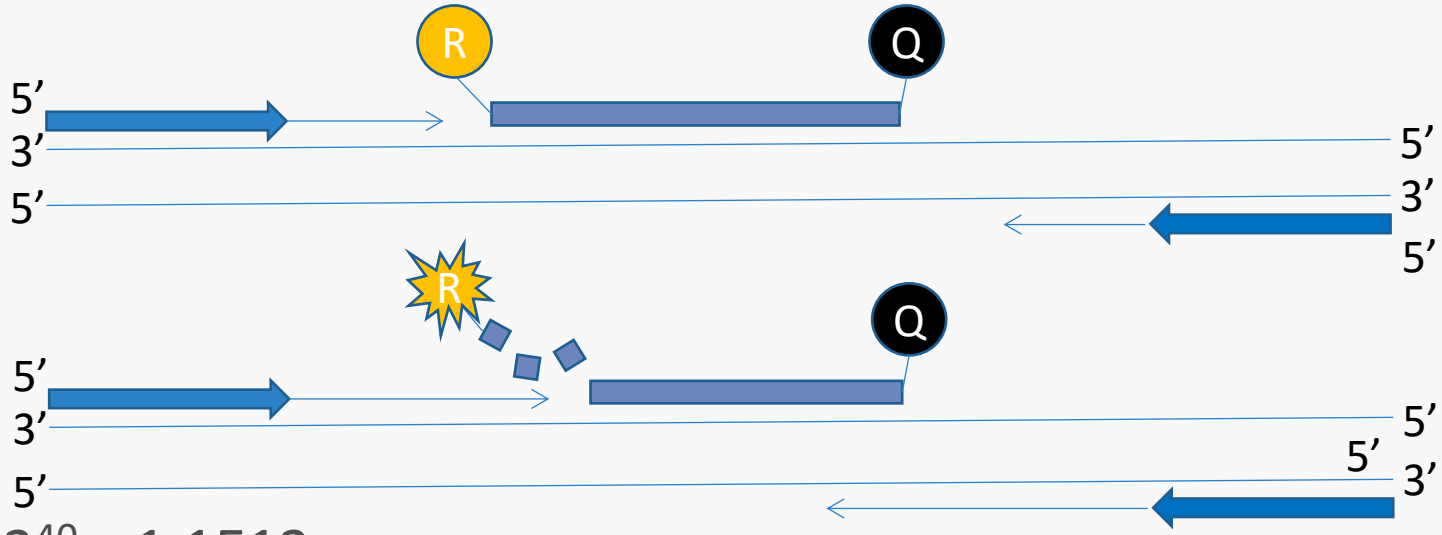
- Essential part of biosafety profile characterization
  - Cell therapies: evaluation of the cell fate and retention in animal models
  - Gene therapies:
    - GT target and non target organs identification
      - Followed by transgene expression assessment in positive organs
    - Potential for germline integration
- Part of the non clinical regulatory package
- Highly sensitive methods recommended in guidelines
  - qPCR

REAL-TIME QPCR ASSAY PRINCIPLE

qPCR: specific amplification of a target DNA sequence using specific oligonucleotides +/- fluorescent probe + Taq DNA polymerase

Temperature cycles

- Denaturation : 95°C
- Annealing - polymerization: 60-65°C



At each cycle:

- DNA quantity x 2 (if PCR efficiency = 100%)

After 40 cycles: Amplification factor = 2^{40} = 1.1E12

Consequences:

- Assay must be very specific
- Beware of contaminations !

charles river

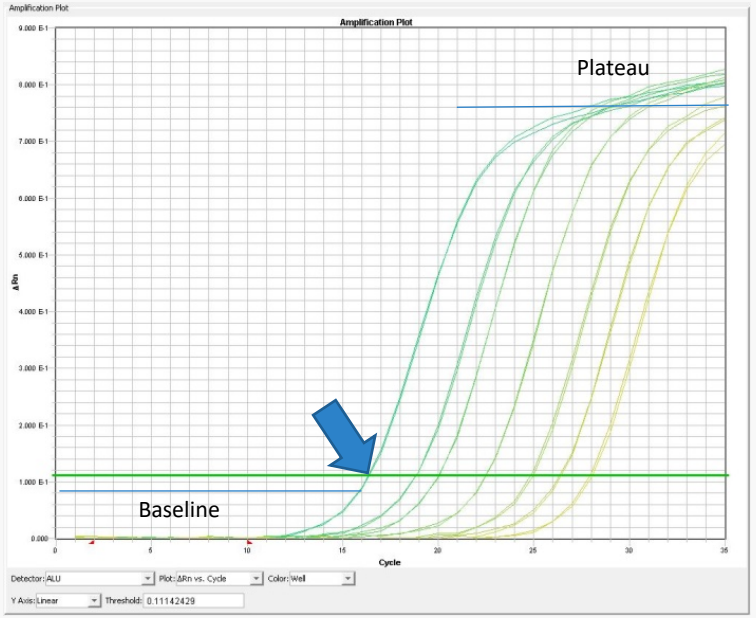
REAL-TIME QPCR ASSAY PRINCIPLE

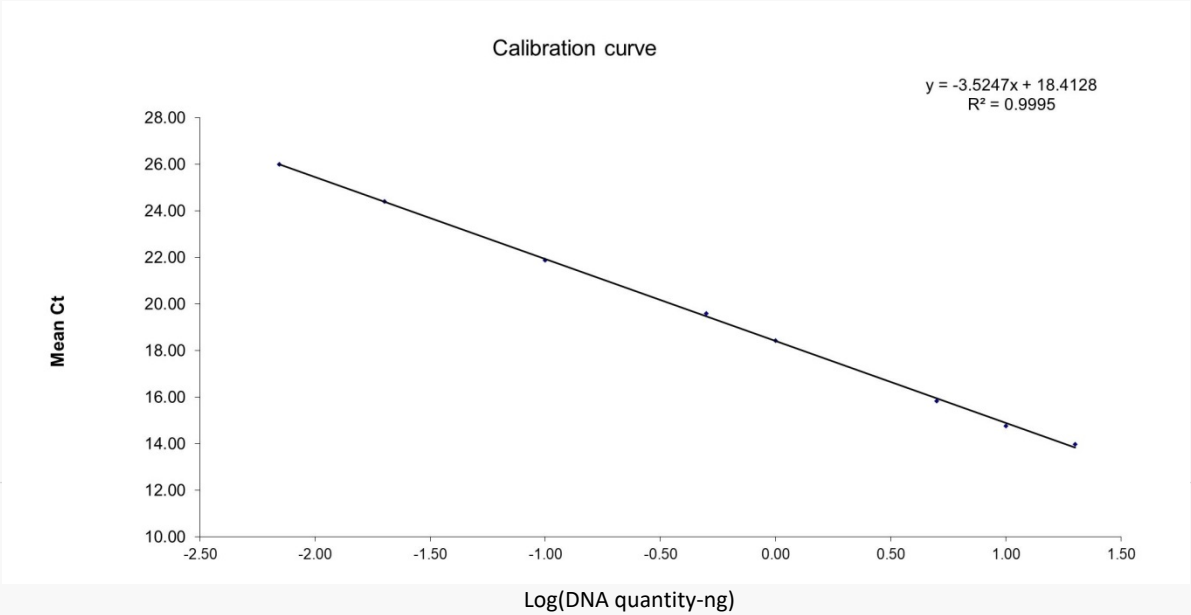
Amplification curve

- Ct value: cycle number threshold in the exponential phase of amplification

Calibration curve: Ct = mLog(x) + b where:

m = -1/log(E +1) with E= PCR efficiency





charles river

QPCR ANALYSES FOR BIODISTRIBUTION & SHEDDING

Some regulatory requirements

- Quantitative, sensitive PCR assay
- Specific detection in both human & animal tissues
- Lower Limit of quantitation ≤ 50 vector copies/1 µg of host DNA
- 3 samples per tissue
  - Incl. a spike with known amounts of vector: eliminate potential false negatives due to PCR inhibition in samples

No guideline for qPCR assay development & validation

- Inspired by Bioanalytical Method Validation guidances ?

charles river

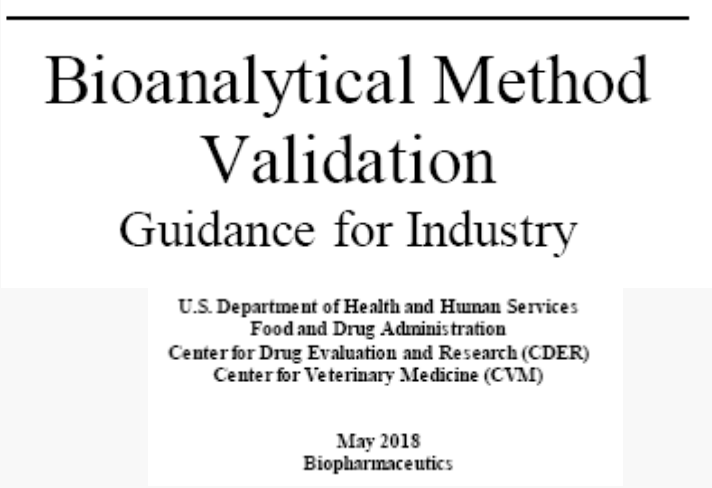
CASE STUDY: EXAMPLE OF A VALIDATION STUDY FOR THE QUANTITATION OF A CELL THERAPY IN RODENTS

- Sponsor: NC Medical Research Inc. Tokyo
- Test item: NCS-01 cells : allogeneic cell therapy for acute strokes derived from mesenchymal stem cells (phase I/II)
- Species for tox/biodistribution study: Sprague-Dawley rat
- qPCR assay transfer from the Sponsor
  - Specific amplification of human *AluY* repeat sequence
    - Subfamily of the *Alu* family – 1.1 M copies per human genome
    - 58.3% of all polymorphic Alus are AluY (a5 and b8)
    - High sensitivity expected
- For PK in blood and biodistribution evaluation in a standard list of tissues
  - Lung, liver, spleen, kidney, heart, testes/ovaries, (thymus), draining lymph nodes, brain, bone marrow and injection site

QPCR VALIDATION CHALLENGES

No dedicated guideline: no consensus on practices: fit-for-purpose, inspired by relevant validation parameters of guidances

- Precision and accuracy determination and acceptance criteria
  - LBA acceptance criteria may not be adequate
  - Precision: intra-duplicate ΔCt, or 5% Ct values etc ?
  - Accuracy: %DEV: 30% ?
- Calibration curve and QCs in the same matrix as the study samples
  - Use of surrogate matrix for ethical reasons ? (Eg: herring sperm DNA)
  - What about RNAs (tissue specific) ?
- Recovery, stability
  - For GT: not possible to mimic viral vector nucleic acids in infected cells
  - Recovery depends on the tissue type
    - Spike tissue or tissue homogenate with known quantities of test item
    - Often requires nucleic acids extraction protocol optimization


Bioanalytical Method Validation

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

May 2018
Biopharmaceutics

GENERAL ASSAY VALIDATION PRINCIPLES APPLICABLE TO QPCR ASSAYS

Reference standards and critical reagents

- Appropriate characterization (identity, purity, stability)
  - Reference standard: commercial human placental DNA

Calibration curve

- at least 6 non zero calibrator levels, in the same matrix as the study samples
  - Surrogate matrix (herring sperm DNA) showed a matrix effect
  - Consideration: matrix = rat genomic DNA, whatever the tissue
  - 8 calibrator levels – in duplicates, prepared in rat liver genomic DNA
    - from 7.00 x 10^{-3} to 20.0 ng of human DNA corresponding to 1.00 to 2857 human genome/µg of rat DNA

Quality control samples

- Five QC levels (LLOQ, L, M, H & ULOQ) for precision and accuracy assessment

GENERAL ASSAY VALIDATION PRINCIPLES APPLICABLE TO QPCR ASSAYS

Accuracy, precision

- Within-run precision and accuracy
  - Six independent sets of freshly prepared QC at five levels

QC level	Human DNA quantity (ng/well)	CV%	%DEV
QC ULOQ	2.00E+01	7.2	10.9
QC High	1.50E+01	5.7	13.5
QC Mid	5.38E-01	7.4	14.8
QC Low	2.10E-02	6.9	8.9
QC LLOQ	7.00E-03	9.9	7.0

- Between-run precision and accuracy
  - Six independent runs of three independents sets of freshly prepared QC at five levels

QC level	Human DNA (ng/well)	CV%	%DEV
QC ULOQ	2.00E+01	4.3	3.1
QC High	1.50E+01	6.4	4.4
QC Mid	5.38E-01	5.1	5.6
QC Low	2.10E-02	7.7	1.5
QC LLOQ	7.00E-03	7.2	3.8

LOD

- 0.0043 pg/µg of rat DNA corresponding to $6.5x10^{-3}$ human genome/µg of rat DNA
- Corresponds to 1 human cell in 33.0×10^6 rat cells

GENERAL ASSAY VALIDATION PRINCIPLES APPLICABLE TO QPCR ASSAYS

Selectivity and specificity

- Assessment in samples from at least 10 individuals: 5 individuals (for ethical reasons)
  - Spiked samples at 3 x LLOQ
  - Blank samples
  - Passed

Dilution effects

- Fit-for-purpose: limiting DNA concentrations in small tissues: matrix DNA dilution
- 3 QC levels spiked in 1.0, 0.50, 0.0625 and 0.02 µg of rat genomic DNA
  - Accuracy and precision passed

Validation of the test item DNA extraction

- Nucleic acids extraction from samples spiked at 3 x LLOQ (5 replicates), then qPCR with PCR inhibition determination in all samples
  - Passed in all tissues, except in blood and testes with recoveries of 26.5 and 17.0%.

GENERAL ASSAY VALIDATION PRINCIPLES APPLICABLE TO QPCR ASSAYS

Stability

- Tissue samples (3 to 5) spiked with 2 levels of the test item, then stored for up to 27 weeks at -80°C
- DNA extraction and qPCR analysis with determination of PCR inhibition in all samples.
  - Passed in all tissues
- Stability in extracted DNA: not assessed

CONCLUSION

Assay validated for its intended use

- Biodistribution study in rats

No guideline for qPCR assay validation: fit-for purpose

- When applicable or relevant, validation guidances followed
  - Calibration curves and QCs
  - Precision and accuracy assessment
- Acceptance criteria may be adapted
- Discussions about practices in the EBF qPCR working group on going

Some specific challenges

- For GT products: difficulties to mimic study samples for recovery and stability assessment (no infection in tissue homogenates)

CONTACT US

Address:
RN 13
27930 Miserey, France

Website:
www.criver.com

Email:
askcharlesriver@crl.com

Phone:
877.CRIVER.1

charles river