

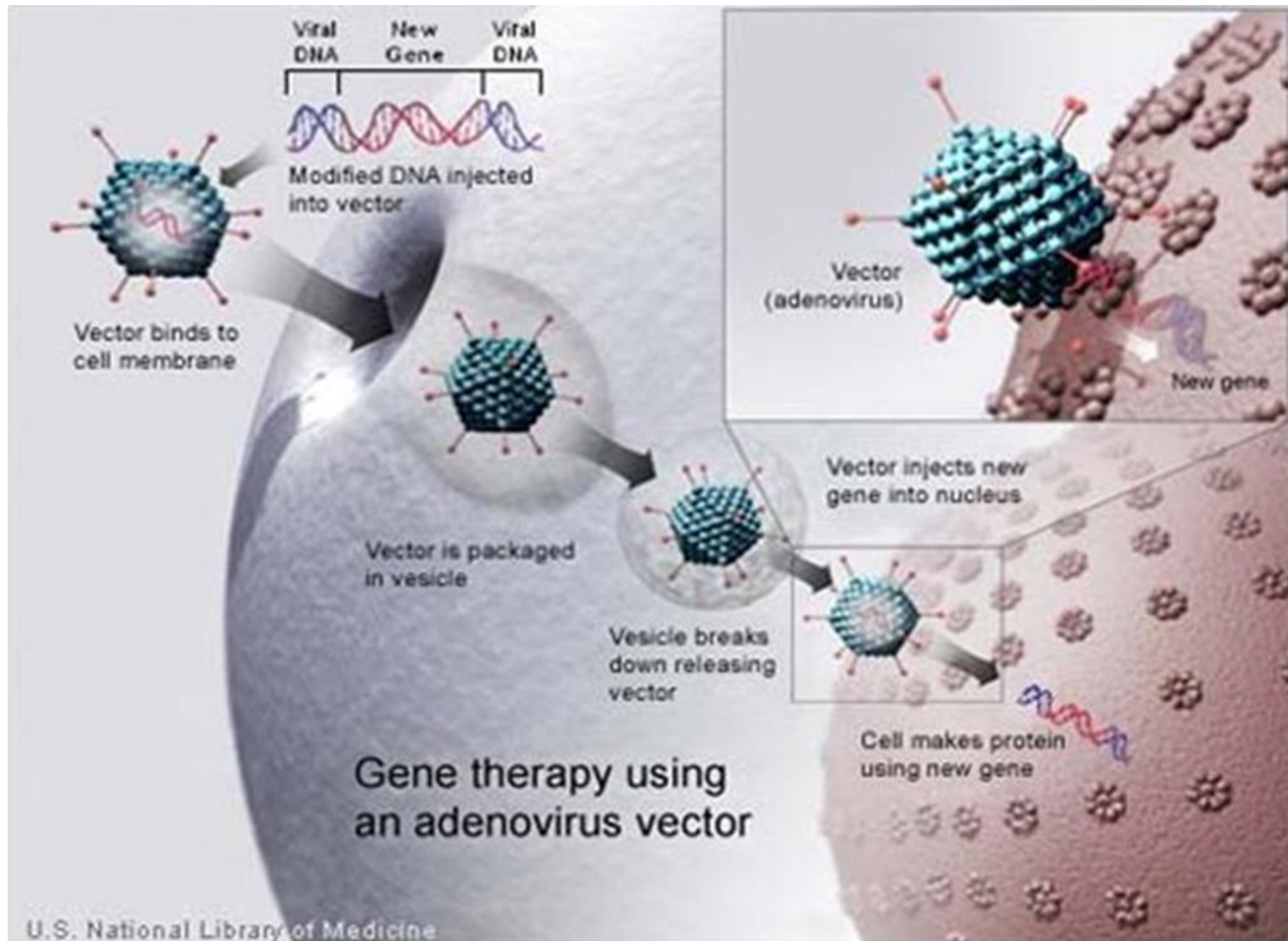
qPCR assay validation for the quantification of gene therapy therapeutics in clinical studies

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**EBF – Focus Workshop: New Modalities and Novel Concepts
in Bioanalysis**

15 May 2018, Lisbon

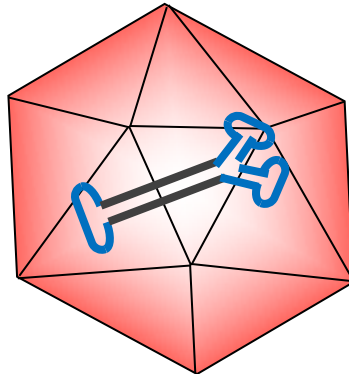
Gene therapy compounds



General bioanalytical strategy for gene therapy projects

EXPOSURE («PK»)

Quantification of vector: detection of vector DNA by qPCR



Quantification of transgene mRNA by qRT-PCR



mRNA



Quantification of protein expressed by the transgene



HUMORAL IMMUNOGENICITY

Semi-quantification of antibodies against viral capsid
(*pre-existing antibodies*)

**see November 2015 presentation at EBF Open Symposium (Barcelona)*

Semi-quantification of anti-protein antibodies*

Biodistribution in clinical and preclinical studies

what is it, why do we do it, what is assessed?

- Main questions to be answered in a Biodistribution Study:
 - Is the vector present in a particular tissue? -> qPCR
 - If so, is the transgene expressed? (not always addressed) -> RT qPCR
- Objective of study is to show absence of vector, not presence of vector
 - Data are used to determine the potential for vector presence in desired target tissues/biological fluids, in non-target tissues/biological fluids, and in the germline
 - The characterization of the vector presence, persistence, and clearance profile can inform the selection of the gene therapy product dosing schedule, the monitoring schedule for various activity/safety parameters.
 - The animal BD study does not need to be performed in the Tox species (e.g. monkey) but can be performed in another species (e.g. rat)
 - Need to assess for the presence of the vector DNA sequences in a panel of tissues collected at multiple time points (few days to several months post-administration)
- ✓ 2006 FDA Guidance For Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events
- ✓ 2008 EMA Guideline on the Non-clinical Studies Required Before First Clinical Use of a Gene Therapy Medicinal Product
- ✓ 2013 FDA Guidance For Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products
- ✓ 2015 FDA Guidance For Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products

**Clinical qPCR assay
validation and application
to assess biodistribution and
viral shedding of a gene
therapy compound locally
administered to patients**

Assay lifecycle

qPCR validation

- ICH Q2 (R1) guideline 'Validation of analytical procedures: text and methodology'
- Linearity, range, precision, limit of quantification, limit of detection with 95% confidence, specificity, accuracy, robustness.

Automated DNA extraction

- QIAGEN QIASymphony SP automated extraction system
- Blood, saliva, 2 tissue types collected on 2 swab types

Sample analysis

- 291 samples
- ABI 7900HT analyzer

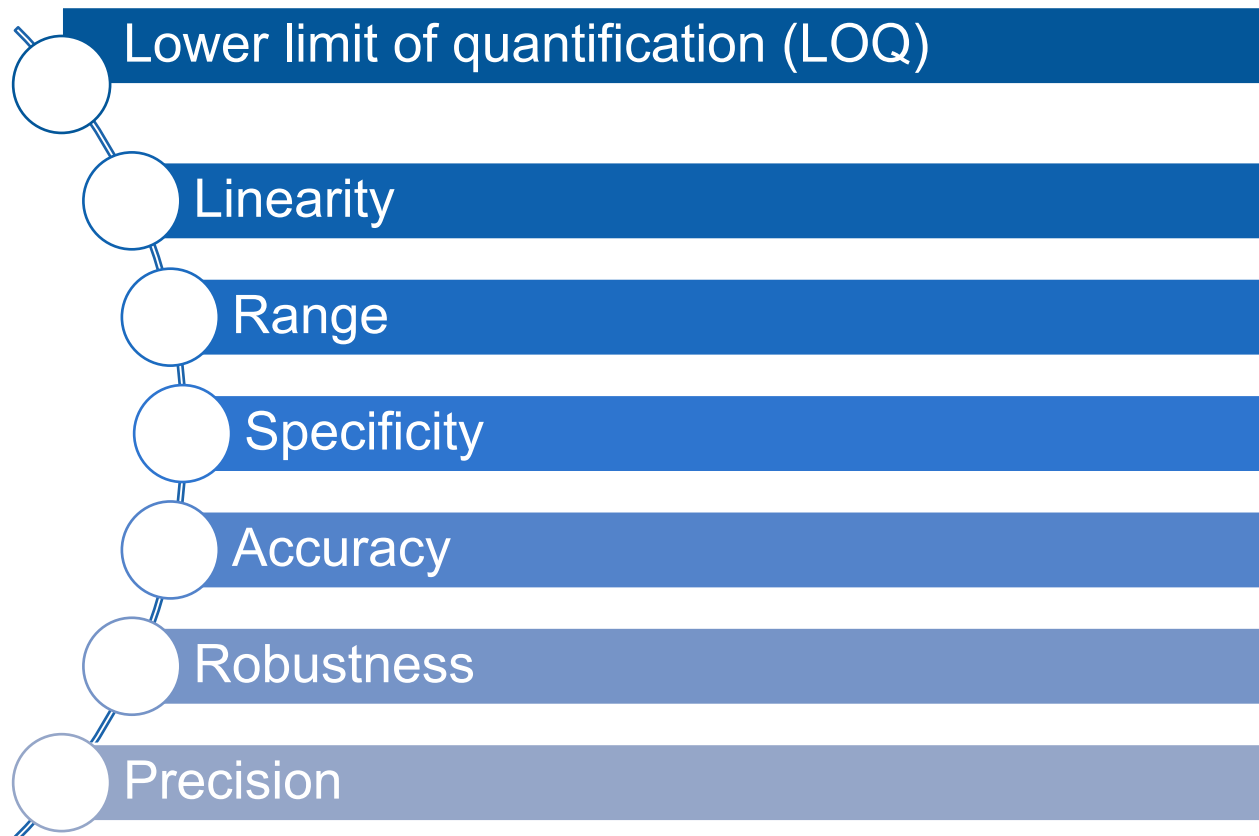
qPCR platform bridging / cross-validation

- ABI 7900HT analyzer not supported anymore by provider
- Bridging with ABI QuantStudio using real samples and QCs

Sample analysis

- 102 samples
- ABI QuantStudio analyzer

Validated qPCR parameters



ICH harmonised tripartite guideline Q2(R1):
Validation of analytical procedures: text and methodology

qPCR validation summary

Method	An automated extraction method and real-time quantitative PCR assay directed at viral vector
Test article	Plasmid (38 kb) encompassing the whole replication-incompetent viral vector engineered to deliver the gene of interest expressed under the control of a tissue-specific promoter.
Amplicon	Primers and probe lie completely within the viral genome sequence, generating an amplicon of 71 basepairs.
Matrix analyzed	Human, rodent and E.coli DNA. Human blood and biofluids (Saliva and two tissues collected on swabs)
Linearity range	1×10^1 to 1×10^7 copies per reaction
LOD	2.37 copies/reaction with 95% fiducial limits of 1.67 to 6.01 copies/reaction
LOQ	10 copies/reaction
Inter assay precision	CV \leq 1.26% for C _T values, \leq 24.62 for quantities
Intra assay precision	CV \leq 1.36% for C _T values, \leq 35.28 for quantities
Robustness	CV \leq 1.53% for operator variation, \leq 1.43% for change in reagents
Specificity and Accuracy	No cross reactivity or inhibition observed in presence of rodent and E.coli genomic DNA
Platform	ABI 7900HT Analyzer

DNA extraction from human blood and biofluids: procedure

Human blood, saliva, two types of swabs collected from two tissues

QIAGEN QIASymphony SP automated extraction system

Extraction efficiency, specificity, accuracy and repeatability: three separate extractions by at least two analysts on each tissue spiked with low amounts of plasmid (10 and 100 copies /reaction)

Low amounts of DNA extracted from saliva and swabs => requires addition of carrier RNA => no OD quantification of the genomic DNA extracted

DNA eluted in 100 μ L (blood) or 110 μ L (swabs and saliva) -> 10 μ L analyzed in qPCR

Recovery calculation

Acceptance criterion: for each swab / tissue type: CV for calculated quantities of the three independent extractions <30% for values > LOQ

DNA extraction from human blood and biofluids: particular focus

Blood extraction

- potential interference with the amount of blood recommended by the manufacturer was previously observed
- => three volumes of blood were processed

Swabs

- match exactly the collection procedure used in the clinics
- => influence of the tissue (swabs collected on 2 different body locations) and of the swab type (wet or dry) were assessed

DNA extraction recovery ranges for theoretical values of

	10 copies/reaction	100 copies/reaction (%CV)
Blood 100 µL	31.5% - 118.1%	55.8% - 76.8% (17.8)
Blood 150 µL	24.9% - 279.9%	29.6% - 96.6% (51.6)
Blood 220 µL	35.0% - 117.9%	42.6% - 47.2% (5.7)
Saliva	37.1% - 120.7%	69.8% - 121.6% (26.9)
Wet Swab tissue 1	84.3% - 140.2%	84.4% - 121.6% (26.1)
Wet Swab tissue 2	28.9% - 143.2%	72.7% - 119.6% (20.3)
Dry Swab*	73.0% - 102%	75.0% - 101% (10.1)

Previous observation confirmed: higher interference with 220 µL blood

No major difference between tissue types

*Absence of tissue: interference from tissue was not re-tested as already tested with wet swabs

Cross-validation of two PCR platforms

ABI 7900HT Analyzer not supported any more by the provider and must be replaced by ABI QuantStudio (QS) Analyzer

STRATEGY

Fresh QCs analyzed on both platforms (n=6 duplicates at six concentration levels)

DNA extracted from real samples, previously analyzed on 7900HT, and stored at -20°C

ACCEPTANCE CRITERIA

Inter-run total error < 10% for QCs

2/3 of values within 30% difference of the mean of both determinations

Negative samples on 7900 platform remain negative on QS

RESULTS

Two platforms successfully bridged

Bias value for 10 copies per reaction => LOQ increased to 30 copies; LOD unchanged

Sample analysis

393 samples analyzed

- 12 pts; 4 tissues
- 291 on 7900HT Analyzer
- 102 on QuantStudio Analyzer
- CV on copy number < 30%

15 Positive (levels > LOQ)

5 detectable levels <LOQ but >LOD

17 trace levels <LOD but >0

356 Negative

Reporting of results: not only numerical values, but also categorization (cf. panel discussion)

Results

Swab @ administration site

- Detectable/trace and positive levels at the administration site: «exposure» confirmation

Swab tissue 1

- Detectable/trace and positive levels two to four days post administration
- Maximal persistence: 15 days post-administration: level <LOD in 1 patient
- No DNA detected in any patient from day 29 on.

Saliva

- Detectable/trace and positive levels two days post-administration
- Maximal persistence: 15 days post-administration: (level <LOD in 1 patient)
- No DNA detected in any patient from day 29 on.

Blood

- No DNA detected at any timepoint analyzed (up to 169 days post administration)

- No DNA detected in any sample collected prior to administration
- «Exposure» confirmed at site of administration
- No DNA detected in blood at any timepoint analyzed
- Decrease of viral concentrations with time in all the other analyzed tissues: no DNA trace detected in any sample from day 29 post-administration.

As a conclusion: questions to the audience for discussion

- Is it a new role for the BA different from the support of large molecule and biologics programs?
- How can we optimize the use and ensure accurate interpretation of the data generated from the BioA lab?

Aim of qPCR in this study: demonstrate the absence of vector

-> Fit for purpose validation of the method

-> Reporting of results: not only numerical values, but also categorization of results < LOQ: optimization or confusion?

- Negative
- <LOQ but >LOD (detectable levels) -> “Conservative reporting”
- <LOD but >0 (trace levels) -> «Ultra-conservative reporting»
- Positive

Main contributors:

- Hazel Staton (Covance CMC, UK)
 - Marie-Hélène Delmotte
- Mark Milton, Richard Colvin and Prafulla Bhad

Thank you