AAV8 Shedding Assay to Support Gene Therapy Clinical Trials

Dr. Wibke Lembke

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Shedding – Context of Use

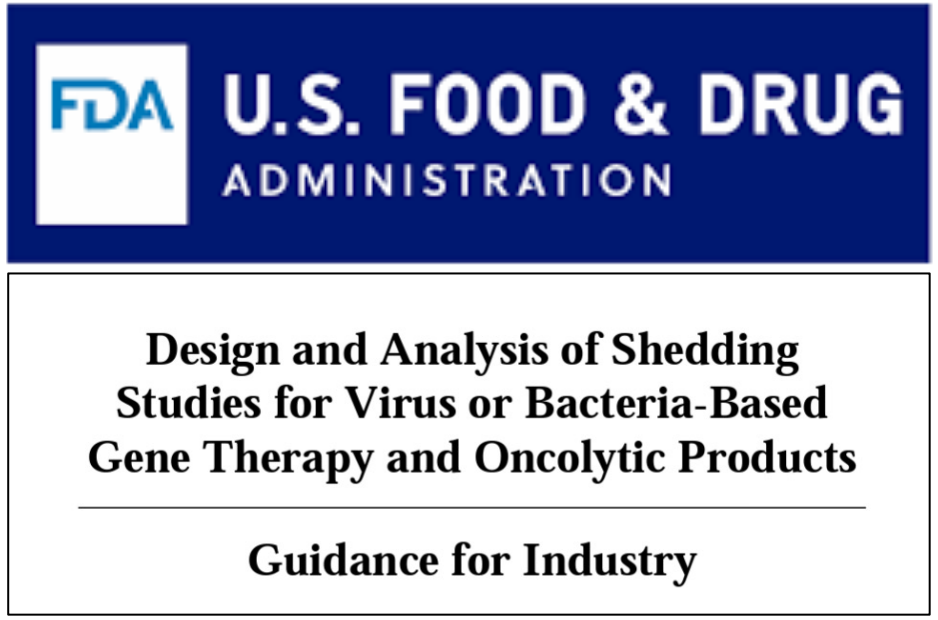
- “Shedding” means release of [Gene Therapy Product] from the patient through one or all of the following ways: excreta (feces); secreta (urine, saliva, nasopharyngeal fluids etc.); or through the skin (pustules, sores, wounds).
- Shedding is defined as the issemination of the virus/vector through secretions and/or excreta of the patient.
- Shedding is distinct from biodistribution, which describes how a product is spread within the patient’s body.
- Assessment of shedding can be utilized to understand the potential risk associated with transmission to third parties and the potential risk to the environment.
- Typical shedding matrices: urine, feces, saliva, semen, swabs

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA U.S. FOOD & DRUG ADMINISTRATION

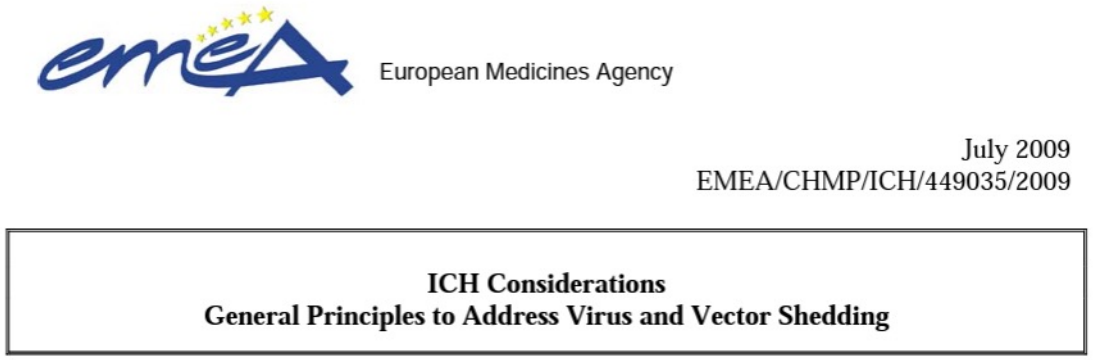
FDA Recommendation on Shedding

- Shedding assay(s) should be demonstrated to be specific, sensitive, reproducible and accurate.
- We recommend testing of clinical samples in a shedding assay in replicates to determine reproducibility.
- The sensitivity of the assay should be determined in terms LOD and the limit of quantitation (LOQ), if using a quantitative assay.
- While the Agency does not expect shedding assays to be validated, the assays should be qualified to meet minimal performance capabilities
- Blood is not typically analyzed for shedding but should be collected as part of pharmacokinetic analysis



EMA Recommendation on Shedding

- Specific, sensitive and reproducible qualified assay, quantitative preferred
- Polymerase chain reaction (PCR) and infectivity are the two assays typically used for the detection of shed virus / vector
- Use of a quantitative PCR (qPCR)-based assay is recommended (sensitive, reproducible, and rapid)


European Medicines Agency

July 2009
EMEA/CHMP/ICH/449035/2009

ICH Considerations
General Principles to Address Virus and Vector Shedding

Validation: Test Pre-defined Parameters with Associated Criteria

COU for Shedding assays is distinct from BD

COU & Scientific Rationale

Assay Development

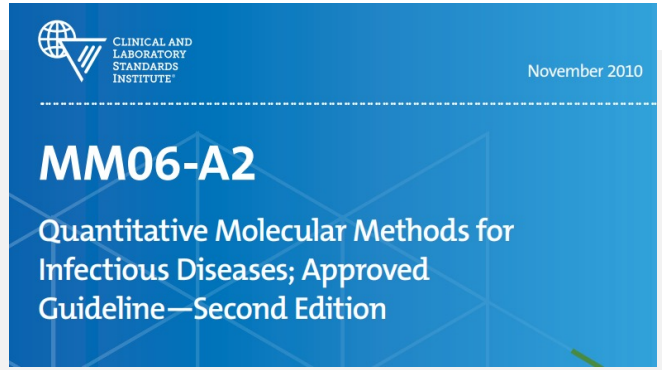
Validation

Bioanalysis

Validation Plan

Validation

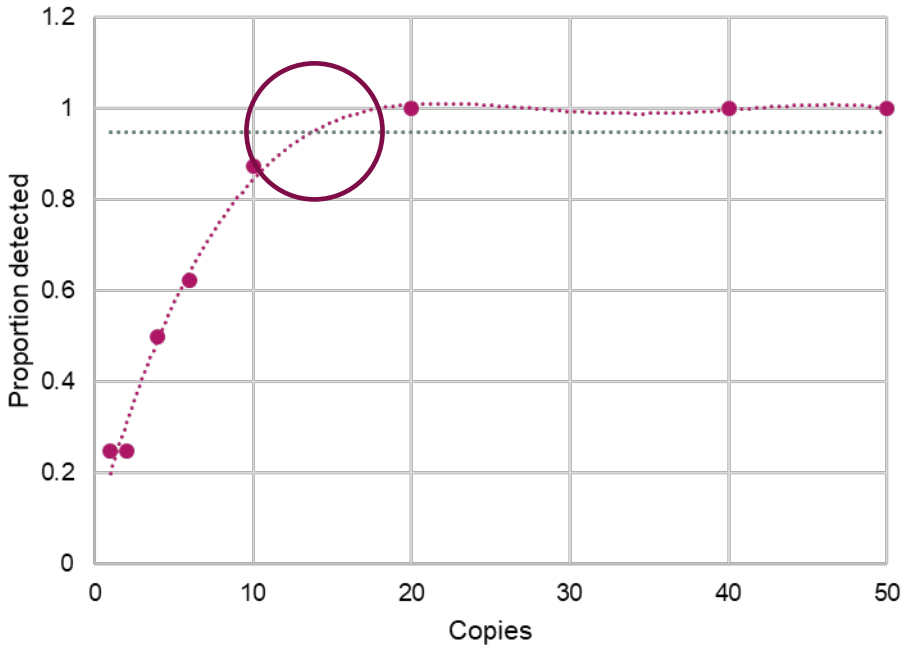
Validation Report



Limit of Detection (LOD): Concept and Calculation

The LOD is estimated as the lowest concentration at which 95% of positive samples can be detected

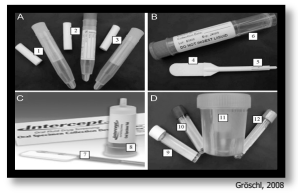
Genomic Copies	n_{total}	$n_{positive}$	$N_{positive}$ / n_{total}
50.00	8.00	8.00	1
40.00	8.00	8.00	1
20.00	8.00	8.00	1
10.00	8.00	7.00	0.875
6.00	8.00	5.00	0.625
4.00	8.00	4.00	0.5
2.00	8.00	2.00	0.25
1.00	8.00	2.00	0.25

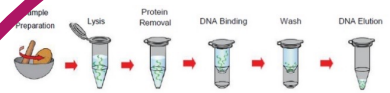


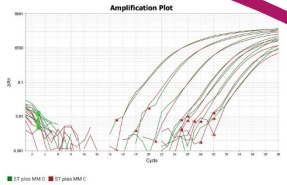
LOD = 15 copies

Shedding: 5 Steps from Sample Collection to Data Reporting

- All steps are equally important to generate reliable and reproducible data sets







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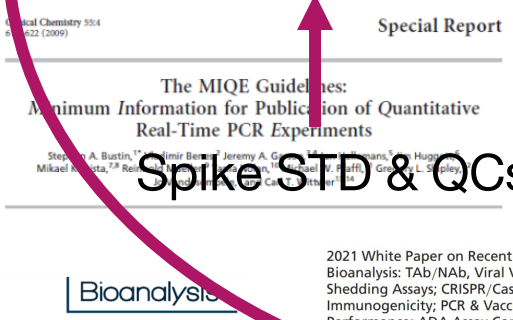
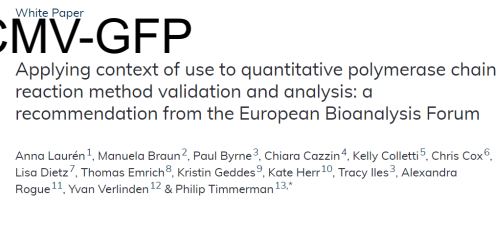
Sample Collection → Sample Handling → DNA Extraction → DNA Evaluation → Data Reporting

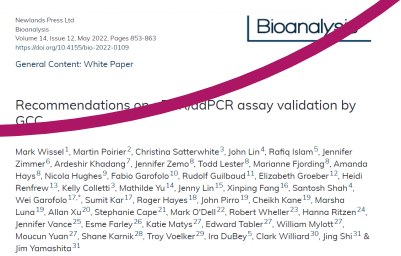
Reference: AAV8 pAV-CMV-GFP

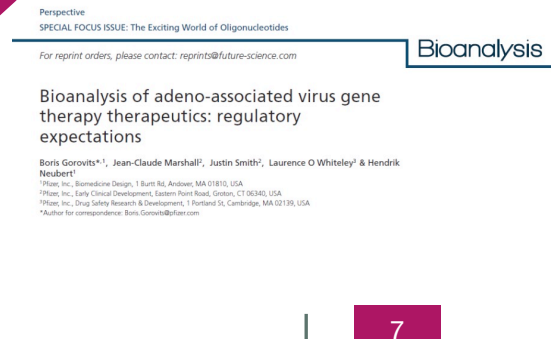
Spike STD & QCs

Validated qPCR method









Extraction Kit Evaluation

Kit \ Matrix	Blood	Plasma	Saliva	Urine	Semen	Feces
Qiagen Blood and Tissue Kit	X	X	X	X	X	
Qiagen Fast DNA Stool Mini Kit						X
Qiagen Viral RNA Mini Kit		X	X	X		
Zymo Research Quick DNA Urine Kit				X		
Zymo Research Quick DNA miniprep Plus Kit	X	X	X		X	X
Promega gDNA Extraction Kit	X	X	X	X	X	X

X = listed in the kit manual or specific protocols are available on the manufacturer's website

Extraction Kit Evaluation

Kit \ Matrix		Blood	Plasma	Saliva	Urine	Semen	Feces
Qiagen Blood and Tissue Kit	Recovery %CV	110% 13%CV	118% 14%CV	92% 13%CV	42% 24%CV	84% 31%CV	
	LOD (GC/µL)	2.7	1.3	1.6	1.4	7.9	
Qiagen Fast DNA Stool Mini Kit	Recovery %CV						85% 23%CV
	LOD (GC/mg)						7.3
Qiagen Viral RNA Mini Kit	Recovery %CV		19% 25%CV	52% 15%CV	88% 12%CV		
	LOD (GC/µL)		6.8	2.5	0.6		
Zymo Research Quick DNA Urine Kit	Recovery %CV				17% 11% CV		
	LOD (GC/µL)				0.9		
Zymo Research Quick DNA miniprep Plus Kit	Recovery %CV	59% 23%CV	45% 33%CV	6% 55.8%CV		37% 32%CV	No recovery
	LOD (GC/µL)	1.3	1.7	12.5		4.9	
Promega gDNA Extraction Kit	Recovery %CV	25% 20%CV	39% 27%CV	28% 18%CV	25% 25%CV	57% 27%CV	No recovery
	LOD (GC/µL)	6.0	3.9	5.3	2.7	11.3	

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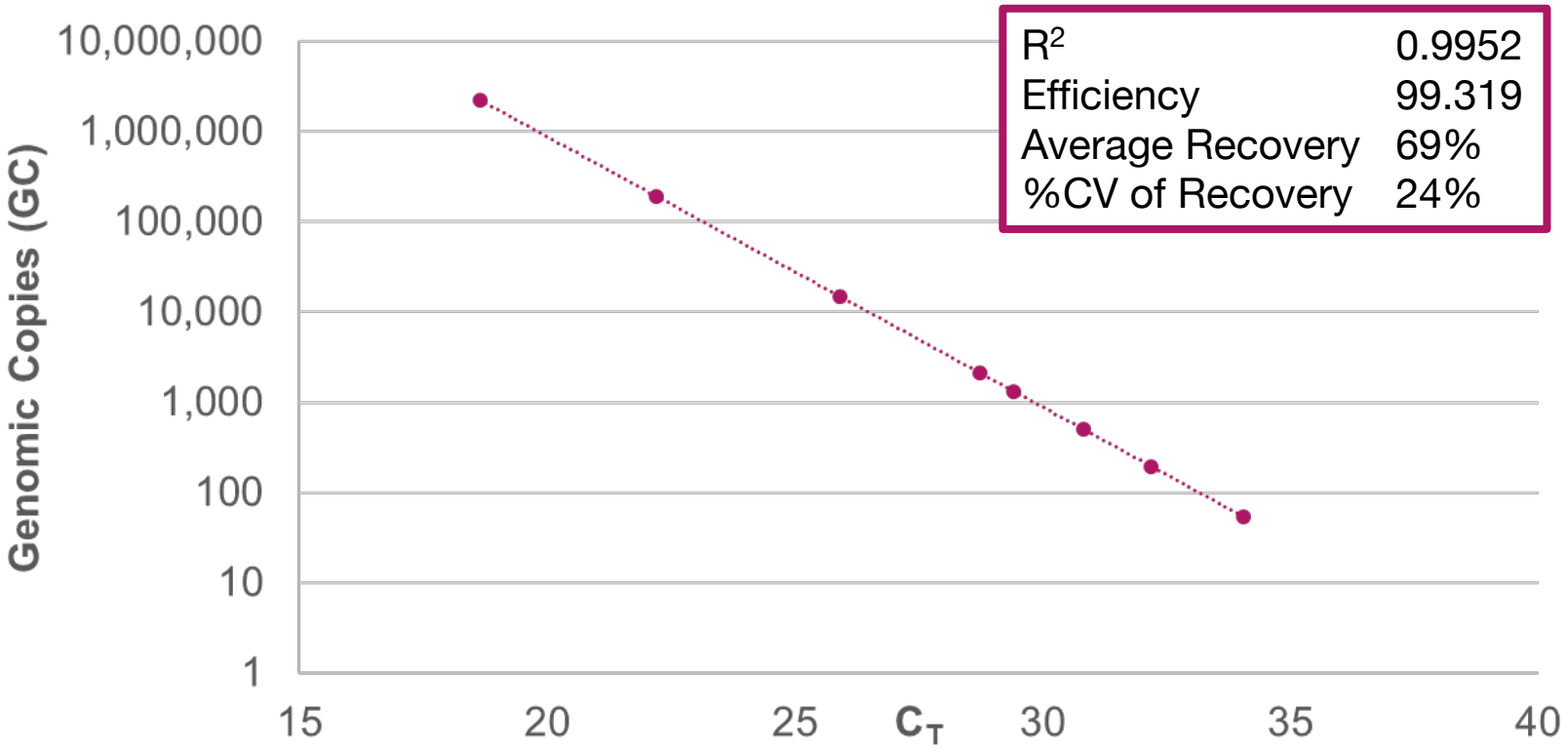
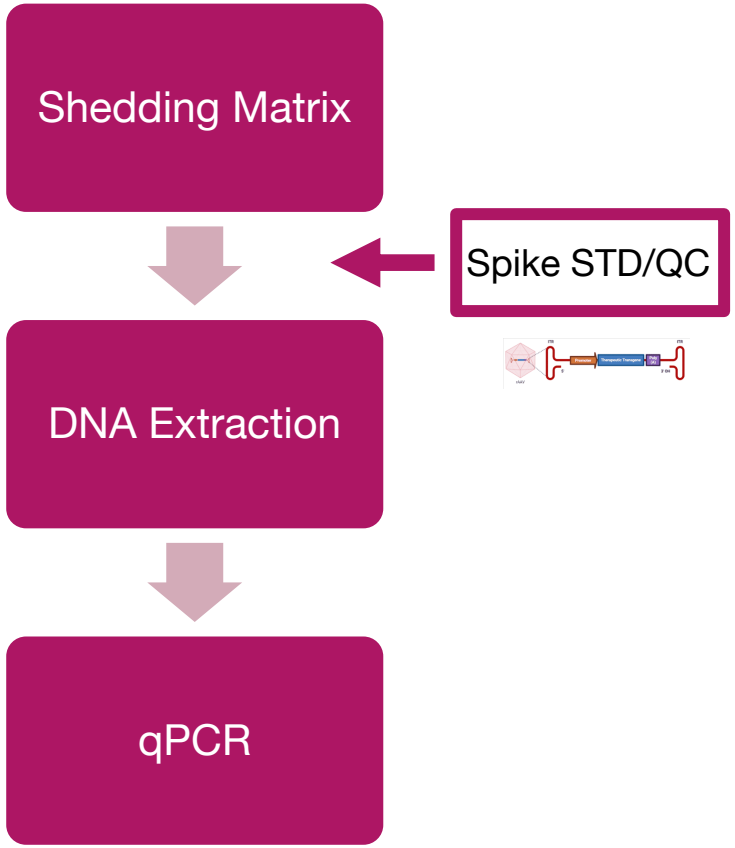
Zymo Research Quick DNA miniprep Plus Kit	Recovery %CV	59% 23%CV	45% 33%CV	6% 55.8%CV		37% 32%CV	No recovery
	LOD (GC/µL)	1.3	1.7	12.5		4.9	
Promega gDNA Extraction Kit	Recovery %CV	25% 20%CV	39% 27%CV	28% 18%CV	25% 25%CV	57% 27%CV	No recovery
	LOD (GC/µL)	6.0	3.9	5.3	2.7	11.3	

STDs: Spiked Before Extraction Using AAV as Reference Item

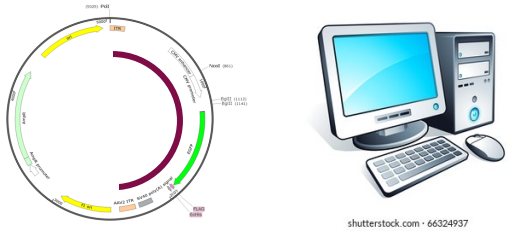
Standard curve at a range of 10^6 to 40 GC





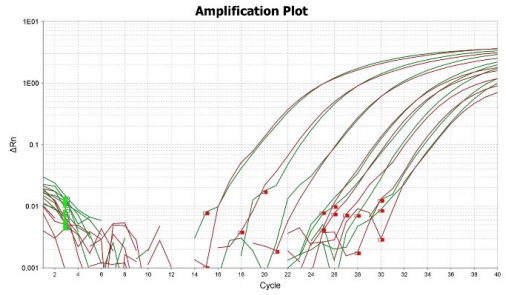
Assay Development Workflow

Design



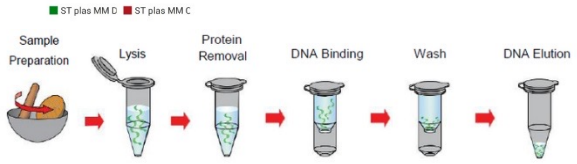
- 3-5 primers/probe sets
- 3 master mixes (MM)

qPCR Optimization



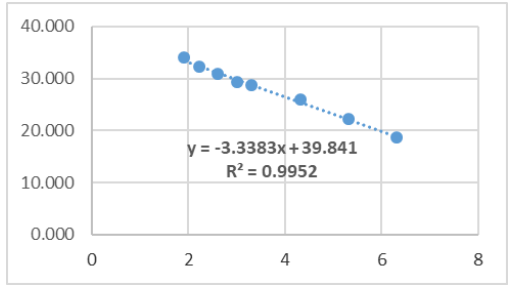
- Define best primer/MM combination
- LLOQ and LOD
- Co-linearity plasmid/viral DNA

DNA Extraction



- Test at least 3 extraction kits per matrix
- AAV viral particle to test recovery

Final Optimization



- STD and QC spiked in prior extraction
- LOD in matrix

Results Reporting Example

Subject	Visit	Test Name	Result	Interpretation
1	Dosing Day -1	AAV8 DNA in Feces	<LOD	Negative
1	Dosing Day 2	AAV8 DNA in Feces	1220	Positive
1	Dosing Day 3	AAV8 DNA in Feces	5200	Positive
1	Dosing week 1 - 11	AAV8 DNA in Feces	…	Positive
1	Week 12	AAV8 DNA in Feces	<LLOQ	Positive
1	Week 13	AAV8 DNA in Feces	<LOD	Negative
1	Week 14	AAV8 DNA in Feces	<LOD	Negative
1	Week 15	AAV8 DNA in Feces	<LOD	Negative
1	Week 16	AAV8 DNA in Feces	No Sample	

Conclusion

Shedding assay has a Unique Context of Use

- Inhibition from different matrixes possible
- Different kits to be evaluated and validated
- Fast turnaround needed to inform on next sample collections from trial participants

Although it has sample collection impact, it is not an IVD

Quantitative PCR Validation to focus on LOD

Extraction kit validation to focus on reproducible recovery and total amount recovered per mL or mg

- Kit insert claims need to be tested experimentally

Acknowledgements

- Johannes Stanta, PhD
- Silvia Moimas, PhD
- Andrea Maddalena, PhD
- Petia Doytcheva, PhD
- Petra Struwe, PhD

Thank you

Determination of the Best Kit for the Different Matrixes (Efficiency and Theoretical LOD)

$$LOD_t = \frac{LOD_q * V_{elu}}{Rec * V_s * V_{pr}}$$

Examples

- Whole blood – Kit C
  - LOD_q = 15 GC
  - Eff = 0.58
  - V_s = 5 µL
  - V_{pr} = 200 µL
  - V_{elu} = 50 µL

$$LOD_t = \frac{15\ GC * 50\ \mu L}{0.58 * 5\ \mu L * 200\ \mu L} \rightarrow 1.3\ GC/\mu L$$

- Faeces – Kit F
  - LOD_q = 15 GC
  - Eff = 0.85
  - V_s = 5 µL
  - V_{pr} = 100 mg
  - V_{elu} = 200 µL

$$LOD_t = \frac{15\ GC * 200\ \mu L}{0.85 * 5\ \mu L * 100\ mg} \rightarrow 7.1\ GC/mg$$

The theoretical LOD (LOD_t) was calculated taking into account the overall recovery (Rec), the processed sample volume (V_{pr}), extraction elution volume (V_{elu}), qPCR sample volume (V_s) and qPCR LOD (LOD_q). For liquid matrixes LOD_t is expressed in GC/uL and for Faeces in GC/mg.

Streamlined Validation Flow

	Experiment	Acceptance criteria
1	Calibration curve, Limit of Quantification (LOQ), Linearity and Range.	R^2 ≥0.990 Amplification efficiency 90% to 110% %CV replicate CT values: ≤3%
2	Specificity	No amplification in presence of human genomic DNA showing that there is no cross-reactivity in the assay.
3	LOD with 95% Confidence	Lowest concentration detected with ≥95% confidence
4	Co-Linearity	Comparable amplification efficiencies between plasmid and viral DNA Slopes ratio between 90% and 110%
5	3x Accuracy and Precision (A&P) using QC Samples	%CV and %Bias: ±35% (±45% at LLOQ)
6	3x Extraction runs for each matrix with QC at 10x LLOQ	Recovery: 50 – 200% %CV and %Bias (total error): ±45%