Dinesh Khokal, PhD, Singapore

- Director, External Affairs, Corporate Quality, JAPAC and Intercontinental – LatAm, Amgen Singapore

GaBI Educational Workshops

1st ASEAN Overview Workshop on
GMP for BIOLOGICALS/BIOSIMILARS

5 August 2018, Furama Resort Da Nang, Vietnam

Validation of viral removal/inactivation and bioanalytical methods

Dinesh Khokal, PhD

5 August 2018

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

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VALIDATION OF VIRAL REMOVAL/INACTIVATION AND BIOANALYTICAL METHODS

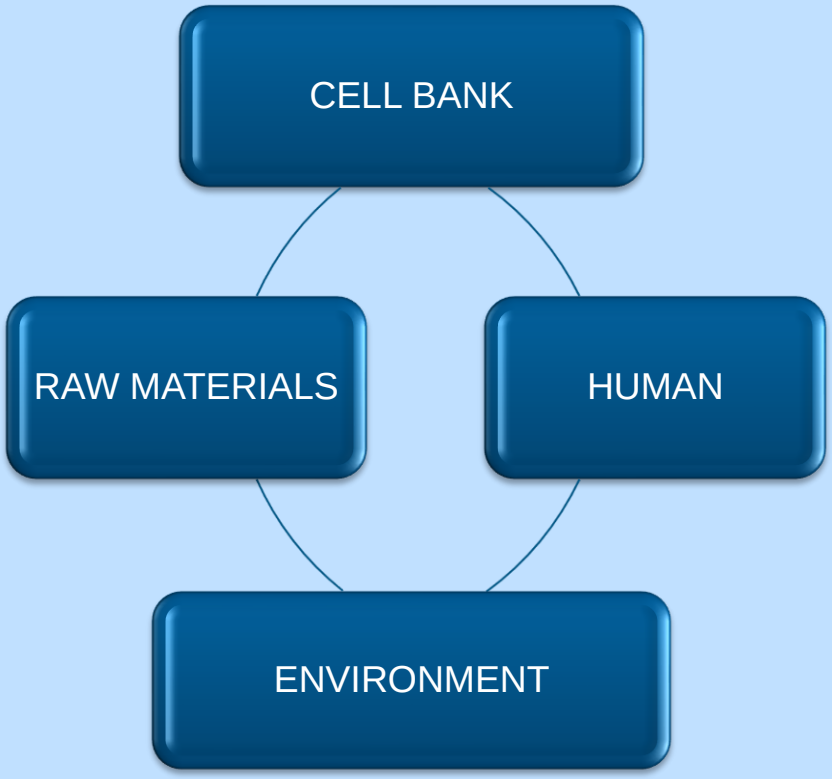
First ASEAN Overview Workshop on cGMP for Biologicals/ Biosimilars
Generics and Biosimilars Initiative (GaBI)
05 August 2018, Da Nang, Vietnam

DINESH KHOKAL, PH.D.
DIRECTOR, EXTERNAL AFFAIRS, JAPAC & LATAM

SYNOPSIS

- **Introduction**
- **Validation of viral removal/ inactivation**
- **Bioanalytical method validation**

POTENTIAL SOURCE OF VIRUS CONTAMINATION



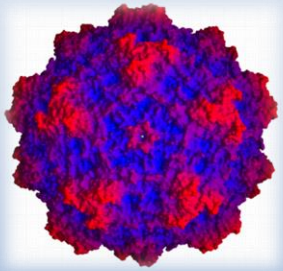
Viral contamination of biotechnology products may arise from the original source of the cell lines or from adventitious introduction of virus during production processes

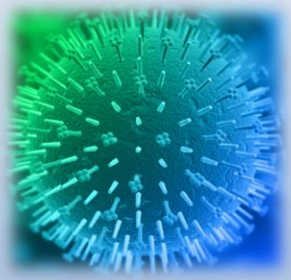
- Risk to all biologics
- Risk due to materials of animal or human origin used
- Number of biologicals have been contaminated
- Virus only identified many years after the product manufacture
- Cause – Contamination of the starting or source material

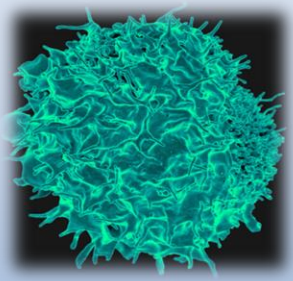
VIRUS CONTAMINATION IN BIO-PROCESSING INDUSTRY

Reported viral contamination

Minute virus of mice (MMV), Retrovirus type 3 (Reo-3), Calicivirus (Feline virus), Circovirus (Porcine virus)







Significant impact

- Product quality
- Facility shutdowns
- Disruption of medicine supply
- Business impact

VIRAL RISK MITIGATION STRATEGIES

- **Main approaches**
  - **Plant Design & contamination control**
  - **Selecting and testing source material**
    - **Control of source materials**
    - **Cell line development**
    - **Virus testing for cell bank**
  - **Testing the clearance capacity of the production processes**
    - **Testing of In-process products**
  - **Testing the product for freedom from detectable viruses**

No approach provides sufficient level of assurance alone

WHY VIRUS CLEARANCE/INACTIVATION VALIDATION?

- No single test is able to demonstrate the presence of all known viruses
- All test systems require a minimum level of viral contamination to record a positive result
- Tests are also limited by statistical considerations in sampling

Establishing the freedom of a biological product from virus will not derive solely from testing but also from a demonstration that the manufacturing process is capable of removing or inactivating them

Validation of the process for viral removal/ inactivation play an essential and important role in establishing product safety

VIRAL CLEARANCE METHODS

Virus inactivation methods

- Chemical methods
  - Low pH incubation
  - Surfactant / Detergent
- Physical methods
  - Heat treatment
  - UV

Virus removal methods

- Precipitation
  - Ammonium sulfate, etc.
- Column Chromatography
  - Ion Exchange
  - Size exclusion
  - Affinity
  - Reverse phase
  - Hydrophobic interaction
- Membrane filtration
- Nanofiltration

SYNOPSIS

- Introduction
- **Validation of viral removal/ inactivation**
- Bioanalytical method validation

AIM OF VIRAL REMOVAL VALIDATION

To demonstrate that manufacturing/ purification processes can eliminate substantially more virus than what may potentially be present in the unprocessed bulk material

To obtain the best reasonable assurance that the product is free of virus contamination

RATIONALE FOR VIRAL VALIDATION

Explanation & justification	• Approach & choice of viruses
Manufacturing conditions	• Representative of wide range of physico-chemical properties
Characterisation of virus	• Viruses that are known to be present • Non-specific “model” viruses
Knowledge on virus	• Virus present in the manufacturing process • Virus that can be cleared to assess product safety
Relevant validation protocol	• Virus tests from the Mater Cell Bank (MCB) • Various steps of production • Final product
Evaluation & characterisation	• Viral clearance from unprocessed bulk • Various process steps studied independently
Virus “spiking“	• Addition of significant amounts of virus to crude material &/or to different fractions obtained during the various process steps
Clearance results	• Log clearance results from orthogonal steps are added together to give overall figure for the process as a whole

CHOICE OF VIRUSES FOR VIRAL CLEARANCE STUDY

A major issue is to determine which viruses should be used

- **Three categories**
  1. "Relevant" viruses
     - known viruses or same species as that are known or likely to be present
  2. Specific "model" viruses
     - substitute viruses, closely related, similar physical & chemical properties
  3. Non-specific "model" viruses
     - viruses displaying significant resistance to physical and/or chemical treatments

OTHER CONSIDERATIONS

Choice of Virus	• Viruses which can be grown to high titer are desirable
Assay	• Availability of an efficient and reliable assay for the detection of each virus
Health hazard	• Certain viruses may pose health hazard to the personnel performing the clearance studies
Introduction of Virus	• Inappropriate to introduce any virus into a production facility because of GMP constraints and safety
Specialized Laboratory	• Viral clearance studies should be conducted in a separate laboratory equipped for virological work
Virology expertise	• Performed by staff with virological expertise • Production personnel involved in designing and preparing a scaled-down version of the purification process

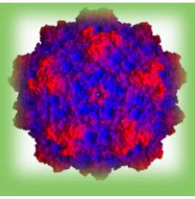
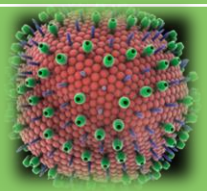
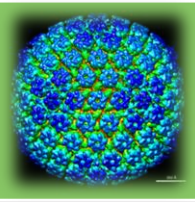
SCALED-DOWN PRODUCTION SYSTEM AND SPIKING

Scale down study	• Validity of the scaling down should be demonstrated
Spiking & sampling	• Ensure spiking experiments and sample collection are relatively straightforward
Simulation of production process	• Level of purification of the scaled-down version should represent as closely as possible the production procedure
Simulation of commercial-scale manufacturing	• Chromatographic equipment, column bed-height, linear flow-rate, flow-rate-to-bed-volume ratio (i.e., contact time), buffer and gel types, pH, temperature, and concentration of protein, salt, and product
Deviations	• Deviations which cannot be avoided should be discussed with regard to their influence on the results

ASSAY METHODS – ACCURACY AND VALIDATION

Assay types	• Wide variety of assay types can be used to detect and quantify viral liter • Each assay type has specific advantages and disadvantages
Accuracy & reproducibility	• Methods must provide accurate and reproducible quantitation of the viral concentration
Variability of assay	• Virus detection assays can result in high variable results due to biological nature of the assay systems
Reliability of results	• Test data generated must provide a reliable estimate of process reduction factors
Expression of results	• Viral concentrations are normally expressed with 95% confidence limit that should be on the order of 5% log of the mean
Main assay types	• Vitro assay to quantitate infectious virus in viral clearance studies: • 1) plaque (or focus) formation assay • 2) cytopathic effect (CPE) assay

VIRUS USED TO VALIDATE PRODUCTS DERIVED FROM MURINE HYBRIDOMA AND CELL LINE

Virus	Genome	Size (nm)	Enveloped?	Resistance
Minute virus of mice (MMV) 	ss-DNA	18-26	No	Very high
Retrovirus type 3 (Reo-3) 	ds-RNA	60-80	No	High
Murine leukemia virus (MuLV) 	ss-RNA	80-130	Yes	Low
Pseudorabies virus (PRV) 	ds-DNA	150-200	Yes	Low-medium

SMALL SCALE VIRAL CLEARANCE STUDY

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5A_R1/Step4/Q5A_R1__Guideline.pdf

TABLE A–1.—EXAMPLES OF VIRUSES WHICH HAVE BEEN USED IN VIRAL CLEARANCE STUDIES

Virus	Family	Genus	Natural Host	Genome	Env	Size (nm)	Shape	Resistance[1]
Vesicular Stomatitis Virus	Rhabdo	Vesiculo-virus	Equine Bovine	RNA	yes	70 x 150	Bullet	Low
Parainfluenza Virus	Paramyxo	Paramyxo-virus	Various	RNA	yes	100–200+	Pleo/Spher	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80–110	Spherical	Low
Sindbis Virus	Toga	Alphavirus	Human	RNA	yes	60–70	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50–70	Pleo/Spher	Low
Pseudo-rabies Virus	Herpes		Swine	DNA	yes	120–200	Spherical	Med
Poliovirus Sabin Type 1	Picorna	Entero-virus	Human	RNA	no	25–30	Icosa-hedral	Med
Encephalomyo-carditis Virus (EMC)	Picorna	Cardio-virus	Mouse	RNA	no	25–30	Icosa-hedral	Med
Reovirus 3	Roe	Orthoreo-virus	Various	DNA	no	60–80	Spherical	Med
SV40	Papova	Polyomavirus	Monkey	DNA	no	40–50	Icosa-hedral	Very high
Parvoviruses (canine, porcine)	Parvo	Parvovirus	Canine Porcine	DNA	no	18–24	Icosa-hedral	Very high

[1] Resistance to physico-chemical treatments based on studies of production processes. Resistance is relative to the specific treatment and it is used in the context of the understanding of the biology of the virus and the nature of the manufacturing process. Actual results will vary according to the treatment. These viruses are examples only and their use is not considered mandatory.

ROBUSTNESS OF VIRAL CLEARANCE IS CHARACTERIZED IN QUALIFIED SMALL SCALE STUDIES USING VIRUS MODEL

VIRUS MODEL CHOSEN IN SMALL SCALE STUDY

FOR ILLUSTRATION ONLY

Virus	Genome	Envelope	Family	Approximate Size (nm)	Resistance to Physicochemical Reagents
xMuLV	RNA	Yes	Retroviridae	80 to 110	Low
PrV	DNA	Yes	Herpesviridae	120 to 200	Low
Reo-3	RNA	No	Reoviridae	60 to 80	Medium
MMV	DNA	No	Parvoviridae	18 to 24	High

- Example of a virus panel comprises 4 representative model viruses having different physicochemical properties, size, and chemical resistance, to demonstrate the robustness of viral clearance capability
- These model viruses include members from each of the four major classes of virus (enveloped or non-enveloped, containing DNA or RNA)
- Two enveloped viruses, xenotropic murine leukemia virus (xMuLV) and pseudorabies virus (PrV), model the retrovirus-like particles found in CHO cells and herpesvirus, respectively
- Non-enveloped Reovirus type 3 (Reo-3) has the ability to infect both human and animal cells. Murine minute virus (MMV) is a model rodent virus which can infect CHO cells

SMALL SCALE VIRAL CLEARANCE STUDY

GE AxiChrom
300 to 1600 mm (Diameter)

SCALE DOWN

GE Hiscale and Tricorn
50 to 5 mm (Diameter)

- Small scale models need to be qualified to represent process in production scale
- Use of worst case process conditions
- Replicate of testing is required due to inherent variability of viral assay
- Load material are spiked with model viruses and clearance is expressed in Log reduction

$$Log_{10}\ Reduction\ Value = Log_{10}\left(\frac{total\ virus_{start}}{total\ virus_{end}}\right)$$

LOG REDUCTION CALCULATION FROM SMALL SCALE STUDY

PROCESS STEP	xMuLV	PrV	Reo-3	MMV
Viral Inactivation	≥ 6	≥ 6	-	-
Chromatography	≥ 6	≥ 6	≥ 6	≥ 6
Nanofiltration	≥ 4	≥ 4	≥ 4	≥ 4
Total	≥ 16	≥ 16	≥ 10	≥ 10

FOR ILLUSTRATION ONLY (VALUE PROVIDED ARE RANDOMLY GENERATED FOR EDUCATION PURPOSE)

Assuming harvest bulk material (20,000L, 2 g/L protein) containing 1 x 10^8 retro-virus like particle (VLP)/ml. For 50% purification yield and a final dose of 100mg protein, the risk of finding the VLP in final dose:

$$\frac{1 \times 10^8 \text{ VLP/ml} \times 20{,}000\text{L} \times 1000\text{ml/L}}{20{,}000\text{L} \times 2\text{ g/L} \times 50\% \times 1000\text{mg/1g} \times 1\text{ dose/ } 100\text{ mg}} = 1 \times 10^{10} \text{ VLP/dose}$$

Risk WITHOUT viral clearance process

≥ 16 LOG REDUCTION OF VIRAL CLEARANCE STEPS

$\leq 1 \times 10^{-6}$ VLP/dose

Risk POST viral clearance process

"Probability to find ≤ 1 Retro-virus particle in 1 million doses"

SYNOPSIS

- Introduction
- Validation of viral removal/ inactivation
- **Bioanalytical method validation**

QUALIFICATION, VALIDATION AND VERIFICATION

Qualification

- Insufficient knowledge of the test's performance to document full validation
- A performance assessment has been made to determine reliability and variability

Validation

- Full knowledge of the test's performance and performance assessment has been made to determine reliability and variability
- Protocol for full validation over a long time frame and with many parameters tested

Verification

- Applied to validated methods that typically appear in compendia
- To demonstrate ability of the method to perform the test according to stated specifications
- Use available standards from NIST, WHO or Pharmacopeia to in test verification

WHY BIOANALYTICAL METHOD VALIDATION?

- To assess the fit-for-purpose appropriate for the intended use
- To ensure that the data are reliable
- To provide critical data to support the safety and effectiveness of drugs and biologic products
- Critical for the quantitative evaluation of analytes (i.e., drugs, including biologic products, and their metabolites) and biomarkers in a given biological matrix (e.g. blood, plasma, serum, or urine)

METHOD VALIDATION PARAMETERS

Test parameters	Description
Sensitivity	Lower limit of detection (LOD), The lowest concentration of an analyte that the analytical procedure can reliably differentiate from background noise
Specificity	Ability to assess unequivocally the analyte in the presence of expected components such as impurities, degraded products and matrix
Precision	Closeness of agreement among measurements obtained from multiple sampling under described conditions
Repeatability	Able to repeat under the same operation conditions, over a short time period
Intermediate precision	Able to obtain the same results within laboratory variations, different days, analysts, equipment, etc.
Reproducibility	Able to obtain the same results among different laboratories variations, different days, analysts, equipment, etc.
Accuracy	Degree of closeness of determined value to the nominal or known true value under prescribed conditions. Accuracy is also sometimes termed trueness.
Quantitation Range	Range of concentration, including upper limit of quantitation (ULOQ) and lower limit of quantitation (LLOQ), that may be reliably and reproducibly quantified through a concentration-response relationship
Linearity	Extent to which the relationship between experimental response value and concentration of the analyte approximates a straight line
Robustness	Ability of the method to deliver accurate, precise results under normal operating-condition variations

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (1)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Sensitivity	**Elements:** • The lowest nonzero standard on the calibration curve defines the sensitivity (LLOQ). **Acceptance Criteria:** • The analyte response at the LLOQ should be ≥ five times the analyte response of the zero calibrator. • The accuracy should be ± 20% of nominal concentration (from ≥ five replicates in at least three runs). • The precision should be ± 20% CV (from ≥ five replicates in at least three runs).	**Elements:** • The lowest nonzero standard on the calibration curve defines the sensitivity (LLOQ). **Acceptance Criteria:** • The accuracy should be ± 25% of the nominal concentration (from ≥ three replicates in at least six runs). • The precision should be ± 25% CV (from ≥ three replicates in at least six runs). • The total error should be ≤ 40%.
Specificity	**Elements:** • The method specificity should be assessed for interference by cross-reacting molecules, concomitant medications, bio-transformed species, etc. **Acceptance Criteria:** • See Selectivity below.	**Elements:** • The method specificity should be assessed for interference by cross-reacting molecules, concomitant medications, bio-transformed species, etc. • Potential interfering materials should be added to calibration curves in buffer. **Acceptance Criteria:** • QCs should meet ± 20%, or 25% at the LLOQ and ULOQ.

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (2)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Accuracy and Precision (A & P)	**Elements:** • A & P should be established with at least three independent A& P runs, four QC levels per run (LLOQ, L, M, H QC), and ≥ five replicates per QC level. A & P Run Acceptance Criteria: • The run should meet the calibration curve acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria. Accuracy: Within-run and between runs: • ± 15% of nominal concentrations; except ± 20% at LLOQ. Precision: Within-run and between runs: • ± 15% CV, except ± 20% CV at LLOQ Total Error: • Not applicable	**Elements:** • A & P should be established with at least six independent A& P runs, five QC levels per run (LLOQ, L, M, H, ULOQ QC), and ≥ three replicates per QC level. A & P Run Acceptance Criteria: • The run should meet the calibration acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria. Accuracy: Within-run and between runs: • ± 20% of nominal concentrations; except ±25% at LLOQ, ULOQ Precision: Within-run and between runs: • ± 20% CV, except ± 25% at LLOQ, ULOQ Total Error: • QCs should be ±30%, except at LLOQ, ULOQ ±40%

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (3)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Selectivity	**Elements:** • Analyze blank samples of the appropriate biological matrix from at least six individual sources. Acceptance Criteria: • Blank and zero calibrators should be free of interference at the retention times of the analyte(s) and the IS. • Spiked samples should be ± 20%LLOQ. • The IS response in the blankshould not exceed 5% of the average IS responses of the calibrators and QCs.	**Elements:** • Investigate parallelism (for endogenous products). • Conduct an analysis of blank samples in the matrix from ≥ 10 individual sources. Acceptance Criteria: • For ≥ 80% of sources, unspiked matrix should be BQL, and spiked samples should be ± 25% at LLOQ, and ± 20% at H QC.
Carryover	**Elements:** • The impact of carryover on the accuracy of the study sample concentrations should be assessed. Acceptance Criteria: • Carryover should not exceed 20% of LLOQ.	• Not applicable

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (4)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Quality Controls (QC)	**Elements:** • For A & P Runs: Four QCs, including LLOQ, low (L: defined as three times the LLOQ), mid (M: defined as mid-range), and high (H: defined as high-range) from at least five replicates in at least three runs • For Other Validation Runs: L, M, and H QCs in duplicates **Acceptance Criteria:** • Refer to A & P Runs, Other Validation Runs, and Stability Evaluations.	**Elements:** • For A& P Runs: Five QCs, including LLOQ, L, M, H, and ULOQ from at least three replicates in at least six runs • For Other Validation Runs: L, M, and H QCs in duplicates **Acceptance Criteria:** • Refer to A & P Runs, Other Validation Runs, and Stability Evaluations.
Other Validation Runs	**Elements:** • ≥ three QC levels (L, M, H) in at least duplicates in each run. **Run Acceptance Criteria:** • Meet the calibration acceptance criteria • ≥ 67% of QCs should be ± 15% of the nominal (theoretical) values, ≥ 50% of QCs per level should be ± 15% of their nominal concentrations	**Elements:** • ≥ three QC levels (L, M, H) in at least duplicates in each run **Run Acceptance Criteria:** • Meet the calibration acceptance criteria • ≥ 67% of QCs should be ± 20% of the nominal (theoretical) values, and ≥ 50% of QCs per level should be ± 20% of their nominal concentrations

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (5)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Recovery	**Elements:** • Extracted samples at L, M, and H QC concentrations versus extracts of blanks spiked with the analyte post extraction (at L, M, and H)	**Elements:** • Need to be demonstrated only if extraction is involved
Stability	**Elements:** • For auto-sampler, bench-top, extract, freeze-thaw, stock solution and long-term stability, perform at least three replicates at L and HQC concentrations. **Acceptance Criteria:** • The accuracy (% nominal) at each level should be ± 15%.	**Elements:** • For auto-sampler, bench-top, extract, freeze-thaw, stock solution/reagent and long-term stability, perform at least three replicates at Land HQC concentrations. **Acceptance Criteria:** • The accuracy (% nominal) at each level should be ± 20%.
Dilution	**Elements:** • QCs for planned dilutions, 5 replicates per dilution factor: o Accuracy: ± 15% of nominal concentrations o Precision: ± 15% CV	**Elements:** • QCs for planned dilutions • Demonstrate dilution linearity • Demonstrate lack of prozone effect, i.e., increasing analyte concentration results in no change or decreased signals compared to the preceding concentration • 5 replicates per dilution factor: o Accuracy: ± 20% of nominal concentrations o Precision: ± 20% CV

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

RECOMMENDATIONS AND ACCEPTANCE CRITERIA FOR BIOANALYTICAL METHOD VALIDATION (6)

Parameters	Validation Recommendations	
	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Calibration Curve	**Elements:** • A blank (no analyte, no IS), a zero calibrator (blank plus IS), and at least six, non-zero calibrator levels covering the quantitation range, including LLOQ in every run. • All blanks and calibrators should be in the same matrix as the study samples. • The concentration-response relationship should be fit with the simplest regression model. **Acceptance Criteria:** • Non-zero calibrators should be ± 15% of nominal (theoretical) concentrations, except at LLOQ where the calibrator should be ± 20% of the nominal concentrations in each validation run. • 75% and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run.	**Elements:** • A blank and at least six, non-zero calibrator levels covering the quantitation range, including LLOQ per validation run. • Calibration curves are usually run in duplicate. • Additional calibrators may be used as anchor points. • All blanks and calibrators should be in the same matrix as the study samples. • The concentration-response relationship is usually fit with a four- or five-parameter logistic model. Other models may be acceptable with justification. **Acceptance Criteria:** • Non-zero calibrators should be ± 20% of nominal (theoretical) concentrations, except at LLOQ and ULOQ where the calibrator should be ± 25% of the nominal concentrations in each validation run. • 75% and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run. • Anchor points should not be included in the curve fit.

Source: US FDA Guidance on Bioanalytical Method Validation, May 2018

ACKNOWLEDGMENT

- Houman Dehghani, Amgen, Keck Course, ‘Control of Adventitious Agent Contamination Risk: Foundation and Historical Perspective’
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- Yusdy Pan, Amgen, “Viral Risk Mitigation in Biologics Manufacturing’

THANK YOU FOR YOUR ATTENTION