

Understanding the Use of Bioassays in a Biomanufacturing Facility

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▶ What is a Bioassay?

A bioassay is an analytical procedure that measures the biological activity of a test substance based on a specific, functional, biological response of a test system

▶ Why we setup a Bioassay?

2.1.2 Biological activity

Assessment of the biological properties constitutes an equally essential step in establishing a complete characterization profile. An important property is the biological activity that describes the specific ability or capacity of a product to achieve a defined biological effect.

A valid biological assay to measure the biological activity should be provided by the manufacturer. Examples of procedures used to measure biological activity include:

- **Animal-based biological assays**, which measure an organism's biological response to the product;
- **Cell culture-based biological assays**, which measure biochemical or physiological response at the cellular level;
- **Biochemical assays**, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions.

Other procedures such as **ligand and receptor binding assays**, may be acceptable.

Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties... A correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies.

(Extracted from ICH Harmonised Tripartite Guideline Q6B: Specifications: test Procedures and Acceptance Criteria for Biotechnological/Biological Products, 10 March, 1999)

- ▶ Assay requirements
- ▶ Compliance requirements
 - Facility & equipment
 - Personnel training
- ▶ Cell-based case study
- ▶ Potency/activity case study

▶ Background information

- Details from sponsor include
 - Target cell line &/or biological effect
 - Measurement of effect
 - Defined interaction between API & target & Sponsor technical information
 - o Cell death
 - o Binding to membrane receptor
 - o Stability of API and its effect
 - o Model that could mimic In-vitro activity?
 - o Changes to methods (late phase programs)
 - o Potential issues with assays (history of test method)
 - o Method details (if available)
 - Assay conditions & calculations

▶ Critical parameters

- Careful consideration to critical reagents, especially cells
- Essential to have robustness around cell parameters before assay development/method validation
 - Cell viability
 - Cell passage
 - Minimum & maximum response
 - Slope
 - EC50 of standard material (if available)
- Implementation of system suitability and/or acceptance criteria that are **relevant**
- Monitoring of these parameters becomes critical as ***troubleshooting tools***

Compliance Requirements

- Facility & Equipment (*)

- ▶ Do we have the appropriate tools/resources to measure the desired effect?
 - Absorbance
 - Fluorescence
 - Luminescence
 - Viability
 - Other detection methods

- ▶ Is there any constraint in developing /implementing a bioassay?
 - Biosafety level assessment
 - PPE for lab personnel
 - Additional reagents?

(*) Ref.: ICH Harmonised Tripartite Guideline Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients 10 November, 2000, sections 4, 12 & 19 (APIs for use in Clinical Trials)

Compliance Requirements

- Personnel Training

- ▶ Evaluate personnel qualifications
- ▶ Review the training program to identify potential gaps in technical capabilities of lab personnel
 - Is there any specific training we can get from sponsor &/or 3rd party collaborators?
- ▶ Why we look into training?

Sec. 211.25 Personnel qualifications. (a)

Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practice (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in current good manufacturing practice shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them.

Extracted from Code of Federal Regulations Title 21, Volume 4 [Revised as of April 1, 2011]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES

SUBCHAPTER C--DRUGS: GENERAL PART 211CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

▶ Cell-based case study

Study 1 – Cell-based Assay (TCID₅₀)

- ▶ **Tissue Culture Infectious Dose** that inhibits 50% the increase in cell density
- ▶ In-Process Assay that measures virus titer
- ▶ Limit Dilution Assay
- ▶ Quantifies the ability of a recombinant virus to inhibit growth in a logarithmically growing population of suspension cells
 - Reports results in terms of virus titer, expressed in pfu/mL.
- ▶ An attempt to offset High Variance in Response Factor by means of **REPLICATION**.
 - Viral Test Samples are Serially Diluted (in triplicate series), and inoculated into logarithmically growing cells (suspension culture).

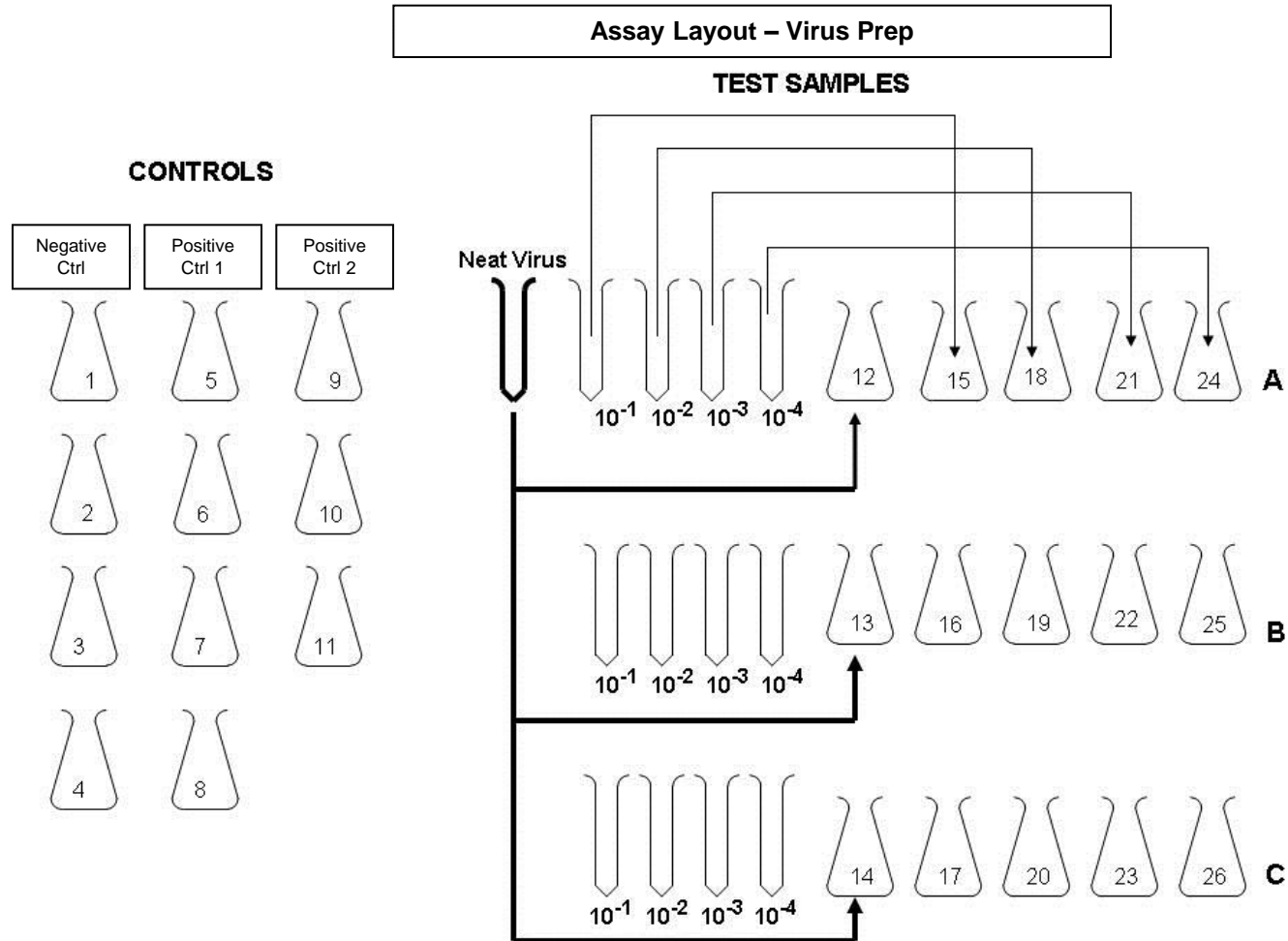
Study 1 – Cell-based Assay (TCID₅₀)

- ▶ Measurement: Tissue Culture Viability (ViCELL XR)

- ▶ Assay Conditions
 - Suspension cells.
 - Cell viability must be $\geq 85\%$ before assay setup
 - Flasks are incubated for 1 dt (~ 24 h).
 - 10 fold serial dilution of virus sample ($1 \times 10^{-1} - 1 \times 10^{-5}$)
 - Validated method covers 20x range ($2 \times 10^8 - 4 \times 10^9$ pfu/mL)

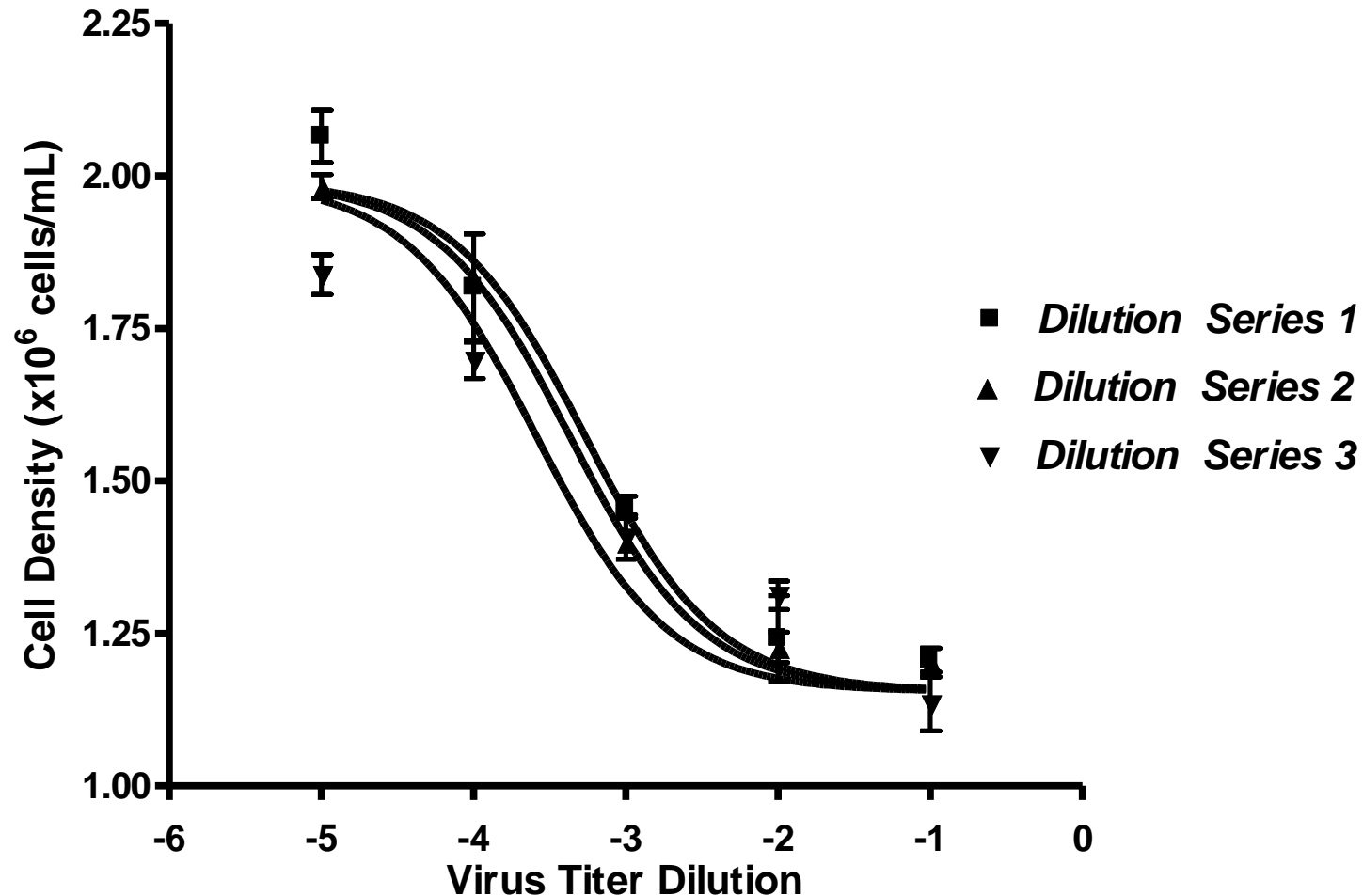
Study 1 – Cell-based Assay (TCID₅₀)

- Assay Setup



Study 1 – Cell-based Assay (TCID₅₀) - cont.

Representative Data (GraphPad PRISM)



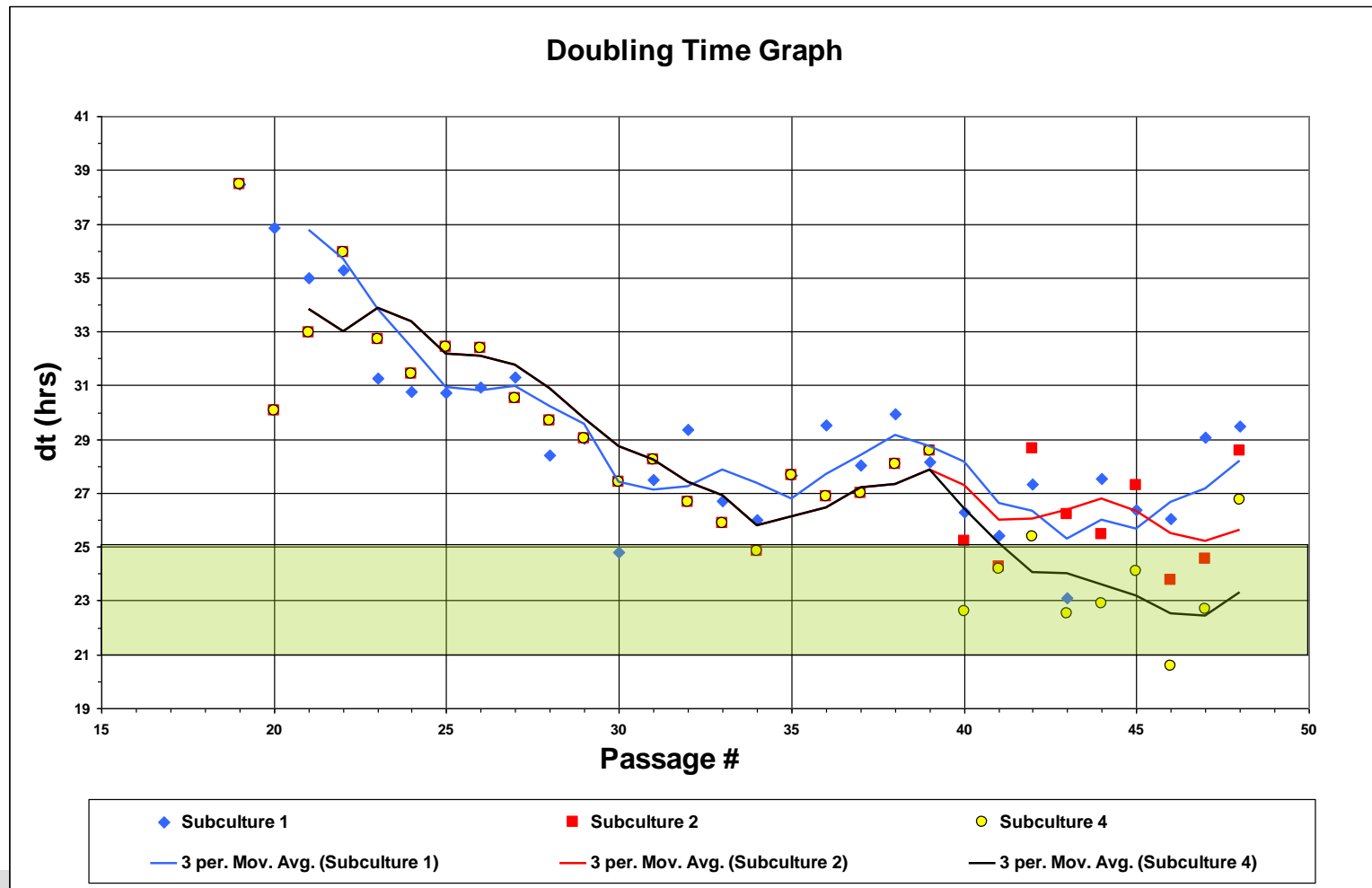
▶ Assay Acceptance / Results

- The acceptance criterion for the curve fit is an r^2 value of ≥ 0.89
- 95% Confidence Interval of $\log(\text{EC}_{50}) \leq 0.5]$
- Range of the titer value is $\geq 2 \times 10^8$ and $\leq 4 \times 10^9$ pfu/mL
- % growth of Positive Control 1 defined per COA
- % growth of Positive Control 2 (EC_{50}) defined per COA

Study 1 – Cell-based Assay (TCID₅₀) - cont.

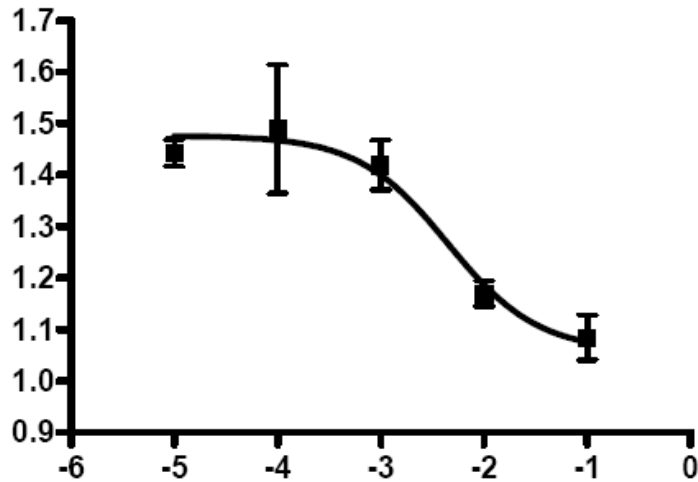
▶ Cell culture data

- Influence of lots of media over cell passage & doubling time

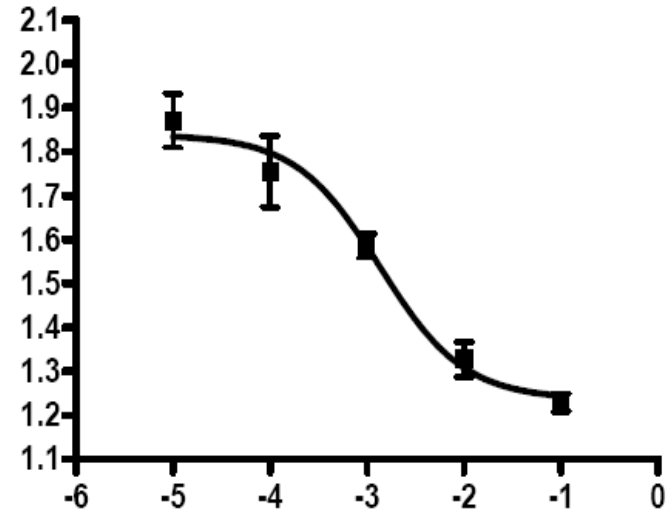


Study 1 – Cell-based Assay (TCID₅₀) - cont.

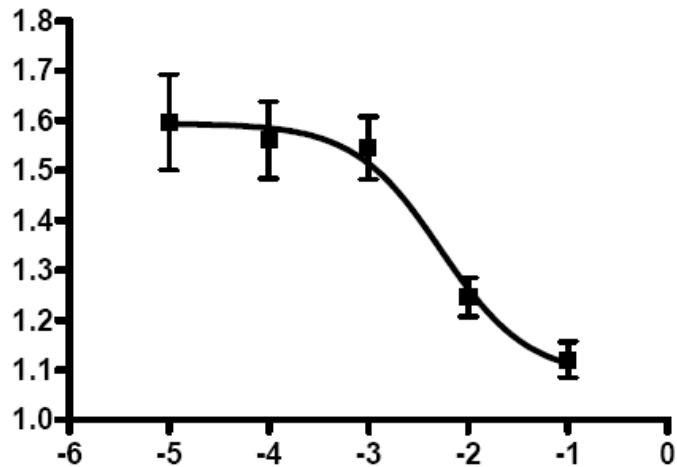
Representative TCID curves



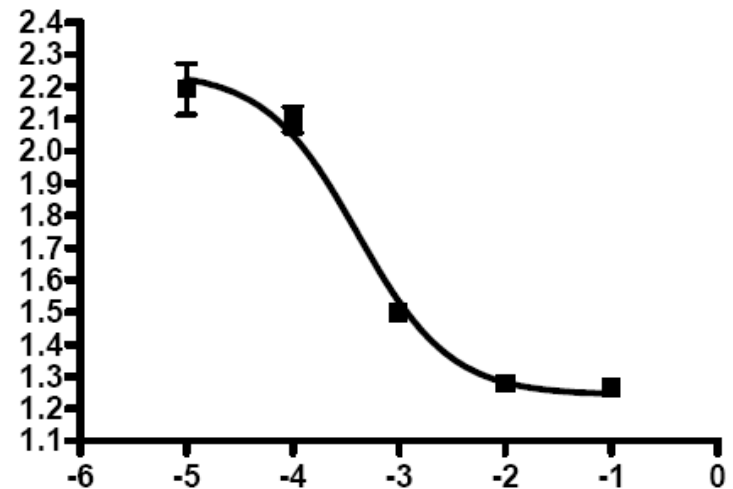
Virus titer: N/A – Invalid Assay



Virus titer: 5×10^8 pfu/mL



Virus titer: 2×10^8 pfu/mL



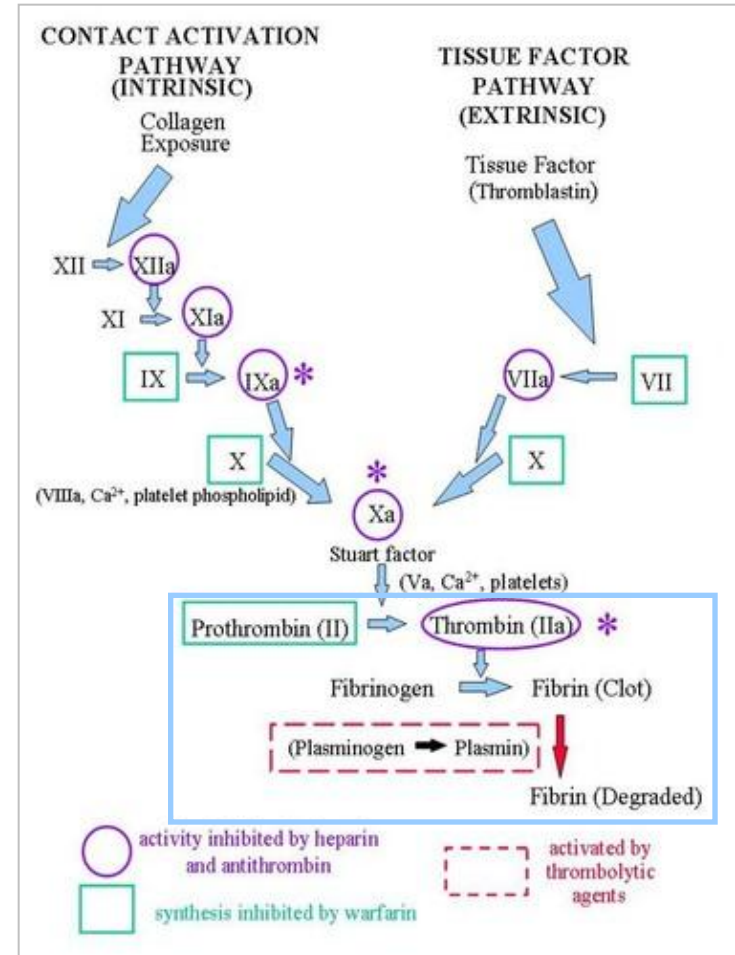
Virus titer: 2×10^9 pfu/mL

▶ Potency/activity case study

Study 2 - Clot Lysis Kinetic Assay

- ▶ Release & Stability indicating assay
- ▶ The principle of the assay is the effect of API material over clot formation via the conversion of fibrinogen to fibrin by thrombin
 - Assay measures the kinetics of lysis of the clot (50% lysis)
 - Evaluates/compares this kinetic to a ctrl sample (ref. material).
 - Result is reported as relative activity to RM (in Units/mL)

coagulation cascade

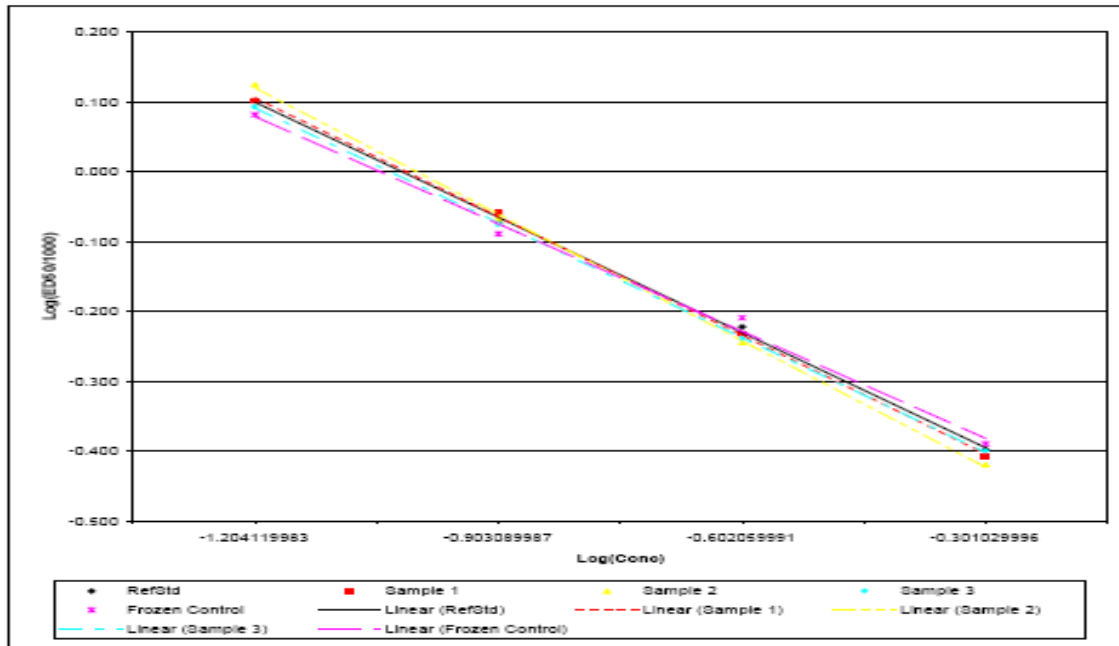
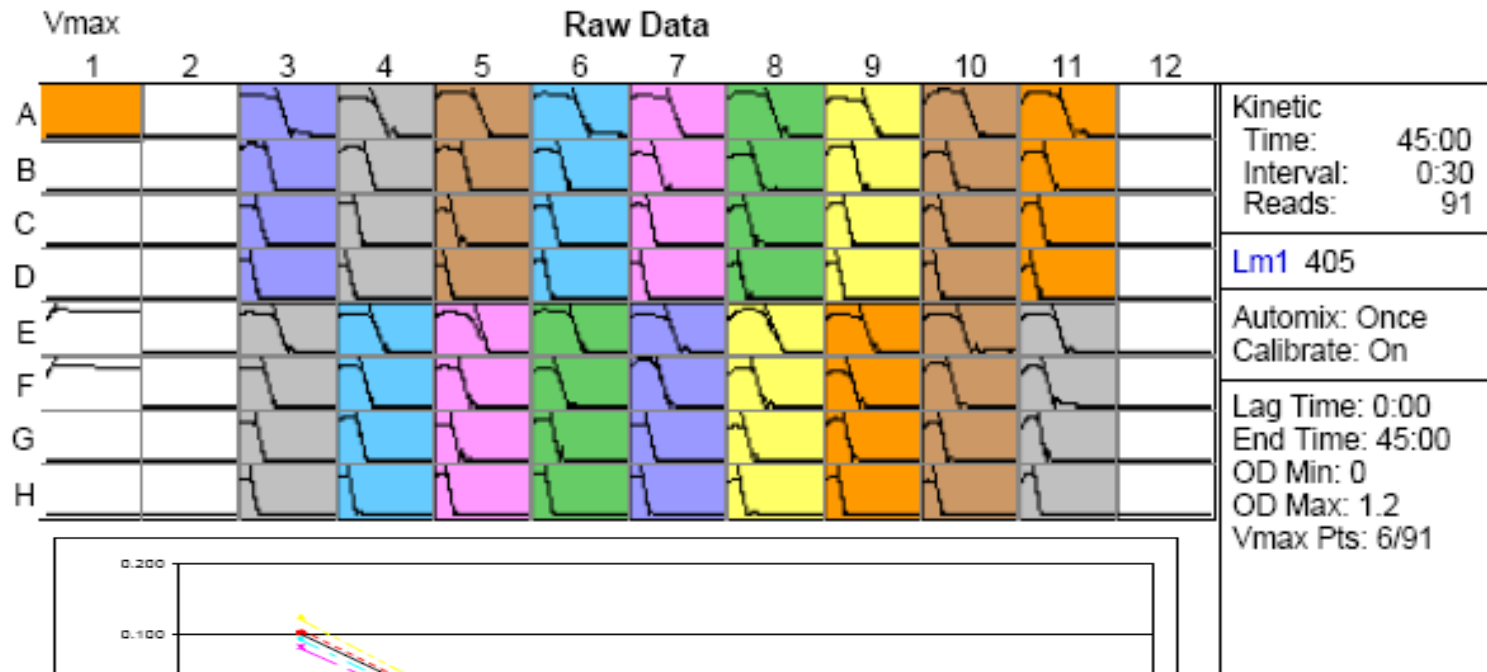


▶ Assay Conditions

- Absorbance readout for 45min kinetic assay (at 30sec intervals)
- Rxn is incubated at 37°C
- p-value for parallelism between sample & ref material must be ≥ 0.01 (CL kinetic slopes of both test sample & RM are not significantly different)
- % CV of replicates $\leq 5\%$

Study 2 - Clot Lysis Kinetic Assay - cont.

Representative data from a CL Kinetic assay



- ▶ ICH Harmonised Tripartite Guideline Q6B: Specifications: test Procedures and Acceptance Criteria for Biotechnological/Biological Products, 10 March, 1999
- ▶ ICH Harmonised Tripartite Guideline Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, 10 November, 2000
- ▶ Code of Federal Regulations Title 21, Part 211 Current Good Manufacturing Practice for Finished Pharmaceuticals [Revised as of April 1, 2011]

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