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## Introduction

- The in vitro EC50 is a key measure of antiviral efficacy.
- Experimental design variations and virus-specific traits can greatly affect EC50 estimates.
- Mechanism-based models help separate the effects of experiments and viral factors on EC50 (Figure 1).

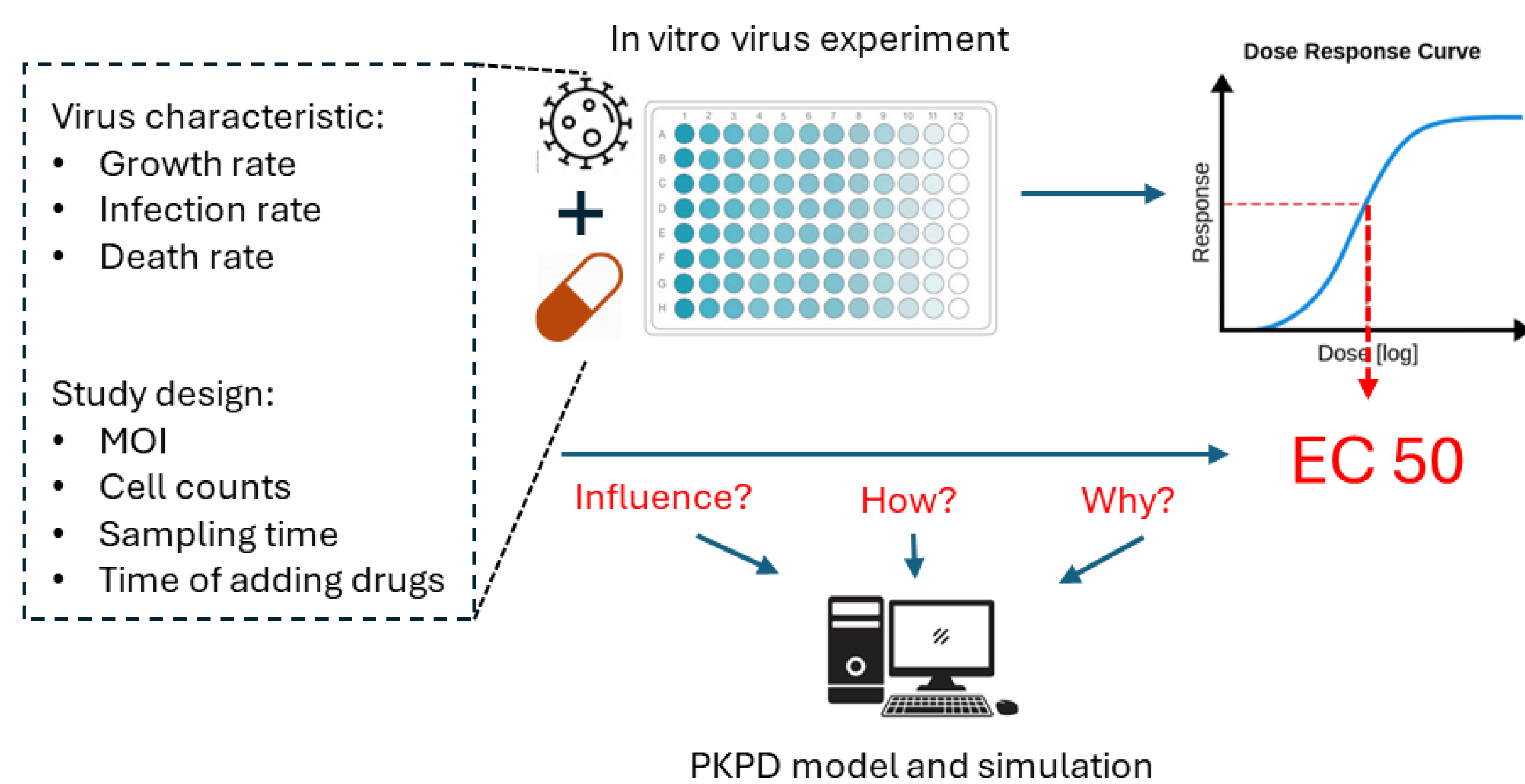


Figure 1. Disentangling Experimental and Biological Influences on EC<sub>50</sub> in Antiviral Drug Evaluation

## Aims

- Aim 1: To determine relationships between empirical EC50 and virus properties including the intrinsic EC50 (in model);
- Aim 2; To explore how experimental designs affect the EC50 calculation in vitro.

## Methods

| Model                                                                                                                                                                              | Experimental design                                                                                                                     | Simulation                                                                                                                   | Calculate EC50                                                                                                               | Application                                                                                                                                            |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>TCL model</li> <li>Cell &amp; virus specific properties: <math>\beta</math>, <math>p</math>, <math>\delta</math>, <math>c</math></li> </ul> | <ul style="list-style-type: none"> <li>MOI</li> <li>Initial cell counts</li> <li>Time of adding drugs</li> <li>Sampling time</li> </ul> | <ul style="list-style-type: none"> <li>Simulate viral load with different doses</li> <li>Plot: Viral load vs Time</li> </ul> | <ul style="list-style-type: none"> <li>Estimate EC<sub>50</sub></li> <li>Plot: Viral load change VS Concentration</li> </ul> | <ul style="list-style-type: none"> <li>Plot: Heatmap of EC50 changes</li> <li>Kendall's tau correlation test</li> <li>Combination influence</li> </ul> |

Figure 2. Framework for Mechanistic Simulation and EC<sub>50</sub> Estimation in Antiviral Evaluation

## Results

- Single factor analysis: infection rate change 10000-fold, EC50 change 30-fold (Figure 3).
- Two factor analysis: infection rate & viral production rate are both positively correlated with EC50 (Figure 4).
- Kendall's Tau coefficient analysis: infection rate & viral production rate have positive influence on EC50. Infected cell clearance rate have negative influence on EC50 (Figure 5).

## Results

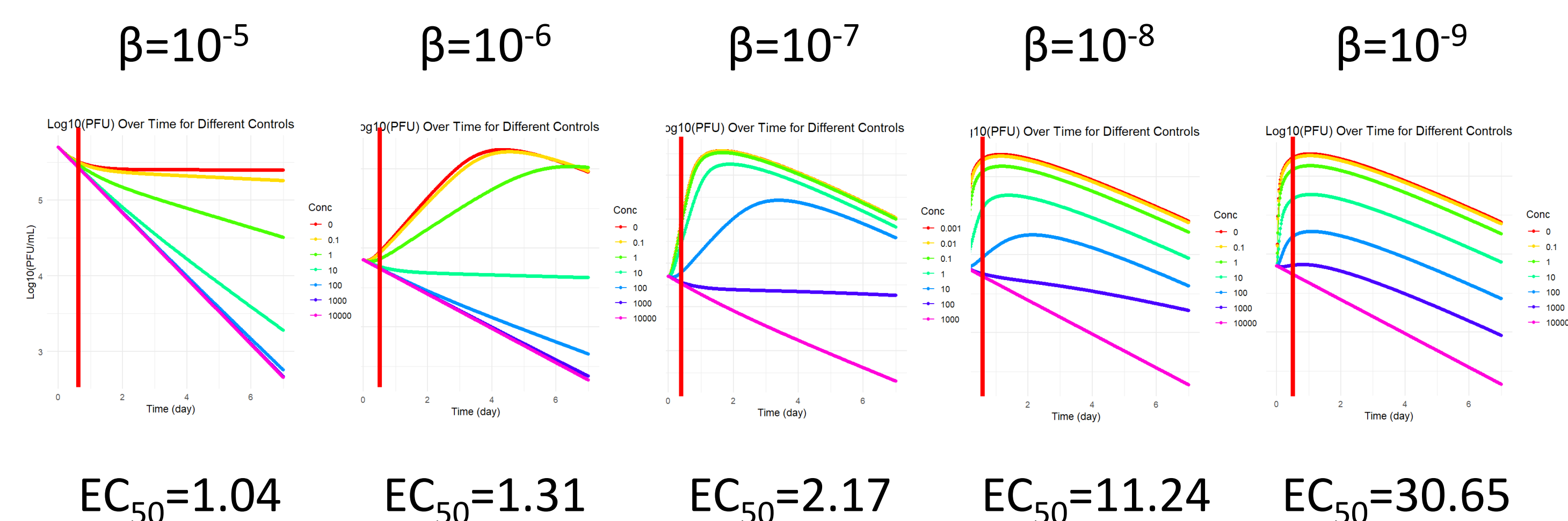


Figure 3. Single factor analysis, estimated EC50 results by different infection rate ( $\beta$ ).

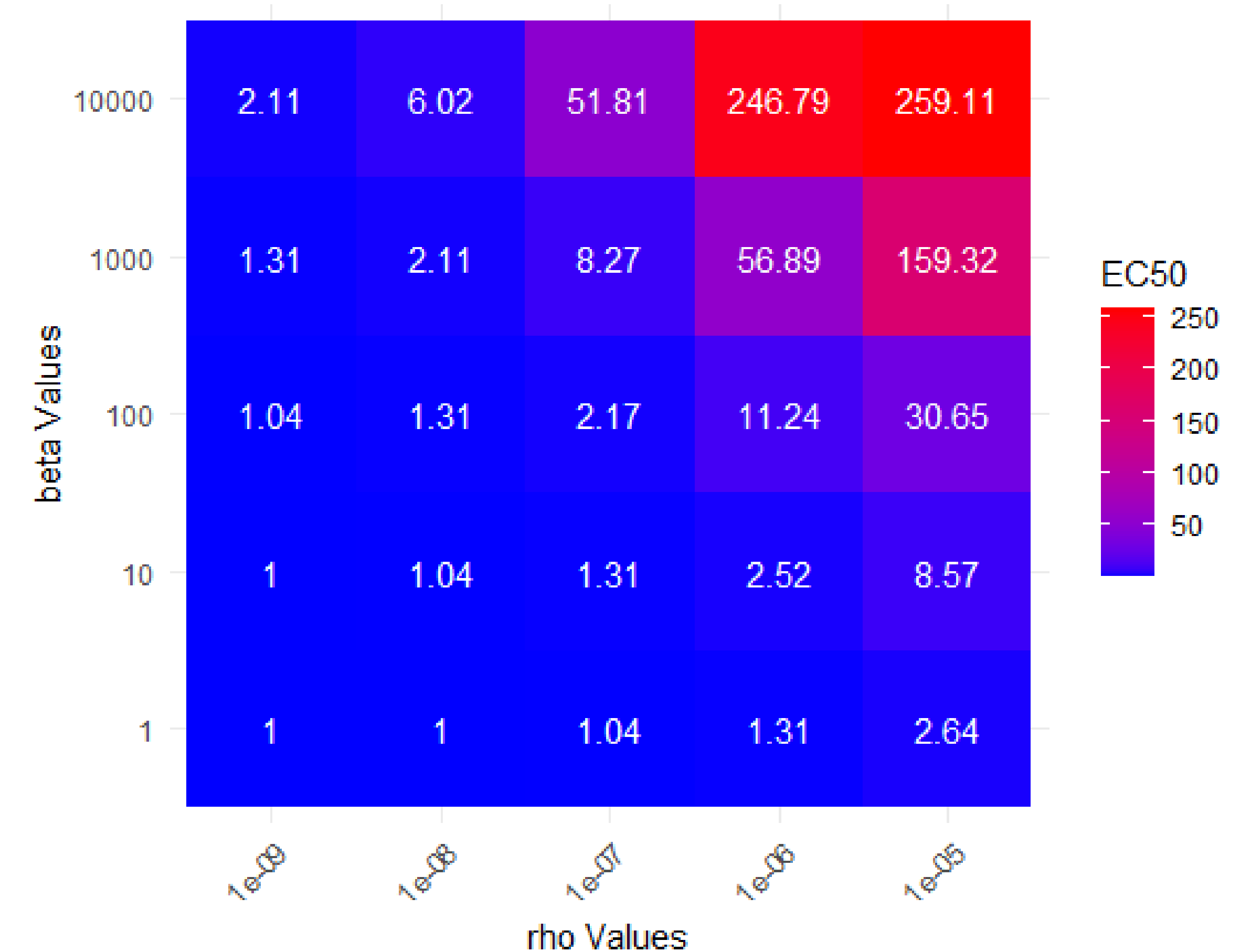


Figure 4. Heatmap of EC50 simulated by different infection rate ( $\beta$ ) & viral production rate ( $\rho$ ).

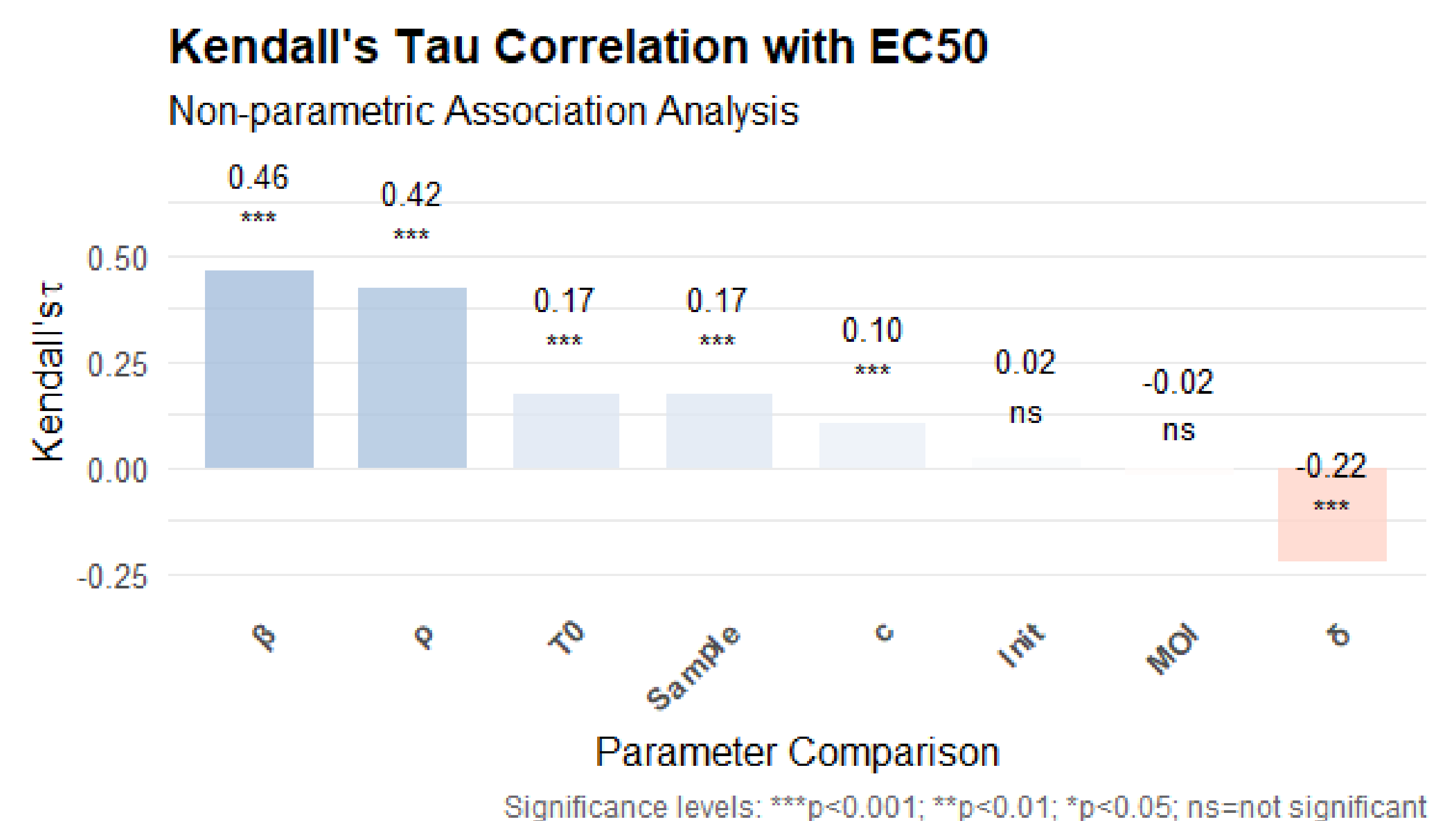


Figure 5. Kendall's Tau coefficient analysis of EC50.  $\beta$ , Infection rate;  $p$ , viral production rate;  $\delta$ , Infected cell clearance rate;  $c$ , Viral clearance rate;  $T_0$ , initial cell counts; MOI, multiplicity of infection; Sample, time of sampling; Init, time of adding drugs.

## Conclusions

- Key factors influencing EC50 estimation are Infection rate and viral production rate.
- These results help optimize experimental conditions and improve the accuracy of pharmacodynamic assessments.