The assessment of novel therapeutics mode of action via cell bioassays and custom cell lines

Alistair Michel, Senior Scientist, Reading Scientific Services Ltd
Matthew Bowdich, Scientist, Reading Scientific Services Ltd

Abstract

Certain therapeutics require mode of action bioassay QC methods to ensure that they meet release specifications before being used in the clinic. This poster describes the preliminary early phase development and qualification of a bioassay executed in a GMP regulated laboratory.

In this case study, a bioassay was developed as a proof of concept for a client using a HPV cancer vaccine based on their proprietary technology.

Step 1: Characterisation of a therapeutic.

Typically, RSSL would recommend and can assist in the full characterisation of the therapeutic before establishing a mode of action bioassay method. However, in certain instances, the bioassay may need to be developed prior to a fully characterised therapeutic being available. In this instance, RSSL would recommend that an early phase qualification of the method rather than a full phase appropriate validation be performed.

Step 2: Identification of a suitable effector cell line.

In this example, it was known that the main T cell epitope for HPV was the $E7_{11\text{-}19}$ epitope, and that this T cell epitope was associated in publications with the HLA A02:01 allele. Therefore, using RSSL's trusted partners, a custom T cell containing the $E7_{11\text{-}19}$ TCR and an IL2-luciferase fusion reporter gene was sourced. Engineering of cell lines can take up to 6 months and when using custom cell lines, it is important to factor in this time frame into project plans.

Step 3: Identification of a suitable target cell line.

For the target cell, this method required a dendritic-like cell line known to express the HLA A02:01. Once sourced, different methods of differentiation were used to determine which method would provide optimal preparation of the target cell line.

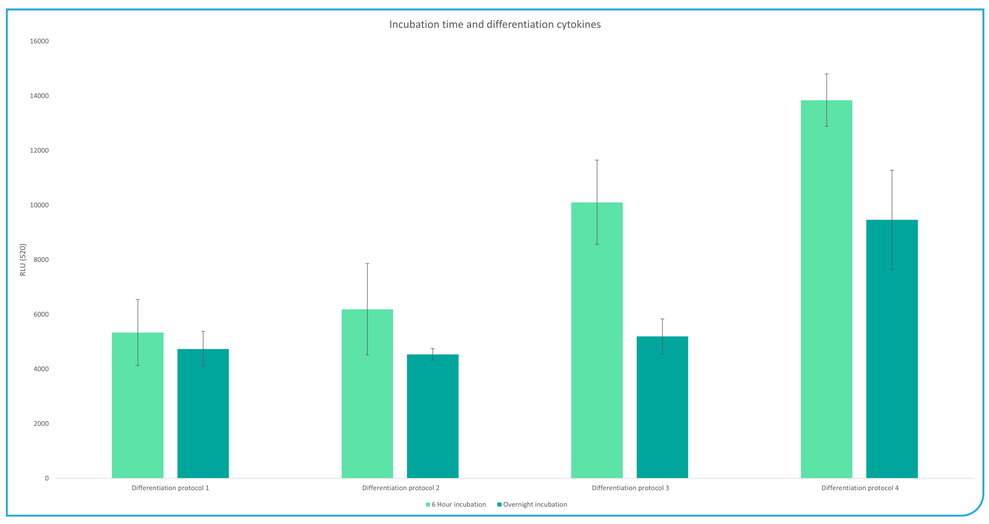

Figure 1: Showing the effect of 4 target cell differentiation protocols on the activation of T cells after 6 hour or overnight incubation with target cell and epitope.

Step 4: Final method design.

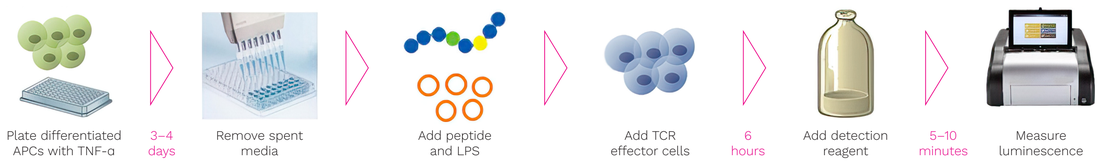

Figure 2: Method schematic.

Step 5: Phase appropriate qualification/validation of method.

Analytical Method Qualification	Analytical Method Validation
Normally done at early phases (e.g. preclinical or before Phase I)	Mandatory for later stages of development
Can also be done before a validation to give an indication of method performance	You don't need to qualify a method before validating
Demonstrates if the design is working or needs more optimisation	

Due to the absence of a final characterised therapeutic, in this example the specificity, linearity and precision (Figures 3–6) of the method was qualified to ensure a reliable bioassay had been produced. Additional validation would need to be required with the final characterised therapeutic before GMP release testing could be performed.

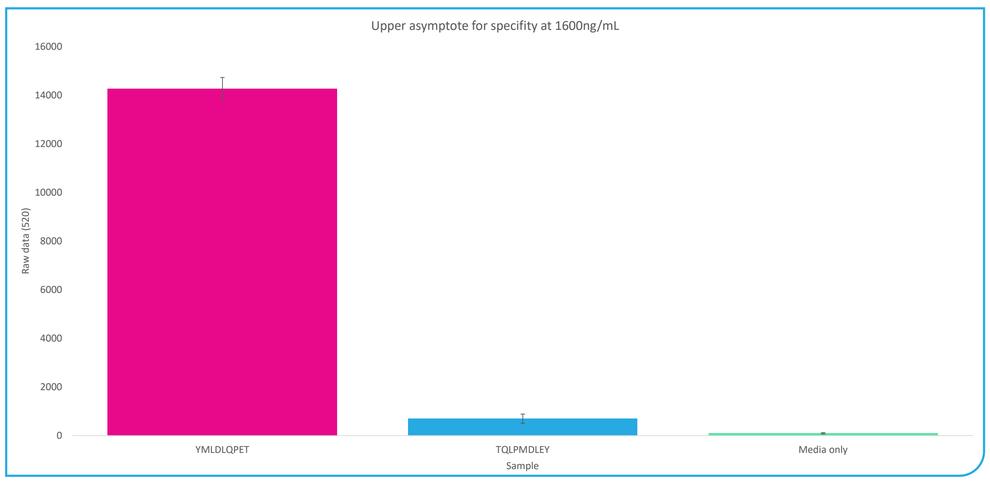

Figure 3: Specificity graph, shows response of cells to positive and negative 9aa peptides at 1600ng/mL (upper asymptote) using the average of the raw data from Assay 6 plate 1 and 2. The error bars show the standard deviation of the same data set.

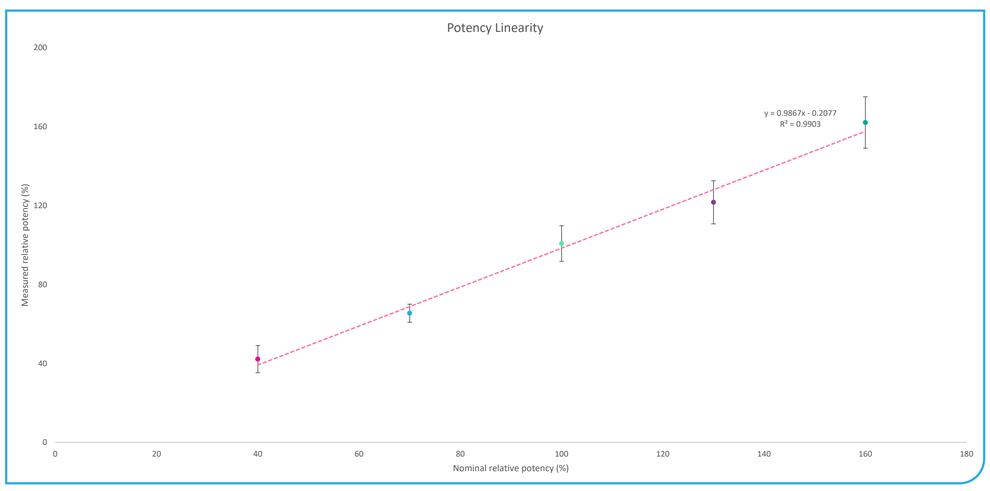

Figure 4: Linearity graph, shows the calculated relative potency from 6 data points (across 3 assays where the nominal value is measured). The error bars are the standard deviations from the same 6 data points.

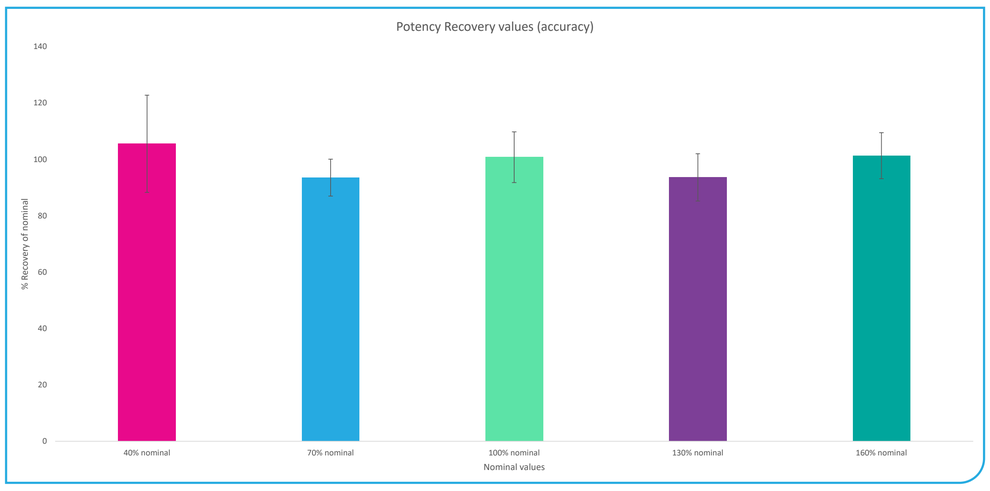

Figure 5: Accuracy graph, shows the recovery using the average of the measured potency from the 6 data points. The error bars show the standard deviation obtained from the recovery for each data point.

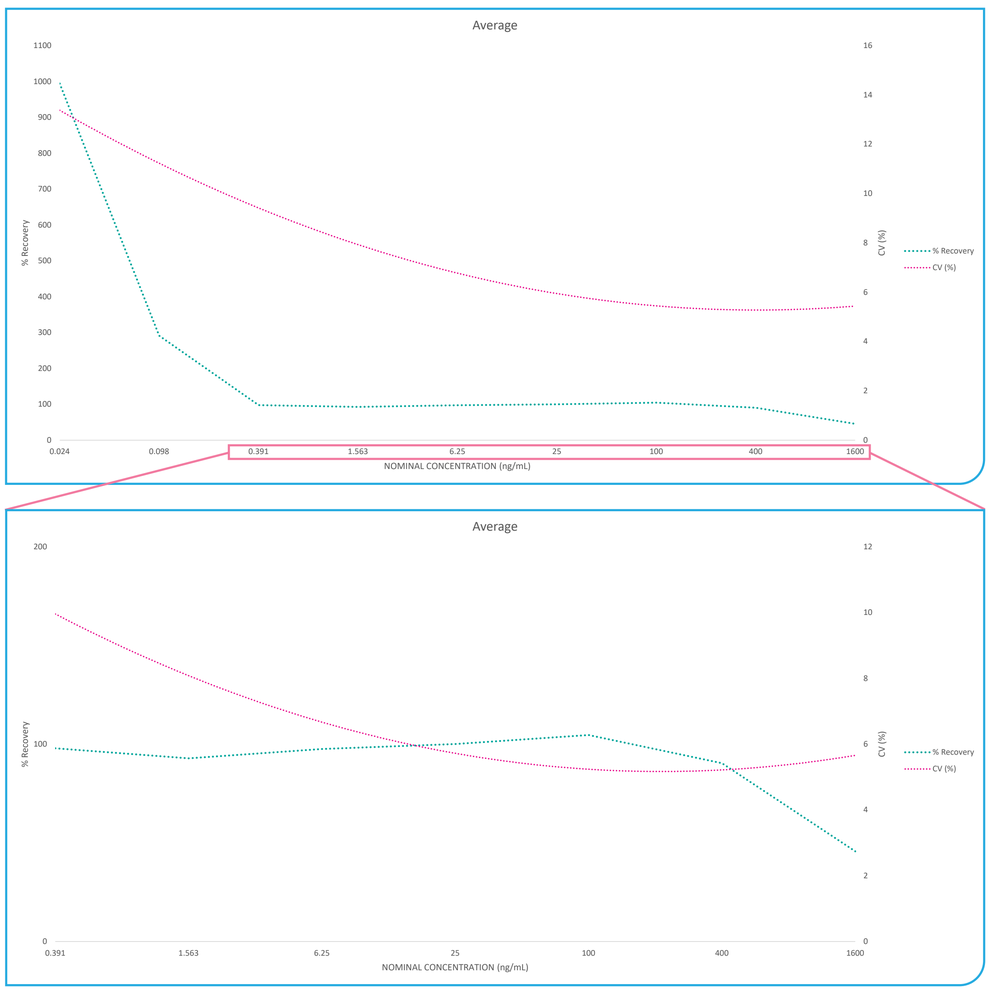

Figure 6: Precision Profile/Standard curve recovery, showing the % recovery (left axis) and %CV (right axis). Upper graph shows all data points; lower graph shows points from 0.391 to 1600 ng/ml.

Reading Scientific Services Ltd
The Reading Science Centre, Whiteknights Campus, Pepper Lane, Reading, Berkshire RG6 6LA • Tel: +44 (0)118 918 4000 • Email: lifesciencesales@rssl.com • www.rssl.com