Utilisation of custom cell lines to determine mode of action of an HPV cancer vaccine

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Abstract

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

A bioassay was developed as a proof of concept for a client using a HPV cancer vaccine based on their proprietary technology.

Step 1: Therapeutic characterisation.

RSSL would recommend and can assist in the full characterisation of therapeutics before establishing mode of action bioassay method. However, in certain instances, a bioassay may need to be developed prior to a fully characterised therapeutic being available. In this instance, RSSL would advise early phase method qualification rather than full phase appropriate validation. The latter approach applied for this case study.

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; this T cell epitope was associated in publications with the HLA A02:01 allele. Therefore, using RSSL's trusted partners, a custom T cell expressing the $E7_{11\text{-}19}$ TCR and containing 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).

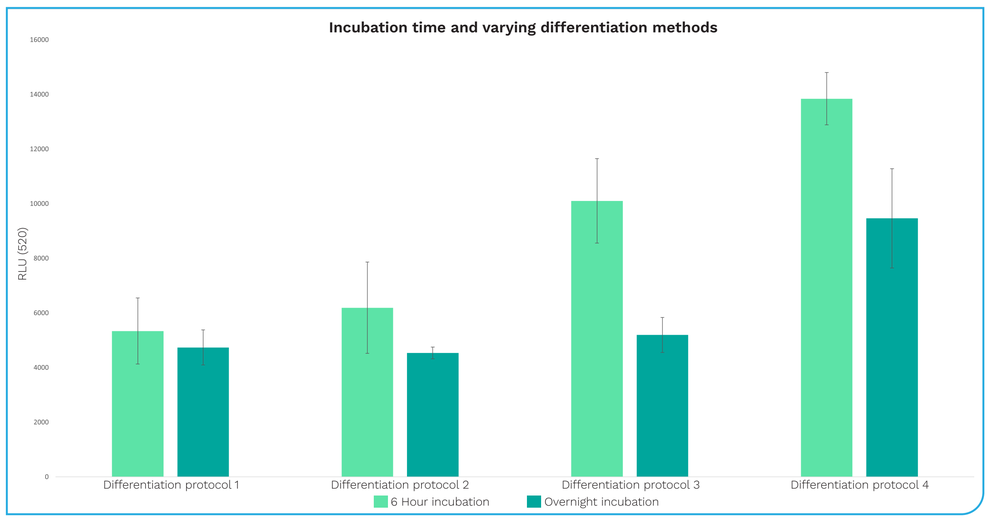

Figure 1: Showing the effect of 4 target cell differentiation protocols on the activation of T cells after 6 hour or overnight incubation with target cell and epitope.

Step 4: Final method design.

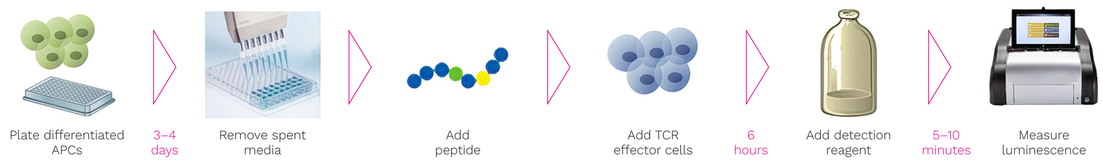

Figure 2: Method schematic.

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)	Performed in accordance with ICH Q2 guidelines
Can also be done before a validation to give an indication of method performance	Mandatory for later stages of development
Demonstrates if the design is working or needs more optimisation	Method does not need to be qualified prior to validation

Due to the absence of a final characterised therapeutic, in this example the specificity, linearity and precision (Figures 3–6) of the method were qualified to ensure bioassay reliability. Validation is required using the final characterised therapeutic before GMP release testing could be performed.

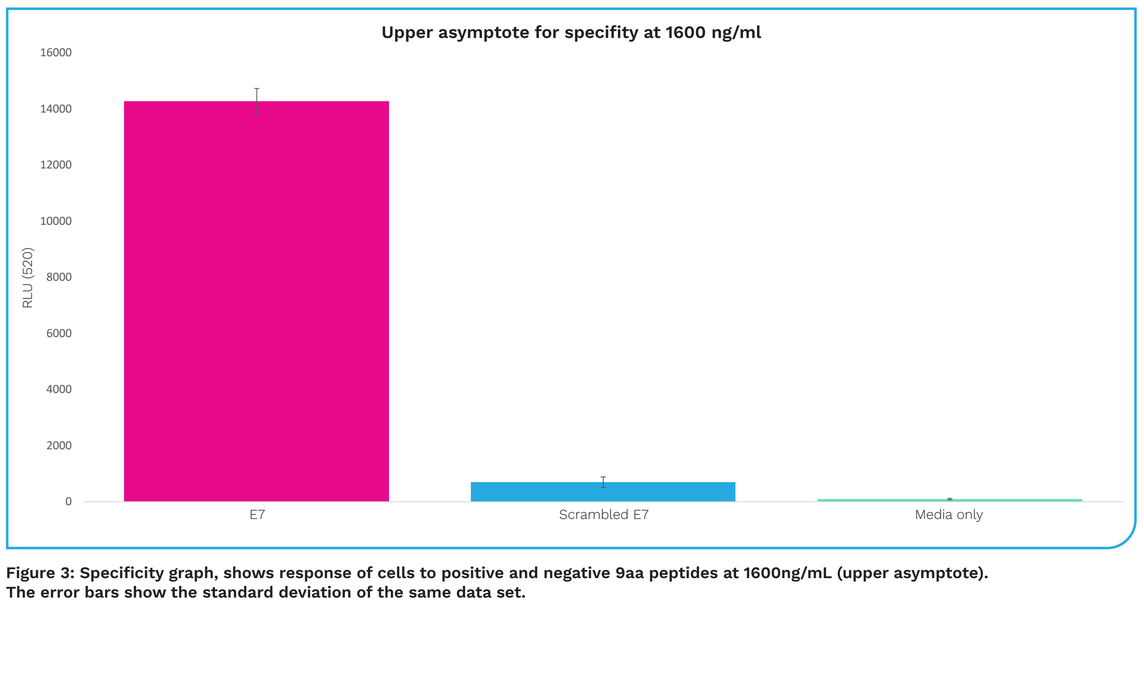

Figure 3: Specificity graph, shows response of cells to positive and negative 9aa peptides at 1600ng/mL (upper asymptote). The error bars show the standard deviation of the same data set.

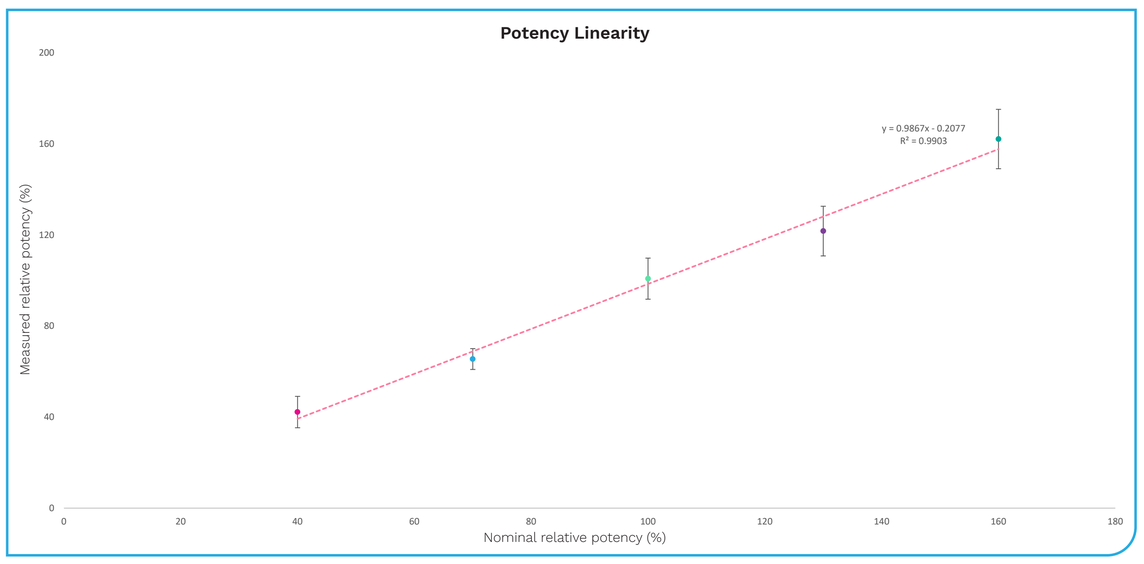

Figure 4: Linearity graph, shows the calculated relative potency from 6 data points. The error bars are the standard deviations from the same 6 data points.

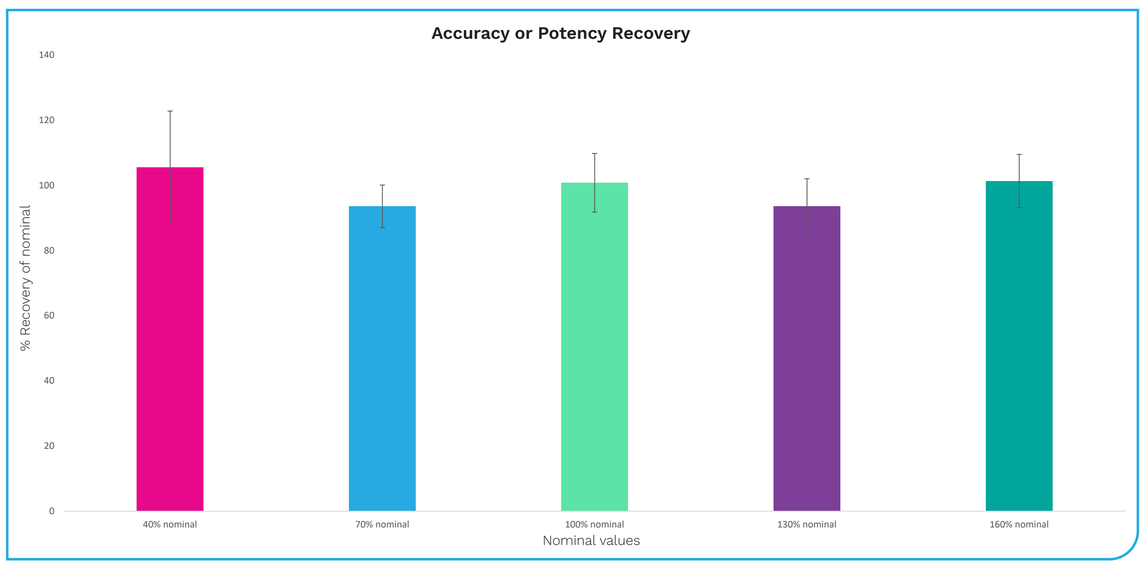

Figure 5: Accuracy graph, shows the recovery using the average of the measured potency from the 6 data points. The error bars show the standard deviation obtained from the recovery for each data point.

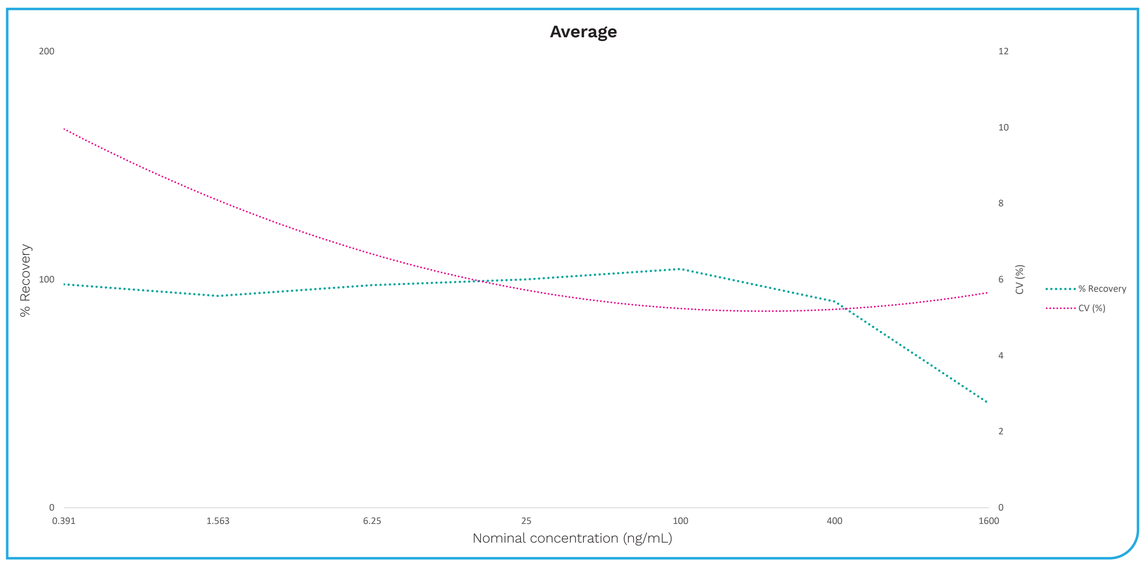

Figure 6: Precision Profile/Standard curve recovery, showing the % recovery (left axis) and % CV (right axis).

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