Preliminary Assessment of Defined Serum-Free Medium in the h-CLAT Method

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INTRODUCTION

The historic use of animal derived products such as Foetal Calf Serum (FCS) in human cell culture raises both scientific and ethical concerns, leading to a growing demand for the elimination of such reagents from laboratories. Use of animal-product-free medium with a defined composition and reduced inter-lot variability is attractive for both cell culture and routine assays and also addresses ethical concerns around the use of human serum in some OECD member countries. Based upon the success of our previously published work to adapt the human cell line activation test (h-CLAT) method to animal-product-free conditions containing human serum, this assay was selected for adaptation to serum-free conditions. This regulatory test measures the expression of CD54 and CD86 surface markers on THP-1 cells in order to model dendritic cell activation, a key step in the skin sensitisation adverse outcome pathway. In order to completely remove serum from the h-CLAT assay conditions, THP-1 cells were adapted for culture in a chemically defined medium (Lonza X-VIVO 15, used to support monocyte growth), before using these cells to test three reference chemicals; DNCB, Lactic Acid and Nickel Sulphate, for both the CV75 (cytotoxicity) and the CD54/CD86 expression assay.

MATERIALS & METHODS

THP-1 cells adapted to animal product-free conditions (RPMI, 0.05mM 2-mercaptoethanol, 10% Human Serum) (Edwards et al., 2018) were first confirmed to be reactive with the h-CLAT reactivity check using three test items (DNCB, Lactic Acid, Nickel Sulphate). THP-1 cells were then transferred into chemically defined Lonza X-VIVO 15 medium (containing 0.05mM 2-mercaptoethanol) either by direct transfer or weaning and seeded at 2x10^5/ml. Weaned cells had the amount of RPMI reduced by 25% with each passage until they were in 100% X-VIVO medium. Cells in X-VIVO and RPMI were counted every 24hrs (+/- 1hr) for 96 hours using a trypan blue exclusion method. THP-1 cells from weaning and direct transfer were then used in both CV75 range finding and h-CLAT reactivity check assays

RESULTS

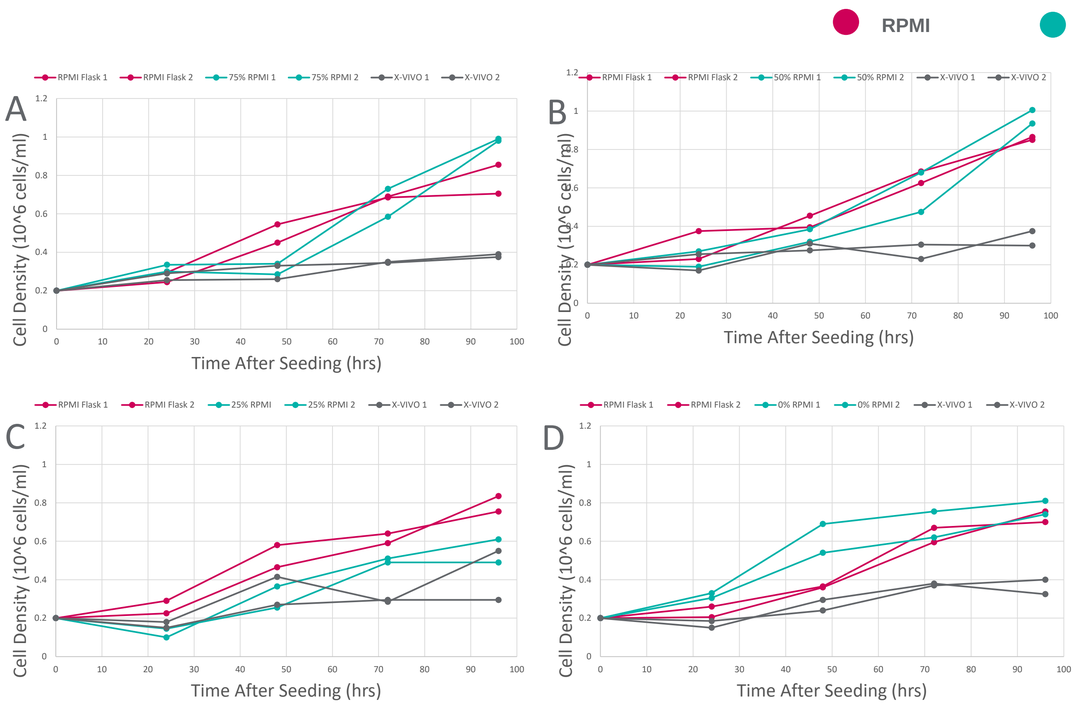

Figure 1: Cell density (viable cells/ml) of THP-1 cells with different culture conditions, recorded every 24 hours

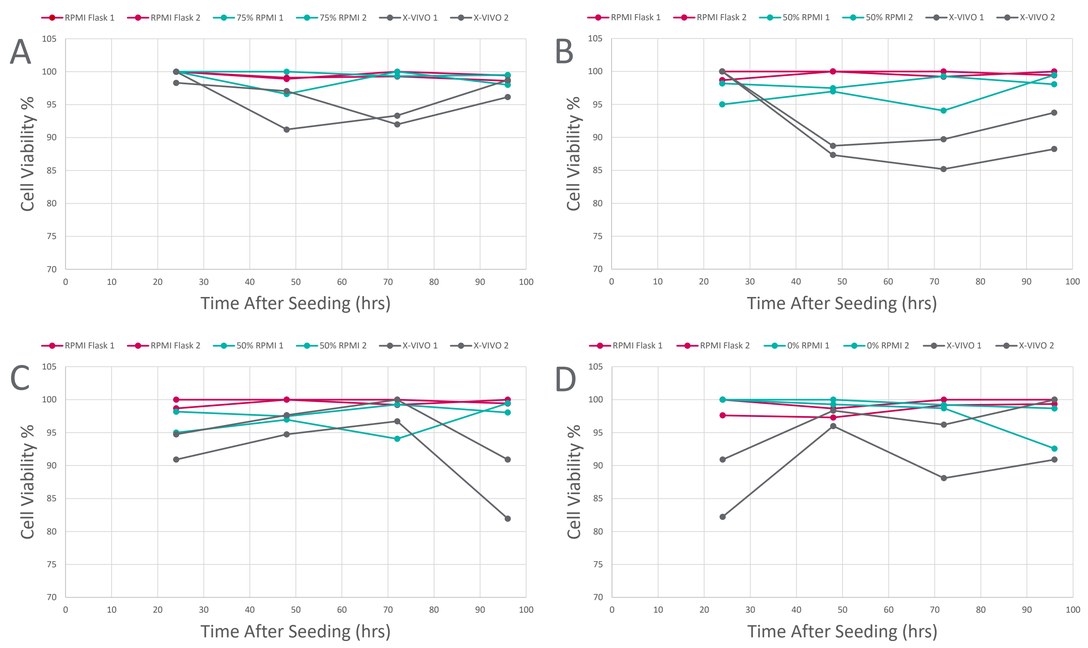

Figure 2: Cell viability (% viable) of THP-1 cells with different culture conditions, recorded every 24 hours

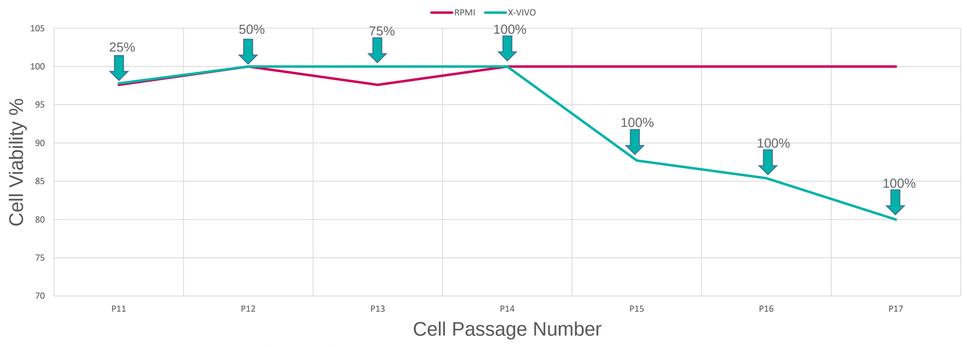

Figure 3: Cell density (viable cells/ml) of THP-1 cells weaned to X-VIVO medium vs RPMI by passage number. Arrows on the graph indicate the percentage of X-VIVO medium used in the cell culture.

Table 1: Summary of CV75 range finding results using weaned 100% X-VIVO 15 THP-1 cells for three reference chemicals. TA1: DNCB, TA2: Lactic Acid, TA3: Nickel Sulphate. *N.B. Results marked * could not have an average CV75 calculated due to low viability.*

	TA1		TA2		TA3	
Viability ≥ 75% at the lowest dose?	74.34	76.89	80.57	74.06	78.62	75.26
Highest dose < 90% viability?	5.67	7.82	14.52	10.65	18.91	20.51
CV75	0.41*		2614.96*		37.52	

Table 2: Summary of h-CLAT reactivity check results using weaned 100% X-VIVO 15 THP-1 cells for three reference chemicals. TA1: DNCB, TA2: Lactic Acid, TA3: Nickel Sulphate.

	Rep 1			Rep 2		
	RFI CD54	RFI CD86	% Viability	RFI CD54	RFI CD86	% Viability
Medium Control	100	100	92	100	100	79
			92			79
			91			77
DMSO Control	71	79	91	117	117	78
			91			77
			90			77
DNCB Positive Control	222	241	54	91	104	74
			56			75
			57			75
Lactic Acid Negative Control	79	83	88	60	68	76
			89			77
			89			76
Nickel Sulphate Positive Control	24	157	38	83	206	60
			38			59
			38			60

DISCUSSION

Initial results for weaning THP-1 cells from RPMI into X-VIVO media appeared promising, with cell density and viabilities comparable to that of standard cells. When transferred directly into X-VIVO medium, reduced cell density and viability was observed (Fig. 1 & 2). Subsequent studies keeping weaned THP-1 cells in 100% X-VIVO medium began to demonstrate reduced viability (Fig. 3), similar to that seen in cells directly transferred into the defined medium. The reduced viability of weaned X-VIVO cells was further demonstrated in both the CV75 and h-CLAT assays (Tables 1 & 2), where cells frequently exhibited low viability. Although the CV75 ranges were met for two out of three reference chemicals, the average CV75 could only be calculated for Nickel Sulphate (TA3), due to low viability. The weaned X-VIVO cells were unable to meet the criteria for passing the h-CLAT reactivity check, suggesting changes to the cell surface markers in addition to reduced viability have occurred. Further work is planned to use new medium optimised for THP-1 growth, LGM-3 (Lonza).

REFERENCES

Edwards *et al.* (2018). Adaption of the human Cell Line Activation Test (h-CLAT) to Animal-Product-Free Conditions. ALTEX. doi:10.14573/altex.1710051.