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Development of a qPCR assay for HHV-7 monitoring

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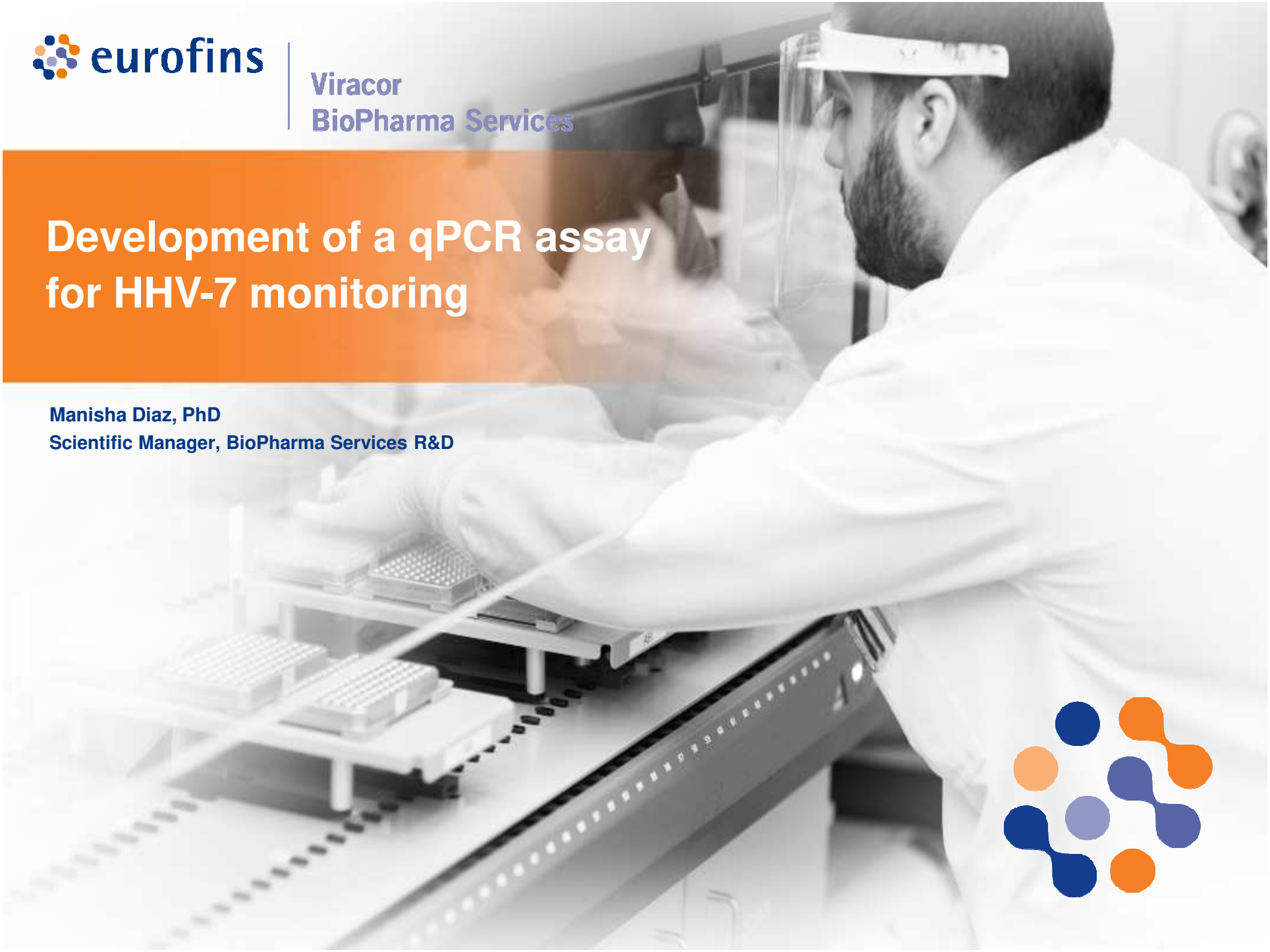

PEGS Boston Virtual: Poster Abstract

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Development of a qPCR assay for HHV-7 monitoring

- **Background**

  Lymphotropic Human Herpesviruses such as HHV-7 infect the majority of the humans (>90% seroprevalence) in the first few years of life resulting in lifelong latency in mononuclear cells. Thus, simple detection of the viral genome by polymerase chain reaction (PCR) is not sufficient to differentiate a latent from active infection. Development of a highly sensitive quantitative polymerase chain reaction (qPCR) assay for monitoring of HHV-7 can therefore serve as a powerful tool in patient management. The objective of this study is to identify the optimal matrix for determination of HHV-7 viral loads.

- **Experimental design**

  Whole blood from 20 donors was collected. In addition, PBMC's from 5 mL of whole blood were isolated using Ficoll overlay then resuspended to a concentration of 6.25 million cells per mL. Nucleic acid was extracted using Qiagen extraction reagents and the nucleic acid concentration was measured using spectrophotometry. HHV-7 viral loads were quantified using Viracor's HHV-7 qPCR assay.

- **Results**

  HHV-7 was detected in 65% (13/20) of whole blood specimens and 70% (14/20) of PBMCs. Mean of HHV-7 viral loads were observed to be higher in PBMCs (2151 copies/mL and 154 copies/µg) when compared to whole blood (1101 copies/mL and 60 copies/µg). Statistical differences were determined using a two-tailed paired test (for copies/mL, $P = 0.0051$ and for copies/µg, $P = 0.0025$).

- **Conclusion**

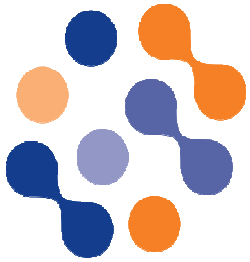
  These results demonstrate the use of PBMCs as a sensitive and suitable marker for monitoring HHV-7.

- **Authors:**

  Manisha Diaz, Mark Wissel, Crystal Marti, Courtney Stoker, Stephanie Schildknecht, Xiaoling Shen, Melissa Sippy

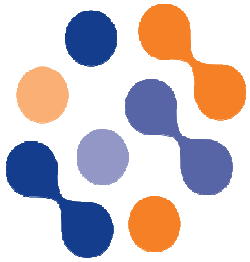
Background

- Lymphotropic Human Herpesviruses such as HHV-7 infect the majority of the humans (>90% seroprevalence) in the first few years of life resulting in lifelong latency in mononuclear cells.
- Simple detection of the viral genome by polymerase chain reaction (PCR) is not sufficient to differentiate a latent from active infection.
- Development of a highly sensitive quantitative polymerase chain reaction (qPCR) assay for monitoring of HHV-7 can therefore serve as a powerful tool in patient management.
- The objective of this study was to identify the optimal matrix for determination of HHV-7 viral loads.



Experimental Design

- Whole blood from 20 donors was collected.
- From the same donors, PBMC's from 5 mL of whole blood were isolated using Ficoll overlay and then re-suspended to a concentration of 6.25 million cells per mL.
- Nucleic acid was extracted using Qiagen extraction reagents and the nucleic acid concentration was measured using spectrophotometry. HHV-7 viral loads were quantified using Viracor's HHV-7 qPCR assay. Eluates were plated in single wells.



Results

- HHV-7 was detected in 65% (13/20) of whole blood specimens and 70% (14/20) of PBMCs.
- Mean of HHV-7 viral loads were observed to be higher in PBMCs (96 copies/mL and 7 copies/µg), when compared to whole blood (55 copies/mL and 3 copies/µg).
- Statistical differences were determined using a two-tailed paired test (for copies/µg, $p = 0.0029$).

Results:

Screening of Whole Blood From 20 healthy donors

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Whole Blood								
Well	Sample ID	Fam none Ct	vic-tam Ct	Conc (ng/uL)	Copies/mL	ng/rxn	Copies/ug	No. of cells/rxn
A2	Whole Blood donor# 234	36.7391	30.3758	71.26	42	712.56	2	100079
A3	Whole Blood donor# 81	Undetermined	31.0199	94.61	Not Detected	946.06	Not Detected	132874
A4	Whole Blood donor# 518	36.0116	32.4372	101.96	69	1019.56	3	143197
B2	Whole Blood donor# 150	35.2236	30.1861	54.66	117	546.56	9	76764
B3	Whole Blood donor# 524	35.2396	32.4649	64.11	116	641.06	7	90037
B4	Whole Blood donor# 500	Undetermined	31.1324	104.11	Not Detected	1041.06	Not Detected	146216
C1	Whole Blood donor# 399	39.1032	30.5720	84.76	8	847.56	0	119039
C2	Whole Blood donor# 169	Undetermined	30.7875	62.31	Not Detected	623.06	Not Detected	87508
C3	Whole Blood donor# 496	Undetermined	31.2185	58.41	Not Detected	584.06	Not Detected	82031
C4	Whole Blood donor# 491	Undetermined	31.1020	65.11	Not Detected	651.06	Not Detected	91441
D1	Whole Blood donor# 105	38.6763	30.3469	59.11	11	591.06	1	83014
D2	Whole Blood donor# 534	36.0953	30.8181	72.16	65	721.56	4	101343
D3	Whole Blood donor# 537	35.4563	31.6464	95.86	100	958.56	4	134629
D4	Whole Blood donor# 523	Undetermined	31.5942	87.76	Not Detected	877.56	Not Detected	123253
E1	Whole Blood donor# 424	38.7988	30.2983	68.26	10	682.56	1	95865
E2	Whole Blood donor# 536	38.6291	31.0766	104.71	12	1047.06	0	147059
E3	Whole Blood donor# 538	38.1544	31.2170	71.86	16	718.56	1	100921
F1	Whole Blood donor# 521	Undetermined	30.7108	73.71	Not Detected	877.56	Not Detected	123253
F2	Whole Blood donor# 533	35.2068	30.6095	86.21	119	862.06	6	121076
F3	Whole Blood donor# 367	37.2459	31.3611	66.31	30	663.06	2	93126

Calculations:

Copies/mL = 10^((Ct-Intercept)/Slope))*1.25

Copies/µg = (Copies/reaction)/(conc of DNA ng/µL*0.01))

No. of cells/reaction = (ng/reaction*1000)/7.12

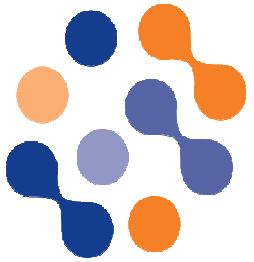
Where,

Ct = Cycle Threshold value

Slope and Intercept = -3.3879 and 41.9076 (stored standard curve generated at Viracor)

ng/µL = concentration of DNA

ng/reaction = concentration of DNA in a PCR reaction



Results:

Screening of PBMCs from 20 healthy donors

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PBMCs								
Well	Sample ID	Fam none Ct	vic-tam Ct	Conc (ng/uL)	Copies/mL	ng/rxn	Copies/ug	No. of cells/rxn
A7	PBMC donor# 234	36.8747	31.5187	54.16	38	541.56	3	76062
A8	PBMC donor# 81	Undetermined	32.0557	78.76	Not Detected	787.56	Not Detected	110612
A9	PBMC donor# 518	35.5178	31.4419	61.96	96	619.56	6	87017
B7	PBMC donor# 150	34.2192	31.9361	58.26	232	582.56	16	81820
B8	PBMC donor# 524	34.3970	31.6585	77.86	206	778.56	11	109348
B9	PBMC donor# 500	Undetermined	31.4209	46.31	Not Detected	463.06	Not Detected	65037
C6	PBMC donor# 399	37.7289	31.6932	62.91	21	629.06	1	88351
C7	PBMC donor# 169	Undetermined	31.5808	54.11	Not Detected	541.06	Not Detected	75992
C8	PBMC donor# 496	Undetermined	31.4964	63.76	Not Detected	637.56	Not Detected	89545
C9	PBMC donor# 491	37.5322	31.3741	55.21	24	552.06	2	77537
D6	PBMC donor# 105	Undetermined	31.6748	93.21	Not Detected	932.06	Not Detected	130907
D7	PBMC donor# 534	35.7230	31.4105	62.51	84	625.06	5	87789
D8	PBMC donor# 537	36.0552	31.5442	50.26	67	502.56	5	70584
D9	PBMC donor# 523	Undetermined	31.0103	51.36	Not Detected	513.56	Not Detected	72129
E6	PBMC donor# 424	35.7599	31.8462	82.41	82	824.06	4	115739
E7	PBMC donor# 536	37.0177	31.2884	46.16	35	461.56	3	64826
E8	PBMC donor# 538	35.4490	31.3618	36.16	101	361.56	11	50781
F6	PBMC donor# 521	37.8218	31.5098	61.91	20	619.06	1	86947
F7	PBMC donor# 533	34.1286	31.6487	52.21	247	522.06	19	73323
F8	PBMC donor# 367	35.5102	31.3760	45.46	97	454.56	9	63843

Calculations:

Copies/mL = 10^((Ct-Intercept)/Slope))*1.25

Copies/µg = (Copies/reaction)/(conc of DNA ng/µL*0.01))

No. of cells/reaction = (ng/reaction*1000)/7.12

Where,

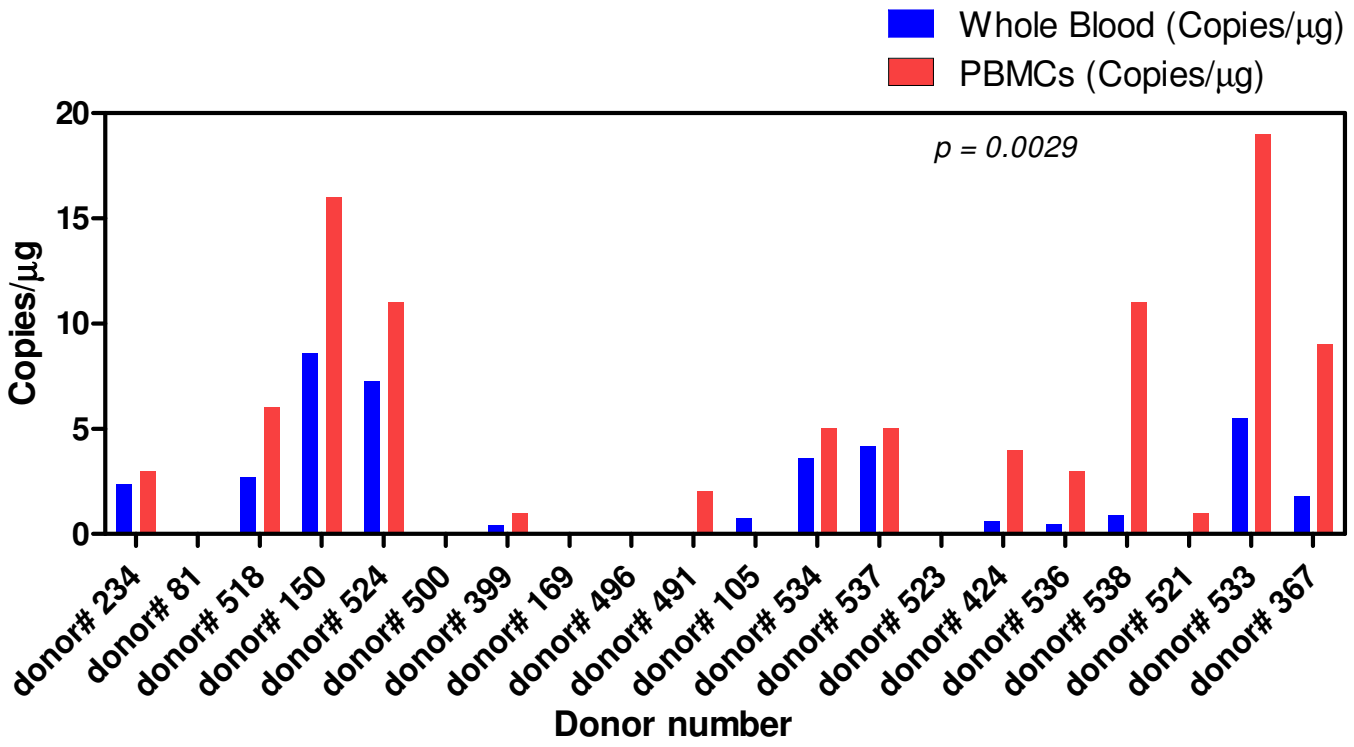
Ct = Cycle Threshold value

Slope and Intercept = -3.3879 and 41.9076 (stored standard curve generated at Viracor)

ng/µL = concentration of DNA

ng/reaction = concentration of DNA in a PCR reaction

Results: Whole Blood vs. PBMCs



Conclusion:

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