

# APPENDIX

## Appendix I

1 . Meltcurve plots for amplification of growth factor , proliferation and differential marker

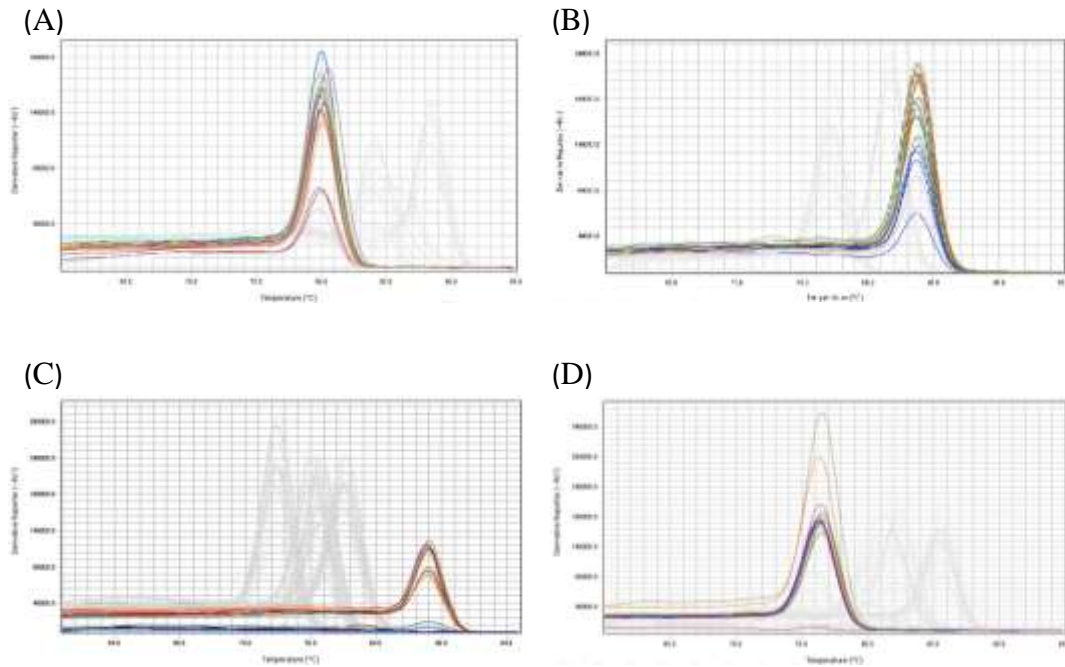


Figure 49 : Meltcurve plots for the amplification of *MKI67* (A), *KRT18* (B), *CCND1* (C), and *VEGFA* (D) in tissue samples. The genes were successfully amplified in the real-time PCR without any amplification of non-specific products.

2 . Meltcurve plots for amplification of immunoregulatory genes

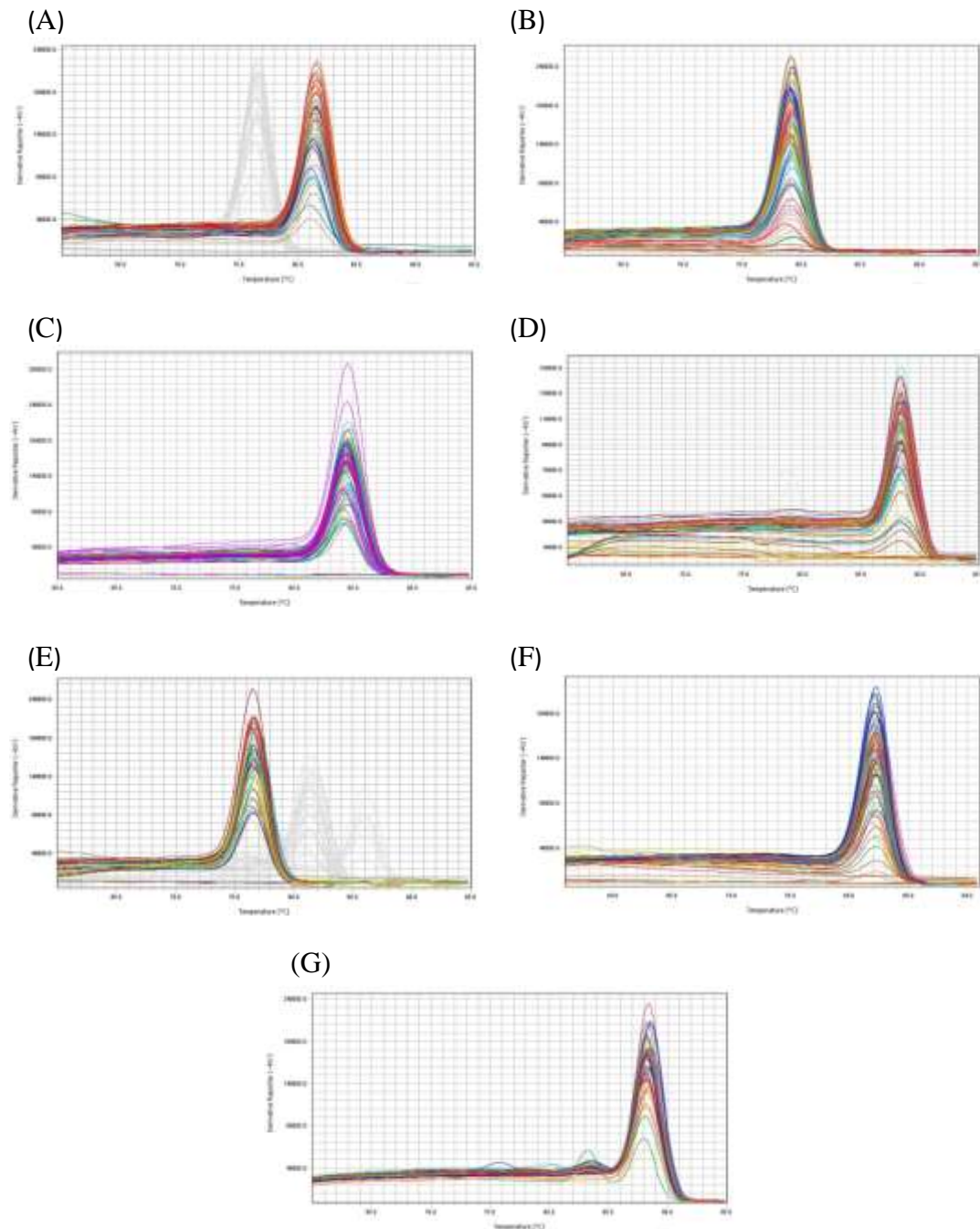


Figure 50: Meltcurve plots for the amplification of *IL-10* (A), *TGF-β* (B), *KIR2DL1* (C), *KIR2DS1* (D), *IFN-γ* (E), *HLA-G* (F), and *GAPDH* (G) in tissue samples. The genes were successfully amplified in the real-time PCR without any amplification of non-specific products.

4. Meltcurves for Pro-Inflammatory Cytokines, Interferones

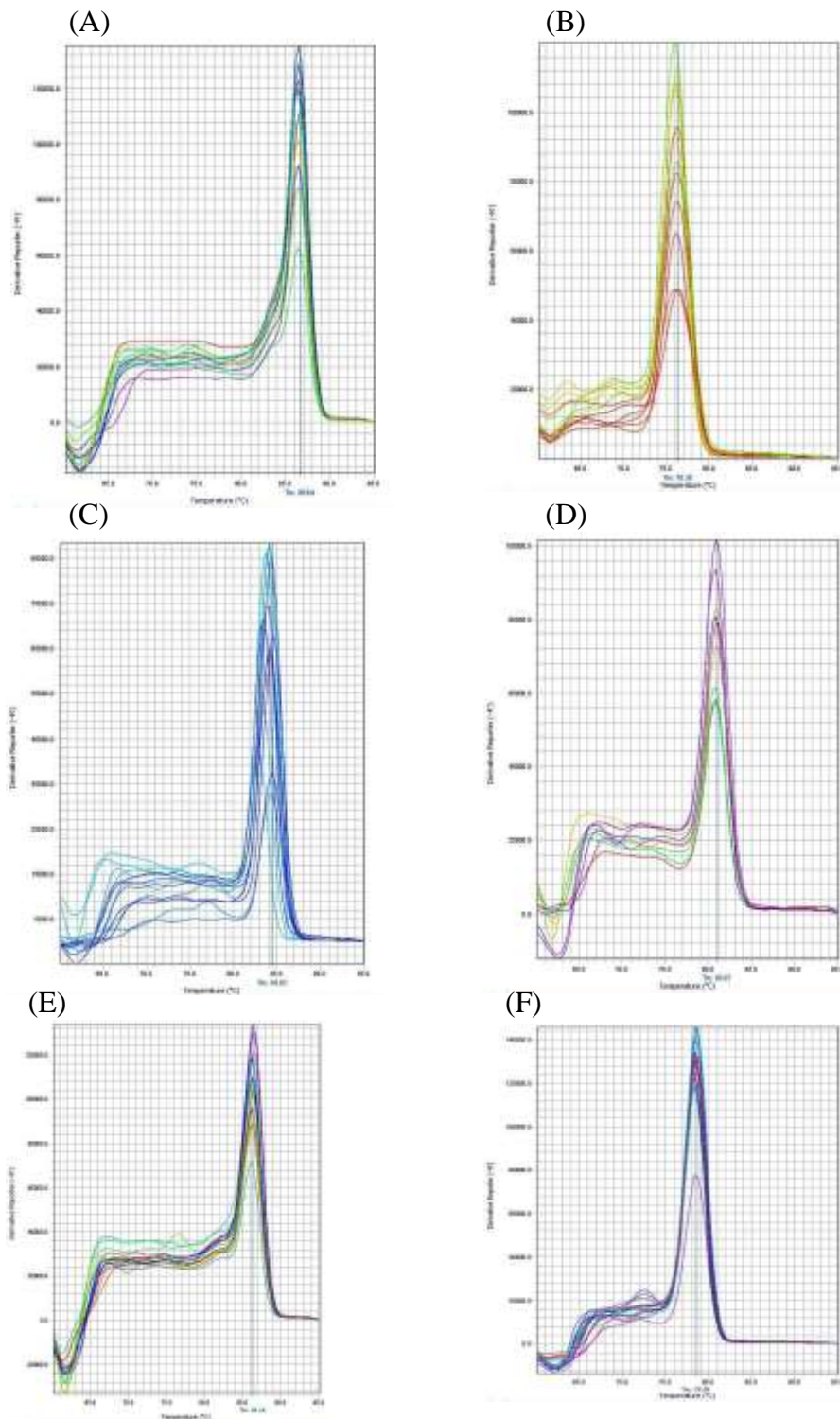
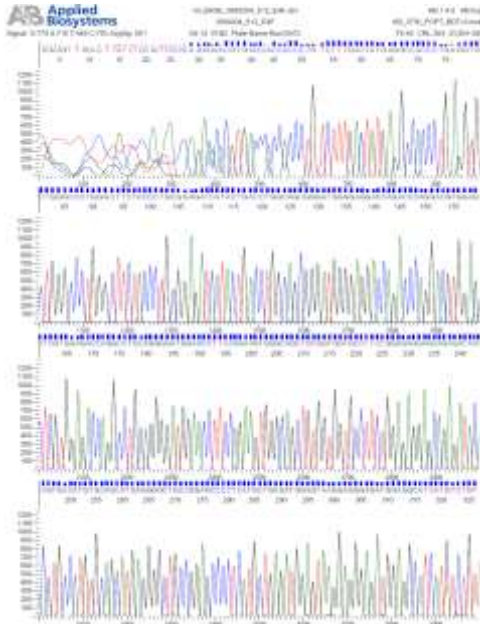


Figure 51 : Meltcurve plots for the amplification of *IL-1B* (A), *IL-2* (B), *IL-15* (C) *IL-21* (D), *DNMT1* (E), *HSF1* in tissue samples. The genes were successfully amplified in the real-time PCR without any amplification of non-specific products.

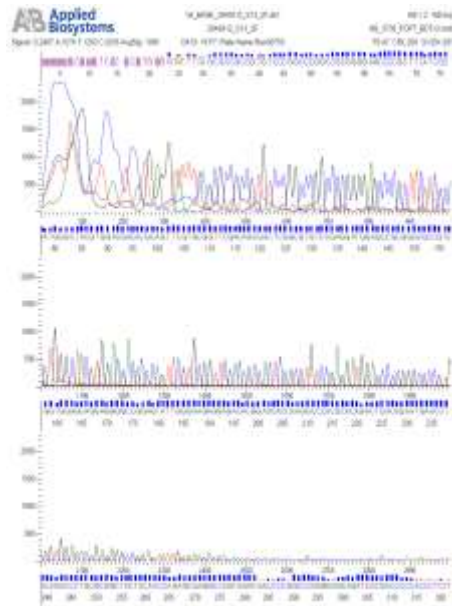
Appendix II

1. Sequencing results of HLA-G exon amplification using cDNA in PCR

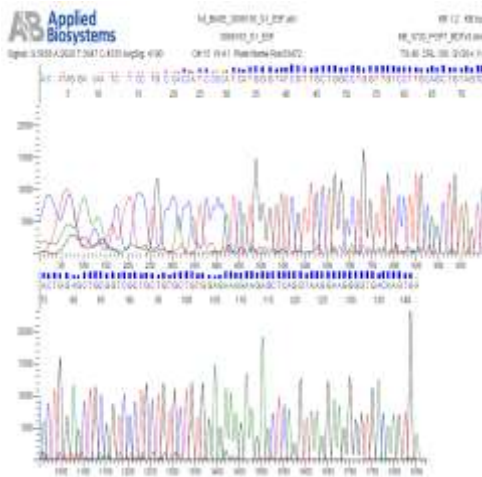
(A)



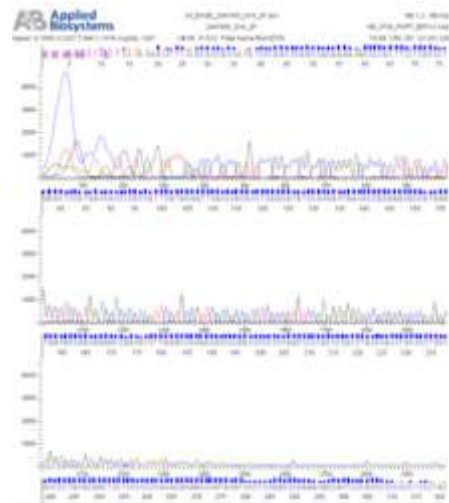
(B)



(C)



(D)



(E)

Sequences producing significant alignments

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Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
<input checked="" type="checkbox"/> Homo sapiens MHC class I antigen (HLA-G) gene HLA-G*01:01:01:01 ADN_129 fl allele complete cds	579	579	98%	3e-161	99.68%	MN620156.1
<input checked="" type="checkbox"/> Homo sapiens MHC class I antigen (HLA-G) gene HLA-G*01:01:01:01 allele complete cds	579	579	98%	3e-161	99.68%	MN524392.1
<input checked="" type="checkbox"/> Homo sapiens isolate DNA-188 MHC class I antigen (HLA-G) gene HLA-G*01:01:01:01 allele complete cds	579	579	98%	3e-161	99.68%	NK415682.1
<input checked="" type="checkbox"/> Homo sapiens isolate DNA-121 MHC class I antigen (HLA-G) gene HLA-G*01:01:01:05 allele complete cds	579	579	98%	3e-161	99.68%	NK415683.1
<input checked="" type="checkbox"/> Homo sapiens isolate DNA-89-91 MHC class I antigen (HLA-G) gene HLA-G*01:04 allele complete cds	579	579	98%	3e-161	99.68%	NK415677.1

(F)

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - exon 2_3 and partial cdx	545	545	99%	3e-151	99.34%	AY561651.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01_ADN_129-N allele - complete sequence	540	540	99%	1e-149	99.01%	MN621156.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	540	540	99%	1e-149	99.01%	MN524397.1
Homo sapiens isolate DNA-388 MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	540	540	99%	1e-149	99.01%	MN415667.1
Homo sapiens isolate DNA-521 MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	540	540	99%	1e-149	99.01%	MN415660.1

(G)

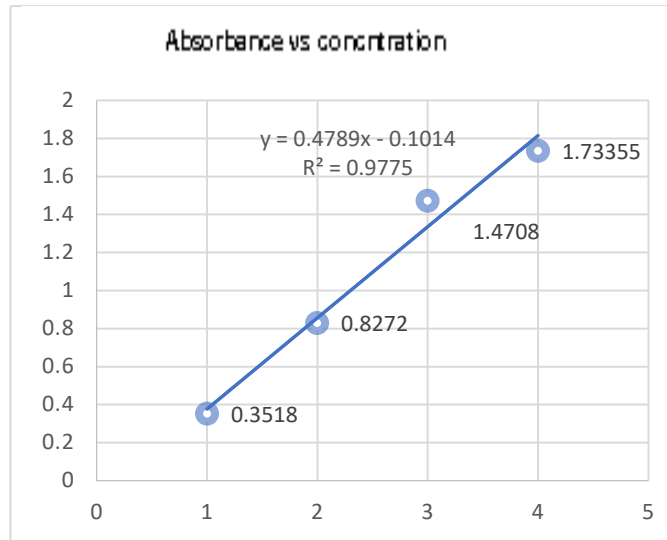
Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01_ADN_129-N allele - complete sequence	573	573	96%	2e-155	99.06%	MN621156.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01_ADN_16N allele - complete cdx	573	573	96%	2e-155	99.06%	MN621156.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	573	573	96%	2e-155	99.06%	MN524397.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	573	573	96%	2e-155	99.06%	MN524397.1

(H)

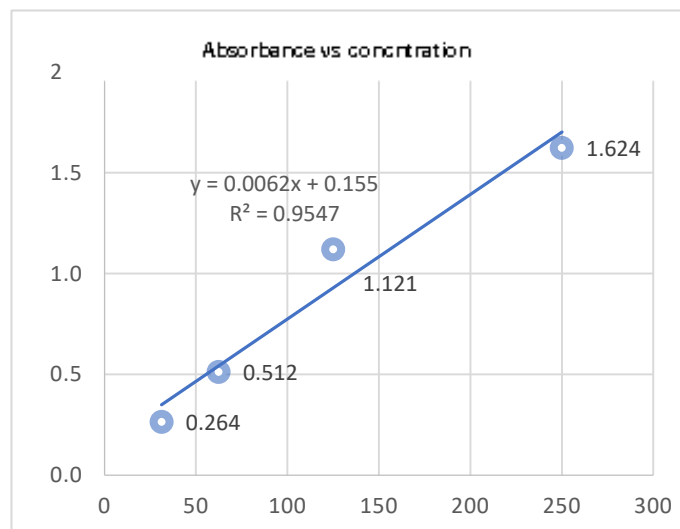
Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01_ADN_129-N allele - complete sequence	296	296	99%	2e-49	100.00%	MN621156.1
Homo sapiens MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	296	296	99%	2e-49	100.00%	MN524397.1
Homo sapiens isolate DNA-388 MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	296	296	99%	2e-49	100.00%	MN415667.1
Homo sapiens isolate DNA-521 MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	296	296	99%	2e-49	100.00%	MN415668.1
Homo sapiens isolate DNA-58-183 MHC class I antigen (HLA-G) gene: HLA-G*01:01:01:01 allele - complete cdx	296	296	99%	2e-49	100.00%	MN415673.1

Figure 52 : Sequencing results for amplicons of HLA-G exon 2, exon 3, exon 4 and exon 5 of HLA-G transcript. Results of sanger sequencing and NCBI blasts confirmed the specificity of band amplification for exon 2 (A and E), Exon 3 (B and F), exon 4 (figure C and G) and exon 5 (D and H).

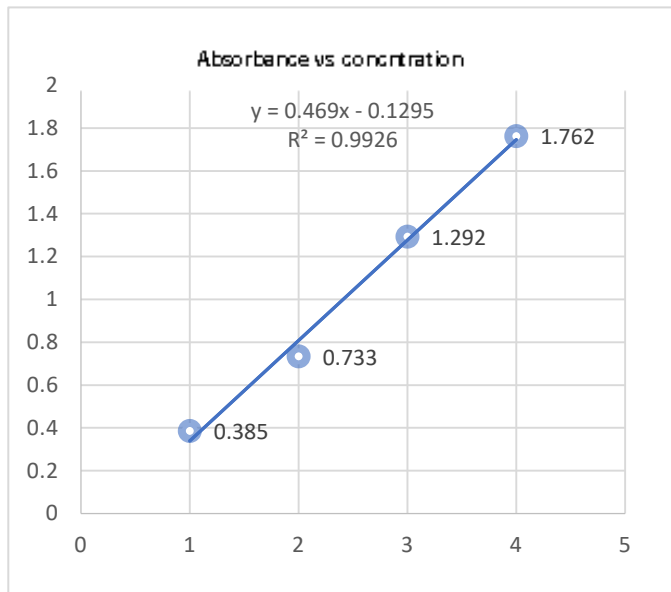
2. Standard graph of calibrators of anticardiolipin antibody and anti  $\beta$ 2 glycoprotein 1 by ELISA



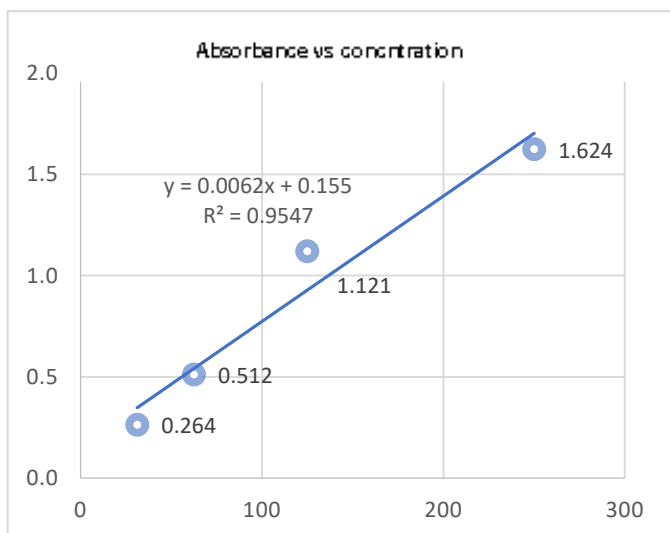
(a)



(b)



(c)



(d)

Figure 53 : Standard graphs of (a) anticardiolipin antibody IgG (b)IgM (C) anti  $\beta$ 2 glycoprotei 1 IgG (d) anti  $\beta$ 2 glycoprotei 1 IgM by ELISA .



# Interaction of autoantibodies and KIR- HLA genotype in relation to pregnancy outcome.

*by* Mayuri Bora

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