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**Chapter 1**

Development and evaluation of conventional PCR based protocols using existing and newly designed primers and their comparison with commercially available kit for the detection of herpes simplex virus

1.1 Introduction
1.2 Materials and methods
1.2.1 Patient selection and samples collection
1.2.2 DNA extraction
1.2.3 Primer designing
1.2.4 Primer synthesis
1.2.5 Positive and negative controls of PCR
1.2.6 PCR protocols for different assays and primers
  1.2.6.1 Amplification using commercial kit
  1.2.6.2 Amplification of UL 30 gene target
  1.2.6.3 Amplification of US3 gene target for differentiation of HSV-1 and HSV-2
1.2.7 Analysis of PCR products
1.2.8 Statistical analysis

1.3 Results
1.4 Discussion & Conclusions

**Chapter 2**

Development of real-time PCR assays (Taqman and SYBR Green) with newly designed and existing primers and evaluation of the developed assays in clinical samples of patients & Development of a quantitative real-time PCR assay for determination of viral load in clinical samples of herpes simplex encephalitis patients

2.1 Introduction
2.2 Materials and methods
2.2.1 Patient selection and samples collection
2.2.2 DNA extraction
2.2.3 Real-time PCR assays
  2.2.3.1 SYBR green real-time PCR
  2.2.3.2 Taqman real-time PCR
  2.2.3.3 HSV-1 and HSV-2 real-time PCR
  2.2.3.4 Quantitative real-time PCR
2.2.4 Statistical analysis

2.3 Results
2.4 Discussion & Conclusions
Chapter 3
Designing and synthesis of antigenic peptides by targeting envelope glycoproteins of herpes simplex virus, development of synthetic peptide based IgM/IgG antibody detection ELISA and evaluation of the developed immunoassay in clinical samples of patients.

3.1 Introduction
3.2 Materials and methods
   3.2.1 Patient selection and samples collection
   3.2.2 DNA extraction and PCR
   3.2.3 Selection and designing of peptides
   3.2.4 Synthesis of peptides
   3.2.5 ELISA
   3.2.6 Positive/negative threshold baseline and observed antibody titer
   3.2.7 Regression analysis and predicted antibody titer
   3.2.8 Statistical analysis

3.3 Results
3.4 Discussion & Conclusions

Chapter 4
Development of an in-house based ELISA for the detection of herpes simplex virus antigen using hyperimmune sera isolated from herpes simplex virus seropositive patients and evaluation of the developed immunoassay in clinical samples of patients.

4.1 Introduction
4.2 Materials and methods
   4.2.1 Patient selection and samples collection
   4.2.2 DNA extraction and PCR
   4.2.3 Anti-HSV IgG detection in sera
   4.2.4 Preparation of anti-HSV
   4.2.5 HSV antigen detection by ELISA
   4.2.6 HSV antigen detection by ELISA with Protein A
   4.2.7 Statistical analysis

4.3 Results
4.4 Discussion & Conclusions

Chapter 5
Production of anti-peptides against the identified potential peptides, development of ELISA for the detection of HSV antigen using anti-peptide antibodies and evaluation of the developed immunoassay in clinical samples of patients.

5.1 Introduction
5.2 Materials and methods
   5.2.1 Patient selection and samples collection
   5.2.2 DNA extraction and PCR
   5.2.3 Antipeptides production
   5.2.4 ELISA for antipeptide titer assay
   5.2.5 ELISA for HSV antigen detection in CSF
   5.2.6 HSV antigen concentration using AP-5 and AP-6
   5.2.7 Statistical analysis