

Laboratory Report: Diagnostic Polymerase Chain Reaction (PCR)

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The development of diagnostic polymerase chain reaction analysis of biopsied ocular tissue is playing an increasingly important role in our care of patients with ocular inflammatory disease. While our development of diagnostic PCR began for analysis of vitrectomy specimens, it is being put increasing use in the analysis of biopsied conjunctiva, sclera and cornea in patients with unusual forms of inflammation in these sites. A publication on just such an instance involving a patient with recurrent nodular scleritis resistant to steroids, to non-steroidal inflammatory drugs, and to methotrexate is currently under review for publication. Polymerase chain reaction analysis of biopsied sclera in this patient (along with immunofluorescence probing as well) showed definitively the presence of varicella zoster virus DNA (and viral protein by immunofluorescence), indicating that this patient had recurrent nodular scleritis on the basis of zoster virus. Treatment with oral Famvir each day completely abolished the recurrent episodes which had been disabling this patient for approximately nine months.

We believe that diagnostic PCR will be increasingly used in the care of patients with ocular inflammatory disease. The oligonucleotide primer pair sequences, designed to identify complimentary sequences of specific microbes, are the critical elements, along with the stringency aspects of the laboratory and the appropriate choice of positive and negative controls, of properly run diagnostic PCR. Few laboratories have made the commitment to develop such technology, but we believe such laboratories will be found increasingly at the university centers around the United States within the next decade.

In hopes of stimulating an exchange of information and the standardization of primer sequences, we publish, with this laboratory report, the oligonucleotide sequences that we currently use for the indicated microbes which can cause uveitis, retinitis, scleritis, conjunctivitis, and keratitis.

PRIMER SEQUENCES

HSV - 1

CAT CAC CGA CCC GGA GAG GGA C
GGG CCA GGC GCT TGT TGG TGT A
product 93 bp DNA segment

VZV

GTT GCG CGG ACG CCT TTC TCC G
CAA TGT CCA GGG TAT CGG AGT ATG
product 203 bp DNA segment

CMV

GTG GAT CTT CTG GTG TGC GTC C
GAC GTA TAT AAC AGC AGC GTG
product 361 bp DNA segment

BORRELIA

CAC ATA TTC AGA TGC AGA CAG AGG TTC TA
GAA GGT GCT GTA GCA GGT GCT GGC TGT
product 390 bp DNA segment

TUBERCULOSIS

TCC GCT TCC AGT CGT CTT CC
GTC CTG GCG AGT CTA GGC CA
product 240 bp DNA segment

TOXOPLASMOSIS

GGA ACT GCA TCC GTT CAT GAG
TCT TTA AAG CGT TCG TGG TC
product 193 bp DNA segment

WHIPPLE'S BACILLUS

5'-GGA ATT CCA GAG ATA CGC CCC CCG CAA
5'-CGG GAT CCT GTG AGT CCC CGC CAT TAC GC
product 154 bp DNA segment

TREPONEMA PALLIDUM

5'-CAG GTA ACG GAT GCT GAA GT
5'-CGT GGC AGT AAC CGC AGT CT
product 500 bp DNA segment