PCR and allele-specific restriction analysis (ASRA) of *pfdhfr* codons 50, 51 and 59

PRIMARY PCR

5 ul of slide/filter-paper extract in total 25 ul PCR

Sense primer FR519-A 5'**GCGCGC**TAATAACTACACATTTA3'

Antisense primer FR519-B 5'**CCCGGG**CTCTTATATTTCAATTT3'

Product size: 147 bp

PCR PROGRAM (ASRA-1)
Primary Denaturing: 95^0C,5 min
Denaturing: 92^0C, 30 sec
Annealing: 45^0C, 30 sec
Extension: 65^0C, 45 sec
Cycles: 45
Final Extension: 72^0C, 15 min

SECONDARY PCR FOR 51 AND 59 CODONS

1-2 ul of primary reaction in total 25 ul secondary PCR

Sense primer FR51-D

5'CTAGGAAATAAAGGAGTATTACCATGGAAATG**G**A3'

Antisense primer FR59-D

5'ATTTTTCATATTTTGATTCATTCACATATGTTGTAACTG**T**AC3'

Product size: 113 bp

PCR PROGRAM (ASRA-2)
Primary Denaturing: 95^0C,5 min
Denaturing: 92^0C, 30 sec
Annealing: 45^0C, 30 sec
Extension: 65^0C, 30 sec
Cycles: 15-25
Final Extension: 72^0C, 15 min

SECONDARY PCR FOR 50 CODON

Sense primer FR519-A **GCGCGC**TAATAACTACACATTTA

Antisense primer FR59-D

ATTTTTCATATTTTGATTCATTCACATATGTTGTAACTG**T**AC

Product size: 128 bp

PCR PROGRAM (ASRA-2)

Primary Denaturing: 95,5 min
Denaturing: 92, 30 sec
Annealing: 45, 30 sec
Extension: 65, 30 sec
Cycles: 15-25
Final Extension: 72, 15 min

RESTRICTION DIGEST

Digests for each were performed separately. 5-8 ul of PCR was digested with 1 unit enzyme overnight at 37^0C in a total 20 ul reaction using New England Biolabs #4 buffer. Products were examined by agarose electrophoresis on 2% NuSeive (FMC, Rockville, MD), with an "uncut" (no enzyme) digest run alongside.

51 codon

Enzyme: EcoRI (New England Biolabs, Beverly, MA)

Codon cleaved: wild (N)

Cleaved product sizes: 35 and 78bp

59 codon

Enzyme: BsrGI (New England Biolabs, Beverly, MA)

Codon cleaved: wild (R)

Cleaved product sizes: 43 and 65bp

50 codon

Enzyme: Tai I (New England Biolabs, Beverly, MA)

Genotype cleaved: mutant (Arg) and not wild (Cys)

Cleaved product sizes: approx. 58 and 70 bp

PCR and allele-specific restriction analysis (ASRA) of *pfdhfr* codons 108 and 164

PRIMARY PCR

Sense primer FR100-A **GGGGGG**CAGTTACAACATATGTGA

Antisense primer FR100-B **GGGGG**CACATTCATATGTACTATTT

Product size:414bp

PCR PROGRAM (ASRA-1)
Primary Denaturing: 95,5 min
Denaturing: 92, 30 sec
Annealing: 45, 30 sec
Extension: 65, 45 sec
Cycles: 45
Final Extension: 72, 15 min

SECONDARY PCR

Sense primer FR108-D
CTAATTCTAAAAAATTACAAAATGT

Antisense primer FR164-D3
TTTCTTTTCTAAAAATTCTTGATAAACAACGGAACCTC**T**TA

Product size: 254 bp

PCR PROGRAM (FR100-2)
Primary Denaturing: 95,5 min
Denaturing: 92, 30 sec
Annealing: 42, 30 sec
Extension: 65, 45 sec
Cycles: 15-25
Final Extension: 72, 15 min

RESTRICTION DIGEST

Digests for each are performed separately
NOTE: for Psi I, use 0.5X NEB#4 buffer and 1x BSA
Analyze products on 2% NuSeive gel

108 codon

Enzyme: Alu I (New England Biolabs, Beverly, MA)

Codon cleaved: wild (Ser) and not mutants (Asn or Thr)

Approx. cleaved product sizes: 46 + 210 bp

Note: BsrI will cut 108 Asn; ScrfI will cut 108 Thr.

164 codon

Enzyme: Psi I(New England Biolabs, Beverly, MA)

Codon cleaved: wild (Ile) and not mutant (Leu)

Approx. cleaved product sizes: 42 and 214bp

PCR and allele-specific restriction analysis (ASRA) of *pfdhfr* codon 16

PRIMARY PCR

Sense primer AMP-1 TTTATATTTTCTCCTTTTTA

Antisense primer FR519-B 5'**CCCGGG**CTCTTATATTTCAATTT3'

Product size: 255 bp

PCR PROGRAM (ASRA-1)
Primary Denaturing: 95,5 min
Denaturing: 92, 30 sec
Annealing: 45, 30 sec
Extension: 65, 45 sec
Cycles: 45
Final Extension: 72, 15 min

SECONDARY PCR

Sense primer SP1 ATGATGGAACAAGTCTGCGAC

Antisense primer FR59-D

5'ATTTTTCATATTTTGATTCATTCACATATGTTGTAACTG**T**AC3'

Product size: 220 bp

PCR PROGRAM (ASRA-2)
Primary Denaturing: 95^0C,5 min
Denaturing: 92^0C, 30 sec
Annealing: 45^0C, 30 sec
Extension: 65^0C, 30 sec
Cycles: 15-25
Final Extension: 72^0C, 15 min

RESTRICTION DIGEST

5-8 ul of PCR was digested with 1 unit enzyme for 5 hours in a total 20 ul reaction using New England Biolabs #2 or 4 buffer. Analyze products on 2% NuSeive gel with an "uncut" (no enzyme) digest run alongside.

16 codon

Enzyme: Mwo I (New England Biolabs, Beverly, MA)

Codon cleaved: wild (Ala) and not mutant (Val)

Cleaved product sizes: 174 and 46 bp