REFERENCES


King, M.W., (mking@medicine.indstate.edu) 1999. Introduction to molecular medicine. [Date of access: 4 June 2001]


APPENDIX I: VOLEETEER INFORMATION FORM

PLEASE NOTE
Write this number on both sample bottles: ______
PRELOADING SAMPLE (Before juice) GREEN STICKER
POST LOADING SAMPLE (After juice) ORANGE/RED STICKER

Name: ________________________________________
Surname: _______________________________________
Sex: Male: _______ Female: ________________
Occupation: ______________________________________
Residential address: ______________________________________
Home province: ______________________ (e.g. Gauteng)

Do you smoke?:
Yes: ________ No: ________

Do you drink?:
Yes: ________ No: ________

Any allergies?: ______________________________________
__________________________________________________

Have you taken any medication in the past 24 hours? (Tick where applicable):
Eggs: _____ Fish: _____ Beans: _____ Broccoli: _____ Cauliflower: _____
Brussel sprouts: _____ Sea food: ______

I acknowledge that:
• A higher dose of a TMA-base containing 600mg TMA will be administered for this
  test (TMA is found in some everyday foods).
• In case of me being a carrier or affected by trimethylaminuria, an unpleasant odour
  may be exhibited.

I give full consent towards this test.
Signature: ______________________________________
Date: ______________________________________
APPENDIX II: INDIVIDUAL INFORMATION FORM

Name: ____________________________________________________________
Date of birth: ____________________________________________________
Sex: ____________________________________________________________

Allergies: ________________________________________________________
_________________________________________________________________
_________________________________________________________________

Clinical symptoms: (e.g.) skin rash, nausea, etc.
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

How often do you suffer from:

<table>
<thead>
<tr>
<th>Condition</th>
<th>More than once a week</th>
<th>Once a week</th>
<th>Once a month</th>
<th>Once in three months</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEADACHES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAUSEA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKIN RASH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYPERTENSION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (Specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Any noticeable unusual body odour:

Childhood: _______
Puberty: _______
Menstruation: _______

Signature: ____________________________
Date: ________________________________
# APPENDIX III: LABORATORY SAMPLE TREATMENT FOR LC-MS

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>20</td>
</tr>
<tr>
<td>TEA</td>
<td>20</td>
</tr>
<tr>
<td>Water:acetonitrile (80:20) with 1% formic acid</td>
<td>960</td>
</tr>
</tbody>
</table>

100µl of the final mixture was used for analysis in the LC-MS.
# APPENDIX IV: PCR PRIMERS FOR FMO3

<table>
<thead>
<tr>
<th>Name</th>
<th>5'-Nucleotide sequence-3'</th>
<th>Exon</th>
<th>Tm value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMO3</td>
<td>GCCAAAGAGCGAATCAAAATAA</td>
<td>2</td>
<td>54.62</td>
</tr>
<tr>
<td>FMO4</td>
<td>TACACTTCCCAACCTATTTTCCT</td>
<td>2</td>
<td>50.89</td>
</tr>
<tr>
<td>FMO5</td>
<td>GACCTGATCATATACCTCATTTA</td>
<td>3</td>
<td>42.69</td>
</tr>
<tr>
<td>FMO6</td>
<td>CAGTAGTAGACATAGACCTTCCT</td>
<td>3</td>
<td>39.31</td>
</tr>
<tr>
<td>FMOE45</td>
<td>TAATTGGTTTGTCCGGACATCATGTCGATC</td>
<td>4</td>
<td>49.66</td>
</tr>
<tr>
<td>FMOE43</td>
<td>GGCAGTGTGAGTCATAATTTAC</td>
<td>4</td>
<td>53.13</td>
</tr>
<tr>
<td>FMO9</td>
<td>TAGCACATTATTGACTGCATC</td>
<td>5</td>
<td>49.89</td>
</tr>
<tr>
<td>FMO10</td>
<td>CCACATTTCATATCACAACCTTTC</td>
<td>5</td>
<td>50.55</td>
</tr>
<tr>
<td>FMO11</td>
<td>GGTATAGATCCATCTACATCAAGA</td>
<td>6</td>
<td>48.89</td>
</tr>
<tr>
<td>FMO12</td>
<td>TTGCTGATGGCTAAATACCTTGA</td>
<td>6</td>
<td>51.20</td>
</tr>
<tr>
<td>FMO13</td>
<td>CTTATCAATTATATATAGCC</td>
<td>7</td>
<td>44.64</td>
</tr>
<tr>
<td>FMO14</td>
<td>GGACCTTGTAAACTAGGATTATTG</td>
<td>7</td>
<td>46.66</td>
</tr>
<tr>
<td>FMO15</td>
<td>GAATTGGTGTTGCTGTCTGAAAAAT</td>
<td>8</td>
<td>49.52</td>
</tr>
<tr>
<td>FMO16</td>
<td>CATAAATTCTCACTTTTCTATGG</td>
<td>8</td>
<td>45.41</td>
</tr>
<tr>
<td>FMO17</td>
<td>ATGTAAATTCTCAGATTATAAA</td>
<td>9</td>
<td>40.66</td>
</tr>
<tr>
<td>FMO18</td>
<td>CTGAATAGAAAAAGCAGGTGG</td>
<td>9</td>
<td>46.82</td>
</tr>
</tbody>
</table>
APPENDIX V: Common mutations in the FMO3 gene.

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutation</th>
<th>Location</th>
<th>Nucleotide number</th>
<th>Codon</th>
<th>Effect on FMO3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A52T</td>
<td>G→A</td>
<td>Exon 2</td>
<td>154</td>
<td>52</td>
<td>Decreases activity (Mild TMAuria)</td>
</tr>
<tr>
<td>M66I</td>
<td>G→T</td>
<td>Exon 3</td>
<td>198</td>
<td>66</td>
<td>Loss of substrate affinity (TMAuria)</td>
</tr>
<tr>
<td>P153L</td>
<td>C→T</td>
<td>Exon 4</td>
<td>472</td>
<td>153</td>
<td>Catalytic activity abolished (TMAuria)</td>
</tr>
<tr>
<td>E158K</td>
<td>G→A</td>
<td>Exon 4</td>
<td>551</td>
<td>158</td>
<td>Diminished substrate affinity in vivo (Benign)</td>
</tr>
<tr>
<td>I199T</td>
<td>T→C</td>
<td>Exon 5</td>
<td>596</td>
<td>199</td>
<td>Mild TMAuria</td>
</tr>
<tr>
<td>V257M</td>
<td>G→A</td>
<td>Exon 6</td>
<td>771</td>
<td>257</td>
<td>(Benign)</td>
</tr>
<tr>
<td>E305X</td>
<td>G→T</td>
<td>Exon 7</td>
<td>913</td>
<td>305</td>
<td>Causes TMAuria</td>
</tr>
<tr>
<td>E308G</td>
<td>A→G</td>
<td>Exon 7</td>
<td>923</td>
<td>308</td>
<td>Decreases activity (Benign)</td>
</tr>
<tr>
<td>E314X</td>
<td>G→T</td>
<td>Exon 7</td>
<td>940</td>
<td>314</td>
<td>Decreases activity (Mild TMAuria)</td>
</tr>
<tr>
<td>R387L</td>
<td>G→T</td>
<td>Exon 7</td>
<td>1160</td>
<td>387</td>
<td>Decreases activity (Mild TMAuria)</td>
</tr>
<tr>
<td>G475D</td>
<td>G→A</td>
<td>Exon 8</td>
<td>1424</td>
<td>475</td>
<td>Mild TMAuria</td>
</tr>
<tr>
<td>R492W</td>
<td>C→T</td>
<td>Exon 9</td>
<td>1474</td>
<td>492</td>
<td>Inactivates FMO3 (TMAuria)</td>
</tr>
</tbody>
</table>