Familial Hypercholesterolemia (FH) Array

Rapid and reliable genetic assessment of patients with suspected FH
Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is characterised by high levels of low density lipoprotein (LDL) and early onset of cardiovascular disease. Three main genes which are known to be associated with FH are low density lipoprotein receptor (LDLR), apolipoprotein B (ApoB) and proprotein convertase subtilisin/kexin-type 9 (PCSK9).

Many patients have mutations in the LDLR gene that encodes the LDL receptor protein, which normally removes LDL from the circulation, or in ApoB, which is the part of LDL that binds with the receptor. PCSK9 encodes an enzyme that is involved in regulating the degradation of the LDL receptor protein.

Patients who have one abnormal copy (heterozygous) of these genes may have premature cardiovascular disease between the ages of 30 and 40. Having two abnormal copies (homozygous) may cause severe cardiovascular disease in childhood. Heterozygous FH is a common genetic disorder; occurring in 1 in 500 people in most countries; whereas homozygous FH is much rarer; occurring in 1 in a million births. Heterozygous FH, when detected early can be successfully treated with statins, bile acid sequestrants or other hypolipidemic agents that lower cholesterol levels. Accurate diagnosis can therefore lead to more tailored treatments and better patient outcomes.

The most common genetic defects in FH are LDLR mutations (prevalence of 1 in 500, depending on the population), ApoB mutations (prevalence of 1 in 1000) and PCSK9 mutations (prevalence less than 1 in 2500). The FH biochip array detects 20 mutations known to influence the function of these three genes, with the majority from the LDLR gene. The FH Array covers 60% of these mutations in the UK and Ireland.

### FH Array I

<table>
<thead>
<tr>
<th>Randox FH No.</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Gene</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>c.10580G&gt;A</td>
<td>p.R3527Q</td>
<td>ApoB</td>
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<td>2</td>
<td>c.2292delA</td>
<td></td>
<td>LDLR</td>
</tr>
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<td>3</td>
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<td>p.D482N</td>
<td>LDLR</td>
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<td>4</td>
<td>c.551G&gt;A</td>
<td>p.C184Y</td>
<td>LDLR</td>
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<tr>
<td>5</td>
<td>c.1845+11C&gt;G</td>
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<td>LDLR</td>
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<td>p.C231X</td>
<td>LDLR</td>
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<td>7</td>
<td>c.933delA</td>
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<td>LDLR</td>
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<td>8</td>
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<td>p.E101K</td>
<td>LDLR</td>
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<td>p.P685L</td>
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<td>p.W483R</td>
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<td>c.1120G&gt;T</td>
<td>p.D374Y</td>
<td>PCSK9</td>
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</tbody>
</table>

Principle of the Familial Hypercholesterolemia Array

The FH array is based on a combination of multiplex PCR and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple wildtype and mutant DNA regions in a number of genes. A unique primer set is designed for each mutation target (and control), which will hybridise to a complementary discrete test region (DTR) on the Randox proprietary biochip array. This combination of Randox priming and spatially organised biochip array technology enables high multiplexing of the assay. Analysis can be completed from template DNA through PCR to data readout in 3 hours.
Intended use

The FH Array is intended for the simultaneous qualitative detection of mutations in 20 targets within the low density lipoprotein receptor gene (LDLR), apolipoprotein B (ApoB) gene and proprotein convertase subtilisin/kexin-type 9 (PCSK9) gene. The sample type is genomic DNA extracted from blood.

Clinical data

Several validation studies were completed using FH samples, with both blinded and un-blinded samples assessed. Total correlation of 98% was observed when using the FH array.

FH Array Protocol

- **Save time and cost**
  - with simultaneous mutation detection using the FH Array

Step 1

Extraction

- Genomic DNA extracted from blood

Step 2

Amplification

- Multiplex PCR reaction

Step 3

Hybridisation

- Amplicon hybridisation/conjugation to biochip array

Step 4

Detection

- Imaging and result processing by Evidence Investigator

Benefits of the Familial Hypercholesterolemia Array

- The FH array is a rapid simple method for determining mutational status
- Samples can be assessed in small batches (as low as 3 samples)
- Easy to interpret results using the Evidence Investigator dedicated software
- Turnaround time of ~ 3 hours
- Streamlined workflow - protocol and reagents optimised for the molecular laboratory
- System can be used to detect single base changes, insertions and deletions, within the same multiplex PCR
- Only 20ng of genomic DNA required
Evidence Investigator
Multiplexing...proven, perfected, evolved

The Evidence Investigator is a semi-automated, benchtop biochip analyser which offers complete patient profiling.

Save time and costs -
Multiplexing reduces time, labour and reagents associated with multiple individual tests

Increase throughput -
For greater laboratory efficiency

Consolidation -
Of immunoassays and molecular diagnostics, improving laboratory efficiency

Result traceability -
Chain of custody features and bar coded reagents

No hidden costs -
Package includes imaging module, PC and imaging software, thermoshaker, biochip carrier handling tray and barcode scanner

Ease of operation -
Straightforward testing procedure, ready-to-use biochips and minimal sample handling

Extensive QC -
Internal quality controls ensure all key assay steps have been performed correctly i.e. amplification

Retrospective reporting -
Enabling additional analysis of previously captured sample data

Ordering Details

<table>
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<tr>
<th>Description</th>
<th>Size</th>
<th>Cat. No.</th>
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<td>54 biochips</td>
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<td>Evidence Investigator Analyser</td>
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<td>EV3602</td>
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</table>

*Note: Extraction reagents are not included

References