Molecular Diagnostics

Precise diagnostics for targeted therapy
Introduction

Randox Biosciences, part of Randox Laboratories, is dedicated to improving health worldwide through scientific discovery, drug development and diagnostics. Spanning four key divisions; Life Sciences, Pharma Sciences, Research and Molecular; Randox Biosciences offers complete tailored solutions for clinical and research use. From initial cultivation of raw materials for assay development, through to providing companion diagnostics, custom and molecular based assays across a range of therapy areas; Randox Biosciences is a trusted partner supplying quality diagnostic solutions to the clinical, life science, pharmaceutical, research and biopharma industries.

Randox Molecular offers diagnostic, prognostic and predictive solutions across a variety of disease areas including sexually transmitted infection, respiratory tract infection, colorectal cancer, familial hypercholesterolemia (FH) and cardiovascular disease (CVD).

Randox Molecular offers a wide range of assay formats including SNP genotyping, pathogen detection, mutation detection and gene expression, optimised for use with the Randox Evidence Investigator semi-automated bench top biochip analyser for improved laboratory efficiency.
SNP Genotyping
Cardiovascular Risk Prediction Array

Rapid multiplex SNP genotyping is based on innovative primer design that can discriminate DNA sequences which differ only at one base. Products amplified will correspond to target portions of DNA from tissue, buccal swabs or blood. Amplified regions are then hybridised to a biochip array with spatially tethered probes complementary to target amplicons. Each position on the biochip array corresponds to a specific SNP genotype and is capable of both multiplexing and determining the zygosity of the sample.

Pathogen Detection
STI Multiplex Array and Respiratory Multiplex Array

Pathogen detection, through nucleic acid (DNA/RNA) analysis offers rapid, sensitive, multiplex detection of viral, bacterial and protozoan pathogens. Following nucleic acid extraction from a broad range of sample types (sputum, urine, swabs etc.) target DNA/cDNA is amplified in a single reaction and subsequently hybridised to a biochip array containing multiple pathogen-specific probes. This rapid, highly sensitive and specific process enables identification of primary and co-infections simultaneously, often in asymptomatic patients and has the capacity for use with many pathogen panels.

Mutation Detection
KRAS BRAF PIK3CA Array and Familial Hypercholesterolemia (FH) Arrays I & II

The assay is based on a combination of multiplex PCR and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple targets in a number of genes. A unique primer set is designed for each target which will hybridise to a complementary oligo-nucleotide probe spotted on a biochip discrete test region (DTR). This combination of priming and spatially organised biochip array technology enables enhanced specificity of the assay.

Gene Expression
Breast Cancer Array (in development)

Individual genes are differentially expressed according to internal and external cellular inputs. Interpretation of the expression levels of one or a number of genes can provide valuable information relating to the physiological health of a cell or associated organ in an individual at that time. Harnessing such gene expression or gene signatures, particularly in a multiplex array, can provide a powerful insight into normal and disease processes. Randox has built upon advances in amplification technology and biochip arrays to create a number of quantitative RNA expression arrays that will enhance clinical decisions and therapy choice, leading to more personalised care for each patient.
Molecular Testing with Evidence Investigator

Rapid, accurate and comprehensive molecular testing

The Evidence Investigator offers complete patient profiling with the most comprehensive test menu on the market. The Evidence Investigator is a compact, semi-automated bench top platform, consolidating immunoassay and molecular diagnostics on a single platform with protein and DNA biochips.

Utilising revolutionary Biochip Array Technology (BAT), the Evidence Investigator allows simultaneous detection of multiple analytes from a single sample for efficient and cost-effective testing.
Minimum batch of three biochips (one strip) which includes patient sample, positive and negative controls.

Ready-to-use, nine biochip carrier 
Randox biochips can support multiple assays per biochip.

1. **Extract DNA**
   Genomic DNA is extracted from patient sample.

2. **Amplification**
   Single tube multiplex PCR reaction.

3. **Hybridisation / conjugation**
   Batches of 3 to 54 biochips are placed in the thermoshaker.

4. **Wash step and addition of signal reagent**
   After washing, signal reagent is added to each biochip before imaging.

5. **Biochip carrier loaded into Evidence Investigator**
   A Charged Coupled Device (CCD) camera inside the Evidence Investigator takes 2 minutes to image each biochip carrier.

6. **Chemiluminescence**
   The chemiluminescent light signal generated from each discrete test region (DTR) is simultaneously detected.

7. **Result reporting**
   Image processing software translates the light signal generated from the chemiluminescent reaction into relative light units (RLUs) and Randox QC-assigned cut-offs allow simple reporting of positive or negative results with no data interpretation or manual processing required.
STI Multiplex Array

Rapid, simultaneous detection of 10 common sexually transmitted infections

Introduction

STIs and related complications, such as infertility or reproductive health problems, represent a significant public health issue in both developed and developing countries. Many infections are asymptomatic and can remain undiagnosed, increasing the risk of unhindered spread. If untreated, STIs can impact fertility, increase risk of ectopic pregnancies and infant mortality.

Antibiotic resistance

Antimicrobial resistance is the largest threat to the control and management of STIs globally. Caused by unrestricted access to antibiotics, overuse and poor quality of antibiotics, as well as genetic mutations within disease organisms, it poses a threat to sexual health worldwide.

The World Health Organisation (WHO), has stated that unless urgent action is taken, therapeutic options for the treatment of STIs will no longer be effective due to the emergence of antimicrobial resistance. With no alternative therapeutic treatments in the pipeline, WHO is calling for increased research and development into pipeline products, as well as greater vigilance on the correct use of antibiotics, increased monitoring and reporting of resistant strains as well as better prevention, diagnosis and control of gonococcal infections.1

The STI Multiplex Array

The Sexually Transmitted Infection (STI) Multiplex Array simultaneously detects 10 bacterial, viral and protozoan infections including primary, secondary and asymptomatic co-infections for a complete infection profile.

A unique primer set is designed for each target which will hybridise to a complementary oligo-nucleotide probe spotted on a biochip discrete test region (DTR). This combination of priming and spatially organised biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template DNA through PCR to data readout in ~6 hours. The array is CE marked for routine clinical use.

According to the World Health Organisation, more than 1 million people acquire a sexually transmitted infection (STI) every day and, each year, 500 million new cases of curable sexually transmitted infections (including syphilis, gonorrhoea, chlamydia and trichomoniasis) occur; therefore early and accurate detection is critical.1

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The STI Multiplex Array detects 10 STIs in one sample

Benefits of the Randox STI Multiplex Array

Product features
- Rapid turnaround time of ~6 hours from extracted genomic DNA to result
- Compatible with various sample matrices including urine and swabs
- 54 patient samples can be processed simultaneously

Benefits to the laboratory
- Simultaneously detecting 10 of the most common sexually transmitted infections provides a complete infection profile, detecting primary, secondary and asymptomatic co-infections in one test for a comprehensive and cost-effective screen, reducing the need for multiple or confirmatory tests associated with single infection detection

Benefits to the patient
- Detection of primary, secondary and asymptomatic co-infections ensures the patient is diagnosed accurately first-time, reducing length of exposure to infection, which can impact fertility and reproductive health and informs prescription of appropriate treatment, including proper use of antibiotics
Antibiotic resistance

In recent years, some pathogens, such as Staphylococcus aureus and Streptococcus pneumoniae have acquired resistance to antibiotics, rendering them ineffective in treating disease. This can largely be attributed to patient misuse of antibiotics as well as inappropriate prescribing by healthcare professionals. For example, antibiotics are ineffective against many respiratory tract infections, particularly viral infections, yet in the UK, RTIs account for 60% of antibiotic prescriptions in primary care.\(^4\) Correct identification and diagnosis of bacterial and/or viral pathogens is therefore critical to inform correct prescribing of antibiotics.

The Respiratory Multiplex Array

The Respiratory Multiplex Array is the most comprehensive screening test for infections of both the upper and lower respiratory tracts, simultaneously detecting 22 bacterial and viral pathogens from a single sputum, lavage or nasopharyngeal sample.

The assay is based on a combination of multiplex PCR and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple targets. A unique primer set is designed for each target which will hybridise to a complementary oligo-nucleotide probe spotted on a biochip discrete test region (DTR). This combination of priming and spatially organised biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template DNA through PCR to data readout in ~6 hours. The array is CE marked for routine clinical use.
Respiratory Multiplex Array detects 22 bacterial and viral pathogens

Influenza A
Influenza B
Human rhinovirus A/B
Human enterovirus A/B/C
Moraxella catarrhalis
Legionella pneumophila
Chlamydophila pneumoniae
Human bocavirus 1/2/3
Human adenovirus A/B/C/D/E
Streptococcus pneumoniae
Haemophilus influenza
Staphylococcus aureus
Mycoplasma pneumoniae
Human respiratory syncytial virus A
Human parainfluenza virus 1
Human parainfluenza virus 2
Human parainfluenza virus 3
Human parainfluenza virus 4
Human coronavirus 229E/NL63
Human coronavirus OC43/HKU1
Human respiratory syncytial virus B
Human parainfluenza virus 2
Human parainfluenza virus 1

Respiratory Multiplex Array protocol

Extraction
RNA and DNA is extracted from broncholveolar lavage, nasopharyngeal swab, sputum or saliva samples

Amplification
Single tube multiplex PCR reaction

Hybridisation
Amplicon hybridisation to biochip array

Detection
Imaging and results processing by Evidence Investigator analyser

~6 HOURS

Product features
- Rapid turnaround time of ~6 hours from extracted genomic DNA to result
- Compatible with various sample matrices including sputum, lavage and nasopharyngeal samples
- Semi-quantitative testing allowing determination of primary infection

Benefits to the laboratory
- Simultaneously identifying the most prevalent pathogens, both viral and bacterial, will provide a rapid and more cost-effective diagnostic tool than current tests that only look for single pathogens

Benefits to the patient
- A more complete infection profile allows identification of the infective agent and detection of co-infections, to inform correct therapeutic treatment, including the appropriate use of antibiotics, and/or physician advice to patients for optimal patient care
- Rapid result reporting reduces the time from presentation of infection to therapeutic intervention, and reduces length of exposure to infection
- Precise, rapid diagnosis allows for early treatment intervention and potentially avoids exacerbations or the need for hospitalisation
- Reduced sample requirement to perform the diagnostic test will be of particular benefit to infants, children and the elderly
Mass gatherings, such as the Hajj increase the likelihood of the spread of infectious diseases. The Kingdom of Saudi Arabia annually hosts over 2 million Muslim pilgrims from around 184 countries during the Hajj pilgrimage, making it one of the largest and most culturally and geographically diverse mass gatherings in the world. Respiratory tract infections (RTIs) are the most common infection transmitted between pilgrims during Hajj, and most pilgrims develop RTIs during their few weeks stay in Makkah and Madinah. The Randox Respiratory Multiplex Array was used to screen for the presence of bacterial and viral upper and lower respiratory tract infections during the 2013 Hajj:


This study examined the presence of co-infections in patients admitted to healthcare facilities in Makkah and Medina, Saudi Arabia, with a primary diagnosis of severe community-acquired pneumonia (CAP) during the 2013 Hajj, using the Randox Respiratory Multiplex Array. The study highlighted the frequency of co-infections in respiratory infections and the importance of using multiplex technology to detect both bacterial and viral pathogens.

Clinical data

Study results revealed the wide range of infections present in the patient cohort.

- 68.4% of patients were confirmed to have a co-infection. Of these, bacterial pathogens were detected in 84.6% and viruses in 80.7%
- 80.7% of samples were positive for more than one respiratory pathogen and 65.3% were positive for both bacteria and viruses


This study sampled the environment in the King Abdul Aziz International (KAAI) Airport, Pilgrims City, Jeddah, during Hajj season to detect respiratory pathogens, using the Randox Respiratory Multiplex Array. 58 environmental samples (18 air samples and 40 surface samples) were tested for the presence of infectious pathogens, of which 8 samples were positive for at least one of the pathogens detectable by the assay. Air samples were negative with the exception of one (5.5%), which tested positive for influenza B virus.

- Of the 40 surface samples, 7 (17.5%) were positive for pathogens
- The most common pathogen contaminants of surfaces were adenovirus (3 of 7, 42.8%) and coronavirus OC43/HKU1 (3 of 7, 42.8%)
- Potentially pathogenic bacteria (e.g., H. influenza, M. catarrhalis) were also present on environmental surfaces
SINCE THEIR DISCOVERY LAST CENTURY, ANTIBIOTICS HAVE REVOLUTIONISED MODERN MEDICINE

IN RECENT YEARS, SOME PATHOGENS HAVE ACQUIRED RESISTANCE TO ANTIBIOTICS, RENDERING THEM INEFFECTIVE IN TREATING DISEASE

WHAT CAN BE DONE TO PREVENT ANTIMICROBIAL RESISTANCE?

**RESIST** ASKING FOR OR USING ANTIBIOTICS UNNECESSARILY

**DESIST** PRESCRIBING ANTIBIOTICS INAPPROPRIATELY

**UTILISE** MULTIPLY DIAGNOSTIC ASSAYS FOR RAPID DETECTION OF PRIMARY, SECONDARY AND ASYMPTOMATIC CO-INFECTIONS, ENABLING ACCURATE, FIRST-TIME DIAGNOSIS AND APPROPRIATE PRESCRIPTION OF ANTIBIOTICS

**Random Respiratory Multiplex Array**
- Simultaneously detects up to 22 bacterial and viral upper and lower respiratory tract infections
- Multiplex technology differentiates between viral and bacterial infection and identifies secondary and asymptomatic co-infections

**Random STI Multiplex Array**
- Simultaneously detects up to 10 bacterial, viral and protozoan pathogens
- Multiplex technology allows identification of multiple asymptomatic co-infections

- **Patient misuse**
  - Asking GPs to prescribe antibiotics unnecessarily
  - Obtaining antibiotics without a prescription
  - Saving antibiotics for future use
  - Sharing antibiotics with other people
  - Unnecessarily repeating courses of antibiotics due to non-compliance

- **Inappropriate prescribing**
  Antibiotics are ineffective against many RTIs, particularly viral infections, yet in the UK, RTIs account for 60% of antibiotic prescriptions in primary care. 

Antimicrobial Resistance in Infectious Diseases
KRAS, BRAF, PIK3CA* Array

Rapid profiling of point mutations in the KRAS, BRAF and PIK3CA genes

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide.\(^1\) Metastatic disease accounts for 40-50% of newly diagnosed patients and is associated with high morbidity.\(^1,2\) Despite recent therapeutic advances, the prognosis for patients with metastatic CRC (mCRC) remains poor.\(^3\) In recent years monoclonal antibodies (moAbs), like cetuximab and panitumumab which target the epidermal growth factor receptor (EGFR), have proven to be effective in combination with chemotherapy or as single agents for the treatment of mCRC.\(^4,5\)

These moAbs block the signal from EGFR inhibiting downstream signalling including KRAS, BRAF and PIK3CA mediated events (see diagram below). However, when KRAS, BRAF and PIK3CA are mutated they are permanently ‘turned on’, permitting downstream events irrespective of anti-EGFR therapy.

**Why test the KRAS, BRAF, and PIK3CA* genes?**

Early studies conducted on mainly heavily pre-treated chemotherapy-refractory patients and also chemotherapy-naive patients with mCRC indicated that only 10-20% of patients clinically benefited from anti-EGFR moAbs.\(^5,6\) Consequently oncogenic activation of EGFR downstream effectors was investigated. Analysis confirmed that patients with mCRC carrying activating KRAS gene mutations do not benefit from anti-EGFR moAb therapy.\(^7,8\) KRAS mutations have since emerged as the major negative predictor of efficacy in patients receiving cetuximab or panitumumab.\(^3\) The occurrence of KRAS mutations however only accounts for approximately 35-45% of nonresponsive patients.\(^3\) The identification of additional genetic determinants of primary resistance to EGFR-targeted therapies in colorectal cancers is therefore important. Recent studies have focused on mutations in BRAF\(^9\) and PIK3CA\(^10\) genes which have been reported to affect patient response to EGFR-targeted moAbs. Furthermore, since KRAS and BRAF mutations are mutually exclusive,\(^11\) targeted testing across multiple genes is advantageous.
The KRAS, BRAF, PIK3CA* Array

The KRAS, BRAF, PIK3CA* Array simultaneously detects 20 point mutations within the KRAS, BRAF and PIK3CA genes. The assay is validated for use with DNA extracted from fresh/frozen and formalin fixed paraffin embedded (FFPE) tissue. Whilst the PIK3CA target is for research use only, the KRAS and BRAF targets are CE marked for routine clinical use.

Whilst designed for colorectal cancer, the KRAS, BRAF, PIK3CA* Array has implications for mutation screening in other cancer types, e.g. lung cancer.

Point mutations detectable by the KRAS, BRAF, PIK3CA* Array

<table>
<thead>
<tr>
<th>KRASCodon</th>
<th>BRAFCodon</th>
<th>PIK3CACodon</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>13</td>
<td>61</td>
</tr>
<tr>
<td>G12A</td>
<td>G13D</td>
<td>Q61K</td>
</tr>
<tr>
<td>G12C</td>
<td>G13D</td>
<td>Q61L</td>
</tr>
<tr>
<td>G12R</td>
<td>G13C</td>
<td>Q61H(1)</td>
</tr>
<tr>
<td>G12D</td>
<td>G13R</td>
<td>Q61H(2)</td>
</tr>
<tr>
<td>G12V</td>
<td></td>
<td>Q61R</td>
</tr>
</tbody>
</table>

KRAS, BRAF, PIK3CA* Array protocol

1. **Extraction**
   - Genomic DNA is extracted from fresh/frozen or FFPE tissue samples

2. **Amplification**
   - Single tube multiplex PCR reaction

3. **Hybridisation**
   - Amplicon hybridisation/conjugation to biochip array

4. **Detection**
   - Imaging and results processing by Evidence Investigator analyser

~3 HOURS

Benefits of the KRAS, BRAF, PIK3CA* Array

**Product features**
- Rapid turnaround time of ~3 hours from extracted genomic DNA to result
- Compatible with various sample matrices including formalin fixed paraffin embedded (FFPE) tissue and fresh/frozen tissue
- Single reaction multiplex PCR coupled to a biochip provides greater mutation coverage
- Detection of 1% mutant in a background of wildtype genomic DNA

**Benefits to the laboratory**
- Simultaneous detection of mutations within the most common genes implicated in colorectal cancer offers an efficient and cost-effective method for determining mutational status and patient response to therapy

**Benefits to the patient**
- Early diagnosis and detection of mutational status informs selection of appropriate treatment regime in cases of colorectal cancer, for which current treatment options are limited. Therefore, identification of the correct treatment pathway for individual patients based on their mutational status is of paramount importance for optimal patient outcomes

*PIK3CA for research use only
Familial Hypercholesterolemia (FH) Arrays I & II

Rapid, simultaneous detection of 40 mutations within the LDLR, ApoB and PCSK9 genes

Introduction

Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is a common autosomal dominant, or inherited, disease which affects the plasma clearance of LDL-cholesterol (LDL-C), resulting in premature onset of cardiovascular disease (CVD) and a higher mortality risk.\(^1\)\(^3\)

Common genetic defects in FH are attributed to mutations in three genes encoding proteins involved in the uptake of LDL-C from the plasma: the low density lipoprotein receptor (LDLR) gene (prevalence of 1 in 500), the apolipoprotein B (ApoB) gene (prevalence of 1 in 1000) and the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (prevalence of less than 1 in 2500).\(^1\)\(^3\)

Patients who have one abnormal gene mutation are known as heterozygous. Heterozygous FH is a common genetic disorder occurring in 1 person in 200-500 in most countries. Homozygous FH occurs when the patient has two abnormal gene mutations, however this is much rarer, with an occurrence of 1 in a million.\(^4\)\(^5\)

Early diagnosis of FH is crucial as by the time the heterozygous FH sufferer enters early adulthood they will have accumulated >20 years of continuous exposure to build up of fatty or lipid masses in arterial walls and are at a hundred-fold greater risk of a heart attack than other young people. Patients with homozygous FH are at such high risk that they may not live beyond childhood into early adulthood.\(^4\)

The UK National Institute for Health and Clinical Excellence (NICE) guidelines published in 2008 recommend that all FH patients be offered a DNA test to confirm the diagnosis and that identified mutations should be used as the basis for cascade testing of first-degree relatives of index cases. Patients newly identified by such screening can then be offered treatment to reduce the risk of premature cardiac events.\(^1\)

Only few countries currently have national genetic screening programs for FH despite evidence demonstrating that implementing such a program is highly cost-effective, particularly for cascade testing of known index cases as roughly 50% will have inherited the mutation.\(^6\)\(^7\)

The Familial Hypercholesterolemia (FH) Arrays I & II

The Familial Hypercholesterolemia (FH) Arrays I & II are rapid, simple and accurate diagnostic tests which enable simultaneous detection of 40 FH-causing mutations (20 mutations per array) within the LDLR, ApoB and PCSK9 genes.

The assay is based on a combination of multiplex PCR and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple targets. A unique primer set is designed for each target which will hybridise to a complementary oligonucleotide probe spotted on a biochip discrete test region (DTR). This combination of priming and spatially organised biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template DNA through PCR to data readout in ~3 hours.

Clinical data

Several validation studies were completed using FH samples, assessing both blinded and un-blinded samples. Total correlation of 98% was observed when using the Familial Hypercholesterolemia Arrays I & II.
**Benefits of the Randox Familial Hypercholesterolemia (FH) Arrays I & II**

**FH Arrays I & II protocol**

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Mutation</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOB FH1</td>
<td>c.10580G&gt;A</td>
<td>p.(Arg3527Gln)</td>
</tr>
<tr>
<td>LDLR FH2</td>
<td>c.2292delA</td>
<td>p.(Ile764Mett62)</td>
</tr>
<tr>
<td>FH3</td>
<td>c.1444G&gt;A</td>
<td>p.(Asp482Asn)</td>
</tr>
<tr>
<td>FH4</td>
<td>c.551G&gt;A</td>
<td>p.(Cys184Tyr)</td>
</tr>
<tr>
<td>FH5</td>
<td>c.1845+11C&gt;G</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH6</td>
<td>c.693C&gt;A</td>
<td>p.(Cys231*)</td>
</tr>
<tr>
<td>FH7</td>
<td>c.933delA</td>
<td>p.(Glu312Serfs*58)</td>
</tr>
<tr>
<td>FH8</td>
<td>c.301G&gt;A</td>
<td>p.(Glu101Lys)</td>
</tr>
<tr>
<td>FH9</td>
<td>c.313+1G&gt;A</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH10</td>
<td>c.1706-1G&gt;A</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH11</td>
<td>c.2029T&gt;C</td>
<td>p.(Cys677Arg)</td>
</tr>
<tr>
<td>FH12</td>
<td>c.2054C&gt;T</td>
<td>p.(Pro685Leu)</td>
</tr>
<tr>
<td>FH13</td>
<td>c.447T&gt;C</td>
<td>p.(Trp483Arg)</td>
</tr>
<tr>
<td>FH14</td>
<td>c.1432G&gt;A</td>
<td>p.(Gly478Arg)</td>
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<tr>
<td>FH15</td>
<td>c.214delG</td>
<td>p.(Asp72Thrfs*134)</td>
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<td>FH16</td>
<td>c.259T&gt;G</td>
<td>p.(Trp87Gly)</td>
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<tr>
<td>FH17</td>
<td>c.897C&gt;T</td>
<td>p.(Arg333Cys)</td>
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<tr>
<td>FH18</td>
<td>c.681C&gt;G</td>
<td>p.(Asp227Glu)</td>
</tr>
<tr>
<td>FH19</td>
<td>c.2061dup</td>
<td>p.(Asn688Glnfs*29)</td>
</tr>
<tr>
<td>FH20</td>
<td>c.1120G&gt;T</td>
<td>p.(Asp374Tyr)</td>
</tr>
<tr>
<td>PCSK9 FH25</td>
<td>c.1150C&gt;T</td>
<td>p.(Gln424*)</td>
</tr>
</tbody>
</table>

**FH Array I mutation coverage**

**FH Array II mutation coverage**

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Mutation</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR FH20</td>
<td>c.1285G&gt;A</td>
<td>p.(Val429Met)</td>
</tr>
<tr>
<td>FH21</td>
<td>c.680_681delAC</td>
<td>p.(Asp227Glyfs*12)</td>
</tr>
<tr>
<td>FH22</td>
<td>c.1187-10G&gt;A</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH23</td>
<td>c.1048C&gt;T</td>
<td>p.(Arg350?)</td>
</tr>
<tr>
<td>FH24</td>
<td>c.1168A&gt;T</td>
<td>p.(Lys390?)</td>
</tr>
<tr>
<td>FH26</td>
<td>c.232C&gt;T</td>
<td>p.(Glu78Glu)</td>
</tr>
<tr>
<td>FH27</td>
<td>c.1587-1G&gt;A</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH28</td>
<td>c.1706-1G&gt;A</td>
<td>p.(=)</td>
</tr>
<tr>
<td>FH29</td>
<td>c.1796T&gt;C</td>
<td>p.(Leu599Ser)</td>
</tr>
<tr>
<td>FH30</td>
<td>c.1436T&gt;C</td>
<td>p.(Leu479Pro)</td>
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<tr>
<td>FH31</td>
<td>c.1747G&gt;A</td>
<td>p.(Asp492Asn)</td>
</tr>
<tr>
<td>FH32</td>
<td>c.1120C&gt;T</td>
<td>p.(Val374Met)</td>
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<tr>
<td>FH33</td>
<td>c.662A&gt;G</td>
<td>p.(Glu222*)</td>
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<td>FH34</td>
<td>c.682G&gt;T</td>
<td>p.(Glu228)</td>
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<tr>
<td>FH35</td>
<td>c.1150C&gt;T</td>
<td>p.(Gln384?)</td>
</tr>
<tr>
<td>FH36</td>
<td>c.938G&gt;A</td>
<td>p.(Cys313Ile)</td>
</tr>
<tr>
<td>FH37</td>
<td>c.1367G&gt;C</td>
<td>p.(Glu452Glu)</td>
</tr>
<tr>
<td>FH38</td>
<td>c.2042G&gt;C</td>
<td>p.(Cys681Ser)</td>
</tr>
<tr>
<td>FH39</td>
<td>c.1618G&gt;A</td>
<td>p.(Ala540Thr)</td>
</tr>
</tbody>
</table>

**Extraction**
- Genomic DNA extracted from blood

**Amplification**
- Multiplex PCR reaction

**Hybridisation**
- Amplicon hybridisation/conjugation to biochip array

**Detection**
- Imaging and results processing by Evidence Investigator analyser

~3 HOURS

**Benefits to the laboratory**
- Developed with leading experts to test for 40 specific FH-causing mutations with ~78% coverage, providing a targeted, cost-effective assay for FH testing. Rapid turnaround time allows results to be reported within days, compared to lengthy NGS screening which can take weeks or months to report results.
- The array consists of two mutation panels, allowing for single panel testing in cases of cascade screening of known mutations for further laboratory cost savings.

**Benefits to the patient**
- Mutational status can be determined rapidly from a single test, with a reduced need for confirmatory testing with NGS.
- Genetic analysis for FH mutations allows for more accurate diagnosis compared to lipid profiling.
Cardiac Risk Prediction Array

Simultaneous genotyping of 20 SNPs for enhanced CHD risk assessment

Introduction

Coronary Heart Disease (CHD) is the leading cause of death in the developed world and its prevention is a core activity for public health systems worldwide. For example, clinical guidelines from the Joint Cardiac Societies and NICE in the UK recommend that patients at greater than 20% risk of CHD in the next ten years should be classified as high risk and considered for intensive lifestyle intervention and lipid lowering therapy, primarily the prescription of statins.

Current CHD risk assessment tools based on common risk factors such as blood pressure and blood cholesterol levels (eg. PROCAM and Framingham) have low predictive value and take no account of genetic predisposition to CHD. Cooper et al reported only 14% of CHD events during a ten year period were predicted by these algorithmic tools. In recent years Genome Wide Association Studies (GWAS) have been carried out to identify genetic variants associated with CHD. This involves comparing millions of loci in the genomes of a population suffering from CHD and a control population. Meta-analysis of such studies has identified 19 variants (referred to as single nucleotide polymorphisms; SNPs) as being associated with CHD. Individually, the presence of an “at risk” variant does not greatly increase the risk of developing CHD. However, the presence of multiple “at risk” alleles can increase the risk of developing CHD two-fold or greater; an effect similar to being a current smoker. Combining genotype information with common risk factors could allow individuals to be more accurately classified therefore preventative therapies and lifestyle advice can be targeted to those who require it most.
The Cardiac Risk Prediction Array

In order to utilise the GWAS findings in a clinical setting, individuals require to be genotyped for each of the 19 CHD “at risk” SNPs. At present this can be a time consuming and expensive process. Together with key opinion leaders in cardiovascular genetics, Randox has developed a rapid array which will allow all 19 SNPs to be genotyped simultaneously, which incorporates a test to identify patients predisposed to statin-induced myopathy. Firstly, a multiplex PCR reaction is performed, where the products amplified correspond to the genotype of the patient sample. The PCR products are then hybridised onto the Cardiac Risk Prediction biochip array and imaged using the Evidence Investigator analyser to identify which PCR products are present. Patient samples can be genotyped within one day.

Benefits of the Cardiac Risk Prediction Array

Product features
- Simultaneous genotyping of 20 SNPs within one day
- 36 patient samples can be processed per kit
- Easy to interpret results using the Randox Evidence Investigator dedicated software

Benefits to the laboratory
- Developed with key opinion leaders in cardiovascular genetics to identify SNPs associated with CHD risk

Benefits to the patient
- Enhanced CHD risk assessment allows for early interventional therapeutic treatment and/or lifestyle changes to improve cardiovascular health and reduce the risk of CHD
- Genetic profiling identifies those patients predisposed to statin-induced myopathy, allowing clinicians to make more informed decisions when prescribing lipid lowering therapies

Cardiac Risk Prediction Array Protocol

1. **Extraction**
   - Genomic DNA extracted from blood/saliva

2. **Amplification**
   - Multiplex PCR reaction

3. **Hybridisation**
   - Amplicon hybridisation/conjugation to biochip array

4. **Detection**
   - Imaging and results processing by Evidence Investigator analyser

The genotype information is then put into an algorithm which weights each SNP and calculates a CHD genetic risk score. The CHD biochip results are combined with common risk factors and an overall CHD risk score is calculated.

Response to statin treatment

A further important SNP which can predict response to particular statin therapies has been included in the array. Individuals who are homozygous (frequency =0.13) for the risk allele are 17 times more likely to suffer from statin-induced myopathy when treated with high doses of simvastatin. Identifying patients with a higher risk of suffering statin-induced myopathy would allow clinicians to make more informed decisions when prescribing lipid lowering therapies.

Benefits to the patient
- Enhanced CHD risk assessment allows for early interventional therapeutic treatment and/or lifestyle changes to improve cardiovascular health and reduce the risk of CHD
- Genetic profiling identifies those patients predisposed to statin-induced myopathy, allowing clinicians to make more informed decisions when prescribing lipid lowering therapies.
References

STI Molecule Array


Respiratory Molecule Array


Antimicrobial Resistance in Infectious Diseases


KRAS, BRAF, PIK3CA* Array


Familial Hypercholesterolemia (FH) Arrays I & II


Cardiac Risk Prediction Array

Understanding drivers of disease is vital in delivering effective patient care. Through the unravelling of the genetic code, healthcare practitioners are able to predict and prevent disease and prescribe appropriate targeted treatments to specific subgroups, for optimal patient outcomes.

Randox Molecular offers a range of assay formats including SNP genotyping, gene expression, pathogen detection and mutation detection across infectious diseases, cardiovascular disease and oncology. Utilising innovative Biochip Array Technology (BAT) for multi-analyte screening of biological samples, our assays provide a complete patient profile from a single sample for rapid, accurate result reporting.