MOLECULAR DIAGNOSTICS
Precise diagnostics for targeted therapy
BIOCHIP ARRAY TECHNOLOGY

- Removable strip of three wells
- Single reaction well
- 25 discrete test regions
1 BIOCHIP
PROVIDING 22 RESULTS THROUGH RAPID AND ACCURATE TESTING
INTRODUCTION

Randox Biosciences is dedicated to advancing 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, molecular and custom 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.

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

Additionally, we can provide a wide range of assay formats including single nucleotide polymorphisms (SNP) genotyping, pathogen detection and mutation detection. The arrays are optimised for use with the Randox Evidence Investigator semi-automated, medium throughput bench top biochip analyser.
PATHOGEN DETECTION

STI and Respiratory Multiplex Arrays

Both arrays detect the most common and frequently requested infections in sexual and respiratory health. These comprehensive, highly sensitive and specific tests enable identification of primary and co-infections simultaneously, often in asymptomatic patients and enable antibiotic stewardship.

MUTATION DETECTION

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

These unique biochip assays permit high discrimination between multiple targets in a number of genes with a rapid turnaround time (3 hours). The arrays enable detection of the most frequently occurring mutations known to cause disease (FH) and adversely affect patient treatment (KRAS, BRAF, PIK3CA). 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).

SNP GENOTYPING

Cardiovascular Risk Prediction Array

This array identifies individuals with a genetic predisposition to coronary heart disease (CHD). The innovative multiplex primers are designed to discriminate DNA sequences which differ only at one base.
Rapid, Accurate & Comprehensive Molecular Testing

The Randox molecular arrays are analysed on the Evidence Investigator analyser. This analyser 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. The technology allows simultaneous detection of multiple analytes from a single sample for efficient and cost-effective testing.

MOLECULAR TESTING WITH EVIDENCE INVESTIGATOR

Each single 9x9mm Biochip acts as the reaction well, replacing the traditional ELISA 96-well plate format.

Each Biochip has 25 Discrete Test Regions (DTRs) and each DTR holds an individual test.

The Investigator quantifies images using Relative Light Units (RLU), generated by a chemiluminescent reaction.

1 patient sample is added to 1 biochip.
EXTRACT DNA/RNA
Extract nucleic acid

HYBRIDISATION
Biotin-labelled amplified PCR product binds to corresponding unique biochip molecular probe

CONJUGATION
Addition of streptavidin labelled-horse radish peroxidase to produce a biotin-streptavidin-enzyme complex

WASH STEP
Rinse away any unbound product

SIGNAL REAGENT
Addition of equal volumes of luminol and peroxide to produce chemiluminescent biochip spot signal

WASH STEP
Rinse away any unbound conjugate

SIGNAL DETECTION
Biochip carrier is inserted to the Evidence investigator imaging platform. The system’s CCD camera analyses the spots on each biochip. Process takes 5 minutes per carrier

RESULT REPORT
Easy to interpret positive/negative report generated automatically by the dedicated Evidence Investigator software

MULTIPLEX PCR
Single tube amplification using Randox multi-target primer sets
STI TESTING

**THE STI MULTIPLEX ARRAY II**

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. 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.

**PRODUCT FEATURES**

- Turnaround time of ~6 hours from extracted nucleic acid to result
- Validated for urine and swab sample matrices
- 53 patient samples can be processed simultaneously

**ANTIBIOTIC RESISTANCE**

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

The World Health Organisation 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, the 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.

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 in the sexually active population. If untreated, STIs can impact fertility, increase risk of ectopic pregnancies and infant mortality. According to the World Health Organisation (WHO), 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.

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**BENEFITS**

- Detection of primary, secondary and asymptomatic co-infections ensures the patient is diagnosed accurately first-time
- Reduces length of exposure to infection, which can impact fertility and reproductive health
- Informs prescription of appropriate treatment, encourages antibiotic stewardship

**LABORATORY**

- Simultaneously detecting 10 of the most common sexually transmitted infections provides a complete infection profile, detecting primary, secondary and asymptomatic co-infections in 1 test for a comprehensive and cost-effective screen, reducing the need for multiple or confirmatory tests associated with single infection detection
- Broadest CE marked STI microarray panel on the market

**RESULTS**

- Neisseria gonorrhoea (NG)
- Mycoplasma genitalium (MG)
- Ureaplasma urealyticum (UU)
- Chlamydia trachomatis (CT)
- Trichomonas vaginalis (TV)
- Haemophilus ducreyi (HD)
- Mycoplasma hominis (MH)
- Treponema pallidum (TP)
- Herpes simplex Virus 1 (HSV-1)
- Herpes simplex Virus 2 (HSV-2)

**STI TESTING**

1. **TEST**

2. **EXTRACTION**
   - Nucleic acid is extracted from urine or urogenital swab samples

3. **AMPLIFICATION**
   - Single tube 10-plex PCR reaction

4. **HYBRIDISATION**

5. **CONJUGATION**

6. **DETECTION**
   - ~6 hours

- Chart illustrating the process from extraction to detection.
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 and RNA through PCR to data readout in ~6 hours.

The array is CE marked for routine clinical use.

Respiratory tract infections are caused by many viral and bacterial pathogens and are the second most common cause of morbidity and mortality worldwide. Acute respiratory diseases (ARD) accounts for more than 4 million deaths annually and are the leading cause of death in developing countries. Viral respiratory infections can occur in epidemics and can spread rapidly within communities across the globe. Every year, influenza causes respiratory tract infections in 5-15% of the population and severe illness in 3-5 million people. Upper respiratory tract infections can lead to acute asthma exacerbations, acute otitis media, and lower respiratory tract infection such as bronchitis, bronchiolitis and pneumonia. Particularly affecting the young, elderly and the immunocompromised, RTIs can result in prolonged hospital stays and represent a significant cost burden to public health systems worldwide.

Antibiotic resistance in recent years, some pathogens, such as Streptococcus pneumoniae have acquired resistance to antibiotics, rendering the drugs 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.

Correct identification and diagnosis of bacterial and/or viral pathogens is therefore critical to inform correct prescribing of antibiotics.
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.
• Reduced sample requirement to perform the diagnostic test will be of particular benefit to infants, children and the elderly.

LABORATORY

• Simultaneously identifies the most prevalent viral and bacterial respiratory pathogens.
• Comprehensive infection panel, providing a more cost-effective approach to diagnostics, compared to single pathogen tests.
• Easy to interpret result report.
• Validated for multiple matrices, providing more options for testing.
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. The array is 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. 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 ~3 hours.

**PRODUCT FEATURES**
- Rapid turnaround time of ~3 hours from extracted genomic DNA to result
- Validated for formalin fixed paraffin embedded (FFPE) tissue and fresh/frozen tissue
- Sensitivity of 1% mutant in a background of wildtype genomic DNA

**WHY TEST THE KRAS, BRAF, AND PIK3CA* GENES?**
Studies have confirmed that patients with mCRC carrying activating KRAS gene mutations do not benefit from anti-EGFR moAb therapy. KRAS mutations have since emerged as the major negative predictor of efficacy in patients receiving cetuximab or panitumumab. The occurrence of KRAS mutations however only accounts for approximately 35-45% of nonresponsive patients. Recent studies have focused on mutations in BRAF and PIK3CA genes which have been reported to affect patient response to EGFR-targeted moAbs. In addition KRAS and BRAF mutations are mutually exclusive, therefore a multi-gene approach to testing would reduce the need for reflex testing.
**BENEFITS**

- 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.

**LABORATORY**

- Rapid simultaneous detection of 20 key mutations
- An efficient and cost-effective method for determining mutational status and patient response to therapy
- Covers 3 common genes implicated in colorectal cancer, reducing the need for reflex testing

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**EXTRACTION**
Genomic DNA is extracted from fresh/frozen or FFPE tissue samples

**AMPLIFICATION**
Single tube 20-plex PCR reaction

**HYBRIDISATION**

**CONJUGATION**

**DETECTION**

- KRAS
  - G12A
  - G12R
  - G12D
  - G12C
  - G12V
  - G13D
  - G13C
  - G13R
  - G13Q
  - Q61K
  - Q61L
  - Q61R
  - Q61H(1)
  - Q61H(2)
  - A146T
  - A146P

- BRAF
  - V600E

- PIK3CA
  - E542K
  - E545K
  - H1047R

**RESULTS**

1 TEST
20 RESULTS

~3 hours
Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is the most common autosomal dominant, or inherited, disease and 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.\(^3\,^5\)

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.

Only a few countries currently have national genetic screening programs for FH despite evidence demonstrating that implementing such programs is highly cost-effective, particularly for cascade testing of known index cases, as statistically 50% will have inherited the mutation.\(^3\,^4\)

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 Hypercholesterolaemia Arrays I & II.\(^5\,^6\)
**BENEFITS**

- Rapid mutational test to diagnose FH, the most commonly inherited lipid disease
- Mutational status can be determined rapidly from a single test, with a reduced need for confirmatory testing with NGS
- Genetic analysis for FH mutations gives a definitive diagnosis compared to lipid profiling

**LABORATORY**

- The array tests for 40 specific FH-causing mutations with ~78% coverage in the UK and Ireland, providing a targeted, cost-effective assay for FH testing. Rapid turnaround time allows results to be reported same day, compared to lengthy NGS screening which can take months
- The array consists of 2 mutation panels, allowing for single panel testing in cases of cascade screening of known mutations for further laboratory cost savings

**PATIENT**

- Rapid mutational test to diagnose FH, the most commonly inherited lipid disease
- Mutational status can be determined rapidly from a single test, with a reduced need for confirmatory testing with NGS
- Genetic analysis for FH mutations gives a definitive diagnosis compared to lipid profiling

**EXTRACTION**

Genomic DNA is extracted from blood

**AMPLIFICATION**

Single tube 10-plex PCR reaction

**HYBRIDISATION**

~3 hours

**DETECTION**

Conjugation

**FH TESTING**

<table>
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<tr>
<th>MUTATION</th>
<th>PROTEIN</th>
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<tbody>
<tr>
<td>APOB</td>
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</tr>
<tr>
<td>c.10580G&gt;A</td>
<td>p.(Arg3527Gln)</td>
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<td>LDLR</td>
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<td>c.2292delA</td>
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<td>c.1444G&gt;A</td>
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<td>p.(Cys184Tyr)</td>
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<tr>
<td>c.1845+11C&gt;G</td>
<td>p.(=)</td>
</tr>
<tr>
<td>c.693C&gt;A</td>
<td>p.(Cys231*)</td>
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<tr>
<td>c.933delA</td>
<td>p.(Glu312Serfs*58)</td>
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<tr>
<td>c.301G&gt;A</td>
<td>p.(Glu101Lys)</td>
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<td>c.313+1G&gt;A</td>
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<tr>
<td>c.1706-1G&gt;A</td>
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<tr>
<td>c.2039T&gt;C</td>
<td>p.(Cys677Arg)</td>
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<tr>
<td>c.2054C&gt;T</td>
<td>p.(Pro685Leu)</td>
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<tr>
<td>c.1447T&gt;C</td>
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<td>c.681C&gt;G</td>
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<td>PCSK9</td>
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<tr>
<td>c.1120G&gt;T</td>
<td>p.(Asp374Tyr)</td>
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</table>

**FH ARRAY I MUTATION COVERAGE**

- APOB
- LDLR
- PCSK9


**FH ARRAY II MUTATION COVERAGE**

- LDLR


**APOLI**

- Genomic DNA is extracted from blood

**AMPLICON**

- Single tube 10-plex PCR reaction

**HYBRIDISATION**

- Conjugation

**DETECTION**

- Results reported same day, compared to lengthy NGS screening which can take months

**FH TESTING**

- The array tests for 40 specific FH-causing mutations with ~78% coverage in the UK and Ireland, providing a targeted, cost-effective assay for FH testing. Rapid turnaround time allows results to be reported same day, compared to lengthy NGS screening which can take months
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**APPENDIX**

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. Clinical guidelines from the Joint Cardiac Societies and NICE in the UK recommend that patients at greater than 10% risk of CHD in the next 10 years should be classified as high risk and considered for intensive lifestyle intervention and lipid lowering therapy, primarily the prescription of statins.\(^1\)

Current CHD risk assessment tools based on common risk factors such as blood pressure and blood cholesterol levels (e.g. PROCAM and Framingham) have low predictive value and take no account of genetic predisposition to CHD.\(^2,3\) Cooper et al reported only 14% of CHD events during a ten year period were predicted by using algorithmic tools.\(^6\)

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.\(^7\) 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.\(^7\)

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 1 day.
**BENEFITS**

- 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.

**RESPONSE TO STATIN TREATMENT**

Dyslipidaemia can be treated with statins such as Simvastatin to reduce elevated levels of circulating LDL cholesterol in the blood. The Cardiac Risk Prediction Multiplex Array detects an important SNP which can predict a patient’s response to particular statin therapies, therefore avoiding unnecessary statin induced effects such as muscle breakdown, myoglobin release and risk of renal failure. 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.\(^8\) Identifying patients with a higher risk of suffering statin-induced myopathy would allow clinicians to make more informed decisions when prescribing lipid lowering therapies.

**GENOMIC DNA**

**AMPLIFICATION**

Single tube 20-plex PCR reaction

**HYBRIDISATION**

**CONJUGATION**

**DETECTION**

< 1 day

\(^8\) The risk allele is...
STI MULTIPLEX ARRAY

ANTIMICROBIAL RESISTANCE IN INFECTIOUS DISEASES

RESPIRATORY MULTIPLEX ARRAY

KRAS, BRAF, PIK3CA* ARRAY
FAMILIAL HYPERCHOLESTEROLEMIA (FH) ARRAYS I & II


CARDIAC RISK PREDICTION ARRAY


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<th>PRODUCT</th>
<th>DESCRIPTION</th>
<th>CAT NO.</th>
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<tbody>
<tr>
<td>Evidence Investigator</td>
<td>Biochip imaging station including 2 thermoshakers &amp; thermal cycler</td>
<td>EV4187</td>
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<tr>
<td>STI Multiplex Array</td>
<td>STI Array Version I 108 biochip kit</td>
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<td>STI Array Version II 108 biochip kit</td>
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