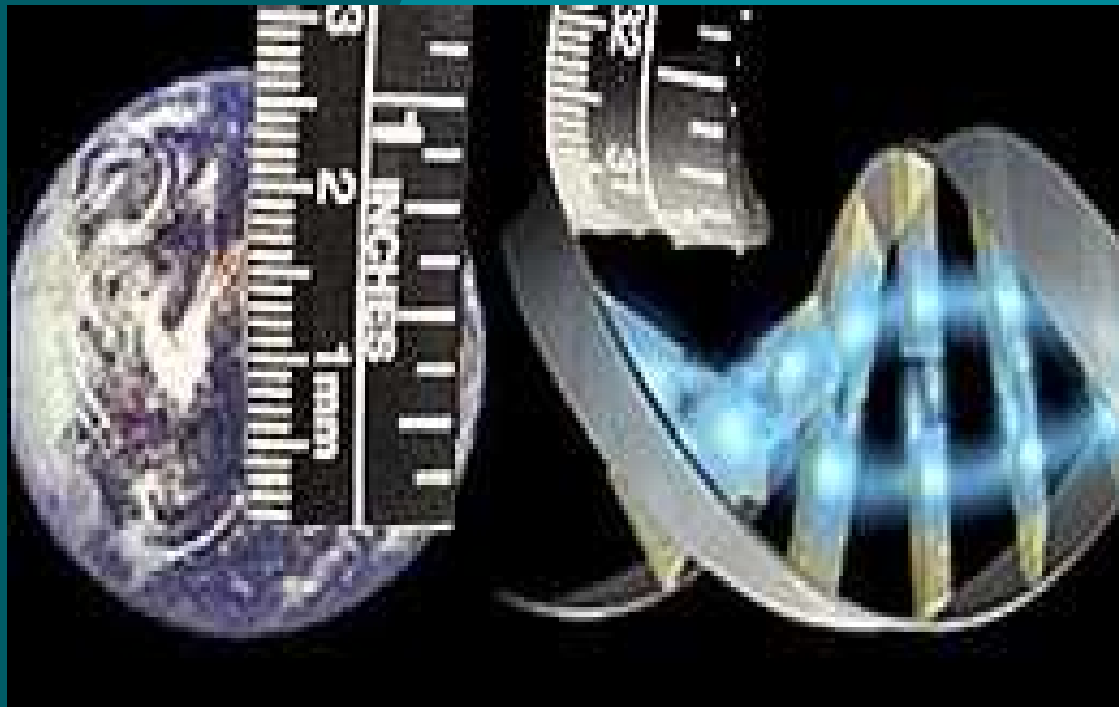


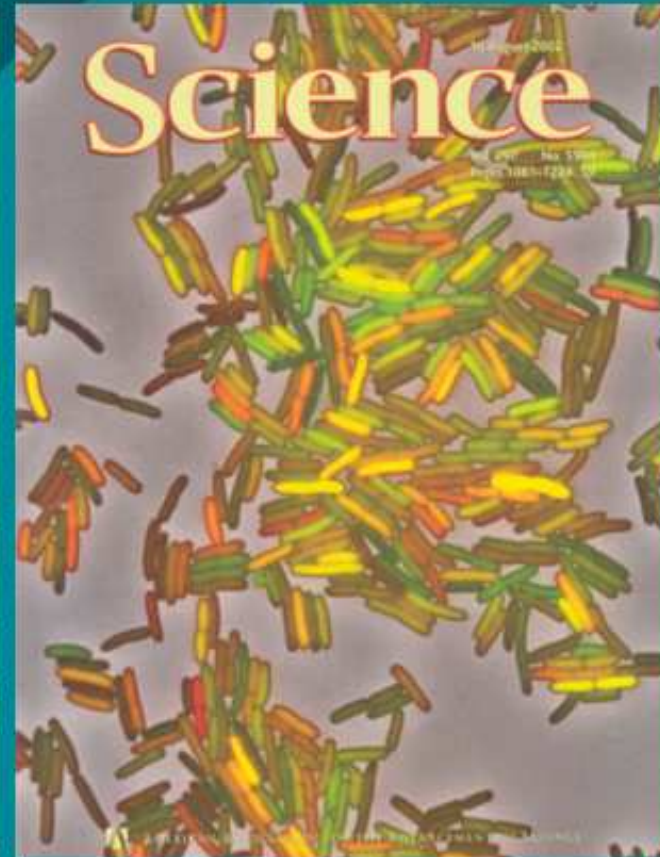
Near Patient Testing

Paul Debenham
LGC



*Setting standards
in analytical science*

DNA analysis promises unrivalled benefits of genetics for all



But must it stay as a laboratory science; could it not come to the point of care?



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Genetic diagnostics at point-of-care: An added value vision

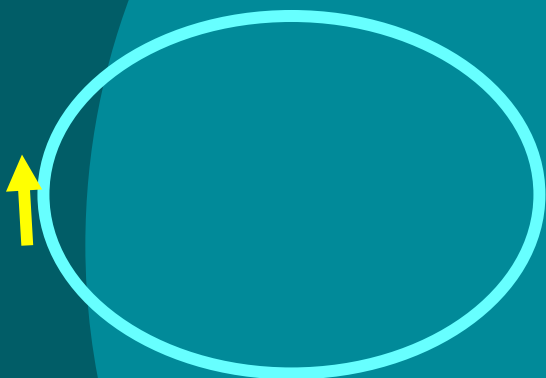
- Technology platform designed to empower health professional - not DIY genetics
- DNA-based definition means the most specific advice and treatment can be achieved at primary care
- Potential to revolutionise treatment of infectious agents - from subjective to objective
- Predict and avoid adverse reactions to medications
- Simple non-invasive sampling

The patient pathway

consultation



sampling



20 minutes
for result

treatment

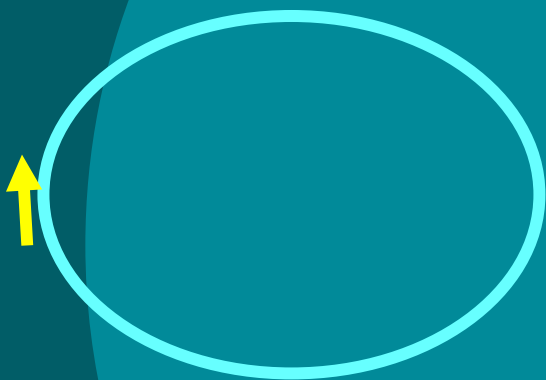


The sample pathway

Sample devices



Transfer to reagents cassette



Chlamydia Test Result

Patient Name: Joe Smith
Patient Address: 3 James Street, London W2 3RX
Sample Device Barcode: 09840830
Date: 05/11/2003

Test Control
Patient Sample

Result



Process for 20
minutes



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Typical Laboratory Process

- Sample Preparation
- PCR Amplification
- PCR products detected and identified
- Results analysis

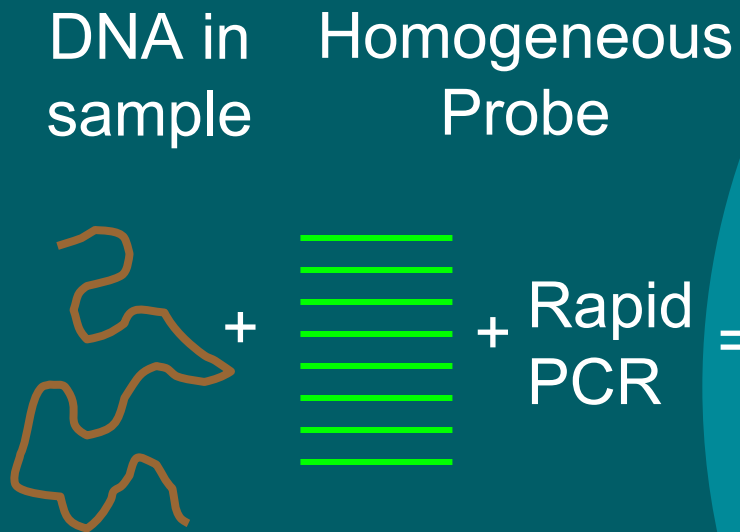


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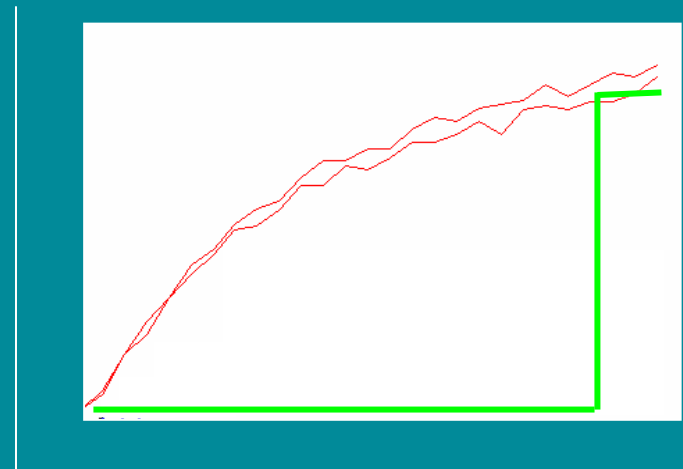
Making DNA analysis where the patient is - progress

- Sample preparation **Eliminate**
- PCR amplification **Accelerate**
- PCR product detection and identification **Simplify**
- Results analysis **Automate**

Probes working after faster PCR amplification



Probe signal



← ~20 minutes for result

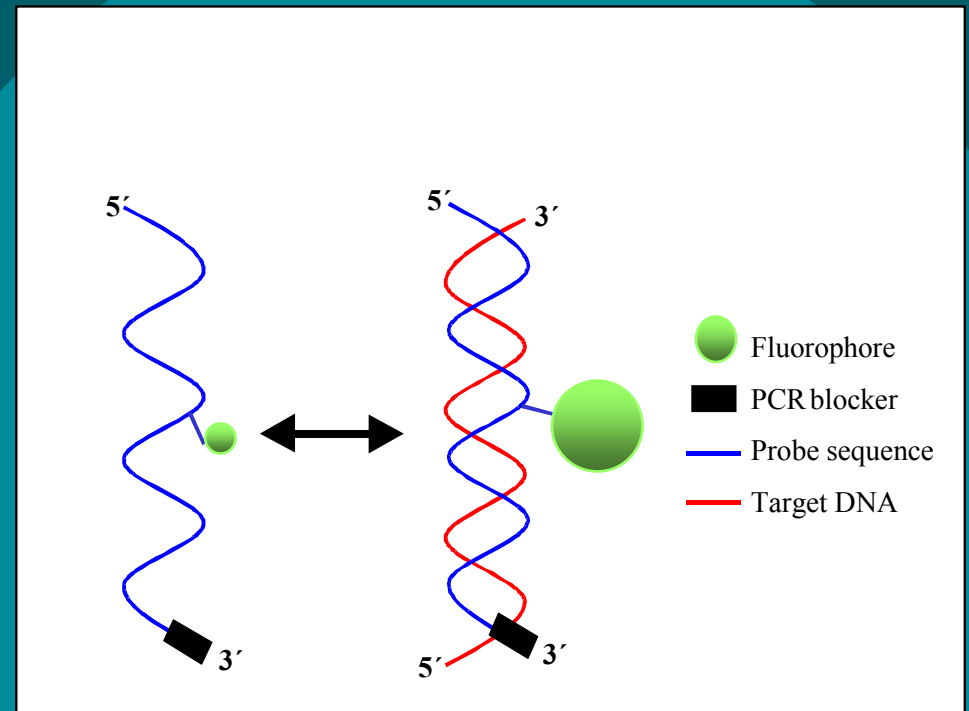
PCR cycles

— Real time

— 2 cycle + melt analysis

HyBeacon™ probes

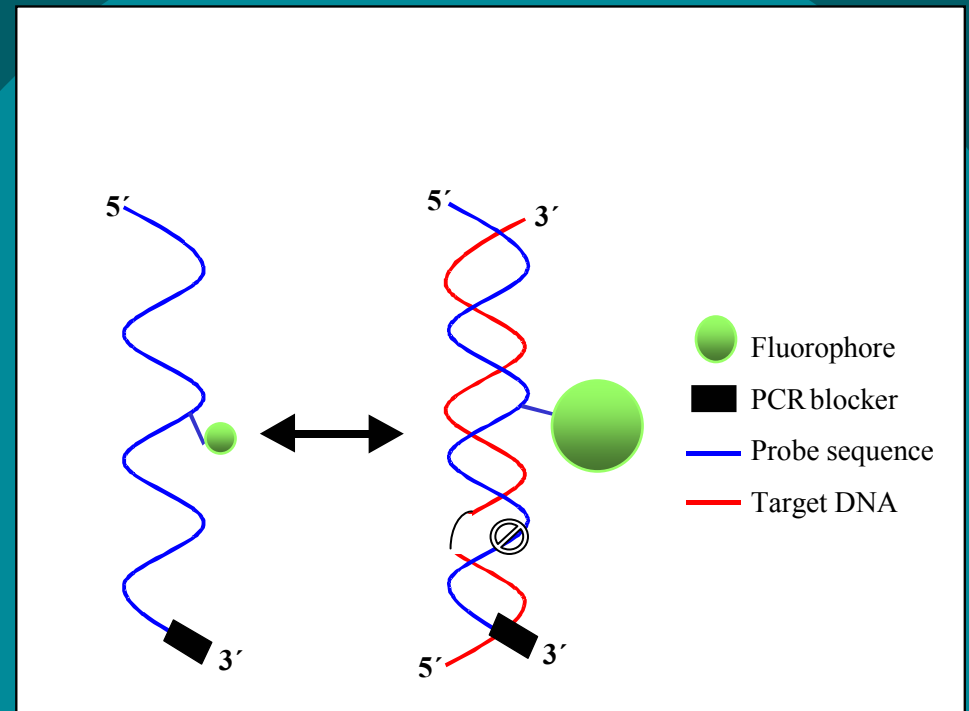
- Simple probe structure, linked only to a single internal 'reporter' fluorescent molecule
- Discovered light emitted increases as probe finds matching DNA
- Can work in PCR thermocycling reaction
- Diagnosis built around a temperature defined increase in light emission linked to binding to matched DNA



t°C

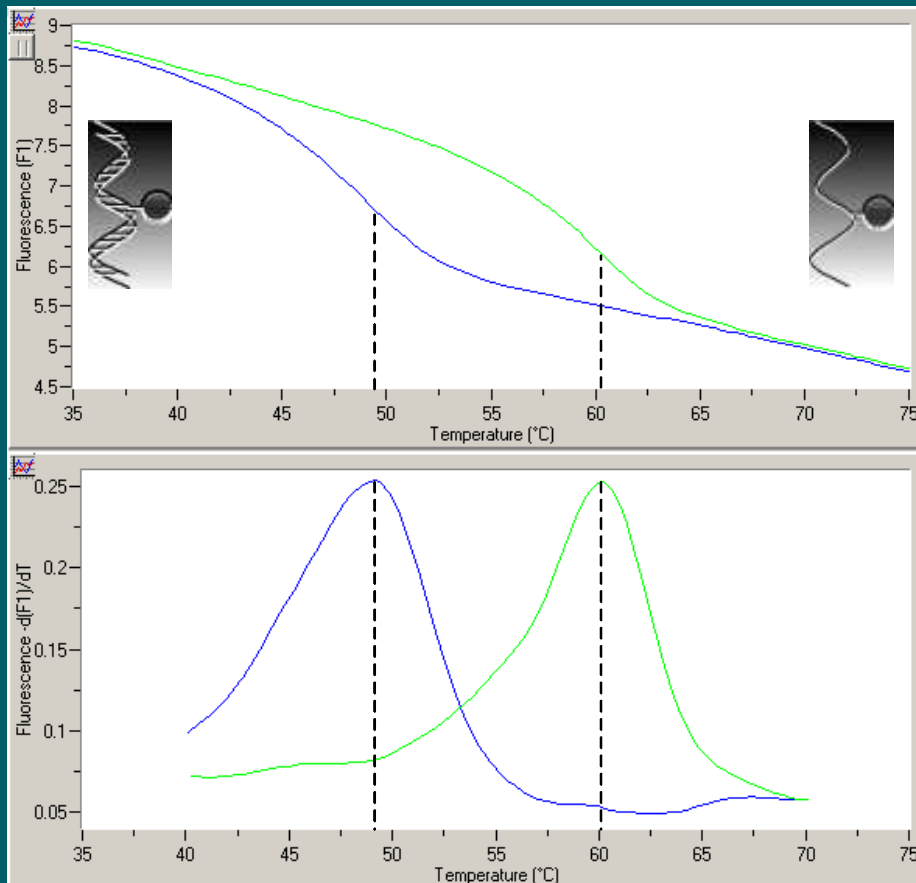
HyBeacon™ probes

- Simple probe structure, linked only to a single internal 'reporter' fluorescent molecule
- Discovered light emitted increases as probe finds matching DNA
- Can work in PCR thermocycling reaction
- Diagnosis built around a temperature defined increase in light emission linked to binding to matched **and unmatched** DNA



T°C -10°C

Melt Peak Analysis



- Melting temperature (T_m) of probe determined by the sequence of the target
- $T_m = 50\%$ hybridised probe
- Target detection & identification
- Magnitude of ΔT_m depends on nature of mismatch
 - Stable - G/G, G/A, G/T
 - Intermediate - T/T, A/A,
 - Destabilising - C/A, C/T, C/C



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Development of a CYP2D6*4 assay

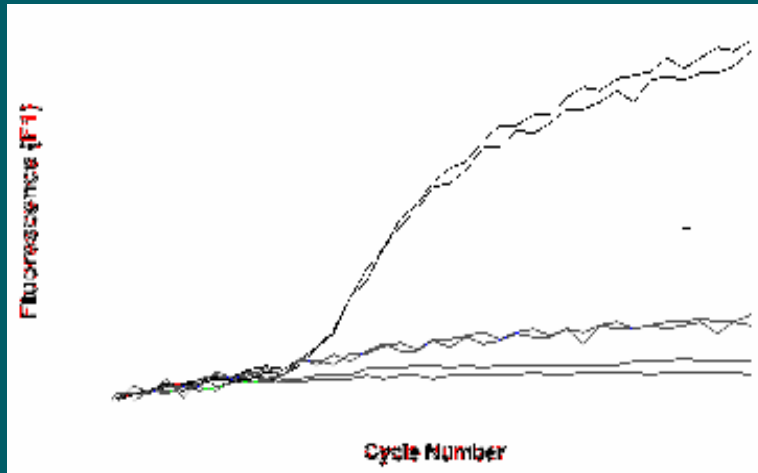


$T_m = 60.4^\circ\text{C} \pm 0.1^\circ\text{C}$

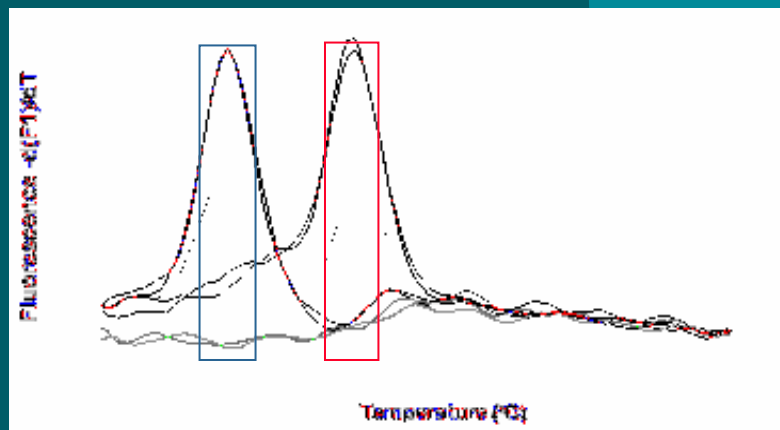


$T_m = 49.3^\circ\text{C} \pm 0.1^\circ\text{C}$

Detection and discrimination of CYP2D6*4 SNP by HyBeacons



- Red** - probe matches patient DNA
- Blue** - probe mismatches patient DNA
- Black** - matched and mismatched DNA present
- Green** - no DNA

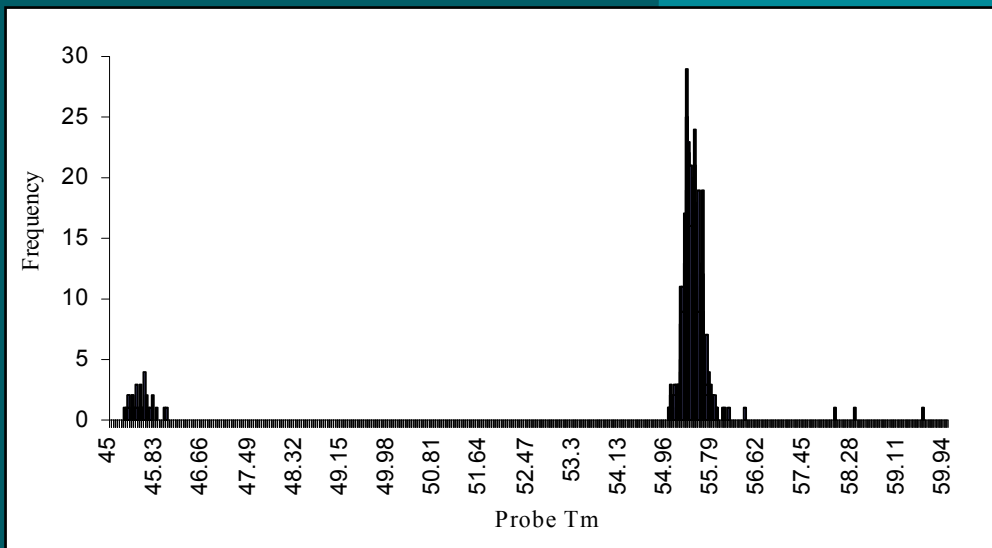
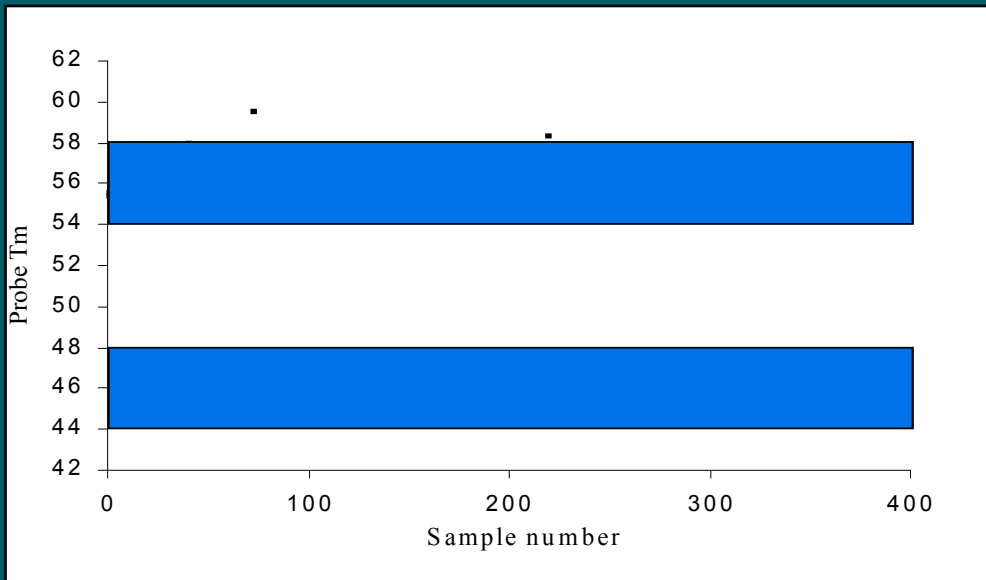


Confirmation test looks at quality of match with temperature



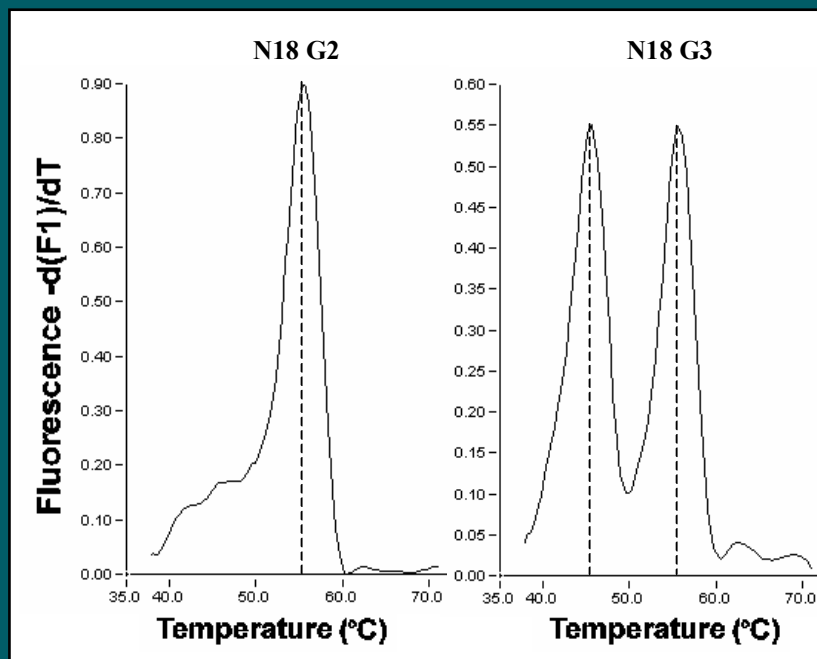
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Factor V Lieden, confidence of genotypes



- Diagnostic zones applied to Tm data
- 99.5% of data points within zones
- No data points between zones.
- Genotypes obtained with at least 99.9% confidence
- Demonstrated kit efficiency, reproducibility, accuracy and robustness

Factor V Leiden Assay



Step 1: Melting Peaks | Step 2: Peak Areas | Extra: Manual Tm

Number of Peaks
 Zero
 One
 Two
 Three

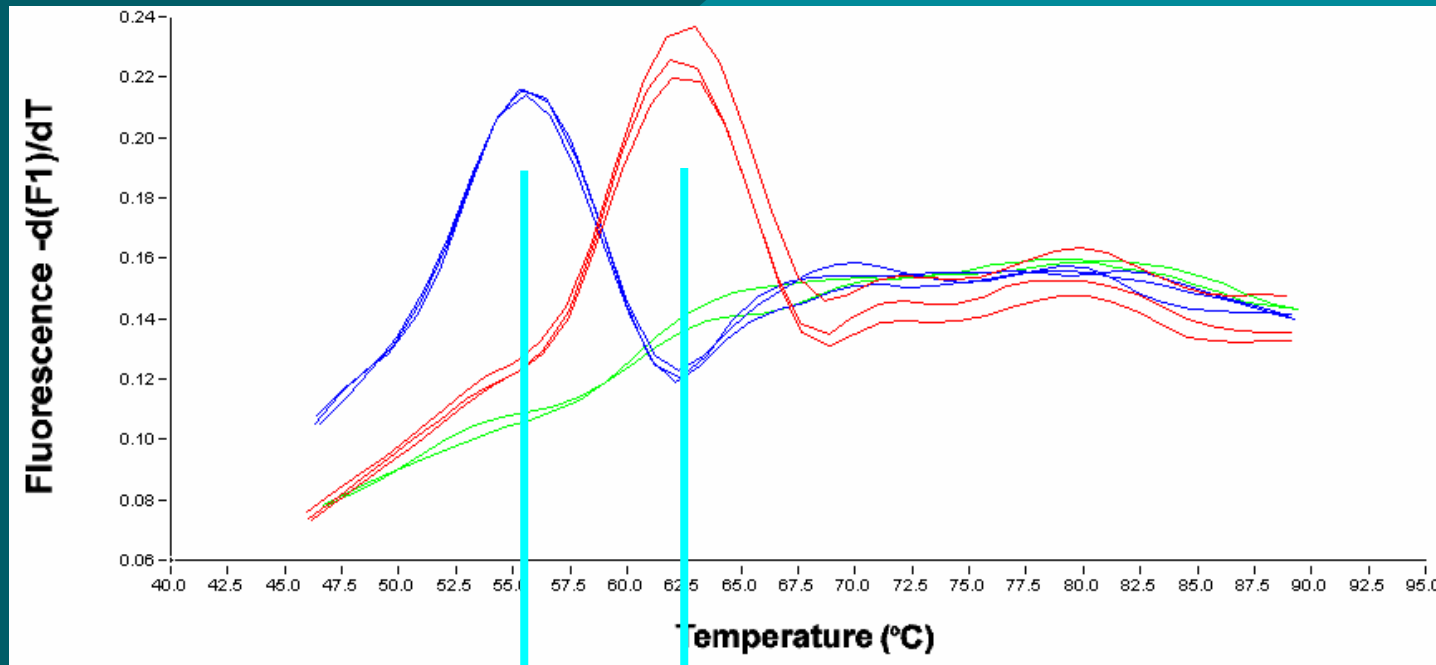
Clear All
 Weighted Fit

	Position	Name	Tm 1	Area 1	SD 1	Tm 2	Area 2	SD 2
—	1	n18 g2	55.34	4.706	2.093			
—	2	Repli. of n18 g2	55.34	4.626	2.070			
—	3	n18 g3	45.32	3.050	2.272	55.52	2.782	2.046
—	4	Repli. of n18 g3	45.31	2.798	2.167	55.57	2.543	1.930
—	5	n18 g4	55.36	4.436	2.108			
—	6	Repli. of n18 g4	55.34	4.215	2.133			
—	7	n18 g5	55.29	5.129	2.222			
—	8	Repli. of n18 g5	55.47	3.256	2.223			
—	9	n18 g6	55.31	4.971	2.180			
—	10	Repli. of n18 g6	55.35	4.569	1.974			
—	11	n18 g7	55.28	3.935	2.166			
—	12	Repli. of n18 g7	55.40	4.258	2.177			
—	13	n18 g8	55.42	5.129	2.195			
—	14	Repli. of n18 g8	55.37	4.787	2.191			
—	15	n18 g9	55.39	2.748	2.178			
—	16	Repli. of n18 g9	55.30	4.905	2.173			
—	17	n18 g10	55.33	5.051	2.134			
—	18	Repli. of n18 g10	55.38	4.656	2.105			
—	19	n18 g11	55.35	4.673	2.171			
—	20	Repli. of n18 g11	55.56	3.022	2.176			
—	21	n18 g12	55.30	4.993	2.226			
—	22	Repli. of n18 g12	55.46	4.818	2.080			
—	23	n18 h1	55.47	4.477	2.105			
—	24	Repli. of n18 h1	55.46	5.146	2.150			
—	25	n18 h2	55.44	4.767	2.055			
—	26	Repli. of n18 h2	55.49	4.081	1.850			
—	27	n18 h3	55.49	4.447	1.883			
—	28	Repli. of n18 h3	55.44	5.002	2.108			
—	29	n18 h4	55.40	3.943	2.011			
—	30	Repli. of n18 h4	55.41	3.949	2.116			
—	31	neg						
—	32	neg						



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Automated diagnosis



Control

Positive



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Development of a Chlamydia trachomatis assay



NNNNNNNCNNNNNNNNN

IIIIIIIIIIIIIIIIII

NNNNNNNNNNNNNGNNNNNNNNNNNNNNNN

$T_m = 55^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$



NNNNNNNCNNNNNNNNN

IIIIIII-III

NNNNNNNNNNNNANNNNNNNNNNNNNNNN

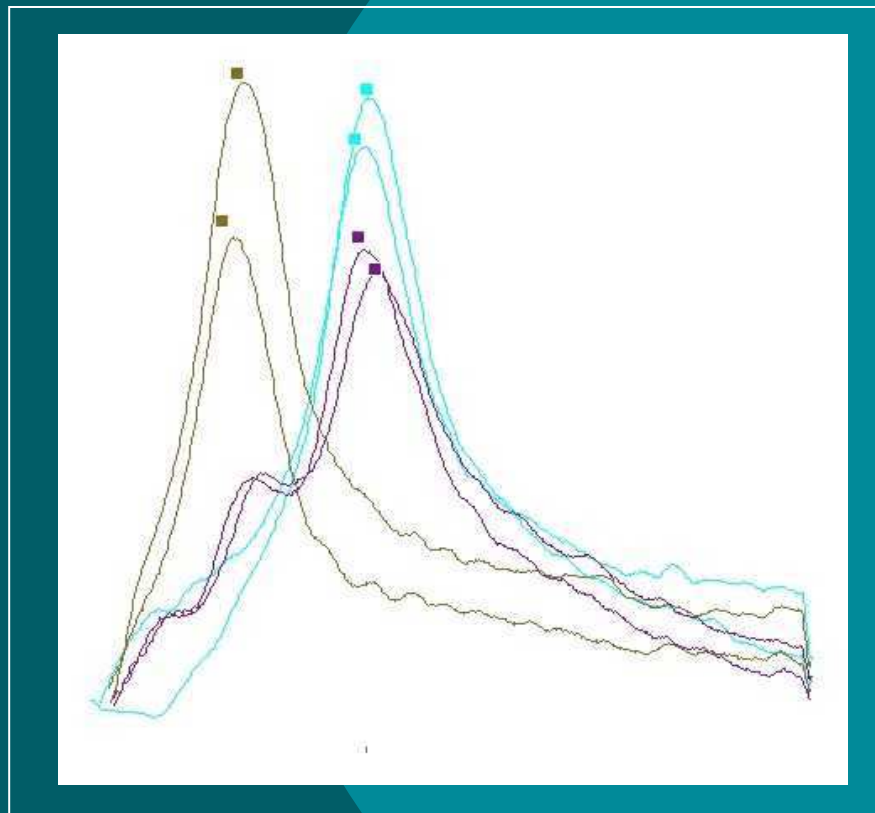
$T_m = 47. ^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$



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Chlamydia diagnostic with internal positive control

Fluorescence $-d(F1)/dT$



Temperature °C



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STR profiling

..GTATGCCATTG ATTG ATTG ATTG ATTGCCGGGCAT..

..GTATGCCATTG ATTG ATTGCCGGGCAT..

Each person has 2 copies of each STR Locus

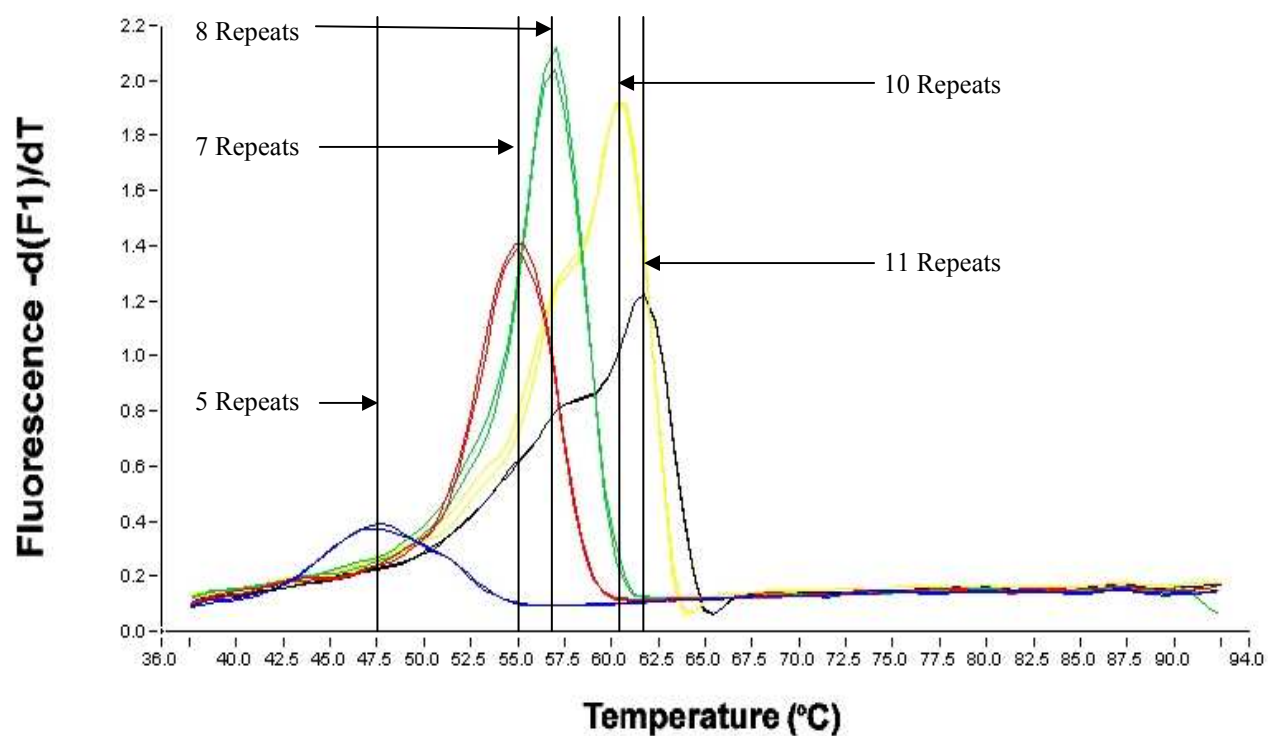


Person A
Type 10,12



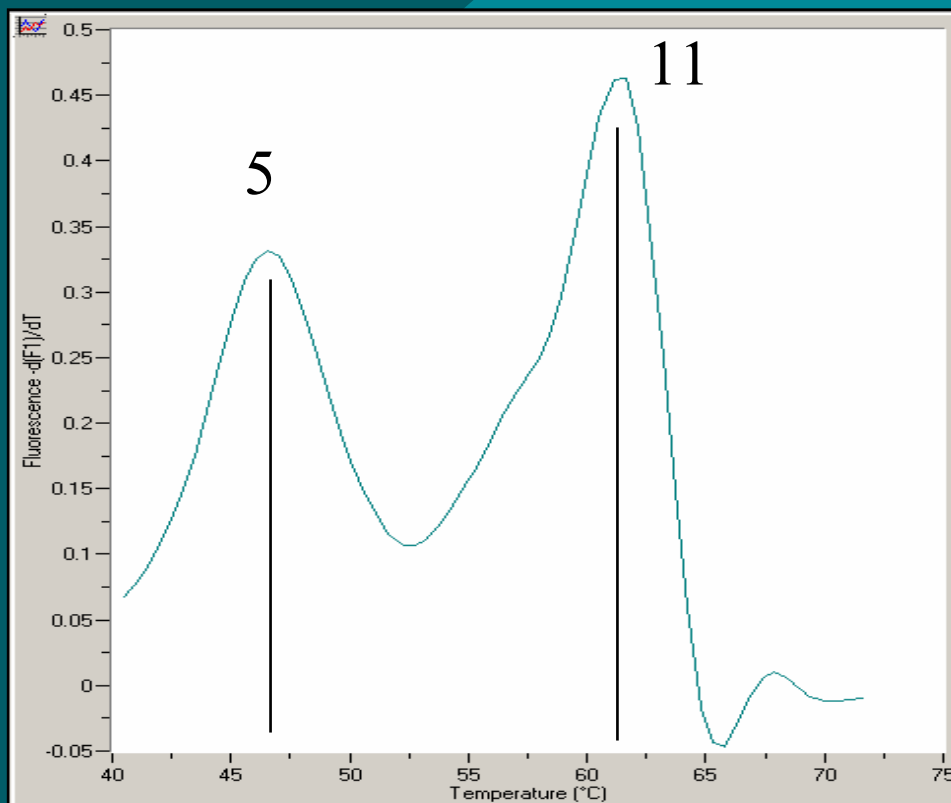
Person B
Type 6,8

Potential of HyBeacons to discriminate STRs of different repeat numbers



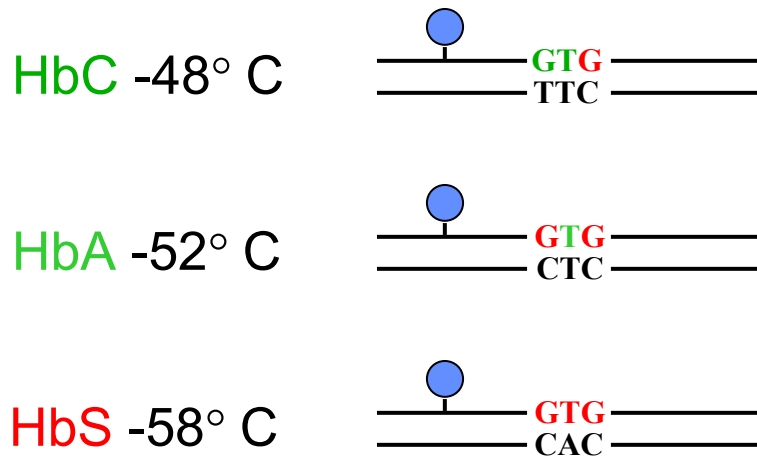
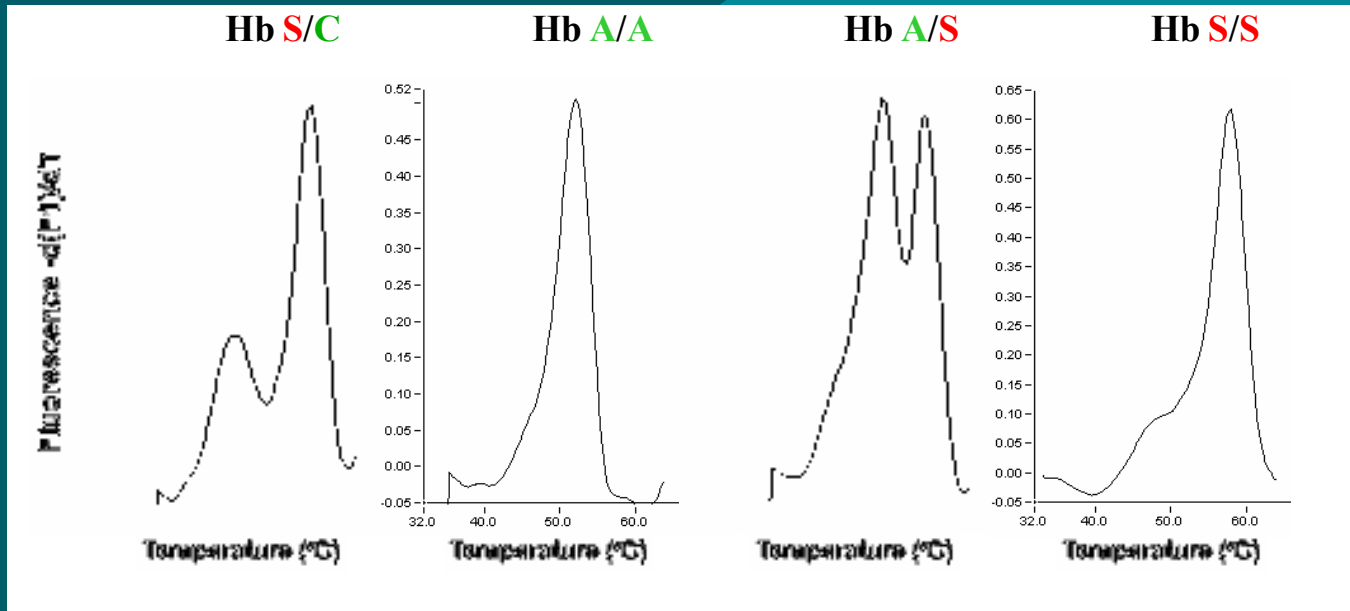
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STR profile direct from saliva



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Sickle Cell Anaemia



Freeze-dried reagents

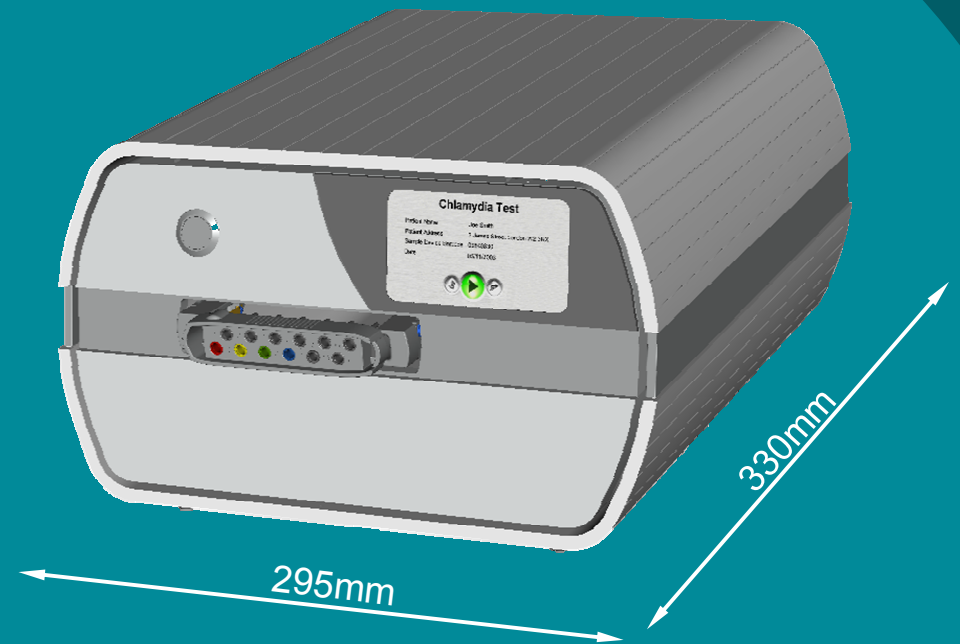


- Sample added directly to Genedrive cartridge
- Sample to result in ~20 minutes



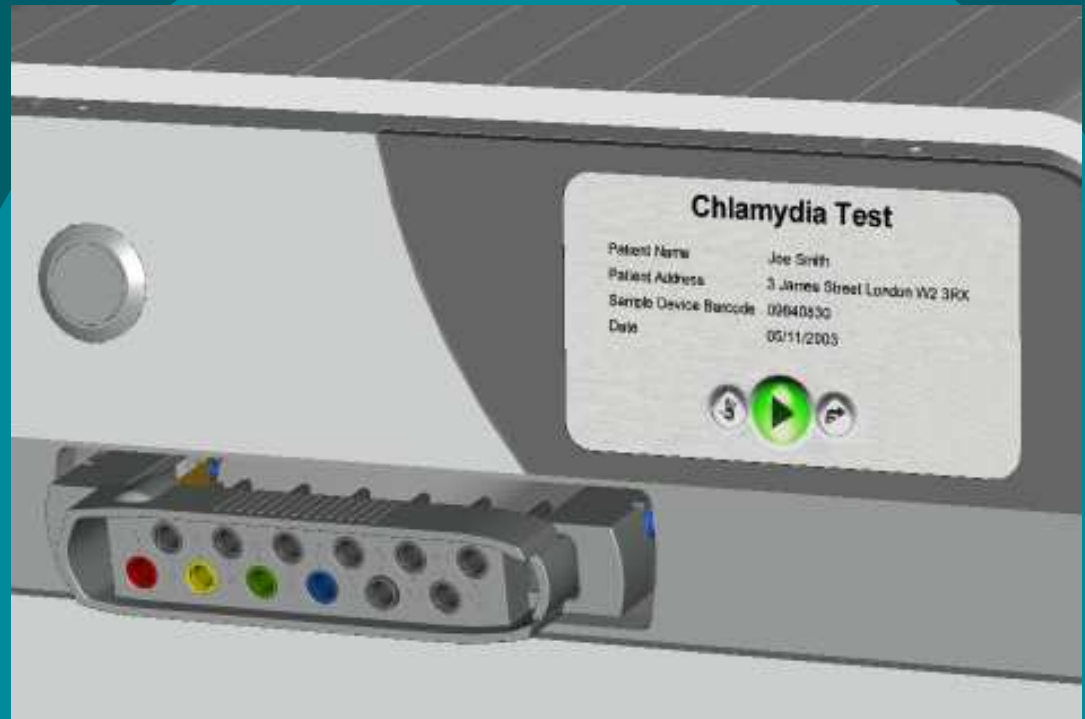
Genedrive[®] - key features

- 24 samples (2x12)
- Single use plastic disposable cartridge
- 50 cycles and melt analysis within 30 minutes
- Compatible with SYBR Green and Fluorescein (495/520nm)
- Highly sensitive optics



End-user Mode

- User interface on instrument
- Scan barcode on cartridge to instruct instrument which assay conditions to perform
- Automated data analysis and characterisation of samples as positive, negative or fail



HyBeacon assays

Target Example	Category
NAT2	Human - DNA
Influenza	Virus - RNA
Adenovirus	Virus - DNA
Streptococcus pneumoniae	Bacterium (Gram +ve) - DNA
Chlamydia trachomatis	Intracellular parasite - DNA



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