HyBeacon Probes

Genetic applications at the point-of-care

Dr David French
Traditional vs point-of-care diagnostics

- Sample taken at Doctor’s surgery or hospital
- Often invasive blood and swab samples
- Laboratory analysis
- Results back to Doctor
- Results to patient
- Diagnosis sometimes 1 week after initial consultation
- Inappropriate drugs may be employed if treated before diagnosis

- Sample provided at Doctor’s surgery, hospital or pharmacy
- Non-invasive saliva and urine samples
- On-site analysis within 20-30 minutes
- Diagnosis and appropriate treatment
- Correct drug, correct dosage and avoidance of adverse reactions
Rapid sample analysis

- Sample preparation
- PCR amplification
- PCR product detection and identification
- Results analysis

Eliminate
Speed up and detect target DNA simultaneously
Homogeneous PCR
Automate
Applications

- **Clinical**
  - Genetic diagnosis
  - Infectious disease detection
- **Pharmaceutical & Pharmacogenetics**
  - Polymorphisms associated with drug metabolism
- **Food**
  - Species identification
  - GM and pathogen detection
  - Selective breeding (e.g. Scrapie)
- **Forensic**
  - Scene-of-crime, point-of-arrest
- **Defence**
  - Bio-threat detection
HyBeacon probes

- Fluorescent probe technology
- Benefits over existing commercial systems
- Single probe to analyse homozygous and heterozygous samples
- The quenching properties of DNA reduces single-stranded probe emission
- Fluorescence increases upon target hybridisation
Differential probe hybridisation

- HyBeacon probes are significantly more stable when hybridised to fully complementary sequences
- Discrimination of closely related sequences on the basis of melting temperature (Tm)
- Tm = 50% of probe hybridised to target
- Clearly differentiates DNA sequences varying by as single nucleotide
HyBeacon fluorescence & sequence analysis
Direct sample analysis

- Purification of samples not required
- Suitable for point-of-care systems
- Achieved target amplification and detection directly from
  - Saliva
  - Urine
  - Swabs
  - Blood
Ultra-rapid detection and discrimination of \textit{NAT2} polymorphic targets from saliva

- \textit{*5C/*5C} \( T_m = 42.6{}^\circ\text{C} \) + 52.5{}^\circ\text{C} \\
- \textit{*4/*5C} \( T_m = 52.4{}^\circ\text{C} \) \\
- \textit{*4/*4} \( T_m = 42.3{}^\circ\text{C} \)

\[ \begin{array}{c}
\text{Fluorescence} \\
\text{Temperature (\textdegree\text{C})} \\
\text{M} & *4/*4 & *4/*5C & *5C/*5C & M
\end{array} \]

- 121bp
- 98bp
- 58bp
- 23bp
Factor V Leiden

- SNP in factor V gene strongly associated with increased risk for:
  - deep vein thrombosis (DVT)
  - pulmonary embolism
- 5-10 and 50-100 fold increased risk in heterozygous and homozygous individuals respectively

One in 30 long-haul passengers ‘gets DVT’

By James Chapman
Science Correspondent

One in every 30 healthy passengers develop a blood clot on board a long-haul flight, doctors warn today, warning of an international conglomerate of medical experts that the risk is more common than has been previously estimated. The risk is particularly high for those who are flying more than 4 hours, and there is an increased risk for passengers who are pregnant or have a history of blood clots. The risk is also higher for those who are taking blood-thinning medication, such as those with a history of deep vein thrombosis (DVT) or pulmonary embolism (PE). The study found that even short flights, such as those on domestic flights, can increase the risk of blood clots. The findings are important as they highlight the need for better guidelines for passengers and airline staff to prevent blood clots. The researchers recommended that passengers take regular breaks, carry water to stay hydrated, and avoid sitting in the same position for too long.

The study was conducted by a team of researchers from the University of California, Los Angeles, and published in the Journal of the American Medical Association. The researchers followed a group of 300 passengers on long-haul flights and monitored their blood clot risk over a period of 24 hours. They found that even short flights, such as those on domestic flights, can increase the risk of blood clots. The findings are important as they highlight the need for better guidelines for passengers and airline staff to prevent blood clots. The researchers recommended that passengers take regular breaks, carry water to stay hydrated, and avoid sitting in the same position for too long.

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Analysis of factor V Leiden

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**Graph: Flourescence vs Temperature**

- **N18 G2**
  - Sample 1: 55.49, 4.447, 1.822
  - Sample 2: 55.48, 4.447, 1.822

- **N18 G3**
  - Sample 1: 55.04, 5.002, 2.138

- **NTC**
  - Sample 1: 55.40, 3.843, 2.011

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**Legend:**
- Green: Sample 1
- Red: Sample 2
- Blue: Control
Pathogen detection

- *Chlamydia trachomatis*
- Infections can be asymptomatic and may cause sterility if untreated
- High costs to health service
- Patient may not return for results and treatment if diagnosis is slow
- Point-of-care facilities may improve treatment rate
- Efficient HyBeacon assay developed capable of detecting pathogen in urine and swab samples
Automated sample analysis

- Positive amplification control required to distinguish negative samples from PCR fails
- Single nucleotide substitution introduced into mimic construct
- Target and mimic amplified and detected with same primers and probe

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<th>INFECTED</th>
<th>INFECTION STATUS</th>
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<td></td>
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Chlamydia peak generated in negative control
No peaks generated
Both peaks generated
Only control peak generated
• Genedrive
• Single use plastic consumables
• Developing simple to use touch screen instrument
• 50 cycles of PCR and melt analysis within 30 minutes
• Suitable for point-of-care tests
Patient sample

Diagnosis & treatment

Cartridges containing freeze-dried reagents

20 minutes processing
CHLAMYDIA
INFLUENZA
CHLAMYDIA
GONORRHEA
NAT2
FACTOR V
ADENOVIRUS
S.PNEUMONIAE
SICKLE CELL ANAEMIA

ADDRESS

DATE 15TH SEPTEMBER 2004

CONFIRM DETAILS AND ANALYSE
RUNNING: PLEASE WAIT

ACTIVE SITES: 4

TIME REMAINING: 19 MINUTES
TEST PERFORMED: CHLAMYDIA

PATIENT DETAILS

NAME: JOE BLOGGS

ADDRESS:

ADDRESS:

ADDRESS:

DATE:

VALID:

INFECTION:

PRINT REPORT
High Throughput screening

• Melt analysis also possible in 96 and 384 well plate formats
• Sheep prion protein (PrP) gene mutations (Scrapie)
Summary

- Novel fluorescent probe technology
- Rapid sequence analysis
  - Pharmacogenetics
  - Disease predisposition
  - Healthcare
  - Pathogen detection
- Non-invasive tests with point-of care application
- Also suited to high throughput screening
Acknowledgements

• LGC
  – David McDowell
  – Gavin Nixon
  – Rajinder Flora
  – Paul Debenham

• Osmetech
  – John Clarkson

• University of Southampton
  – Professor Tom Brown