The Addition of Pancreatitis Associated Protein (PAP) in a Two-Tier IRT/DNA Screening Strategy for Cystic Fibrosis is Less Effective in Programs that Screen at 48 hours of Age.

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<table>
<thead>
<tr>
<th>Summary Statistics</th>
<th>2008/2009</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Budget</strong></td>
<td>36.5 million</td>
</tr>
<tr>
<td><strong>Emergency attendances</strong></td>
<td>55,502</td>
</tr>
<tr>
<td>- Women</td>
<td>20,850</td>
</tr>
<tr>
<td>- Children</td>
<td>35,652</td>
</tr>
<tr>
<td><strong>Admissions</strong></td>
<td>41,595</td>
</tr>
<tr>
<td>- Women</td>
<td>19,480</td>
</tr>
<tr>
<td>- Children</td>
<td>22,115</td>
</tr>
<tr>
<td><strong>Births</strong></td>
<td>5,895</td>
</tr>
<tr>
<td><strong>Beds</strong></td>
<td>316</td>
</tr>
<tr>
<td>- Women</td>
<td>123</td>
</tr>
<tr>
<td>- Children</td>
<td>220</td>
</tr>
<tr>
<td>- ICU/SC</td>
<td>54</td>
</tr>
<tr>
<td><strong>Average bed Occupancy</strong></td>
<td>91.5%</td>
</tr>
</tbody>
</table>
Neonatal Screening Laboratories in Australia

Each year 265,000 Australian babies are screened at or near 48hrs in 5 Specialist Paediatric Hospital Centres. Metabolic Clinic with specialist clinical expertise for treatment and monitoring of IEM.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Australia</th>
<th>South Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (,000 km²)</td>
<td>7,682</td>
<td>984</td>
</tr>
<tr>
<td>Population (millions)</td>
<td>21.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Residents/km²</td>
<td>2.33</td>
<td>1.4</td>
</tr>
<tr>
<td>Urban Pop’n (millions)</td>
<td>1.2</td>
<td>86%</td>
</tr>
<tr>
<td>Health Costs ($m)</td>
<td>$41,742</td>
<td></td>
</tr>
<tr>
<td>Health Costs/Person</td>
<td>$2,333</td>
<td></td>
</tr>
</tbody>
</table>

Newborn screening laboratories in Australia within Paediatric Tertiary Hospitals
AIMS of the Study

➢ To determine the value of adding Pancreatitis-Associated protein (PAP) in a newborn screening strategy for CF.

➢ Does adding PAP to an existing two-tier IRT/DNA strategy improve CF screening:
  - through review of the:
    • correlation between PAP and CF
    • association between elevated PAP and CFTR carriers
    • correlation of the level of PAP :
      » with birth weight & age at collection
      » Specifically at, or near 48h of age
Two-Tier IRT/DNA CF Screening Strategy

- A two-tier IRT/DNA screening strategy is in use in all Australian\New Zealand newborn screening laboratories
  - Has been in operation in South Australia since December 1989.

Two-Tier IRT/DNA CF Screening Strategy

Screening Strategy relies upon:

- **First Tier**: Generous Immunoreactive Trypsin (IRT) cut-off point
  - Top 1%

- **Second Tier**: High frequency of common CFTR mutations
  - p.F508del ~ 72% of CF Chromosomes in our population

Two-Tier IRT/DNA CF Screening Strategy

Screening Strategy relies upon:

- **First Tier**: Generous Immunoreactive Trypsin (IRT) cut-off point
  - Top 1%
- **Second Tier**: High frequency of common CFTR mutations
  - p.F508del ~ 72% of CF Chromosomes in our population

Detection/miss rate

- **Predicted up to 6% of CF neonates missed**
  - IRT<99th centile
  - no CFTR mutations
- **Sweat-testing requires expertise**
  - Sufficient number of tests (ideally centralised)
  - Appropriate age-related normal ranges (>4 weeks old to adults)
- Co-ordinated, timely Genetic Counselling

SA NEONATAL CF SCREENING PROGRAMME

Two-Tier IRT/ DNA Screening Strategy

1st Tier:
- IF IRT $\geq 99^{th}$ CENTILE

2nd Tier:
- 2 mutations
  - confirm with sweat-test
    - CF
    - 2 known mutations
- 1 mutation
  - diagnostic sweat-test
    - CF
    - 1 known mutation
- no mutation
  - no follow up
  - CF carrier
  - CF like disease
SA NEONATAL CF SCREENING PROGRAMME

Two-Tier IRT/ DNA Screening Strategy

1st Tier: IF IRT \geq 99^{th} \text{ CENTILE}

- GENOTYPE
  - 2 mutations
    - confirm with sweat-test
      - CF
        - 2 known mutations
  - 1 mutation
    - diagnostic sweat-test
      - CF
        - 1 known mutation
      - no mutation
        - no follow up
      - CF carrier
        - CF like disease
**Sweat Test in IRT/DNA Screened Population**

Screened cohort with IRT > 99th centile and one or two CFTR mutations.
Sweat Test in IRT/DNA Screened Population

Screened cohort with IRT>99th centile and one or two CFTR mutations

- **Normal**
- **Equivocal**
- **CF**
## South Australian CF Screening Programme Performance Data

<table>
<thead>
<tr>
<th>Description</th>
<th>Number Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants screened</td>
<td>477,904</td>
</tr>
<tr>
<td>IRT &lt;99th</td>
<td>472,169</td>
</tr>
<tr>
<td>DNA mutation analysis performed</td>
<td>5,735 (1.2%)</td>
</tr>
<tr>
<td>No identifiable mutation</td>
<td>5,243</td>
</tr>
<tr>
<td>Two identifiable mutations</td>
<td>94</td>
</tr>
<tr>
<td>One identifiable mutation</td>
<td>398</td>
</tr>
<tr>
<td>Sweat test positive</td>
<td>42</td>
</tr>
<tr>
<td>Sweat test negative</td>
<td>356</td>
</tr>
<tr>
<td>Carrier frequency</td>
<td>1 in 13</td>
</tr>
<tr>
<td>Total number of CF infants detected</td>
<td>136</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>34%</td>
</tr>
<tr>
<td>Missed (presentation 2 -12 years of age)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>Normal IRT</td>
<td>3</td>
</tr>
<tr>
<td>Elevated IRT no identified CF mutation</td>
<td>4</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95%</td>
</tr>
<tr>
<td>Apparent incidence of detected CF infants</td>
<td>1: 3,515</td>
</tr>
<tr>
<td>Prenatal diagnosis and termination</td>
<td>26</td>
</tr>
<tr>
<td>Overall prevalence of CF</td>
<td>1: 2,770 (162 cases)</td>
</tr>
</tbody>
</table>
**NSW CF Screening Programme performance data**

<table>
<thead>
<tr>
<th>Description</th>
<th>Number Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babies screened</td>
<td>925,094</td>
</tr>
<tr>
<td>Tested by PCR</td>
<td>10,275</td>
</tr>
<tr>
<td>CF</td>
<td>296</td>
</tr>
<tr>
<td>p.F508del/p.F508del</td>
<td>168</td>
</tr>
<tr>
<td>p.F508del/other</td>
<td>113</td>
</tr>
<tr>
<td>Terminations</td>
<td>8 (up to 1999)</td>
</tr>
<tr>
<td>Apparent incidence</td>
<td>1:3,000</td>
</tr>
<tr>
<td>Missed, False negatives</td>
<td>18 (5%)</td>
</tr>
<tr>
<td>Normal IRT</td>
<td>6</td>
</tr>
<tr>
<td>Elevated IRT no p.F508del CFTR mutation</td>
<td>12*</td>
</tr>
<tr>
<td>p.F508del/other, negative sweat test</td>
<td>595</td>
</tr>
<tr>
<td>Carrier frequency</td>
<td>1 in 13</td>
</tr>
<tr>
<td>Expected overall number of CF</td>
<td>354</td>
</tr>
</tbody>
</table>

*Data provided by Dr. Veronica Wiley NSW Newborn Screening Programme*
Neonatal Screening for CF

- Pancreatitis-Associated Protein (PAP) has been reported to be elevated in newborn infants with CF
Neonatal Screening for CF

- Pancreatitis-Associated Protein (PAP) has been reported to be elevated in newborn infants with CF.
  - Sarles et al / Pediatr. 147, 302-305 2005

**Suggested IRT/PAP CF screening strategy**

- All newborns are tested for IRT:
  - Those with levels >50mg/L tested for PAP.
  - Those with PAP > 1.8ng/mL and with PAP > 1.0ng/mL, and IRT > 100ng/mL Recalled for sweat-testing
Pancreatitis-associated protein (PAP)- a screening marker for CF?

- PAP
  - A lectin-related secretory protein present in small amounts in normal pancreas and over expressed during the acute phase of pancreatitis.
  - In animal models PAP is constitutively expressed in the intestinal tract, but not in other tissues. PAP mRNA could not be evidenced in liver, stomach, salivary glands, brain, kidney or testis.
  - Its pattern of expression during severe pancreatic aggression suggests that it might be a stress protein involved in the control of bacterial proliferation.
  - PAP has been suggested to be a marker of ‘pancreatic sufficiency’ in individuals with CF
Two-Phase Study Design

- **Phase I:** to determine South Australian newborn population statistics for PAP.
Two-Phase Study Design

– **Phase I**: to determine South Australian newborn population statistics for PAP.

– **Phase II**: to include selected samples from the screening programmes in other Australian states (NSW, QLD & VIC) to form a screen cohort of ~195,000 samples.

• **Selected for CFTR mutational analysis**
  - Top 1% and/or >2.5MoM of IRT values

• Determination of PAP & repeat IRT in South Australia on coded whole blood-spot samples

• Stratify by:
  » CF mutational analysis
  » Sweat-test negative
  » CFTR carriers
Australian PAP Study Phase I

Phase I

• Modification of PAP assay (MucoPAP®, DYNABIO) to use Eu³⁺ labelled strepavidin.

• South Australian Newborn Population
  – Establish normal PAP population distribution and determine levels for the 90ᵗʰ, 95ᵗʰ & 99ᵗʰ centiles

• Cohort
  – 2,885 unselected newborn specimens
    » Normal population statistics
    » CFTR carriers
Population distributions for IRT and PAP on the same samples

- **SA population**
  - Establish population reference intervals for IRT and PAP on 2,885 consecutive blood-spot samples
    - 90th, 95th & 99th percentiles
  - Stratify against age at collection birth weight preterm and low gestational age.

<table>
<thead>
<tr>
<th>PAP Percentiles</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>90</th>
<th>95</th>
<th>97</th>
<th>99</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.085</td>
<td>0.135</td>
<td>0.21</td>
<td>0.48</td>
<td>0.59</td>
<td>0.70</td>
<td>1.07</td>
<td>2,530</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IRT Percentiles</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>90</th>
<th>95</th>
<th>97</th>
<th>99</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.0</td>
<td>12.0</td>
<td>18.0</td>
<td>37.0</td>
<td>45.0</td>
<td>50.8</td>
<td>61.0</td>
<td>2,530</td>
</tr>
</tbody>
</table>
Correlation of IRT and PAP against Age at Collection & Birth Weight

(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations.)
“Clinical Study” Phase II

- Participation by the NSW, QLD & VIC Neonatal Screening Laboratories
  - Provided prospectively collected coded dried blood-spot samples
    - *Selected IRT population* $\geq 99^{th}$ centile and or $>2.5$ MoM
      - 3 blood spots for each case sent as a weekly batch to the SANSCL laboratory for analysis.
        » Estimated 1x IRT & 2 x PAP
    - Statistical analysis
      - To ascertain sensitivity & specificity
Phase II Cohort

• “Clinical study” cohort (N=1,979 specimens) with
  IRT≥ 99th centile and/or >2.5MoM

  – 1,812 No CFTR mutations
  – 119 with a single CFTR mutation
  – 48 specimens from infants with CF (47)
Phase II: IRT versus PAP

Phase II: IRT versus PAP

90th 95th 99th

500
400
300
200
100
0

Immunoreactive Trypsin (IRT; µg/L whole blood)

Pancreatitis Associated Protein (PAP; µg/L)

N=1,979
(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested).
IRT comparison between different groups

Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested.
PAP comparison between different groups

(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested)
PAP comparison between different groups

(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested)
PAP comparison between different groups

(Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested)
PAP comparison between different groups

Plot represents blood-spot samples from the unpartitioned 2,888 in addition to selected cases where N08 represents 8 CFTR mutations tested.
Correlation between IRT and PAP in Neonates with CF

<table>
<thead>
<tr>
<th>Allele Combination</th>
<th>Immunoreactive Trypsin (IRT; µg/L whole blood)</th>
<th>Pancreatitis Associated Protein (PAP: µg/L)</th>
</tr>
</thead>
</table>

90th 95th 99th

99th 95th 90th
Correlation between IRT and PAP in Neonates with CF (p.F508 del homozygous)
Correlation between IRT and PAP in Neonates with CF

Immunoreactive Trypsin (IRT; µg/L whole blood) vs. Pancreatitis Associated Protein (PAP; µg/L)

- p.F508del/X
  - Age at collection
    - 1 = day 2
    - 2 = day 9

- X/X
  - (No CFTR mutations)

90th 95th 99th

0 100 200 300 400 500

0 1.0 2.0 3.0 4.0
IRT and PAP in Infants with CF at Age of Collection

(IRT (not statistically significant at the p<0.05 level)
(Non-parametric K-W median test)
IRT and PAP in Infants with CF at Age of Collection

IRT

<table>
<thead>
<tr>
<th>Age at Collection (days)</th>
<th>IRT (µg/L whole blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

(not statistically significant at the p<0.05 level)

(Non-parametric K-W median test)

PAP

<table>
<thead>
<tr>
<th>Age at Collection (days)</th>
<th>PAP (µg/L whole blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

(statistically different at p<0.05)
Additional studies of PAP level in Neonates with CF

Pancreatitis Associated Protein (PAP µg/L whole blood)

Age at Collection (Days)
Additional studies of PAP level in Neonates with CF

Pancreatitis Associated Protein (PAP µg/L whole blood)

Age at Collection (Days)

- p.F508del/p.F508del
- p.F508del/p.G551D

99th percentile: 5.0 µg/L
95th percentile: 1.0 µg/L
## IRT/DNA versus IRT/PAP/DNA

<table>
<thead>
<tr>
<th>Total</th>
<th>1,978</th>
</tr>
</thead>
<tbody>
<tr>
<td>From a projected newborn screened population of ~195,000</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>IRT ≥ 99th percentile</th>
<th>PAP ≥ 95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR Carrier</td>
<td>119</td>
<td>25</td>
</tr>
<tr>
<td>CFTR carrier Frequency</td>
<td>1 in 16</td>
<td>1 in 80</td>
</tr>
</tbody>
</table>

### CF

<table>
<thead>
<tr>
<th>CFTR genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 p.F508del/p.F508del</td>
</tr>
<tr>
<td>2 p.F508del p.85E</td>
</tr>
<tr>
<td>1 p.F508del p.G542X</td>
</tr>
<tr>
<td>1 p.F508del p.G511D</td>
</tr>
<tr>
<td>10 p.F508/X</td>
</tr>
<tr>
<td>2 p.R553X/X</td>
</tr>
<tr>
<td>1 p.F508 del/p.262 263delT</td>
</tr>
<tr>
<td>2 p.F508del/p.N1303K</td>
</tr>
<tr>
<td>1 p.F508del/p.R1157H</td>
</tr>
<tr>
<td>1 p.F508del/1078delT</td>
</tr>
<tr>
<td>1 X/X</td>
</tr>
</tbody>
</table>

### Detected

<table>
<thead>
<tr>
<th>46</th>
<th>37</th>
</tr>
</thead>
</table>

### Missed by primary analyte

<table>
<thead>
<tr>
<th>1</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>X/X</td>
<td></td>
</tr>
</tbody>
</table>
Better discrimination by using a product of IRT */ PAP

Evidence that IRT and PAP are independent markers of CF
- Combination of IRT & PAP may provide better discrimination
  - IRT * PAP
  - IRT – (IRT * PAP) (Proposed by M Stopsack, Dresden Germany at the 7th ISNS, August 2011)
? Better discrimination by using a product of IRT */ PAP

Given that IRT and PAP show independence as markers of CF
• Combination of IRT & PAP may provide better discrimination
  • IRT * PAP
  • IRT – (IRT * PAP) (Proposed by M Stopsack, Dresden Germany at the 7th ISNS, August 2011)
Summary

- PAP in dried whole blood-spots:
  - Elevated in a percentage of sick-preterm infants.
  - Independent of IRT level in non-CF infants.
  - No discernable correlation with either birth weight or age at collection in normal (non-CF) infants.
  - Levels decline on storage at room temperature.
  - Levels appear to increase over time in infants with CF.
Summary

- Phase II Clinical Study.....ongoing

✓ PAP reduces the number of infants identified as a CFTR carrier
  ✓ For p.F508del 1 in 80 versus the 1 in 16 as seen with IRT≥99th centile.

  ✓ Reduce the number of sweat-tests performed.
  ✓ Likely to reduce the number with equivocal sweat test and mild “CF” disease.
  ✓ Reduce cost of sweat-testing
Phase II Clinical Study.....ongoing

- PAP reduces the number of infants identified as a CFTR carrier
  - For p.F508del 1 in 30 versus the 1 in 13 as seen with IRT≥99th centile.
    - Reduce the number of sweat-tests performed.
    - Likely to reduce the number with equivocal sweat test and mild “CF” disease.
    - Reduce cost of sweat-testing

- Evidence that an elevation of PAP in infants with CF is independent of both the IRT & CFTR genotype.

- PAP is elevated in most infants with CF.
  - BUT a significant number of infants with CF have a PAP <90th centile on samples collected at 2 days of age.
Summary

- PAP in dried blood-spots:
  - This study does not support the clinical utility of adding PAP to our single-sample IRT/DNA protocol, given our early age of sample collection (<48 hours).
Summary - continued

- CF Programmes may find PAP *useful*-  
  - that collect samples at a later age, >72 hours of age  
  - that adopt a 2\textsuperscript{nd} specimen screening strategy

- CF Programmes are *unlikely* to find PAP useful-  
  - That collect a single sample at or near 48 hours,  
    (optimal for MSMS screening)

- A complex algorithm would be required to develop an IRT/PAP/DNA CF screening strategy
Acknowledgment

- South Australian Neonatal Screening Centre (SANSC)
- Clinical Director Genetic & Molecular Pathology
  - Janice Fletcher
- Queensland (QLD) Newborn Screening Laboratory
  - Andrew Thomas
- New South Wales (NSW) Newborn Screening Laboratory
  - Veronica Wiley
- Victorian (VIC) Newborn Screening Laboratory
  - James Pitt
  - Nick Tsianitos

- PerkinElmer Life & Analytical Sciences, Turku, Finland.
  - Marika Kase
  - Petri Huhtinen
- INSERM Marseille, France/DYNABIO S.A
  - J-C Dagorn
p.F508del carrier frequency in elevated IRT

(G Travert, Caen France)
A possible IRT/PAP/DNA CF Screening Strategy?

DBS

IRT

< 95th %ile

≥95th %ile

≥99th %ile

≥99.5th %ile

PAP < 90th %ile

PAP ≥90th %ile

PAP ≥??th %ile

PAP >95th %ile

DNA mutation analysis

No CFTR mutations

1 CFTR mutation

2 CFTR mutations

CF screening NEGATIVE

Sweat-Test

Negative

Positive

CF Screening POSITIVE referral CF centre