Implementation of Cytocell Aquarius FAST FISH Prenatal Enumeration probe kit

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Introduction

• Chromosomal abnormalities are responsible for more than 50% of first trimester spontaneous abortions
• Numerical chromosome imbalances (aneuploidies) are the most common cause of spontaneous abortion
  – aneuploidies of 13, 18, 21, X, Y account for 95% of the chromosomal aberrations
• Rapid aneuploidy detection methods allow prenatal test results to be available within 3 days
  – Abnormal results may be important in clinical decision-making
  – Normal results alleviate parental anxiety
• Current ACGS Best practice guidelines
  – 95% of rapid test results reported within 3 calendar days of sample receipt in the laboratory
Introduction

• Why introduce Cytocell FAST FISH Prenatal Enumeration Probe kit
  – General Hospital Maternity Unit
    • Fetal anomaly screening for Down's, Edward's and Patau's Syndromes
    • Samples are often received later Thursday p.m.
  – Issues with previous commercial prenatal FISH probes
  – Possibility to repeat an uninformative/failed FISH test and still report it on time
  – To provide preliminary aneuploidy detection results as soon as possible
Objective

- Implement Cytocell Aquarius FAST FISH Prenatal Enumeration Probe kit on different types of sample.

The probe set is intended for the detection and quantification of chromosomes 13, 18, 21, X and Y in interphase nuclei of uncultured amniotic fluid cells by FISH.
Validation

• Current best practice guidelines
  • FISH probes to be used on interphase nuclei only must be validated on metaphase chromosomes

• *In house* validation
  – Known FISH normal/abnormal cases
    – Probe signals visible in interphase nuclei
    – Correct signal patterns
    – FISH Quality Score (0-3)
      » 0=FAIL
      » 1=Signals require use of single filters/capturing on an image analysis system to confirm presence
      » 2=Signals visible on double filter (single filter maybe used for reassurance)
      » 3=Signals very bright, easily visible on double filter/triple filter
# Validation

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample type</th>
<th>Previous result</th>
<th>Correct chromosome localisation</th>
<th>Probes visible in interphase nuclei</th>
<th>FISH Quality Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val_1</td>
<td>Cultured lymphocytes</td>
<td>Known FISH Normal case</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_2</td>
<td>Uncultured amniocytes</td>
<td>Known FISH Normal case</td>
<td>N/A</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Val_3</td>
<td>Uncultured amniocytes</td>
<td>Known FISH Normal case</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_4</td>
<td>Uncultured amniocytes</td>
<td>Known FISH Abnormal case (T21)</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_5</td>
<td>uncultured chorionic villus</td>
<td>Known FISH Abnormal case (T21)</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_6</td>
<td>uncultured chorionic villus</td>
<td>Known FISH Abnormal case (T18)</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_7</td>
<td>uncultured chorionic villus</td>
<td>Known FISH Abnormal case (T13)</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_8</td>
<td>uncultured cord material</td>
<td>Known FISH Normal case</td>
<td>N/A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>Val_9</td>
<td>Cultured lymphocytes</td>
<td>Known FISH Normal case</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
</tr>
</tbody>
</table>
Validation

Figure 1: Validation results on different samples: cultured lymphocytes, Normal (A, B); uncultured amniocytes, T21(C); uncultured chorionic villus material, T18 (D); uncultured chorionic villus material, T13 (E); uncultured cord material, Normal (F, G).
MATERIALS AND METHODS

• Preparation of uncultured cells for interphase FISH

• Fluorescence in situ hybridization
  – Cytocell Aquarius FAST FISH Prenatal Enumeration Probe kit
    • according to manufacturer instructions

• Signal interpretation
  • Slides are scored blind by 2 independent analysts
  • One of the analysts must be a state registered scientist
  • Each scorer notes the quality of the hybridisation and the FISH quality score
RESULTS

• Prenatal FAST FISH was performed on 26 samples
  • 16 amniotic fluids (61,5%)
  • 10 chorionic villus (38,5%)
• Postnatal FAST FISH was performed on 2 blood samples
  • Newborns referred for microarray studies
• 14/72 (19,4%) prenatal samples had to be repeated with prenatal FAST FISH probes (4 ½ months period)
  • One amended report
    – Previous commercial probe: 2G0R2A signal pattern consistent with a female fetus
    – Karyotype: 45,X
    – Cytocell FAST FISH Prenatal probe: 1G0R2A signal pattern consistent with Monosomy X
• All samples were reported within 3 calendar days
## RESULTS

- Details of patients with chromosome abnormalities by FAST FISH

<table>
<thead>
<tr>
<th>Case N.</th>
<th>Sample Type</th>
<th>Indication</th>
<th>Other reason(s)</th>
<th>FISH</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>362</td>
<td>Uncultured chorionic villus</td>
<td>Abnormal ultrasound scan</td>
<td>Hydrops</td>
<td>Abnormal female signal pattern with Trisomy 21</td>
<td>47,XX,+21</td>
</tr>
<tr>
<td>383</td>
<td>Uncultured chorionic villus</td>
<td>Abnormal ultrasound scan</td>
<td>Cystic hygroma</td>
<td>Tetraploid signal pattern [60%] Diploid signal pattern [40%]</td>
<td>46,XY [30]</td>
</tr>
<tr>
<td>2628</td>
<td>Uncultured amniocytes</td>
<td>First trimester screening</td>
<td>NITP positive for T18</td>
<td>Abnormal female signal pattern with Trisomy 18</td>
<td>47,XX,+18</td>
</tr>
<tr>
<td>2632</td>
<td>Uncultured amniocytes</td>
<td>Abnormal ultrasound scan</td>
<td>Bilateral talipes, raised nuchal fold</td>
<td>Abnormal male signal pattern with Trisomy 21</td>
<td>47,XY,+21</td>
</tr>
<tr>
<td>403</td>
<td>Uncultured amniocytes</td>
<td>Abnormal ultrasound scan</td>
<td>Cystic hygroma</td>
<td>Abnormal female signal pattern with Trisomy 21</td>
<td>47,XX,+21</td>
</tr>
<tr>
<td>408</td>
<td>Uncultured amniocytes</td>
<td>Abnormal ultrasound scan</td>
<td>Ventricle disproportion, prominent bowel, narrow chest, abnormal cardiac thoracic ratio</td>
<td>Abnormal signal pattern with Monosomy X .ish Y(SRYx1,DYZ3x0)</td>
<td>46,XY [70]</td>
</tr>
<tr>
<td>2701</td>
<td>uncultured lymphocytes</td>
<td>Microarray studies</td>
<td>Upward slating eyes, broad face, increased nuchal fold, thin upper lip</td>
<td>Abnormal female signal pattern with Trisomy 21</td>
<td>47,XX,+21</td>
</tr>
</tbody>
</table>
CONCLUSION

• Successful implementation of FAST FISH Prenatal kit in the service
  • Validated on 3 different fetal sample types and blood samples
• No samples went over the recommended 3 calendar days
  • Improvement on our expected time to provide preliminary results
• 98.6% of prenatal FAST FISH results were concordant with their karyotype
2h hybridisation rescue but...

• ~ 20% repeat rate - why?
  – Critical step in performing FISH is the successful co-denaturation of sample and probe
  – Failure to achieve specified co-denaturation temperature for the recommended time can lead to low fluorescence signals or generate unclear signal patterns
Thermobrite mapping

- 12 temperature tags were used to map the Thermobrite hybridisers at each slide position
- The majority of the slide positions gave sub-optimal readings (<74ºC)

**Recommendation:** modify programs
- Blood samples: 75ºC, 3min
- PETS: 75ºC, 6min
Thank you.

Questions?