Quality Assurance in a FISH-based PGD program

Dr. Leeanda Wilton
Genetic and Molecular Research
Melbourne IVF

lwilton@mivf.com.au

MELBOURNE IVF

Definitions

Quality assurance

Aims to ensure that quality outcome is built into the system before the work is done

Quality control

Aims to ensure that quality outcome did occur after the work was done

MELBOURNE IVF

PGD at Melbourne IVF

- More than 12 years clinical experience
  - Aneuploidy testing
  - Chromosome rearrangements
  - Monogenic disease
  - Gender selection for medical indications
- Will describe our laboratory based QA programs

but QA is about continuous improvement
- FOR ALL

MELBOURNE IVF

External QA providers

- ISO and others
- Can create appearance of QA
  - Assessments can be superficial
  - Focus is on having a document not necessarily accuracy of content
- Can pass these assessments without having true QA
- Much of QA is intuitive

Key features of QA

- Education and training
- Documentation
- Protocols
- Protocol and document review
- Laboratory maintenance
- Testing
- External quality assurance programs

Imperative that all staff have input in QA processes – ownership and commitment

Education and Training

- Essential to employ competent staff
  - Previous experience in PGD not essential
  - Technical competence is essential
  - Rigorous questioning of referees
- Establish internal training programs and competency assessment
  - At beginning of employment
  - Ongoing for all staff

Documentation

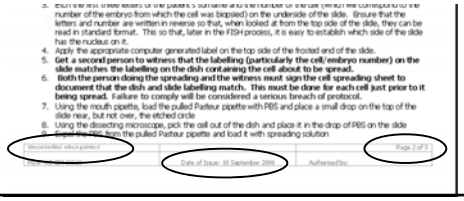
- ☐ Explanation of purpose
- ☐ Clarity
- ☐ Consistency
- ☐ Access
- ☐ Amendment restricted
- ☐ Document control
- ☐ Document review

Cover page for all documents - example

Work Instruction Document - example

Document control

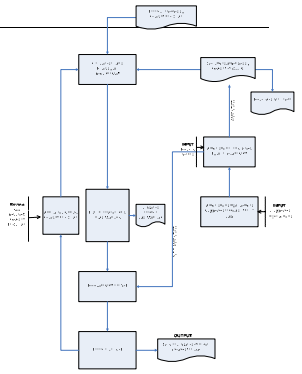
- All documents lodged on Melbourne IVF intranet
- Each department has own section
- No access for non-PGD staff
- Easy access for all relevant staff
- Amendment restricted
- Uncontrolled when printed
- No photocopies!


number of the embryo from which the cell was biopsied) on the underside of the slide. Ensure that the letters and number are written in reverse so that, when looked at from the top side of the slide, they can be read in standard format. This so that, later in the FISH process, it is easy to establish which side of the slide has the nucleus on it.
4. Apply the appropriate computer generated label on the top side of the frosted end of the slide.
5. **Get a second person to witness that the labelling (particularly the cell/embryo number) on the slide matches the labelling on the dish containing the cell about to be spread.**
6. **Both the person doing the spreading and the witness must sign the cell spreading sheet to document that the dish and slide labelling match. This must be done for each cell just prior to it being spread.** Failure to comply will be considered a serious breach of protocol.
7. Using the mouth pipette, load the pulled Pasteur pipette with PBS and place a small drop on the top of the slide near, but not over, the etched circle
8. Using the dissecting microscope, pick the cell out of the dish and place it in the drop of PBS on the slide
9. Expel the PBS from the pulled Pasteur pipette and load it with spreading solution

Uncontrolled when printed | Date of Issue: 10 September 2009 | Page 2 of 3 | Authorised by:

Protocol review

- Need clear process for protocol review
- At least annually
  - But more frequently if required
- Input and continuous improvement by all relevant staff encouraged
  - Ownership of and commitment to change
  - Good educational opportunity
- Clear communication of change

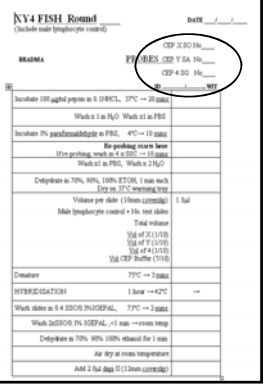


Avoid protocol "creep"

MELBOURNE IVF

Case specific protocol adherence documented

- Assurance of exactly what has been done for each case
- Standard format for each FISH protocol
- Tick that each step followed
  - Easy
  - Consistent
- Probe batch number recording
- Witnessing that correct probes applied



XY4 FISH Round

DATE ___/___/___

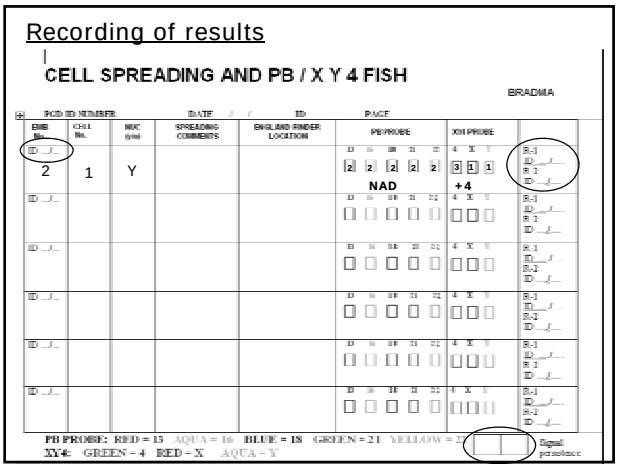
(Include male lymphocyte control)

BRADMA

PROBES: CEP X SO No.___ CEP Y SA No.___ CEP 4 SO No.___

Incubate 100 µg/ml pepsin in 0.1NHCL, 37°C → 20 mins		
Wash x 1 in H_2O Wash x1 in PBS		
Incubate 1% paraformaldehyde in PBS, 4°C → 10 mins		
Re-soaking starts here If re-probing, wash in 4 x SSC → 10 mins		
Wash x1 in PBS, Wash x 2 H_2O		
Dehydrate in 70%, 90%, 100% ETOH, 1 min each Dry on 37°C warming tray		
Volume per slide (10mm coverslip) Male lymphocyte control + 1% test slides Total volume Vol of X (1/10) Vol of Y (1/10) Vol of 4 (1/10) Vol CEP Buffer (7/10)	1 fµl	
Denature	75°C → 3 mins	
HYBRIDISATION	1 hour → 42°C	→
Wash slides in 0.4 SSC/0.3%IGEPAL, 72°C → 2 mins		
Wash 2xSSC/0.1% IGEPAL, <1 min → room temp		
Dehydrate in 70% 90% 100% ethanol for 1 min		
Air dry at room temperature		
Add 2 fµl dapi II (12mm coverslip)		

Recording of results



CELL SPREADING AND PB / X Y 4 FISH

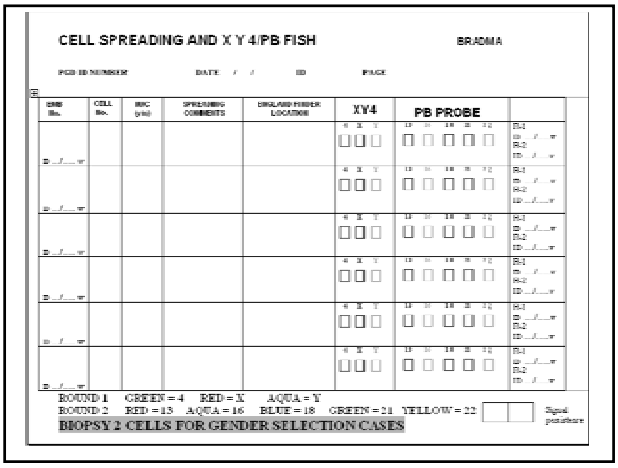
BRADMA

PCD ID NUMBER: DATE: / / ID: PAGE:

EMB No.	CELL No.	NUC (y/n)	SPREADING COMMENTS	ENGLAND FINDER LOCATION	PB PROBE	XH PROBE	
ID: .../...							R-1 ID:.../... R-2 ID:.../...
2	1	Y			2 2 2 2 2	3 1 1	
					NAD	+4	
ID: .../...							R-1 ID:.../... R-2 ID:.../...
ID: .../...							R-1 ID:.../... R-2 ID:.../...
ID: .../...							R-1 ID:.../... R-2 ID:.../...
ID: .../...							R-1 ID:.../... R-2 ID:.../...
ID: .../...							R-1 ID:.../... R-2 ID:.../...

PB PROBE: RED = 15 AQUA = 16 BLUE = 18 GREEN = 21 YELLOW = 22 Signal persistence

XY4: GREEN = 4 RED = X AQUA = Y



CELL SPREADING AND X Y 4/PB FISH

BRADMA

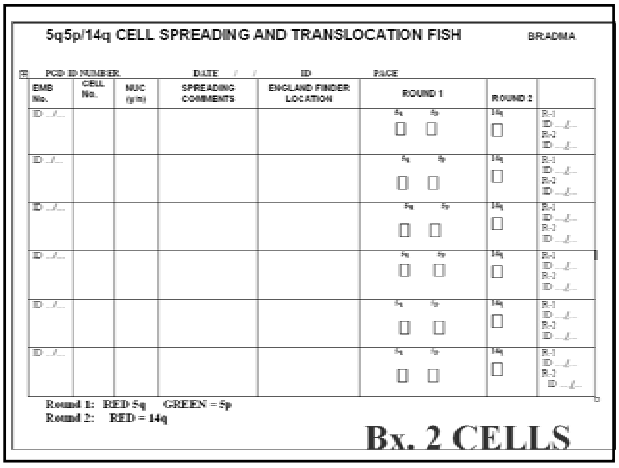
PCD ID NUMBER: DATE: / / ID: PAGE:

EMB No.	CELL No.	NUC (y/n)	SPREADING COMMENTS	ENGLAND FINDER LOCATION	XY4	PB PROBE	
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'
ID: .../... 'n'							R-1 ID:.../... 'n' R-2 ID:.../... 'n'

ROUND 1 GREEN = 4 RED = X AQUA = Y

ROUND 2 RED = 15 AQUA = 16 BLUE = 18 GREEN = 21 YELLOW = 22 Signal persistence

BIOPSY 2 CELLS FOR GENDER SELECTION CASES



5q5p/14q CELL SPREADING AND TRANSLOCATION FISH

BRADMA

PCD ID NUMBER: DATE: / / ID: PAGE:

EMB No.	CELL No.	NUC (y/n)	SPREADING COMMENTS	ENGLAND FINDER LOCATION	ROUND 1	ROUND 2	
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...
ID: .../...					5q 5p	14q	R-1 ID:.../... R-2 ID:.../...

Round 1: RED 5q GREEN = 5p

Round 2: RED = 14q

Bx. 2 CELLS

Equipment QA

- Regular (at least annual) maintenance of equipment
  - FISH – accuracy of temperature is critical!
  - Ovens, hotplates, hybridisation chambers
  - Imaging microscope
    - Changing and centering of lamp
  - Pipettes
  - Fridges/freezers
- Maintenance logs and registers should be kept
- Regular shut-down of laboratory is advisable
  - Equipment maintenance
  - Cleaning

MELBOURNE IVF

QA of consumables

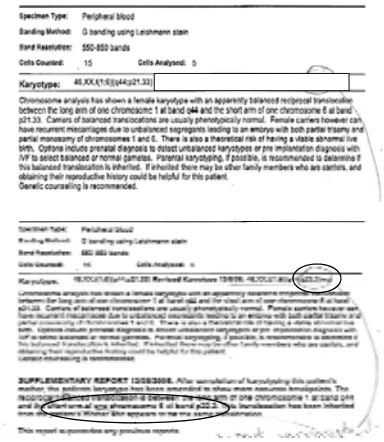
- FISH probes
  - Every new arrival should be tested well before clinical use
    - Can't rely on QA documentation provided by manufacturer
    - Transport problems can occur
    - Test results must be documented
  - Expiry dates of probes
    - At Melbourne IVF we discard probes that are past expiry date – even if they are still working

MELBOURNE IVF

Translocation cases

- Test FISH probes on carrier's lymphocytes
  - Confirm translocation
  - Identify cross-hybridisation
- Mistakes in karyotypes can happen!

MELBOURNE IVF



Specimen Type: Peripheral blood
Banding Method: G banding using Leishmann stain
Band Resolution: 550-650 bands
Cells Counted: 15 Cells Analysed: 5
Karyotype: 46,XX,t(1;6)(q44;q21.33)

Chromosome analysis has shown a female karyotype with an apparently balanced reciprocal translocation between the long arm of one chromosome 1 at band q44 and the short arm of one chromosome 6 at band p21.33. Carriers of balanced translocations are usually phenotypically normal. Female carriers however can have recurrent miscarriages due to unbalanced segregants leading to an embryo with both partial trisomy and partial monosomy of chromosomes 1 and 6. There is also a theoretical risk of having a viable abnormal live birth. Options include prenatal diagnosis to detect unbalanced karyotypes or pre implantation diagnosis with IVF to select balanced or normal gametes. Prenatal karyotyping, if possible, is recommended to determine if this balanced translocation is inherited. If inherited there may be other family members who are carriers, and obtaining their reproductive history could be helpful for this patient.
Genetic counselling is recommended.

Specimen Type: Peripheral Blood
Banding Method: G banding using Leishmann stain
Band Resolution: 550-650 bands
Cells Counted: 15 Cells Analysed: 5

SUPPLEMENTARY REPORT 13/08/2008. After completion of karyotyping this patient's mother, the paternal karyotype has been amended to show more accurate breakpoints. The reciprocal balanced translocation is between the long arm of one chromosome 1 at band q44 and the short arm of one chromosome 6 at band p22.3. This translocation has been inherited from the patients mother and appears to be the same translocation.

This report supercedes any previous reports.

MELBOURNE IVF

Specimen Type: peripheral blood
Banding Method: G banding using Leishman stain
Band Resolution: 550-650 bands
Cells Counted: 15 Cells Analysed: 5
Karyotype: 46,XX,t(1;6)(q44;q21.33)

Chromosome analysis has shown a female karyotype with an apparently balanced reciprocal translocation between the long arm of one chromosome 1 at band q44 and the short arm of one chromosome 6 at band p21.33. Carriers of balanced translocations are usually phenotypically normal. Female carriers however can have recurrent miscarriages due to unbalanced segregants leading to an embryo with both partial trisomy and partial monosomy of chromosomes 1 and 6. There is also a theoretical risk of having a viable abnormal live birth. Options include prenatal diagnosis to detect unbalanced karyotypes or pre implantation diagnosis with IVF to select balanced or normal gametes. Prenatal karyotyping, if possible, is recommended to determine if this balanced translocation is inherited. If inherited there may be other family members who are carriers, and obtaining their reproductive history could be helpful for this patient.
Genetic counselling is recommended.

SUPPLEMENTARY REPORT 13/08/2008. After completion of karyotyping this patients mother, the paternal karyotype has been amended to show more accurate breakpoints. The reciprocal balanced translocation is between the long arm of one chromosome 1 at band q44 and the short arm of one chromosome 6 at band p22.3. This translocation has been inherited from the patients mother and appears to be the same translocation.

SUPPLEMENTARY REPORT 14/08/2008 - Due to a typographical error, the chromosome 6 breakpoint in the "revised karyotype" has been corrected from p22.3 to p21.3

This report supercedes any previous reports.

That is correct. Will get blood to check.

From a highly reputable, accredited cytogenetics laboratory

MELBOURNE IVF

Meetings of laboratory staff

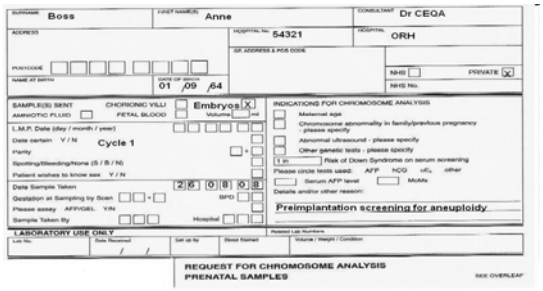
- External risk management assessment of FISH program
- Regular staff meetings
- Informal agenda on whiteboard
  - Junior staff not intimidated
    - comfortable raising issues
    - Reticent staff actively asked to contribute
  - Encourages education
    - Interesting case discussion
  - Encourages contribution
- Minutes kept and circulated

MELBOURNE IVF

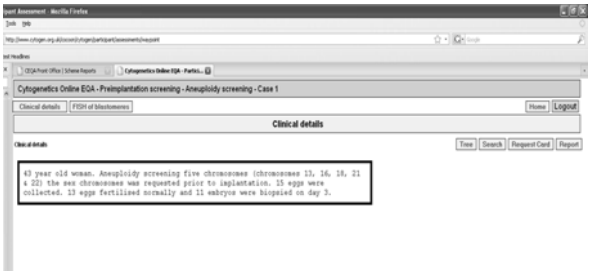
External QA program in FISH

- Initiated and driven by ESHRE PGD Consortium/Ros Hastings
- Cytogenetic European Quality Assurance (CEQA)
- Has existed for some years for routine cytogenetics
- First single blastomere/PGD pilot EQA recently completed
- Need to be registered
  - Nominal fee required

Referral information

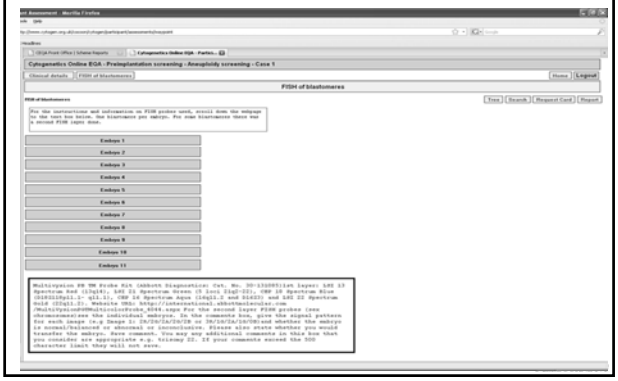


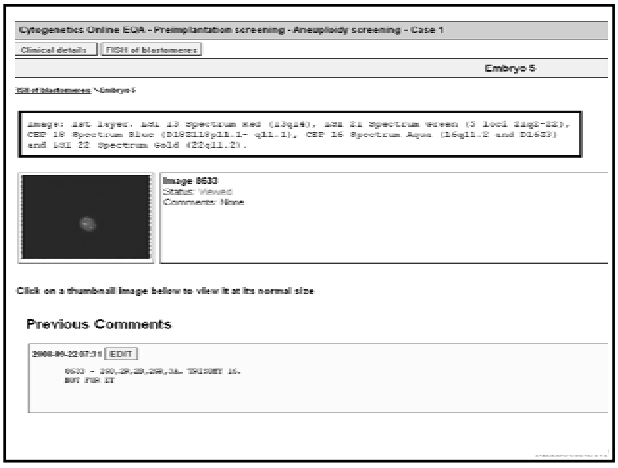
Clinical information



MELBOURNE IVF

FISH probe information





EQA scheme – pilot 2008

- Two cases
  - Aneuploidy testing
  - Chromosomal translocation
- Test individual lab interpretation with broader group of scientists

MELBOURNE IVF

EQA scheme – pilot 2008

- Excellent initiative
  - As many labs as possible should participate in future schemes
- Difficulties
  - 2-D image
  - Static
  - Can't remove colour planes
  - Some images were poor resolution
- Isn't perfect but significantly better than no external QA

MELBOURNE IVF

Quality assurance

- Essential part of practice
  - Particularly with widespread use of PGD
- Good for all
  - Patients
  - Staff
  - Referral centres
- Good for reputation and acceptance of PGD

MELBOURNE IVF

Thank you!