# Quality Assurance in a FISH-based PGD program

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# 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

# PGD at Melbourne IVF

- More than 13 years clinical experience, 1200 cycles
  - Aneuploidy testing
  - Chromosome rearrangements
  - Monogenic disease
  - Gender selection for medical indications

- Will describe our laboratory based QA programs for FISH

*but* QA is about continuous improvement
*- FOR ALL*

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# 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
- Can have good QA by setting up own quality management system
- Much of QA is intuitive

# Key features of QA

- Education and training
- Documentation
- Protocols
- Protocol and document review
- Procurement and service provision
- 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
- Covered by Dr. Harper

# Continuing technical improvement (CTI)

- Peer review of authorised practical tasks
- Performed every 3-6 months
- Individual staff responsible for
  - Initiation of reviews
  - Record keeping

- CTIs reviewed by lab manager/director
- Retraining offered if required
- Each scientist “authorised” to perform individual techniques
  - Authorisation can be revoked

**Continuing Technical Improvement**

**Protocol:** **Analysis of FISH signals**

**Controlled document:** MIVF-WI-GM-0005

**Scientist:**

**Observer:**

**Date:**

| | | | |
|---|---|---|---|
| Set up of microscope and software | ☑ | ☒ | N/A |
| Patient identification | ☑ | ☒ | N/A |
| Location of nucleus | ☑ | ☒ | N/A |
| Analysis and assessment of signals | ☑ | ☒ | N/A |
| Accuracy of paperwork completion | ☑ | ☒ | N/A |
| Witnessing of report | ☑ | ☒ | N/A |
| Storage of slides | ☑ | ☒ | N/A |

**Overall assessment of adherence to protocol:**

**Comments:**

# Documentation

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

# Cover page for all documents - example

Work Instruction Guide
Cell Spreading for FISH
Date of Issue: 18 September 2008

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## Document Control

| | |
|---|---|
| Document Type: | Work Instruction Guide |
| Document ID: | MIVF-WI-GM-00010 |
| Version: | Version 1.00 |
| Author: | Leeanda Wilton |
| Document Addendums: | None |

**Amendment to this document**

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Any questions related to this document should be directed to michael.lofts@mivf.com.au

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These documents or any that are referenced are only current in web browser format and become uncontrolled the moment the document has been printed.

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Failure to comply with these rulings may result in disciplinary action.

## Document Summary

A summary of the procedure being covered in this document should be entered here.

## Applicability

Information on who the policy is applicable to should be entered here.

# Work Instruction Document - example

Work Instruction Guide
Cell Spreading for FISH
Date of Issue: 18 September 2008

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## Cell Spreading for FISH

**Stock solutions:**

*1% Tween 20*
0.5ml of Tween 20
49.5 of distilled or deionised water
Mix gently
Store at 4°C
*1N HCl*
Use commercially diluted product
Store at 4°C

**Spreading Solution:**

*Must be made up fresh on day of cell spreading*
8.9ml of distilled or deionised water
1.0 ml of 1% Tween 20 stock solution
0.1ml of 1N HCl stock solution

**You will need:**

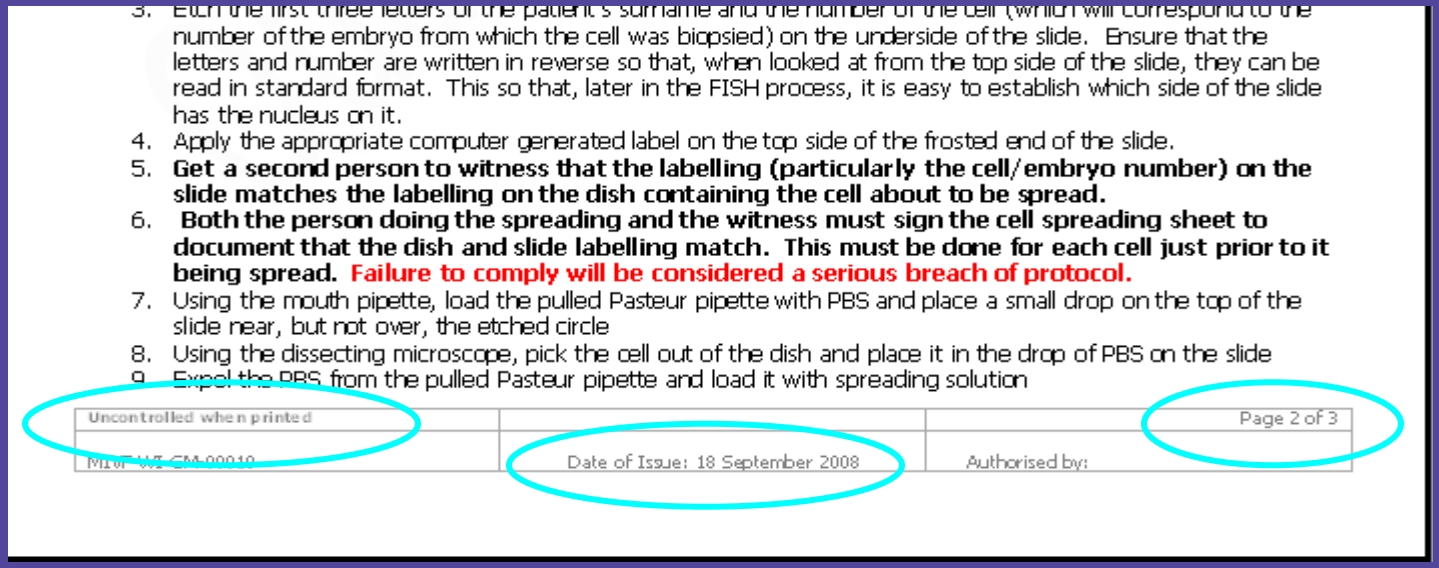
Dissecting microscope
Inverted microscope
Poly-l-lysine coated slides (commercial or in-house)
Phosphate buffered saline (PBS)
Pulled pasteur pipette with flame polished tip
Mouth pipette
Diamond pencil
Cell spreading paperwork with date and patient details completed
70%, 90% and 100% ethanol
Coplin jars
Computer generated slide labels with patient's name, IVF number, date and appropriate embryo numbers

**Method:**

1. Place dish containing cell to be spread onto stage of dissecting microscope. **Under no circumstances should more than one dish containing a cell be on the stage of the microscope at any time.**
2. Using the diamond pencil etch a small circle (~2-3mm diameter) on the underside of the slide. Ensure the "P" on the slide is the right way up (commercial slides only)
3. Etch the first three letters of the patient's surname and the number of the cell (which will correspond to the

# 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!

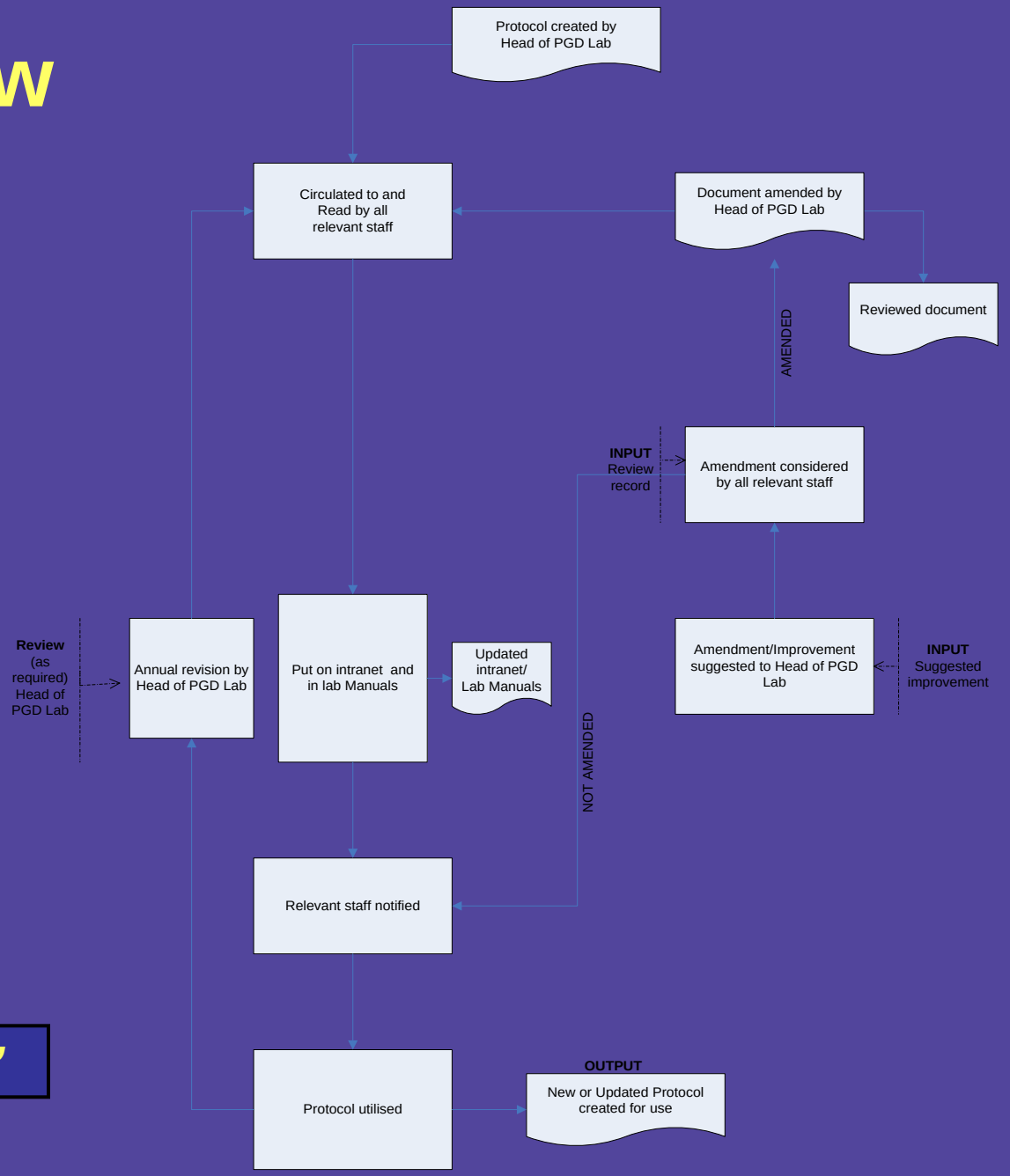


3. Etch the first three letters of the patient's surname and the number of the cell (which will correspond to the number of the embryo from which the cell was biopsied) on the underside of the slide. Ensure that the letters and number are written in reverse so that, when looked at from the top side of 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

# Protocol review

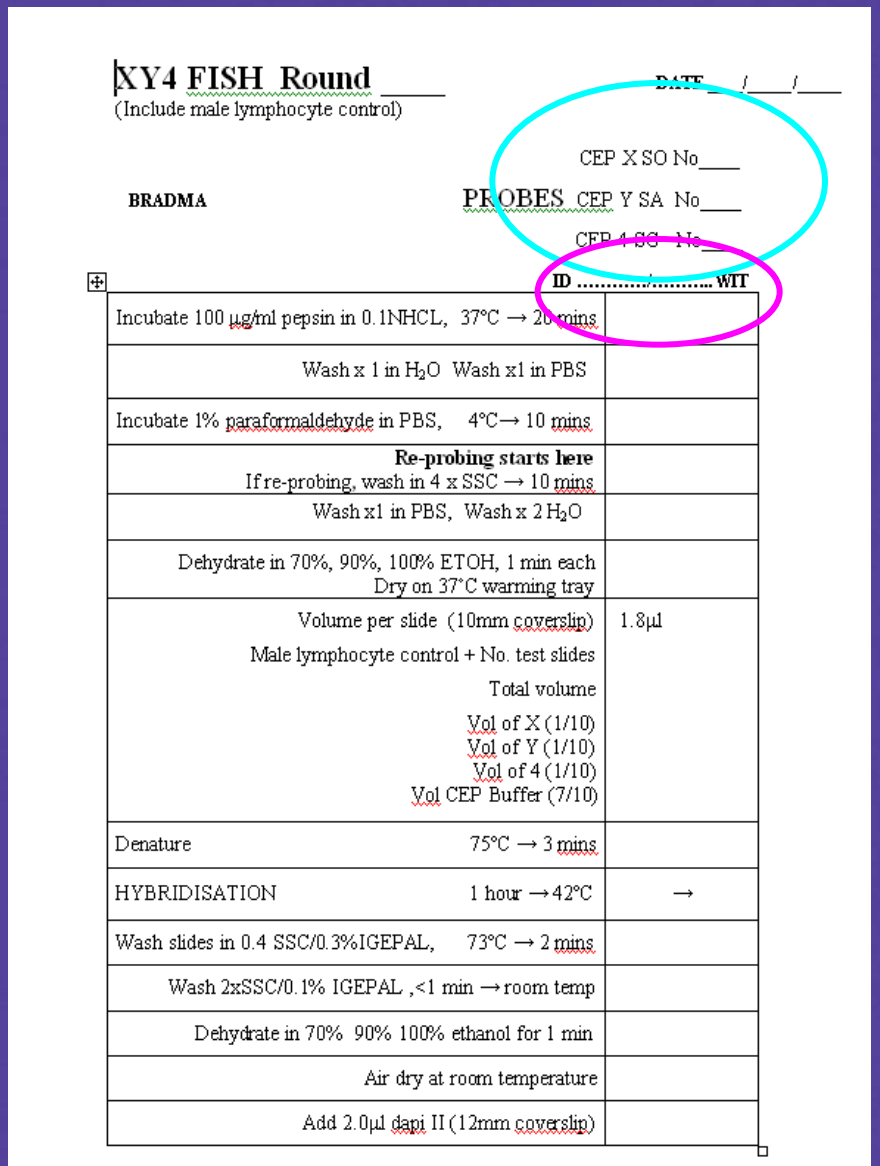
- Need clear process for protocol review
- At least annually
  - But more frequently if required
- Input and continuous improvement by all relevant staff encouraged
  - Ownership of and commitment to change
  - Good educational opportunity
- Clear communication of change

***Avoid protocol "creep"***



# 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)

CEP X SO No____

BRADMA PROBES CEP Y SA No____

CEP 4 SG No____

ID ..... WIT

| | |
|---|---|
| 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-probing 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)<br>Male lymphocyte control + No. test slides<br>Total volume<br>Vol of X (1/10)<br>Vol of Y (1/10)<br>Vol of 4 (1/10)<br>Vol CEP Buffer (7/10) | 1.8µl |
| Denature 75°C → 3 mins | |
| HYBRIDISATION 1 hour →42°C | → |
| Wash slides in 0.4 SSC/0.3%IGEPAL, 73°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 2.0µl dapi II (12mm coverslip) | |

# Procurement

- All products and services supplied to Melbourne IVF must meet defined criteria:
  - Quality - specification
  - Supply – reliability
  - Cost – competitive
- All suppliers have to be approved
- All suppliers regularly evaluated
- Monitored and audited by Procurement Committee
  - Supply problems reported to this committee

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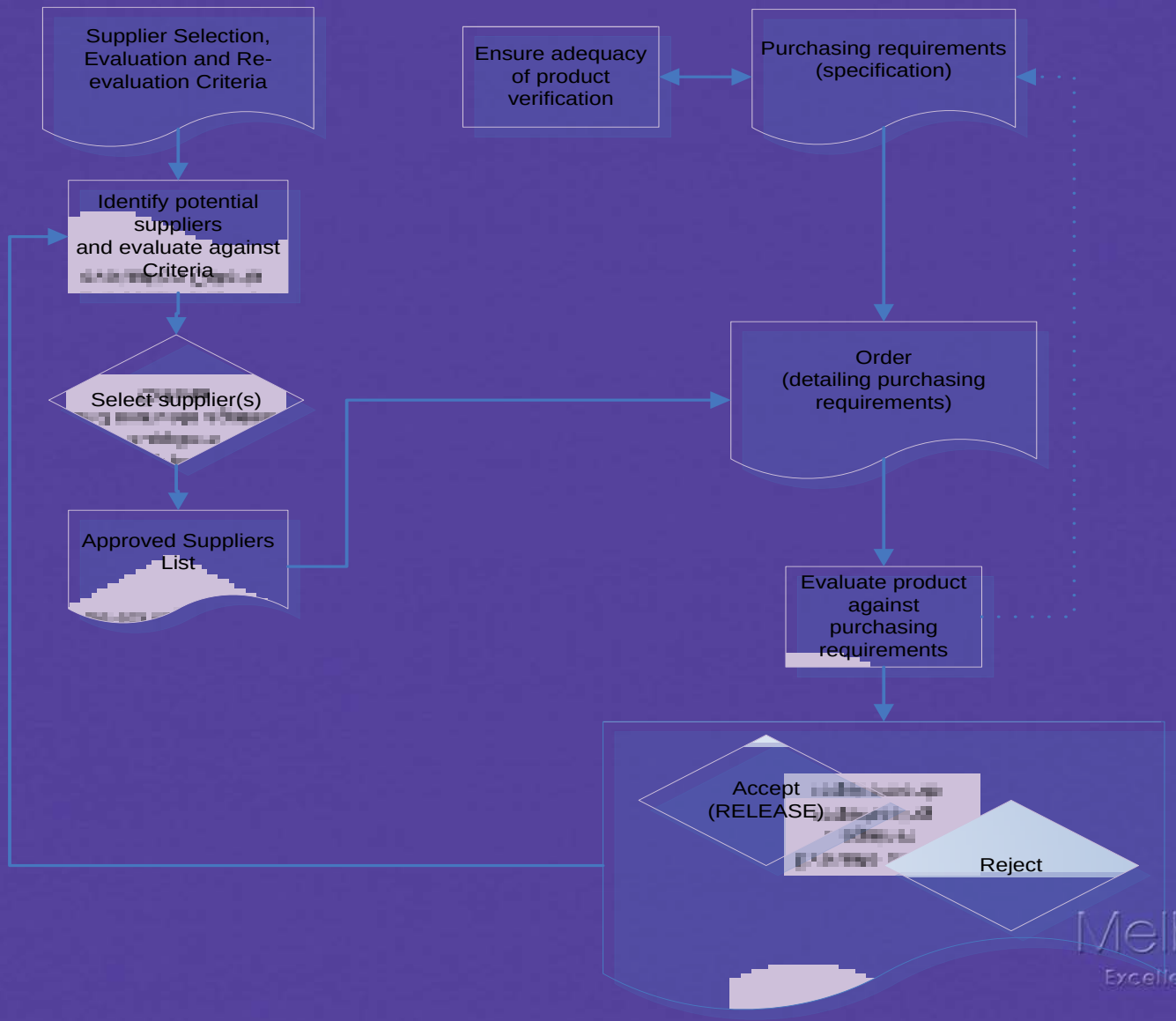
# Procurement process

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# 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
  - Centrifuges
- Maintenance logs and registers should be kept
- Regular shut-down of laboratory is advisable
  - Equipment maintenance
  - Cleaning

# 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

# Translocation cases

- Test FISH probes on carrier's lymphocytes
  - Confirm translocation
  - Identify cross-hybridisation
  - Signal persistence into second round

- Mistakes in karyotypes can happen!

**Specimen Type:** Peripheral blood

**Banding Method:** G banding using Leishmann stain

**Band Resolution:** 550-850 bands

**Cells Counted:** 15 **Cells Analysed:** 5

**Karyotype:** 46,XX,t(1:6)(q44;p21.33) [redacted]

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. Parental 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.

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**SUPPLEMENTARY REPORT 13/08/2008:** After completion of karyotyping this patient's mother, the patients 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 p22.2. This translocation has been inherited from the patient's mother and appears to be the same translocation.

This report supercedes any previous reports.

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**SUPPLEMENTARY REPORT 14/08/2008 :** Due to a typographical error, the chromosome 6 breakpoint in the "revised karyotype" has been corrected from q22.2 to p22.2

This report supercedes any previous reports.

This is correct. Will get blood to check.

From a highly reputable, accredited cytogenetics laboratory

# Meetings of laboratory staff

- External risk management assessment of FISH program
- Regular staff meetings
  - Particularly important as group gets bigger
- 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

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# 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
- Need to be registered
  - Nominal fee required

Tools Help

http://www.ceqa-cyto.eu/cytoc/Home

Latest Headlines

CEQA | Cytogenetics Online EQA - Participant ...

CEQA Cytogenetic European Quality Assessment

EuroGentest
Supported by EuroGentest network

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## Welcome to CEQA

**Cytogenetics European Quality Assessment (CEQA) website.**

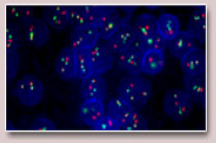
This is the Cytogenetics European Quality Assessment (CEQA) website. The website gives information about the EQA scheme and in addition allows you to register or access the online EQA service.

For more information about the scope of the scheme please use the menu bar.

If you are already registered for the scheme please click on the 'Login' box to access the current EQAs.

If this is the first time you have visited the website and you wish to register for the scheme, please click on the 'Register' box. There is a registration fee to cover adminstration costs.





**Register**

Click here to register with CEQA

**Login**

Click here to login to EQA system

Home | About CEQA | External Quality Assessment | Contact Us

Cytogenetics Online EQA - Preimplantation screening - Aneuploidy screening - Case 1

Clinical details | FISH of blastomeres

Embryo 5

FISH of blastomeres \ Embryo 5

Image: 1st layer. LSI 13 Spectrum Red (13q14), LSI 21 Spectrum Green (5 loci 21q2-22), CEP 18 Spectrum Blue (D18Z118p11.1- q11.1), CEP 16 Spectrum Aqua (16q11.2 and D16Z3) and LSI 22 Spectrum Gold (22q11.2).

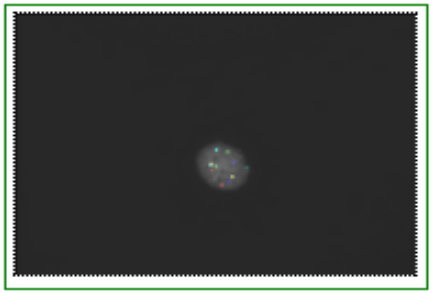


**Image 8633**
Status: Viewed
Comments: None

**Click on a thumbnail image below to view it at its normal size**

## Previous Comments

**2008-09-22 07:31** EDIT

8633 - 2G0,2R,2B,2GR,3A. TRISOMY 16.
NOT FOR ET

# EQA scheme – pilot 2008, 2009

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

# EQA scheme

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

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# Quality assurance

- Essential part of practice
  - Particularly with widespread use of PGD
  - Can be implemented step-by-step
    - Start with protocols/SOPs
- Good for all
  - Patients
  - Staff
  - Referral centres
- Good for reputation and acceptance of PGD

# Thank you!

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