Quality Management in PCR

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Outline

5.2	Accommodation & environmental conditions
5.3	Laboratory equipment
5.4	Pre-examination procedures
5.5	Examination procedures
5.6	Assuring quality of examination procedures
5.7	Post-examination procedures
5.8	Reporting of results
4.10	Corrective action
4.11	Preventive action
4.12	Continual improvement

5 Technical requirements

5.2 Accommodation & environmental conditions

5.2.4 Laboratory design & environment suitable for tasks

- primary sample collection / examinations
- energy sources, ventilation, water, waste disposal

5.2.5 Monitor, control and record environmental conditions

5.2.9 Storage space and conditions

- samples, documents, supplies, records, results

5.3 Laboratory equipment

5.3.1 The laboratory shall have equipment required

5.3.5 Operated by authorized personnel only

PCR

5.2 Accommodation & environmental conditions

5.2.4 Laboratory design

- Work up / PGD clinical cycles / IVF unit
- Pre PCR (Primary reactions / Split reactions)
  - Restricted access / Responsibility
  - Documentation and training for use
  - Positive pressure / UV decontamination
  - Cleaning / waste disposal
- Post PCR

5.2.5 Monitor, control and record environmental conditions

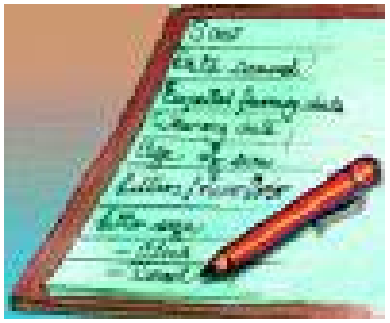
- Restricted access / Monitor and record contamination rates

5.2.9 Storage space and conditions

- Storage of Pre PCR reagents procedures
- Samples, consented genomic DNA, post PGD product
- Workup reports, PGD reports, electrophoretograms

5.3 Laboratory equipment

5 Technical requirements



5.4 Pre-examination procedures

5.4.1 Request form has information to identify

- patient / authorized requester / examination

5.4.2 Specific instructions for the proper collection

5.4.5 Primary samples shall be

- traceable individual / not accepted if lacking identification

5.4.6 Monitor samples to check transportation

- time frame / specified temperature interval / safety

5.4.7 Record

- all samples received / date & time of receipt / receiving officer

5.4.8 Criteria for acceptance/rejection of primary samples

- final report indicate / if problem / if caution interpreting result

5.4.14 Samples stored for a specified time

- Suitable conditions / repetition of the examination

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5.4 Pre-examination procedures

5.4.1 Request form

- Disorder / Mutation reports / tested affected relatives

5.4.2 Sample collection

- Bloods from couples / Bloods from relatives / Blastomeres
- Tubes for collection - Lithium heparin / EDTA / ALB /PK

5.4.5 Primary samples

- Bloods
  - Name / Disorder / Code
  - Samples from relatives / consent
- Blastomeres
  - Who does tubing?
  - Transport PGD

PCR

5.4 Pre-examination procedures

5.4.6 Monitor samples to check transportation

- Transport PGD
  - Contact person in IVF lab and PGD lab
  - Tracking with courier

5.4.7 Record

- Bloods received / Fate
- Tubing table for Blastomeres

5.4.8 Criteria for acceptance/rejection of primary samples

- Bloods for genomic DNA / single cell isolation
  - identification / date taken
- Tubing table for blastomeres

5.4.14 Samples stored for a specified time

- Genomic DNA from consented couples – IQC
- Single cells for control samples in PGD cycle

PCR – Tubing table

University College London	F003: Tubing table for a PCR case
UCL Centre for PGD	Page 1 of 1
Document Number: F003	Date of Issue: 22/12/08

Tubing Table

Patient Name				
Patient Number	ACU/PGD		EC Date	
Disorder			Affected	
Cycle			Date	
Biopsy			Tubing	
Labelling				
Start time			Finish time	

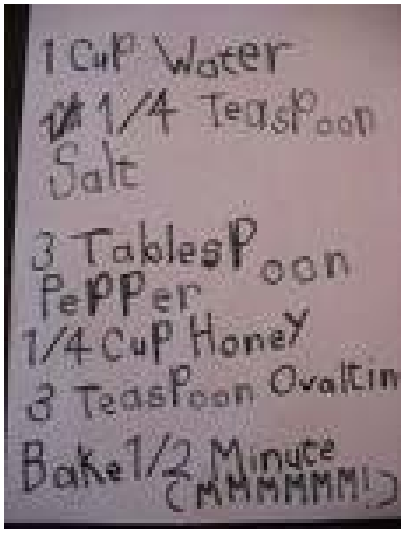
Embryo number	Embryo grade	Embryo comment	Cell number	Cell comment	Nucleus seen during biopsy

5 Technical requirements

5.5 Examination procedures

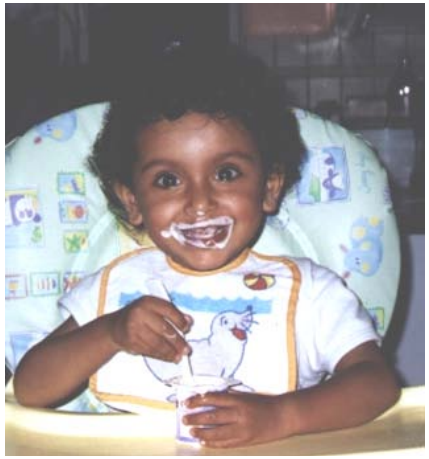
5.5.1 If in-house examination procedures used

- appropriately validated
- fully documented



5.5.2 Document

- purpose of the examination
- principle of the procedure used for examinations
- performance specifications (sensitivity specificity)
- required equipment and reagents
- quality control procedures
- reportable interval of examination results
- alert/critical values
- laboratory interpretation
- potential sources of variability



Workup Report

Work up report and protocol summary for a PCR PGD Case

Referral Reason –

Information provided by

Patient Name	Female	Male	Relative
Date of birth			
Mutation			

Date samples received –
Date DNA extracted-

ACU/PGD number –

RESULTS

Marker	Female Partner	Male Partner	Relative	Comments

Date	Marker 1	Marker 2	Mutation	Cells with Diagnosis %
Efficiency %				
ADO %				

Workup Completed by:

Name: _____ Signature: _____ Date: _____

Protocol authorized for application in treatment cycle by:

Name: _____ Signature: _____ Date: _____

PROTOCOL SUMMARY

Cell lysis

Mastermix – add µl of 1M NaOH/KOH plus µl of Water according to table to g of already aliquoted DTT
aliquot 2.5µl of this into each tube
Dissociation Buffer- Add 40ul BSA to 960ul of DB

After tubing - -80°C for 1 hour
then 65°C for 10 mins

PCR

Multiplex reaction – Loci amplified, X Round

	Stock	Volume/Tube	Concentration in tube
Primer 1 Forward (label)	50µM	0.µl	0.µM
Primer 1 Reverse	50µM	0.µl	0.µM
Primer 2 Forward (label)	50µM	0.µl	0.µM
Primer 2 Reverse	50µM	0.µl	0.µM
Primer 3 Forward (label)	50µM	0.µl	0.µM
Primer 3 Reverse	50µM	0.µl	0.µM
dNTP	10mM	0.5µl	0.2mM
HiFi Buffer (with Mg++)	15mM	2.5µl	1.5mM
Mg++			
Tricine		2.5µl	
Glycerol			
Enzyme	5U/µl	0.25µl	1.25U
Water		To make up to final volume of 21.5 µl	

Checklist for a PGD case

Check list prior to a PGD clinical case and PGD day

Prepared by:

Patient Name	Female	Male
Date of birth		
Mutation		

ACU/PGD number –

Reagent	Date Tested	Batch No	Comment
DTT			
NaOH			
Dissociation buffer			
BSA batch			
Sterile water			
Expand High Fidelity PCR polymerase (HiFi)			
10xPCR buffer for Hifi			
$MgCl_2$			
dNTP's			
Tricine			
Glycerol			
Primers for PCR:			
500 ROX Size standard			

DNA Controls:	Mutation: Date tested	Marker 1: Date Tested	Marker 2 Date Tested
Maternal DNA No:			
Paternal DNA No:			
Relative DNA No:			
Normal DNA No:			
Normal DNA No:			
Single cells	**Efficiency**	**ADO**	

Examination forms

Patient Name			
Patient Number	ACU/PGD	EC Date	
Disorder		Affected	
Cycle		Date	
Operator		Observer	

	1	2	3	4	5	6	7	8	9	10	11	12	
A													A
B													B
C													C
D													D
E													E
F													F
G													G
H													H
	1	2	3	4	5	6	7	8	9	10	11	12	

	Lot Number	Vol/sample	No. Of samples	Total volume
HiDi				
Size Standard				

5 Technical requirements

5.6 Assuring quality of examination procedures

5.6.1 internal quality control systems

5.6.2 laboratory shall determine the uncertainty of results

5.6.4 participation in EQA

- clinically relevant challenges
- mimic patient samples
- checking the entire examination process
- pre- and post-examination procedures

5 Technical requirements

5.7 Post-examination procedures

5.7.1 Authorized personnel shall

- systematically review the results of examinations
- evaluate them with the clinical information
- authorize the release of results

5 Technical requirements

5.8 Reporting of results

5.8.1 Laboratory management responsible for

- formatting reports
- manner to be communicated from the laboratory

5.8.6 Copies or files of reported results retained

5.8.13 Documented procedures for

- release of examination results
- who may release results and to whom
- guidelines for release of results directly to patients

5.8.14 Policies and practices for

- telephone / electronic results reach only authorized receivers
- Verbal results followed by a properly recorded report

4 Management requirement

4.10 Corrective action

4.10.1 Process to determine cause of problem

4.10.2 Document implemented changes

4.10.3 Monitor results of corrective action

4.10.4 Doubt on compliance - audit of activity

4.11 Preventive action

4.11.1 Process to identify improvements (technical / QMS)

- review procedures, trend- and risk-analyses, EQA

4.12 Continual improvement

4.12.1 All procedures reviewed at regular intervals

4.12.2 Evaluate effectiveness of action by review / audit

4.12.4 Quality indicators for contribution to patient care

PCR Key Quality Indicators

- Protocol on single lymphocytes
  - Efficiency
  - ADO rate
- Contamination rates PGD blanks
- Cell diagnosis rate
- Embryo diagnosis rate
- Follow up of spare embryos
- Follow up of pregnancies / Babies

Quality Management in PCR

- Standardized formatting of
  - Data received
  - Results generated
  - Reports
- Traceability of
  - Samples received
  - PCR products from workups / PGD cases
- Enables
  - Monitoring of KQIs
  - Can lead to continual improvement of service