Examination Process

Receiving samples, Standard operating procedures and risk assessments

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Stages in the clinical PGD examination process include

- Preparation of laboratory & reagents before the test
- Receipt & logging-in of samples
- Embryo-cell lysis step
- First PCR set-up
- Post (1st) PCR steps
- Evaluation of results
- Reporting the PGD cycle results

Assuming that

- The assay design is robust & validated (pre-examination stage)
- There are appropriately trained staff, properly calibrated equipment & quality-controlled reagents

Then major risks in PCR-based PGD include:

- Sample mislabelling /misidentification
- Contamination
- Clerical errors during reporting

Sample mislabelling /misidentification

Receipt of cycle samples: Checklist

- Check the cycle identification on accompanying sheet matches identification of expected cycle
- Check numbers on biopsy tubes are clear and in the correct order
- Check the blanks from IVF unit have been included and clearly labelled
- Check all the samples/blanks are in appropriate condition e.g. no tubes have opened in transit; Note any irregularities

Sample mislabelling /misidentification

Apply a comprehensive, robust labelling system throughout all stages of the examination process

- Unique identifier
- Clear (legible) and indelible labeling
  - Printed sticker labelling systems superior to pencil or pen
- Confirmation of tube labeling
  - Witnessing
  - Bar-coding or radio frequency identification

Major risks in PCR-based PGD:

- Sample mislabelling /misidentification
- Contamination
- Clerical errors during reporting

Contamination

Source	Stage of process
Cells of maternal or paternal origin (cumulus cells/sperm)	Embryo fertilization & biopsy
Operator	a) Embryo biopsy b) Cell lysis c) PCR steps (especially first PCR)
Carry-over	PCR steps

Contamination

- Assuming
  - Embryo fertilization has precluded contamination by cells of maternal and /or paternal origin
  - Embryo biopsy has precluded operator contamination

then the remaining examination process must include precautions to preclude operator & carry-over contamination

Operator contamination

Can be minimized if the operator

- Wears clean gloves, clean gown (no gaps between gloves and gown), face mask etc
- Changes gloves frequently not only between steps but also even between handling sample tubes and reagent tubes

Carry-over contamination

In order to preclude (or at least minimize):

- Perform all stages of examination process in separate areas
- Use exclusive lab equipment
- Clean & UV-treat all lab space and equipment before (& after!) each cycle
- Use stringently prepared one-use reagent aliquots (note all batch numbers throughout)
- Include negative controls & blanks at all stages

Monitoring Contamination

Even if the most stringent conditions are applied, chance contamination may occur in individual sample tubes. Thus all assays should include an internal control to monitor contamination in each cell sample.

Thus, in the assay design, polymorphic microsatellite markers should be included alongside the disease-specific assay (pre-examination stage)

The observation of spurious or supernumary allele sizes in relation to those expected to be transmitted by the parents indicates contamination in the specific tube (sample)

Post PCR analysis

- According to the method of choice, following the PCR step(s), samples, controls & blanks are processed to assign genotypes and check for contamination (options of methods described earlier in workshop)
- The results should be evaluated, preferably by 2 (experienced) scientists, recorded on results sheet & signed
- If there are discrepancies between results assigned by each scientist, a third should give an opinion and/or the genotyping process repeated
- Once the genotype of each embryo has been determined the report is prepared

Major risks in PCR-based PGD:

- Sample mislabelling /misidentification
- Contamination
- Clerical errors during reporting

Reporting of results

The report should include

- Referring IVF lab, date, time & condition of samples on receipt, name of couple & disease for which PGD cycle performed etc
- The outcome per sample tube, with EXACTLY the same identification as used by IVF lab:
  - Result achieved – yes or no
  - Status relative to the disease - unaffected or affected (for monogenic recessive disease it is not necessary to differentiate between unaffected-carrier & unaffected-normal)
- The entire report is double-checked by second scientist
- The checked report is given immediately to the IVF lab /centre so that the couple can be consulted & the embryo transfer can be performed

Reporting of results

Must preclude clerical errors and chance of misinterpretation by IVF lab.

- Before writing report, recheck work & results sheets for the cycle
  - Final check-list
- Report the result for each sample tube using EXACTLY the same identification as used by IVF lab
- The IVF lab should confirm receipt of examination report

Risk assessment for accuracy & reliability of PGD results

To recap, the pitfalls in a PGD examination process include

- General: Sample identification, clerical
- Specific to PCR-based PGD: Contamination, Allele drop-out (ADO)

Once pitfalls have been identified & their likely occurrence minimized, a risk assessment for accuracy & reliability of the protocol used in the examination process can be made

Risk assessment for accuracy & reliability of PGD results

Figures for risk assessment can be acquired from the protocol performance

- During protocol-assay trials
- Through internal audit (untransferred embryo follow-up)
- Through pregnancy & baby follow-up
- Through EQA

Risk assessment for accuracy & reliability of PGD results

Try to quantify:

- The influence of ADO on false postive or (especially) false negative rates
  - This is especially critical for autosomal dominant disease when testing for presence/absence of disease associated mutation
  - The more linked sites included, then the lower the risk of misinterpreting genotype status of cell(s) representing each embryo due to ADO (see pre-examination stage)
- The likelihood of contamination
- The likelihood of clerical error

ADO & misdiagnosis risk for autosomal recessive disease when both parents carry same mutation (5%ADO)

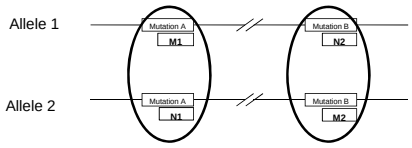
Combinations	Diagnosis		Possibility	
			Correct diagnosis (Unaffected embryo)	**Misdiagnosis** (rejection of unaffected embryo)
M/N	Not affected	Transfer	0.95	
M	Affected	No transfer		0.025
N	Not affected	Transfer	0.025	
		TOTAL	**0.975**	**0.025**

If parents carry an identical mutation, then the detection of a N allele ensures the transfer of an unaffected embryo

Theoretically there is no risk of unacceptable misdiagnosis based on mutation analysis alone

M = mutant allele; N = normal allele

ADO & unacceptable misdiagnosis risk for autosomal recessive disease - analysis of two different mutations



Assuming mutation detection method shows status at both alleles for each locus (mutation)

If ADO is 5%, then for each mutation, we will see:
M/N in 95% of analyses,
M alone in 2.5%, and
N alone in 2.5%

Unacceptable misdiagnosis risk for autosomal recessive disease if ADO is observed to be ~5%

Combinations		Diagnosis		Possibility	
Locus 1	Locus 2			**Correct diagnosis** (Rejection of affected embryo)	**Misdiagnosis** (transfer of affected embryo
M1/N1	M2/N2	Affected	No transfer	0.9025	
M1/N1	M2	Affected	No transfer	0.0238	
M1/N1	N2	Unaffected	Transfer		0.0238
M1	M2/N2	Affected	No transfer	0.0238	
M1	M2	Affected	No transfer	0.0006	
M1	N2	Unaffected	Transfer		0.0006
N1	M2/N2	Unaffected	Transfer		0.0238
N1	M2	Unaffected	Transfer		0.0006
N1	N2	Unaffected	Transfer		0.0006
			TOTAL	**0.9507**	**0.0493**

Locus 1 (Exon A)	
M1/N1	95%
M1	2.5%
N1	2.5%

Locus 2 (Exon B)	
M2/N2	95%
M2	2.5%
N2	2.5%

Risk assessment for accuracy & reliability of PGD results

- For an autosomal recessive disease
  - Assuming 25% of embryos will be affected
  - Then if ADO is 5%, when 2 mutations are involved (in the same locus), then the chance of unacceptable misdiagnosis is 25% of 4.9% = 1.2%

Thus for every assay, analysis of at least 2 loci linked to the disease will reduce the risk of misdiagnosis due to ADO to a minimum (<1%)

Unacceptable misdiagnosis risk for autosomal dominant disease if ADO is observed to be ~5%

Combinations	Diagnosis		Possibility	
			Correct diagnosis (Rejection of affected embryo)	**Misdiagnosis** (transfer of affected embryo)
M/N	Affected	No transfer	0.95	
M	Affected	No transfer	0.025	
N	Not affected	Transfer		0.025
		TOTAL	**0.975**	**0.025**

Risk assessment for accuracy & reliability of PGD results

- For an autosomal dominant disease
  - Assuming 50% of embryos will be affected,
  - Then if ADO is 5% for a single locus, the chance of unacceptable misdiagnosis is 50% of 2.5% =1.25%

Thus for every assay, analysis of at least 2 loci linked to the disease will reduce the risk of misdiagnosis due to ADO to a minimum (<1%)

Standard operating procedures and checklists

These are necessary to

- Support correct laboratory procedures & examination conditions by operators
- Optimize accuracy & reliability of PGD results

Standard operating procedures and checklists

As required for all accredited clinical diagnostic laboratory procedures

Standard operating procedure for …….

- **SOP EDITION No, DATE OF ISSUE,** Etc.
- **PURPOSE OF EXAMINATION**
- **INTRODUCTION/PRINCIPLES**
- **HEALTH AND SAFETY**
- **PERSONNEL**
- **EQUIPMENT AND SPECIAL FILES**.
- **CONSUMABLES AND SPECIAL FILES**
- **CHEMICALS & REAGENTS AND SPECIAL FILES**
- **METHOD**
  - *Sample receipt*
  - *Etc for all steps/stages*

Standard operating procedures and checklists

Checklist for ……e.g. PGD case

- Patient information
- ART information
- Biopsy information
- Genotyping protocol information
- Reporting
- Embryo transfer
- Follow-up of spares
- Pregnancy

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