Reporting of results

Francesco Fiorentino

"GENOMA" – Molecular Genetics Laboratory

Rome - Italy

ISO requirements for reporting of results

5.8.1 Laboratory management responsible for

- formatting reports
- manner in which it is to be communicated from the laboratory

The policy needs to include details of:

- who reports the results and on what forms
- the confidentiality of the reports
- the internal (detailed laboratory report of the PGD case)
- the external report (the summary report for the IVF unit)
- how the reports are authorized and by whom
- how the results are reported.

ISO requirements for reporting of results

5.8.3 Results shall be legible, without mistakes and reported to persons authorized to receive and use this kind of medical information. The report shall also include:

- clear, unambiguous identification of the examination
- the identification of the laboratory that issued the report
- unique identification of the patient and destination of the report
- name or other unique identifier of the requester and the requester's address
- date and time of sample collection and time of receipt by the laboratory
- date and time of release of report
- sample type
- reference intervals
- interpretation of results
- identification of the person authorizing the release of the report
- signature or authorization of the person checking or releasing the report

ISO requirements for reporting of results

5.8.6 Copies or files of reported results shall be retained by the laboratory, as long as medical relevant or as required by national requirements

5.8.11 Laboratory management shall establish turnaround times for each examination.

- turnaround time shall reflect clinical needs
- There shall be a policy for notifying the requester when an examination is delayed
- This procedure should be developed in collaboration between IVF and PGD lab personnel

Releasing of examination results

5.8.13 The laboratory shall have clearly and documented procedures for releasing of examination results, including:

- designated personnel responsible for releasing the results
- to whom the results can be released
- guidelines for release of results directly to patients

- It is recommended not to release the information on the embryos to be transferred verbally (by phone) to prevent a misunderstanding which could result in the transfer of the wrong embryo.
- only emailed, faxed or hardcopy reports should be used in PGD.
- it may be useful to email a provisional report. This will be useful information when discussing the results with the patients and deciding which embryos to replace.
- However, a hardcopy report signed by the authorizing personnel is needed by the IVF unit before embryo transfer is conducted.
- It is a good practice for the PGD team to be available to discuss the report with the IVF team

REPORTING OF RESULTS IN PGD

Pre- PGD Practice Run

- Pre-examination procedure
- Transport PGD

PGD Work -up

- Pre-examination procedure

PGD Cycle

Post-examination Procedure

- Internal report
- Report for the IVF unit

REPORTING OF RESULTS

Pre- PGD Practice Run

Pre-PGD Practice Run

- In DNA-based PGD, DNA contamination can cause a misdiagnosis and PCR inhibition can determine a diagnosis failure.
- The biopsy/tubing procedure used at IVF centres should be evaluated before performing the first case by scheduling a “practice run” (i.e. a dummy run test on spare blastomeres), in order to assure that:
  - the number of tubes with no cells is acceptable
  - a result can be obtained (no PCR inhibition)
  - contamination is not introduced into the specimens.

Pre-PGD Practice Run report

Date of report: **Time:**

IVF unit details

Referring Centre:	**IVF unit ID no.:**
Department:	**Address:**
City	**Country:**
Referring physician(s):	**Receiver(s) of the report:**

Pre-PGD Trial

Analysis Performed:	Pre-PGD dummy run on spare blastomeres	**Trial:**	1
Method of Analysis:	Minisequencing analysis + Fluorescent PCR of STR markers		
Gene investigated:	HBB **OMIM:** +141900	**Trial ID no.:**	

IVF data

IVF cycle Ref. No.:		**Biopsy date:**		**Time:**	
Specimen type:	Blastomeres	**Date sample Received:**		**Time:**	
No. of cells analysed:	10	**No. of blanks:**	10		

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Pre-PGD Practice Run report

Blast. No	PCR Signal	Blank. No	Contamination
1	YES	1	NO
2	YES	2	NO
3	YES	3	NO
4	YES	4	NO
5	YES	5	NO
6	YES	6	NO
7	YES	7	NO
8	YES	8	NO
9	YES	9	NO
10	YES	10	NO

Gene/ marker name	No. Cells tested	No. Cells with PCR Failure	No. Cells with positive PCR	Amplification rate (%)	No. Cells with ADO	ADO rate (%)	No. of Blanks	No. of PCR signals	Contamination rate (%)
D11S4146	10	1	9	90,0%	0	0,0%	0	0	0,0%
D11S988	10	0	10	100,0%	0	0,0%	0	0	0,0%
HBB ex. 1	10	1	9	90,0%	1	11,1%	0	0	0,0%
D11S1338	10	0	10	100,0%	1	10,0%	0	0	0,0%
D11S1997	10	0	10	100,0%	1	10,0%	1	1	0,0%
D11S1331	10	1	9	90,0%	0	0,0%	0	0	0,0%
Total	**60**	**3**	**57**	**95,0%**	**3**	**5,3%**	0	0	0,0%

Pre-PGD Practice Run report

CONCLUSION:

The results obtained are within the expected range for single cell PCR (Thornhill et al., 2005). The IVF unit can proceed to the clinical PGD cycle.

Person performing Pre-PGD dummy run testing:

Name _____ **Qualification** _____ **Signature** _____

Results verified and validated by:

Name _____ **Qualification** _____ **Signature** _____

Head of the Laboratory:

Name _____ **Qualification** _____ **Signature** _____

REPORTING OF RESULTS

PGD
Work -up

PGD Work-up report requirements

- Name of laboratory issuing results
- Name of the receiver of the report
- Unequivocal identification of the couple (2 identifiers per individual)
- Specimen information – type of sample and date of sampling (also time if appropriate)
- Methods being performed and markers used
- Disease being tested
- Mutations given in Human Genome Variation Society (HGVS) mutation nomenclature
- Accession number of reference sequence (including version number) given when stating mutations
- Clearly presented results with appropriate interpretative comments
- Appropriate reference intervals
- Error rates clearly stated
- Identification of individual providing results and authoriser
- Date of report

Date of report:

IVF unit details

Referring Centre:	
Department:	
Address:	
City / Country:	**IVF unit ID no.:**
Referring physician:	
Receiver of the report:	

PGD work-up details

Genetic disorder of concern:	Beta Thalassemia			**OMIM:**	+141900
Mode of inheritance:	Autosomal recessive				
Method of Analysis:	Minisequencing analysis + Fluorescent PCR of STR markers				
PGD Strategy:	Direct mutation testing + linkage analysis by STR markers genotyping				
Gene investigated:	HBB	**OMIM:**	+141900	**Ref. Seq. Accession No.:**	
Mutation(s) involved:	**Male partner:**	IVS1-5(G>C)	**Female partner:**	IVS1-5(G>C)	

Patients' details

Patient Name (female partner):	XX XX XX			**DOB:**	12/04/1982
Disease status:	carrier			**Sample's ID:**	C2268
Patient Name (male partner):	XY XY XY			**DOB:**	12/04/1982
Disease status:	carrier			**Sample's ID:**	C2267
Patient Name:	XXXXXXXX			**DOB:**	01.01.2002
Relationship to patients:	daughter	**Disease status:**	affected	**Sample's ID:**	C2266
Indication for PGD:	Beta Thalassemia carriers	**Couple's ID no.:**			
Other indication:					

Case Summary

PGD Work-up report requirements

Mutation(s) confirmation

The pre-PGD workup started with blood sample analysis of the prospective parents for mutation confirmation and informativity testing of the polymorphic markers included in the assay. Additional family members or affected children of the couple (if any) may also be tested as the individual situation warrants. The results of mutation analysis are shown in Table I:

Table I

Patient Name	Relationship	Gene Region	Mutation analysis results
XX XX XX	Female partner	HBB ex. 1	IVS1-5(G>C) / WT
XY XY XY	Male partner	HBB ex. 1	IVS1-5(G>C) / WT
XXXXXXXX	Daughter	HBB ex. 1	IVS1-5(G>C) / IVS1-5(G>C)

PGD Work-up report requirements

Informativity testing of linked STR markers

STR haplotyping for family members (father, mother and an affected member of the family, if any) was performed, in order to identify the most informative STR markers linked to the disease causing gene, to be used in the following clinical PGD cycle.
A panel of 5 different informative STR markers were selected. Genotyping results of the selected markers and their distance from the gene are shown in Table II.

Table II

Gene / Marker	Dye Label	Chr. band	Chr. Location (Mb)	Distance from the gene (Mb)	Genotyping results					
					Male Partner (C2268)		Female Partner (C2267)		Affected Daughter (C2267)	
D11S4146	HEX	p15.4	3.7	1.5	**158**	**168**	**168**	**170**	**158**	**168**
D11S988	FAM	p15.4	4.5	0.7	**135**	**124**	**135**	**122**	**135**	**135**
HBB ex. 1		p15.4	5.2	0	**IVS1-5**	**WT**	**IVS1-5**	**WT**	**IVS1-5**	**IVS1-5**
D11S1338	FAM	p15.4	5.9	0.7	**120**	**126**	**128**	**126**	**120**	**128**
D11S1997	FAM	p15.4	6.3	1.1	**144**	**144**	**144**	**140**	**144**	**144**
D11S1331	HEX	p15.4	7.5	2.3	**146**	**146**	**152**	**148**	**146**	**152**

PGD Work-up report requirements

Optimisation of multiplex PCR on single cells

Multiplex PCR was designed to amplify simultaneously the selected linked STR markers which were found to be informative for the couple during informativity testing.

Parameters such as amplification efficiency, allele drop-out (ADO) and contamination rates for each marker/locus used in the multiplex PCR were also determined on the couples' own single buccal cells. A summary of the amplification results is shown in Table III. None of the blank controls included in the single cell test series displayed amplification signals, indicating absence of contamination. In view of these results, the assay was considered to be robust enough to proceed to clinical application.

Gene/ marker name	No. Cells tested	No. Cells with PCR Failure	No. Cells with positive PCR	Amplification rate (%)	No. Cells with ADO	ADO rate (%)
D11S4146	50	2	48	96.0%	2	4.2%
D11S988	50	1	49	98.0%	3	6.1%
HBB ex. 1	50	2	48	96.0%	3	6.3%
D11S1338	50	3	47	94.0%	2	4.3%
D11S1997	50	3	47	94.0%	1	2.1%
D11S1331	50	1	49	98.0%	3	6.1%
Total	**300**	**12**	**288**	**96.0%**	**14**	**4.9%**

PGD Work-up report requirements

CONCLUSION:

PGD can be offer to this couple by using the above protocol. The estimated misdiagnosis rate is **<1%**.

NOTE: The accuracy of the results is based on the assumption that samples received were correctly identified, family relationship are true and clinical diagnosis of relatives is correct.

Person performing Diagnosis:

Name _____ **Qualification** _____ **Signature** _____

Head of the Laboratory:

Name _____ **Qualification** _____ **Signature** _____

REPORTING OF RESULTS

PGD Cycle

PGD cycle Report

Items that should be included in the PGD cycle report which is sent to the IVF unit

- Name (and address) of the laboratory issuing results (PGD lab)
- Name (and address) of the receiver of the report (IVF unit)
- Name and number of the report form (as used for document control)
- Unequivocal identification of the couple (2 identifiers per individual, Name and DOB)
- Unique patient number (Couple's ID no.)
- Unique cycle identifying number (PGD cycle ID no.)
- Disease being tested
- Methods being performed
- Mutations given in Human Genome Variation Society (HGVS) mutation nomenclature
- Accession number of reference sequence (including version number) given when stating mutations
- Date of the egg collection
- Specimen information (type of sample; date/time of biopsy)
- Clearly presented results (ideally in tabulated form) with appropriate interpretative comments with clear indication of whether embryos should or should not be transferred (highlighting of abnormal results)
- Appropriate reference intervals
- Error rates clearly stated
- Identification of the person performing the diagnosis and the witness
- Identification of the person verifying the results and authorizing the release of the report and their signature
- Pagination to include the actual and total number of pages
- Date of report

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Date of report: **Time:**

IVF unit details

Referring Centre:	**IVF unit ID no.:**
Department:	**Address:**
City	**Country:**
Referring physician(s):	**Receiver(s) of the report:**

PGD details

Analysis Performed:	Preimplantation genetic diagnosis of Beta Thalassemia	**Trial:**	1
PGD cycle ID no.:	**Couple's ID no.:**		
Method of Analysis:	Minisequencing analysis + Fluorescent PCR of STR markers		
PGD Strategy:	Direct mutation testing + linkage analysis by STR markers genotyping		
Genetic disorder of concern:	Beta Thalassemia	**OMIM:**	+141900
Gene investigated:	HBB **OMIM:** +141900	**Ref. Seq. Accession No.:**	
Mutation(s) involved:	**Male partner:** IVS1-5(G>C)	**Female partner:**	IVS1-5(G>C)

Patients' details

Patient Name (female partner):	XX XX XX	**DOB:**	12/04/1982
Disease status:	carrier	**Sample's ID:**	C2268
Patient Name (male partner):	XY XY XY	**DOB:**	12/04/1982
Disease status:	carrier	**Sample's ID:**	C2267
Indication for PGD:	Beta Thalassemia carriers		
Other indication:			

IVF data

IVF cycle Ref. No.:	**OPU date:**	**Biopsy date:**	**Time:**
Specimen type:	Blastomeres **Date sample Received:**	**Time:**	
No. COC:	**No. MII:** **No. Fertilised:**	**No. thawed embryos:**	
No. Survived embryos:	**Tot. embryos for PGD:**	**No. Biopsied embryos:**	

PGD cycle Report: Results

		RESULTS			
Embryo No.	Blast. No	HBB gene IVS1-5(G>C)	Linked Markers (Chr.11)	FINAL DIAGNOSIS	Transferable
1	1A	Heterozygote	disomy	CARRIER	Y
	1B	Heterozygote	disomy		
2	2A	NR	NR	NO DIAGNOSIS	N
3	3A	Homozygote	disomy	AFFECTED	N
	3B	Homozygote	disomy		
4	4A	Heterozygote	disomy	CARRIER	Y
	4B	Heterozygote	disomy		
5	5A	Heterozygote	disomy	CARRIER	Y
	5B	Heterozygote	disomy		
6	A	WT	disomy	NORMAL	Y
	B	WT	disomy		
7	A	Heterozygote	disomy	CARRIER	Y
	B	Heterozygote	disomy		
Blanks	Absence of amplification				

WT: Wild Type; **NR**: No Results; **Y**: Yes**; N**: No.

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PGD cycle Report: Conclusion

CONCLUSION:

Embryo **3** resulted to be **AFFECTED**. The transfer of this embryos is **discouraged.**

Embryos **1, 4, 5** and **UF2** resulted to be **CARRIER**. Embryo **UF1** resulted to be **NORMAL**. Their transfer is **RECOMMENDED**.

No diagnosis have been achieved for embryo **2** because of an amplification failure for all the markers. Embryo cryopreservation for further analysis is **RECOMMENDED**.

TECHNICAL NOTES: The STR markers **D11S4146, D11S988, D11S1997**, **D11S1331** and **D11S1338,** which are linked to the HBB gene, were used for ADO and contamination detection. The estimate **misdiagnosis rate is < 1%**. Further technical details are provided in the PGD work-up report.
The accuracy of the results is based on the assumption that samples received were correctly identified, family relationship are true and clinical diagnosis of relatives is correct.
Reference intervals: **Normal** (no gene mutations); **Carrier** (heterozygote, i.e. presence of one mutation only in one of the chromosomes); **Affected**: (Homozygote, i.e. presence of the same mutation in both chromosomes or Compound Heterozygote, i.e. both chromosomes are carrying one of the mutations).

Person performing Diagnosis:
Name _____ **Qualification** _____ **Signature** _____

Witness:
Name _____ **Qualification** _____ **Signature** _____

Results verified and validated by:
Name _____ **Qualification** _____ **Signature** _____

Head of the Laboratory:
Name _____ **Qualification** _____ **Signature** _____

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PGD INTERNAL REPORT

Report of Preimplantation Genetic Diagnosis

Name and address of PGD Centre

Name and address of IVF unit

This form needs two unique patient identifiers, e.g. name and dob. It also needs a laboratory ID reference which is unique to that couple and cycle, e.g. the patient's hospital number and PGD cycle number

Patient number:
Lab ID (unique identifier):
Date of egg collection or date of biopsy:
Time and date sample received in PGD lab:
Time and date report issued:

Maternal and paternal details need to be filled in as appropriate using full ISCN 2009 for abnormal karyotype or disorder (OMIM number), gene (OMIM number), accession number of reference sequence (plus version number), Mutation(s) using Human Genome Variation Society (HGVS) nomenclature

Maternal name:
Maternal dob:
Maternal karyotype or mutation

Paternal name:
Paternal dob:
Paternal karyotype or mutation:

Brief details of protocol used as such FISH probes, *markers used, genetic distance of markers used in testing, parental haplotypes, error rates clearly stated* etc.

Person performing Diagnosis: (Name and signature)
Witness/checker: (Name and signature)

Report

Embryo code	Details of analysis	Overall result	Transferable yes or no

Signed: Head of laboratory: (Name, signature and date)

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Report to be sent to the IVF unit

Report of Preimplantation Genetic Diagnosis

Name and address of PGD Centre

Name and address of IVF unit

Patient number:
Lab ID (unique identifier):
Date of egg collection or date of biopsy:
Time and date sample received in PGD lab:
Time and date report issued:

Maternal name:
Maternal dob:
Maternal karyotype or disorder/mutation:

Paternal name:
Paternal dob:
Paternal karyotype or disorder/mutation:

Person performing Diagnosis: (Name and signature)
Witness/checker: (Name and signature)

Summary of results:
The results of the biopsy and FISH/PCR from blastomeres (or polar bodies) from embryos xx, xx, xx and xx showed a normal pattern and so these embryos may be considered for transfer *(complete as appropriate)*

Report

Embryo code	Overall result	Transferable *yes or no*

Signed by PGD lab head: (Name and signature)

Information on which embryos were transferred, any resulting pregnancy and delivery need to be reported to the PGD laboratory.

PGD is based on X cells removed from each embryo. The accuracy of the result is estimated to be XX. The accuracy of the results is based on the assumption that :- samples received were correctly identified, family relationships are true and clinical diagnosis of relatives is correct.

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Thank you……..

Francesco Fiorentino
fiorentino@laboratoriogenoma.it