

The Preimplantation Genetic Diagnosis International Society (PGDIS)

Guidelines for good practice in PGD: programme requirements and laboratory quality assurance

Preimplantation Genetic Diagnosis International Society

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Abstract

The Preimplantation Genetic Diagnosis International Society (PGDIS) was organized in October 2002, with the purpose of encouraging and co-ordinating research, education and training in this multidisciplinary field, requiring a close collaboration of obstetricians, fertility specialists, embryologists and human geneticists. One of the major tasks of PGDIS is to advance the safety and accuracy of PGD and to encourage its adoption into clinical practice for improvement of genetic practices and reproductive medicine. In this context, PGDIS published voluntary guidelines applicable for any centre offering PGD in 2004, and these guidelines are now being updated and extended based on the present extensive PGD experience. The application of these guidelines is intended to further benefit patients and provide guidance to the laboratory staff. As in previous guidelines, PGDIS presents this document being aware that differences in national regulations exist that can affect local PGD practice. The document contains recent consensus points of general application that promote quality biopsy procedures and laboratory practice, enabling PGD centres to offer an improved clinical outcome to their patients. A variety of aspects related to a safe working system have been taken into consideration, based on the assumption that a quality programme depends on the co-operation of the whole PGD team.

Keywords: good laboratory practice, guidelines, PGD, PGDIS, protocol, quality assurance

Disclaimer for clinical guidelines

This guideline is designed primarily as an educational resource for centres offering PGD to help them to provide quality medical services. Adherence to these guidelines is completely voluntary and does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures or tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinician and laboratory should apply their own professional judgment to the specific clinical circumstances presented by the individual patient or specimen.

Clinicians and laboratories are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this guideline. They also are advised to take notice of the date this guideline was adopted, and to consider other medical and scientific information that becomes available after that date.

Introduction

Preimplantation genetic diagnosis (PGD) is currently an established procedure, allowing at risk couples to have an unaffected child without facing prenatal diagnosis and termination of pregnancy. The detection and avoidance from transfer of embryos with genetic abnormalities is also an alternative to the traditional selection of the embryos based on morphologic criteria.

The Preimplantation Genetic Diagnosis International Society (PGDIS) was organized in October 2002, with the purpose of encouraging and coordinating research, education and training in this multidisciplinary field, requiring a close collaboration of reproductive medicine physicians, fertility specialists, embryologists and human geneticists (Kuliev and Verlinsky, 2004). One of the major tasks of PGDIS is to advance the safety and accuracy of PGD and to encourage its application into clinical practice for improvement of genetic practices and reproductive medicine.

In this context, PGDIS drafted voluntary guidelines in 2004, applicable for any centre offering PGD (PGDIS, 2004). The

application of these guidelines was intended to benefit patients and provide guidance to the laboratory staff. These guidelines are now updated in the light of current developments in PGD, which has presently been applied in approximately 30,000 clinical cycles performed for 170 different conditions (**Table 1**). This has resulted in the birth of thousands of children free of the conditions for which PGD was performed, with a comparable prevalence of abnormality to that in the general population, further supporting the safety of PGD. PGD is currently performed for single gene disorders, late onset disorders with genetic predisposition, chromosomal disorders, including aneuploidy and structural rearrangements, and HLA typing to improve the access to HLA matched stem cell transplantation.

PGDIS presents this document being aware that differences in national regulations exist that can affect the local PGD practice. The document contains consensus points within PGDIS, concerning general applications which promote quality laboratory practice, enabling PGD centres to offer the good clinical outcome to their patients. A variety of aspects related to a safe working system have been taken into consideration, based on the assumption that a quality programme depends on appropriate co-operation of the multidisciplinary team involved.

1 PGD indications

PGDIS considers that PGD is indicated for the following purposes:

1.1 For carriers of Mendelian disorders in order to have an unaffected offspring without facing prenatal diagnosis and clinical termination of pregnancy

1.2 For HLA typing with the purpose of conceiving a sibling that is a match to an older sibling who requires stem cell therapy (Verlinsky and Kuliev, 2006).

1.3 For carriers of translocations or other structural chromosome abnormalities in order to have an unaffected offspring without facing prenatal diagnosis and termination of pregnancy, to reduce the risk of miscarriages (Munné *et al.* 1998a, 2000; Verlinsky *et al.* 2005), and/or to improve the chance of unaffected conception in infertile carrier couples (Otani *et al.* 2006) (further discussion in Appendix A.1). This indication includes recurrent pregnancy loss (RPL) caused by translocations.

1.4 For idiopathic RPL. Although the prognosis to conceive a child after standard treatment is good, couples wanting to reduce the trauma, pain and side effects of recurrent miscarriages can use PGD to reduce the risk of miscarriage (Werlin *et al.* 2003; Munné *et al.* 2005).

1.5 For infertile patients. Several sub-indications have been proposed:

1.5.1 PGD has been shown to significantly reduce trisomic conceptions (Colls *et al.*, 2007). Thus, PGDIS considers this a valid indication regardless of maternal age and number of embryos produced.

1.5.2 PGD has also been shown to reduce significantly

spontaneous abortions in infertile couples undergoing IVF (Munné *et al.*, 1999, 2006). Thus, for couples wanting to prevent the risk of miscarriages, PGDIS considers this a valid indication regardless of maternal age and number of embryos produced.

1.5.3 PGD has been proposed as a method to increase take-home baby rates in certain subgroups of IVF patients (see Appendix A.2). Although there have been contradictory studies, rigorous analysis of methodology used in those studies (see Appendix A.2) has led PGDIS to nonetheless consider that the procedure, if performed adequately, is not detrimental, and thus can be performed in centres following best practice methods as described in Section 8.

2 Set-up of a PGD programme

PGD is based on the oocyte or embryo biopsy and DNA analysis of the biopsied material by PCR or fluorescent in-situ hybridization (FISH). The biopsy procedures presently range from the first and second polar body (PB1 and PB2) sampling, to a single blastomere removal at the cleavage stage, or blastocyst biopsy, which also provides a possibility of a confirmatory diagnosis following the PB or blastomere analysis (De Boer *et al.*, 2004; Verlinsky and Kuliev, 2005). Although the methods involved in achieving PGD accuracy will differ depending on circumstances, in many occasions a reliable diagnosis can be achieved by using two or three different methods, especially when more than one indication for PGD is involved.

A PGD programme requires the involvement of several units. Communications between these units should be clear, informative and kept under strict quality control.

2.1 IVF centre. All patients entering a PGD programme should be informed about the procedure, the expected results, the experience in the specific centre, and the limits of the technique. Specific informed consent forms must be discussed with patients; their written authorization to perform the treatment should be required.

2.2 Genetic counselling. Evaluation of couple's reproductive history and adequate genetic counselling should be part of the preliminary evaluation for all PGD patients, particularly for couples at high genetic risk due to chromosome structural abnormalities or monogenic diseases.

2.3 IVF laboratory. The laboratory should be organized according to the guidelines for good practice in IVF laboratory prepared by the ESHRE Embryology Special Interest Group (Gianaroli *et al.*, 2000). All laboratory procedures and protocols should be collected in a manual that is kept updated by the laboratory director; the current version should be available for utilization by every member of the staff. This enables strict uniformity of treatment. The procedures for single embryo culture, cell biopsy, and transfer of the selected embryos on the basis of the genetic results are additional aspects expressly related to the PGD programme.

2.4 Genetic laboratory. Protocols for single-cell genetic analysis or analysis of biopsied cells, both by FISH and PCR, should

be available, as should be unique identification systems for patients and for each cell or biopsy.

2.5 QC protocols should be in place for each genetic analysis protocol. These should include error rates calculated on at least an annual base. Error rates should be measured by reanalysis with the same protocol of cells from non-replaced previously diagnosed embryos.

3 Patient management

Patients should be informed of the procedure to be applied in their specific case and on the expected results. If the requested genetic analysis is not performed by that centre, information on tests available in other centres should be provided.

3.1 PGD feasibility for gene defects. The first step is to verify if the specific genetic analysis is feasible, either targeting the mutation in question or using the linkage analysis which allows PGD for any genetic disease, irrespective of the availability of specific sequence information (see Appendix A.3).

3.2 Reproductive counselling. The pertinent documentation on the reproductive history of the couple should be analysed and, if necessary, completed with additional examinations (e.g. sperm analysis, karyotype, hysteroscopy).

3.3 PGD set-up. Before initiating hormonal stimulation and PGD for single-gene disorders and translocations, a work-up should be performed to establish the feasibility of the assay and develop a specific design for each couple, before performing the actual PGD (see Appendix A.4).

4 IVF stimulation protocols

As PGD represents a method of embryo selection, a good response to hormonal stimulation is an important prerequisite for a successful PGD cycle.

4.1 Poor responders should be informed of their reduced success rate.

4.2 Stimulation should be devised to increase the optimal number of retrieved oocytes.

An embryo freezing programme should be available in case cryopreservation of generated embryos is necessary.

4.3 Fertile patients undergoing a PGD cycle should be advised to have protected intercourses in order to avoid the risk of spontaneous pregnancy.

4.4 When starting the IVF/PGD cycle, the couple should be required to sign an informed consent that is specific for the requested PGD (e.g. PGD for a given single-gene disorder(s), PGD for translocations, PGD for aneuploidy, PGD for only HLA matching). The consent should provide explicit information on the current error rate in that PGD centre and pertinent recommendation (e.g. conventional prenatal diagnosis in case of pregnancy).

5 IVF and insemination

5.1 In PGD performed for aneuploidy and translocations using FISH, there are no restrictions on the technique of insemination. Conversely, for carriers of single gene disorders where PCR will be applied, or for molecular (CGH, DNA microarrays) assessment of chromosome status, ICSI is recommended in order to avoid sperm contamination. For the same reason, removal of corona cells should be especially meticulously performed.

5.2 Single-embryo culture is compulsory after biopsy has been performed.

6 Source of DNA for PGD and biopsy methods

6.1 Types of cells to biopsy

6.1.1 First and second polar body (Verlinsky and Kuliev, 2005). In PGD for aneuploidy, polar bodies can be removed simultaneously from pronuclear zygotes, whereas in PGD for single gene disorders they must be removed sequentially. PGD on a polar body only permits diagnosis of female-related defects. In polar body fragmentation, extreme attention is required for complete recovery.

6.1.2 Blastomere biopsy from day 3 embryos can be used for any type of PGD indication.

6.1.3 Blastocyst biopsy of trophectoderm cells. The clinical application of this technique is very recent and only limited data have been reported (De Boer, 2004). Therefore, its applicability on a large scale needs validation.

6.2 Embryo biopsy procedure

6.2.1 Significant experience is required from the person responsible for biopsy of embryos. PGDIS recommends that this procedure be learned from expert laboratories or practitioners, and periodic retraining undertaken using discarded embryos. PGDIS discourages clinical embryo biopsy by personnel performing the technique sporadically or having little prior experience.

6.2.2 Before initiating PGD, the centre should validate that performance of the biopsy procedure does not have a deleterious effect on the embryos viability.

6.2.3 A single blastomere should be removed from embryos that have already entered the third cleavage division (at least 5 blastomeres). Biopsy of two cells should be avoided (see Appendix A.2).

6.2.4 Presence of a clearly visible nucleus guides the selection of the blastomere to be biopsied.

6.2.5 Three methods for opening the zona pellucida have been reported: mechanical, chemical, laser. It is recommended that only one breach in the zona be made in order to avoid loss of

blastomeres during the procedure or embryo entrapment during hatching. Procedures resulting in large zona openings (>60 µm), excessive embryo squeezing, excessive heat from unaligned lasers, can be detrimental and should be avoided.

6.2.6 Biopsy medium. As for other micromanipulation procedures, the medium used should be the same as that in which the embryo was cultured, with the exception of being Ca-Mg-free to avoid a ‘shock’. Ideally these embryos should be biopsied in this media in a short period of time (about 1 min).

6.2.7 Sucrose could supplement the media in order to shrink the cells and allow the use of smaller zona pellucida openings.

6.2.8 Speed of the biopsy procedure is critical. It is recommended that one person do the embryo biopsy and a second perform dish change-over of embryos in order to minimize the time the embryos are outside the incubator.

6.2.9 Adequate number of incubators are recommended to minimize opening and closing the incubator, and hence producing temperature fluctuations.

6.2.10 Biopsied cells should be intact to ensure good quality results.

6.2.11 Following biopsy, validation by two persons involved in dish preparation and embryo culture is recommended in order to guarantee the unique correspondence between the embryo biopsied and the biopsied cell tested. Special attention must be paid in clearly identifying and labelling, especially if the genetic analysis is to be performed in a laboratory different from that performing biopsy.

6.2.12 Single-embryo culture is thereafter compulsory.

6.2.13 If biopsied cells are sent from IVF centres to PGD reference laboratories, embryologists in the IVF centres should be aware of the necessary conditions to retrieve and prepare the samples for delivery.

6.2.14 A declaration from the IVF laboratory should accompany the samples stating that correspondence between blastomere and embryo identification was verified.

6.3 Polar body biopsy procedure

6.3.1 Mechanical polar body biopsy is recommended, with several differences from the procedure of embryo biopsy (Verlinsky and Kuliev, 2005).

7 PCR for single-gene disorders

PGD is presently applied to approximately 170 different conditions with the range of indications is gradually expanding (see Appendix A.5).

7.1 To initiate PGD for single gene disorders the following procedures are recommended:

7.1.1 Confirmation of the detected mutations in the DNA in that

family, for which purpose a variety of strategies exist.

7.1.2 Confirmation also is required in X-linked disorders, for which PCR analysis should be used for identification of specific mutation or linked markers.

7.1.3 Identification of closely linked markers that can be included in the reaction, as determined by studying family members. Definition of minimal requirements exists (Rechitsky *et al.*, 2001; Sermon, 2002).

7.1.4 Availability of exogenous DNA-free reagents.

7.1.5 Availability of a dedicated, fully equipped area for PGD.

7.1.6 Identification of the appropriate positive and negative controls.

7.1.7 Haplotyping in male de-novo mutations and in the absence of offspring for testing.

7.1.8 Test validation on single cells (e.g. buccal cells, lymphocytes, discarded or donated blastomeres), using the following recommended guidelines:

7.1.8.1 Amplification efficiency not less than 85%

7.1.8.2 Application of the protocols of simultaneous detection of the causative gene as well as highly polymorphic markers closely linked to the gene tested this will detect preferential amplification and allele-drop out (ADO) (see Appendix A.6).

7.1.8.3 Use of the polymorphic markers to detect and avoid extraneous DNA contamination, which should be no greater than 5%.

7.1.9 PGD reference laboratories: IVF centres sending samples to PGD reference laboratories for the first time should do a test (dry) run in order that the PGD reference laboratory can verify that amounts of DNA contamination are tolerable in PCR samples and that the number of tubes with no cells is acceptable.

7.2 To perform a clinical case for PGD of gene defects the following steps are recommended:

7.2.1 Recommended strategy includes nested PCR and fluorescent PCR in a multiplex reaction, with one blank for every cell and use of positive controls (i.e. parents’ blood carrying the same genotype) along the clinical cycle.

7.2.2 PCR set-up. The preliminary phase must be satisfactorily completed, with results interpreted preferably by two independent readers before starting the clinical case.

7.2.3 Both alkaline lysis and proteinase K/SDS (potassium/sodium dodecyl sulphate) have been reported to give comparable results and are equally acceptable.

7.2.4 The PCR results should be read by two independent readers. The only embryos that are given a diagnosis are those in which results are concordant.

8 PGD for chromosomal disorders (FISH)

8.1 Cell fixation needs to be performed before PGD analysis of chromosome disorders through fluorescence in-situ hybridization (FISH):

8.1.1 Methanol: acetic acid is recommended to minimize signal overlap and associated errors, and to reduce the loss of micronuclei and DNA fibres (Velilla *et al.*, 2004). No fixative drops should be added once the cytoplasm has broken. Appropriate stereoscope optics and mirrors are required to see the tridimensionality of this process.

8.1.2 The presence of nuclei and lack of cytoplasm should be confirmed before sending the slide for FISH analysis, using a phase contrast scope.

8.1.3 Significant experience is required from the person fixing these cells. PGDIS recommends that this procedure be learned from expert laboratories or practitioners, with periodic retraining undertaken using day 2 embryo mouse blastomeres.

8.2 To perform FISH for PGD of chromosomal disorders, these procedures should be followed:

8.2.1 Probes should be tested on normal and trisomic cell lines to evaluate the efficiency, especially in the case of 'home-brew' probes.

8.2.2 For probe validation, FISH diagnosis should be verified on at least 95% of the analysed cells alluded to in Section 8.1.

8.2.3 In PGD for aneuploidy, ideally an aggregate set of eight chromosome probes is recommended, including chromosomes X, Y, 13, 15, 16, 18, 21, 22, whose aneuploidies are mostly represented in spontaneous abortions.

8.2.4 In PGD for translocations, the adequate combination of probes should be tested on carriers' lymphocytes in order to validate the ability to detect unbalanced rearrangements (Munné *et al.*, 2000; Gianaroli *et al.*, 2002). A minimum of two distal and one proximal, or two proximal and one distal, probes should be used to detect all potential segregations.

8.2.5 Scoring criteria should be established for the interpretation of FISH signals (Munné *et al.*, 1998b; Magli *et al.*, 2001).

8.2.6 Qualified personnel with genetic expertise and having a PhD or MD degree should direct and supervise each clinical case.

8.2.7 FISH signals should be recorded by two independent readers. Results should be compared and a consensus reached in the case of discordance. If discordance occurs, the use of 'no result rescue' (NRR) is recommended (see Section 8.2.8).

8.2.8 NRR (Magli *et al.*, 2001; Colls *et al.*, 2007) is recommended to reduce the number of cells with dubious results and reduce errors. NRR consists on reanalysing a cell with no clear results for a specific chromosome with a probe binding to the same chromosome but to a different locus.

8.2.9 PGD reference laboratories: IVF centres sending samples to PGD reference laboratories for the first time should do a test run ('dry-run') so the PGD reference lab can verify the quality of the cells fixed for each person fixing cells. Training should be sought if fixation techniques are not of the standards of the reference laboratory as stated in Section 8.1.

8.2.10 Psychological counselling. Psychological support should be extended to PGD couples who have a history of reproductive failure.

9 Embryo transfer

The IVF laboratory must receive from the genetic laboratory a written report of the performed analysis. A diagnosis should be reported for any tested embryo. Embryos recommended for transfer should be clearly indicated.

9.1 Information to patients. Prior to transfer, patients must be informed of results obtained. This is recommended also in cases in which no transferable embryos are detected.

9.2 Given that the error rate after single-cell analysis is not negligible, conventional prenatal diagnosis should be recommended to confirm the analysis performed on a single cell.

9.3 From clinic to clinic certain variables exist and are acceptable: transfer medium and catheter, use of ultrasound guidance, day of transfer, number of embryos to be transferred. General recommendations include soft catheter, very gentle mode of transfer and number of embryos transferred (it is recommended not to exceed two).

9.4 Selection of embryos for transfer. A double-check point is recommended at the time of transfer to verify both patient identification and embryo selection.

9.5 Transfer of non-biopsied or non-diagnosed embryos is generally not recommended; however, this can be considered in aneuploidy testing if no other options exist and the couple is suitably informed.

10 Spare embryos

10.1 Cryopreservation of PGD normal embryos with regular development. Initially, cryopreservation on day 3 of biopsied embryos had a low success rate (Joris *et al.*, 1999; Magli *et al.*, 1999), but improved survival rates were later reported (Jericho *et al.*, 2003). Expanding culture to blastocyst is another way to minimize the number of spare embryos needing cryopreservation. A 'double-check point' is recommended at the time of freezing in order to verify patient and embryo identification.

10.2 Non-transferred, non-cryopreserved embryos should be used for confirmation of PGD results, specifically by re-analysing all blastomeres of these embryos. A systematic re-analysis should be performed to determine efficiency of the technique and estimate the current one-cell error rate, which is recommended as less than 10%.

11 Follow-up of pregnancies

Although the birth of healthy children is the aim of PGD, the reduction of the risk of miscarriages is also the important objective for the couples with translocations and/or recurrent pregnancy loss. Therefore, follow-up of clinical pregnancies is pivotal for evaluation of the efficiency of the technique.

11.1 Implantation rate, calculated as the proportion of viable embryos per the number of embryos transferred.

11.2 Clinical pregnancy rate, calculated as the number of embryos with heart beat per number of transfers, and take-home baby rate.

11.3 Clinical pregnancy loss (spontaneous abortion) rate, calculated as proportion of pregnancy losses per number of clinical pregnancies. The analysis of the aborted fetus is recommended as it helps determine the correctness of PGD results, i.e. *in-vivo* efficacy.

11.4 Follow-up of infants. Identification of major and minor malformations will help determine the effects related to the biopsy procedure. An extended follow-up would be especially valuable for evaluation of long-term effects possibly derived from the invasiveness of the technique. Definitions and recommended protocols are available (Simpson and Liebaers, 1996)

12 Quality control and assurance: outcome measures

Because the results of PGD depend on the experience and quality of the multidisciplinary work, each affecting significantly the overall outcome, the results from the PGD programme should be monitored on a systematic basis from each individual centre. Outcome measures include the following:

12.1 Proportion of cells overtly damaged during embryo biopsy.

12.2 Proportion of cells for which a diagnosis was not obtained.

12.3 Misdiagnosis rate per embryo and per pregnancy calculated *in vitro*.

12.4 Misdiagnosis rate calculated *in vivo*.

12.5 Implantation rate.

12.6 Clinical pregnancy loss rate.

12.7 Clinical pregnancy rate.

12.8 Take-home baby rate.

12.9 Multiple pregnancy rate (monozygotic versus dizygotic).

12.10 Minimum number of pre-clinical assays to be performed in a given laboratory before clinical application.

12.11 Criteria to assess the competence of the staff involved in the different steps of PGD.

13 Future perspectives

Novel techniques and PGD applications have been proposed in recent years for improving the performance of PGD: multiple displacement amplification (MDA), metaphase conversion for second polar body and blastomeres, DNA microarrays and chip technology. Valuable information is deriving from preliminary reports, but additional data are necessary before considering these procedures as part of the standard clinical application.

Table 1. List of diseases and genes for which PGD has been performed (correct on 22 November 2007).

<i>Disease</i>	<i>MIM number</i>	<i>Inheritance</i>	<i>Gene name/symbol</i>	<i>Protein name</i>	<i>Location</i>
Achondroplasia; ACH	100800	AD	FGFR3	Fibroblast growth factor receptor 3 [precursor]	4 p16.3
Acyl-CoA dehydrogenase, medium-chain, deficiency	201450	AR	ACADM	Acyl-CoA dehydrogenase, medium-chain specific, mitochondrial [precursor]	1p31
Acyl-CoA dehydrogenase, very long-chain; ACADVL	609575	AR	ACADVL	Acyl-coenzyme A dehydrogenase, very long chain	17p13-p11
Adenosine deaminase deficiency; ADA	102700	AR	ADA	Adenosine deaminase	20q13.11
Adenomatous polyposis of the colon; APC	175100	AD	APC	Adenomatous polyposis coli protein	5 q21-q22
Adrenoleukodystrophy; ALD	300100	XL	ABCD1	Adrenoleukodystrophy protein	Xq28
Albinism, ocular, type I; OAI	300500	XL	OAI	G-protein coupled receptor 143	Xp22.3
Alopecia universalis congenita; ALUNC	203655	AR	HR	Hairless protein	8 p21.2
Alpers diffuse degeneration of cerebral gray matter with hepatic cirrhosis	203700	AR	POLG	Mitochondrial DNA polymerase gamma	15q25
Alpha 1 antitrypsin deficiency (AAT)	107400	AR	SERPINA1	Alpha-1-antitrypsin [precursor]	14q32.1
Alport syndrome, X-linked; AITS	301050	XL	AMMECR1	AMME syndrome candidate gene 1 protein	Xq22.3
Amyloidosis I, hereditary neuropathic	176300	AD	TTR	Transthyretin [precursor]	18q11.2-q12.1
Androgen receptor; AR (testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)	313700	XL	AR	AR protein	Xq11-q12
Aneuploidies by STR genotyping					
Angioedema, hereditary; HAE					
Ataxia-telangiectasia; AT	106100	AD	SERPING1	Plasma protease C1 inhibitor precursor	11q11-q13.1
Basal cell nevus syndrome; BCNS (Gorlin)	208900	AR	ATM	Serine-protein kinase ATM	11q22-q23
Blepharophimosis, ptosis, and epicanthus inversus; BPES	109400	AD	PTCH	Patched protein homolog 1	9q22.1-31
Blood group – Kell–Cellano system	110100	AD	FOXL2	Forkhead box protein L2	3 q23
Brachydactyly, Type B1; BDB1	110900	AD	KEL	Kell blood group glycoprotein	7 q33
Brain tumour, posterior fossa of infancy, familial	113000	AR	ROR2	Receptor tyrosine kinase-like orphan receptor 2	9q22
Breast cancer, familial	601607	AD	SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily B member	22q11.2
Breast cancer, familial	113705	AD	BRCA1	Breast cancer type 1 susceptibility protein	17q21
Breast cancer, familial	600185	AD	BRCA2	Breast cancer type 2 susceptibility protein	13q12.3
Bruton agammaglobulinaemia tyrosine kinase; BTK	300300	XL	BTK	Bruton agammaglobulinaemia tyrosine kinase	Xq21.33-q22
Canavan disease	271900	AR	ASPA	Aspartoacylase	17pter-p13
Ceroid lipofuscinosis, neuronal 2, late infantile; CLN2	204500	AR	CLN2	Tripeptidyl-peptidase I [precursor]	11p15
Charcot-Marie-Tooth disease, axonal, type 2E	607684	AD	NEFL	Neurofilament triplet L protein	8 p21
Charcot-Marie-Tooth disease, demyelinating, type 1A; CMT1A	118220	AD	PMP22	Peripheral myelin protein 22	17p12
Charcot-Marie-Tooth disease, demyelinating, type 1B; CMT1B	118200	AD	MPZ	Myelin P0 protein [precursor]	1q23.3
Charcot-Marie-Tooth disease, X-linked, 1; CMTX1	302800	XL	GJB1	Gap junction beta-1 protein	Xq13.1
Cholestasis, progressive familial intrahepatic 2	603201	AR	ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11	2q24
Chondrodysplasia punctata 1, X-linked recessive; CDPX1	302950	XL	ARSE	Arylsulfatase E	Xp22.3

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<i>Disease</i>	<i>MIM number</i>	<i>Inheritance</i>	<i>Gene name/symbol</i>	<i>Protein name</i>	<i>Location</i>
Choroideraemia; CHM	303100	XL	CHM	Rab proteins geranyltransferase component A 1	Xq21.2
Citrullinaemia, classic	215700	AR	ASS	Argininosuccinate synthase	9q34.1
Collagen, type IV, alpha-5; COL4A5	303630	XL	COL4A5	Collagen, type IV, alpha 5	Xq22.3
Colorectal cancer, hereditary non-polyposis, type 1; HNPCC1	120435	AD	MSH2	DNA mismatch repair protein Msh2	2p 2-p21
Colorectal cancer, hereditary non-polyposis, type 2; HNPCC2	609310	AD	MLH1	DNA mismatch repair protein Mlh1	3 p21.3
Congenital adrenal hyperplasia (CAH)	201910	AR	CYP21A2	Cytochrome P450 XXIB	6 p21.3
Craniofacial dysostosis, type I; (CFD1)	123500	AD	FGFR2	Fibroblast growth factor receptor 2 [precursor]	10q26.13
Curarino syndrome	176450	AD	HLXB9	Homeobox protein HB9	7q36
Cutis laxa, autosomal recessive, type I	219100	AR	FBLN4	EGF-containing fibulin-like extracellular matrix protein 2	11q13
Cystic fibrosis; CF	219700	AR	CFTR	Cystic fibrosis transmembrane conductance regulator	7q31.2
Cystinosis, nephropathic; CTNS	219800	AR	CTNS	Cystinosis	17p13
Darier–White disease; DAR	124200	AD	ATP2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	12q23-q24.1
Deafness, neurosensory, autosomal recessive 1; DFNB1	220290	AR	GJB2	Gap junction protein connexin-26	13q11-q12
Diamond–Blackfan anaemia; DBA	105650	AD	RPS19	40S ribosomal protein S19	19q13.2
Dysautonomia, familial	223900	AR	IKBKAP		9q31
Dystrophia myotonica 1	160900	AD	DMPK	Myotom-in-protein kinase	19q13.2-q13.3
Early-onset familial Alzheimer disease	104760	AD	APP	Amyloid beta A4 protein [precursor]	21q21.3
Ectodermal dysplasia 1, anhidrotic; ED1	305100	XL	EDI	Ectodysplasin A	Xq13.1
Ectodermal dysplasia, anhidrotic	224900	AR	EDAR	Tumour necrosis factor receptor superfamily member EDAR [precursor]	2q 11-q13
Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 1; EEC1	129900	AD	p63	Tumour protein 63	7 q 11.2-q21.3
Emery–Dreifuss muscular dystrophy, autosomal recessive; EDMD3	604929	AR	LMNA	Lamin A/C	1q21.2
Emery–Dreifuss muscular dystrophy, X-linked; EDMD	310300	XL	EMD	Emerin	Xq28
Epidermolysis bullosa dystrophica, Pasini type	131750	AR	COL7A1	Collagen alpha 1(VII) chain [precursor]	3 p21.3
Epidermolysis bullosa lethalis	226650	AR	LAMB3	Laminin, beta 3	1q32
Epidermolysis bullosa simplex and limb-girdle muscular dystrophy	226670	AR	PLEC1	Plectin 1, intermediate filament binding protein 500kDa	8q24
Epiphyseal dysplasia, multiple, 1; EDM1	132400	AD	COMP	Cartilage oligomeric matrix protein [precursor]	19p13.1
Exostoses, multiple, type I	133700	AD	EXT1	Exostosin-1	8 q24.11q24.13
Fabry disease	301500	XL	GLA	Alpha-galactosidase A [precursor]	Xq22
Facioscapulohumeral muscular dystrophy 1A; FSHMD1A	158900	AD	FRG1	FRG1 protein	4 q35
Fanconi anaemia, complementation group C; FANCC	227645	AR	FANCC	Fanconi anaemia group C protein	9q22.3
Fanconi anaemia, complementation group E; FANCE	600901	AR	FANCE	Fanconi anaemia, complementation group E	6p22-p21
Fanconi anaemia, complementation group F; FANCF	603467	AR	FANCF	Fanconi anaemia group F protein	11p15
Fanconi anaemia, complementation group G	602956	AR	XRCC9	DNA-repair protein XRCC9	9p13
Fanconi anaemia, complementation group J	609054	AR	BRIP1	Fanconi anaemia group J protein	17q22
Fanconi anaemia, complementation group A; FANCA	227650	AR	FANCA	Fanconi anaemia group A protein	16q24.3
Fragile site mental retardation 1	309550	XL	FMRI	Fragile X mental retardation 1 protein	Xq27.3

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Disease	MIM number	Inheritance	Gene name/symbol	Protein name	Location
Fragile site, folic acid type, rare, FRA(X)(q28); FRAXE	309548	XL	FMR2	Fragile X mental retardation 2 protein	Xq28
Friedreich ataxia 1; FRDA	229300	AR	FRDA	Frxataxin, mitochondrial precursor	9q13
Galactosaemia	230400	AR	GALT	Galactose-1-phosphate uridylyltransferase	9p13
Gangliosidosis, generalized GM1, type I	230500	AR	GLB1	Galactosidase, beta 1	3p21.33
Gaucher disease, type I	230800	AR	GBA	Glucosylceramidase [precursor]	1q21
Glomovenous malformation (GVM)	138000	AD	TIE2/TEK	Protein receptor tyrosine kinase	1p22-p21
Glutaric acidemia I	231670	AR	GCDH	Glutaryl-coenzyme A dehydrogenase	19p13.2
Glycogen storage disease type VI	232700	AR	PYGL	Glycogen phosphorylase, liver form	14q21-q22
Haemoglobin-alpha locus 1; HBA1	141800	AR	HBA1	Haemoglobin alpha chain	16pter-p13.3
Haemoglobin-alpha locus 2; HBA2	141850	AR	HBA2	Haemoglobin alpha subunit	16pter-p13.3
Haemoglobin-beta locus; HBB	141900	AR	HBB	Haemoglobin beta chain	11p15.5
Hemophagocytic lymphohistiocytosis, familial, 2	603553	AR	PRF1	Perforin 1 [precursor]	10q22
Hemophilia A	306700	XL	F8	Coagulation factor VIII [precursor]	Xq28
Hemophilia B	306900	XL	F9	Coagulation factor IX [precursor]	Xq27.1-q27.2
HLA matching genotyping					
Homocystinuria due to deficiency of N(5,10)-methylene tetrahydrofolate reductase activity	236250	AR	MTHFR	Methylenetetrahydrofolate reductase	6 q21.3
Hoyeraal-Hreidarsson syndrome; HHS	300240	XL	DKC1	H/ACA ribonucleoprotein complex subunit 4	Xq28
Huntington disease; HD	143100	AD	HD	Huntingtin	4 p16.3
Hurler syndrome	607014	AR	IDUA	Alpha-L-iduronidase [precursor]	4 p16.3
Hydrocephalus, X-linked; L1CAM	308840	XL	L1CAM	Neural cell adhesion molecule L1 [precursor]	Xq28
Hyperinsulinemic hypoglycaemia, familial, 1; HHHF1	256450	AR	ABCC8	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	11p15.1
Hypophosphatasia, infantile	241500	AR	ALPL	Alkaline phosphatase, tissue-non-specific isozyme [precursor]	1p36.1-34
Hypophosphatemic rickets, X-linked dominant	307800	XL	PHEX	Phosphate regulating endopeptidase homolog	Xp22.2-p22.1
Hypophosphatemic rickets, X-linked dominant	307800	XL	PHEX	Phosphate regulating endopeptidase homolog	Xp22.2-p22.1
Immunodeficiency with hyper-IgM, type 1; HIGM1	308230	XL	TNFSF5	Tumour necrosis factor ligand superfamily member 5	Xq26
Incontinentia pigmenti; IP	308300	XL	IKBKG	NF-kappa-B essential modulator	Xq28
Isovaleric acidemia; IVA	243500	AR	IVD	Isovaleryl coenzyme A dehydrogenase	15q14-q15
Krabbe disease	245200	AR	GALC	Galactocerebrosidase [precursor]	14q31
Leigh syndrome; LS	185620	AR	SURF1	Surfeit locus protein 1	9q34.2
Leukoencephalopathy with vanishing white matter; VWM	603896	AR	EIF2B2	Translation initiation factor eIF-2B beta subunit	14q24
Li-Fraumeni syndrome 1; LFS1	151623	AD	TP53	Cellular tumour antigen p53	17p13.1
Loeys-Dietz syndrome; LDS	609192	AD	TGFBR2	Transforming growth factor, beta receptor II (70/80kDa)	3p22
Long-chain 3-hydroxyacyl-coa dehydrogenase deficiency;HADHA	600890	AR	HADHA	Trifunctional enzyme alpha subunit, mitochondrial [precursor]	2p 3

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<i>Disease</i>	<i>MIM number</i>	<i>Inheritance</i>	<i>Gene name/symbol</i>	<i>Protein name</i>	<i>Location</i>
Machado–Joseph disease; MJD	109150	AD	ATX3	Machado–Joseph disease protein 1	14q24.3-q31
Marfan syndrome; MFS	154700	AD	FBN1	Fibrillin 1 [precursor]	15q21.1
Meckle-Gruber Syndrome	249000	AR	MKS1	Meckelin	17q23
Metachromatic leukodystrophy	250100	AR	ARSA	Arylsulfatase A [precursor]	22q13.31-qter
Metaphyseal chondrodysplasia, Schmid type; MCDS	156500	AD	COL10A1	Collagen, type X, alpha 1	6q21-q22
Methylmalonic aciduria and homocystinuria (MIMACHC)	277400	AR	MMACHC	Methylmalonic aciduria and homocystinuria protein	1p34.1
Microcoria-congenital nephrosis syndrome	609049	AR	LAMB2	Laminin beta-2	3p21
Migraine, familial hemiplegic, 1; FHM1	141500	AD	CACNA1A	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	19p13.2-p13.1
Morquio syndrome, non-kerat sulfate-excreting type	252300	AR	GALNS	Galactosamine (N-acetyl)-6-sulfate sulfatase	16q24.3
Mucopolysaccharidosis type II (Hunter) Hunter–McAlpine craniostenosis syndrome	309900	AD	IDS	Iduronate 2-sulfatase [precursor]	Xq28
Multiple acyl-CoA dehydrogenase deficiency; MADD	231680	AR	ETFA	Electron-transfer-flavoprotein, alpha polypeptide	15q23-q25
Multiple endocrine neoplasia, type I; MEN1	131100	AD	MEN1	Multiple endocrine neoplasia I	11q13.1
Multiple endocrine neoplasia, type IIa; MEN2A	171400	AD	RET	Ret proto-oncogene	10q11.2
Muscular dystrophy, Becker type; BMD	300376	XL	DMD	Dystrophin	Xq21.2
Muscular dystrophy, Duchenne type; DMD	310200	XL	DMD	Dystrophin	Xq21.2
Myotubular myopathy 1; MTM1	310400	XL	MTM1	Myotubularin	Xq28
N-acetylglutamate synthase deficiency	237310	AR	NAGS	N-acetylglutamate synthase	17q21.31
Neurofibromatosis, type I; NF1	162200	AD	NF1	Neurofibromin	17q11.2
Neurofibromatosis, type II; NF2	101000	AD	NF2	Merlin	22q12.2
Neuropathy, hereditary sensory and autonomic, type III; HSAN3	223900	AR	IKBKAP	Kinase complex-associated protein	9q31
Niemann–Pick disease	257220	AR	NPC1	Niemann–Pick C1 protein	18q11-q12
Norrie disease; NDP	310600	XL	NDP	Norrin	Xp11.4-p11.3
Oculocutaneous albinism, type I; OCA1	203100	AR	TYR	Tyrosinase [precursor]	11q14-q21
Oculocutaneous albinism, type II; OCA2	203200	AD	OCA2	P protein	15q11.2-q12
Omenn syndrome	603554	AD	RAG1	V(D)J recombination-activating protein 1	11p13
Optic atrophy 1; OPA1	165500	AD	OPA1	Dynamin-like 120 kDa protein, mitochondrial [precursor]	3 q28-q29
Ornithine transcarbamylase deficiency	311250	XL	OTC	Ornithine carbamoyltransferase, mitochondrial [precursor]	Xp21.1
Osteogenesis imperfecta congenita; OIC	166200	AD	COL1A2	Collagen alpha 2(I) chain [precursor]	7 q22.1
Osteogenesis imperfecta congenita; OIC	166200	AD	COL1A1	Collagen alpha 1(I) chain [precursor]	17q21.31-q22
Osteopetrosis, autosomal recessive	259700	AR	TCIRG1	Vacuolar proton translocating ATPase 116 kDa subunit a isoform 3	11q13.4-q13.5
Osteopetrosis, autosomal recessive	259700	AR	TCIRG1	T-cell, immune regulator 1	11q13.2
Pancreatitis, hereditary; PCTT	167800	AD	PRSS1	Protease, serine, 1 (trypsin 1)	7q32-qter17q34
Pelizaeus–Merzbacher-like disease; PMLD	311601	XL	PLP1	Myelin proteolipid protein	Xq22
Peutz–Jeghers syndrome; PJS	175200	AD	STK11	Serine/threonine kinase 11	19p13.3

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MIM number	Inheritance	Gene name/symbol	Protein name	Location
		Phenylketonuria	Phenylalanine-4-hydroxylase	12q22-q24.2
		Polycystic kidney disease 1; PKD1	Polycystin 1 precursor	16p13.3
		Polycystic kidney disease 2; PKD2	Polycystin 2	4 q22.1
		Polycystic kidney disease, autosomal recessive; ARPKD	Polycystic kidney and hepatic disease 1 [precursor]	6 p12.3
		Popliteal pterygium syndrome; PPS	Interferon regulatory factor 6	1q32-q41
		Propionic acidemia	Propionyl coenzyme A carboxylase, alpha polypeptide	13q32
		Retinitis pigmentosa	Rhodopsin	3q21-q24
		Retinitis pigmentosa 3; RP3	Retinitis pigmentosa GTPase regulator	Xp21.1
		Retinoblastoma; RB1	Retinoblastoma-associated protein	13q14.1-q14.2
		Rett syndrome; RTT	Methyl-CpG-binding protein 2	Xq28
		Rhesus blood group, CcEe antigens; RHCE	CcEe antigens	1p36.2-p34
		Rhesus blood group, D antigen; RHD	D antigen	1p36.11
		Sandhoff disease	Beta-hexosaminidase beta chain [precursor]	5 q13
		Sickle cell anaemia	Haemoglobin beta chain	11p15.5
		Smith-Lemli-Opitz syndrome; SLOS	7-Dehydrocholesterol reductase	11q12-q13
		Sonic hedgehog; SHH	Sonic hedgehog protein [precursor]	7 q36
		Spinal muscular atrophy, type I; SMA1	Survival motor neuron protein	5 q12.2-q13.3
		Spinocerebellar ataxia 1; SCA1	Ataxin 1	6p23
		Spinocerebellar ataxia 2; SCA2	SCA2 protein	12q24
		Spinocerebellar ataxia 6; SCA6	Voltage-dependent P/Q-type calcium channel alpha-1A subunit	19p13
		Spinocerebellar ataxia 7; SCA7	Ataxin-7	3 p21.1-p12
		Stickler syndrome, type I; STL1	Collagen, type II, alpha 1	12q13.11-q13.2
		Succinic semialdehyde dehydrogenase deficiency	Succinate semialdehyde dehydrogenase, mitochondrial [precursor]	6 p22
		Symphalangism, proximal; SYM1	Noggin [precursor]	17q22
		Tay-Sachs disease; TSD	Beta-hexosaminidase alpha chain [precursor]	15q23-q24
		Torsion dystonia 1, autosomal dominant; DYT1	Torsin A [precursor]	9q34
		Treacher Collins-Franceschetti syndrome; TCOF	Treacle protein	5 q32-q33.1
		Tuberous sclerosis type 1	Hamartin	9q34
		Tuberous sclerosis type 2	Tuberin	16p13.3
		Tyrosinaemia, type I	Fumarylacetoacetate hydrolase (fumarylacetoacetase)	15q23-q25
		Ulnar-mammary syndrome; UMS	T-box 3	12q24.1
		Von Hippel-Lindau syndrome; VHL	Von Hippel-Lindau disease tumour suppressor	3 p26-p25
		Wiskott-Aldrich Syndrome; WAS	Wiskott-Aldrich syndrome protein	Xp11.23-p11.22
		Zellweger syndrome; ZS	Peroxisomal membrane protein 3, 35kDa	8q21.1
		Zellweger syndrome; ZS	Peroxisome biogenesis factor 1	7 q21-q22

Appendix A.1

Spontaneous abortion rates after PGD of translocations are reduced to about 10% and birth rates increased to about 90% (Munné *et al.* 1998, 2000) compared with 29 and 71% respectively, following evaluation and treatment of concomitant factors (Stephenson and Sierra, 2006).

Appendix A.2

Rationale

The potential of preselection of euploid embryos for transfer is based on the well established fact that approximately half of oocytes and embryos tested in poor prognosis IVF patients are chromosomally abnormal (Kuliev *et al.*, 2003; Magli *et al.*, 2007; Munné *et al.*, 2007a).

PGD was thus proposed as a method to increase take-home baby rates (Munné *et al.*, 1993), specifically for women 35 and older with a minimum of 6 biopsiable embryos (Munné *et al.*, 2003). In addition, PGD for infertility has been applied for other poor prognosis IVF patients, including repeated IVF failures.

Limitations and mosaicism

The accuracy of PB or embryo biopsy approaches may be limited, because oocyte testing does not detect errors from paternal meiosis, fertilization related abnormalities and mitotic errors, whereas a single biopsied blastomere might not represent the actual karyotype of the embryo. This is because while approximately 30% abnormal embryos are mosaics, mosaicism alone causes only about 5% of diagnostic errors because in most mosaics almost all cells are abnormal (Colls *et al.* 2007). A third alternative could be preselection of embryos for transfer may be performed by a combination of mechanical zona opening and sequential PB1, PB2 and blastomere sampling (Cieslak *et al.* 2006).

Factors affecting clinical success

Except for a few series, the existing experience suggests significant positive impact of aneuploidy testing on the reproductive outcome of poor prognosis IVF patients (Gianaroli *et al.*, 1999, 2001; Munné *et al.*, 1999, 2003, 2006). Applied presently in over 20,000 IVF cycles in the effort to pre-select the embryos with highest potential to result in pregnancy, PGD for chromosomal disorders has demonstrated the positive clinical impact through the improved implantation and pregnancy rates, reduction of spontaneous abortions and improved take home baby rate in poor prognosis patients, including those of advanced reproductive age, repeated IVF failures and recurrent spontaneous abortions.

Although there is strong evidence that PGD for chromosomal aneuploidy will potentially contribute to the pregnancy outcome of the poor prognosis IVF patients, the actual impact will depend on success in adhering to the guidelines in this document. Transfer of incorrectly tested abnormal embryos will lead to implantation and pregnancy failures; thus, testing of the products of conception obtained after spontaneous abortions

may be also useful. Further improvement of the accuracy and completeness of the chromosomal analysis will be necessary to improve impact of the procedure on the clinical outcome. The beneficial effect of PGD depends on minimizing biopsy damage.

The lack of positive effect of aneuploidy testing in two of the three published randomized trials (RCT) (Staessen *et al.*, 2004; Platteau *et al.*, 2005) may be due to potential detrimental effect of two blastomere removal, which definitely reduced the implantation potential of the biopsied embryos to the extent that could not be bridged even by preselection of aneuploidy free embryos (Cohen *et al.*, 2007). In the other RCT that failed to detect the positive effect, a single blastomere was tested, but biopsy *per se* significantly (56%) decreased implantation rate (Mastenbroek *et al.*, 2007). If biopsy is not done appropriately, PGD selection may not compensate for that damage. In addition, not all the chromosomes recommended in these guidelines were tested. There further must have been poor selection of cases given a mean number of only 4.8 embryos, fewer than the 6.0 recommended for eligibility (Cohen and Grifo, 2007; Munné *et al.*, 2007b,c; Kuliev and Verlinsky 2008).

Although further randomized controlled studies will still be required to quantify in more detail the clinical impact of the pre-selection of aneuploidy free zygotes for embryo transfer, any participating centre must first be able to verify that the biopsy procedure in their hands does not have undue deleterious effect on embryos viability. In the absence of the appropriate randomized controlled studies, the positive impact of PGD was demonstrated by the comparison of reproductive outcome in the same patients with and without PGD (Gianaroli *et al.*, 2004; Verlinsky *et al.*, 2005).

[TABLE 1 HERE]

Appendix A.3

This approach might be more universal, for it makes it possible to track the inheritance of the mutation without actual testing for the gene itself.

Appendix A.4

Depending on the mutation studied, different primer systems are designed, with special emphasis on eliminating false priming to possible pseudogenes, for which purpose the first-round primers are designed to anneal to the regions of non-identity with the pseudogene. This preparatory work may require single sperm typing for the establishment of the paternal haplotypes, thus dictating linked marker analysis in addition to mutation testing, especially in cases of paternally derived dominant conditions.

Appendix A.5

The most frequent indications remain cystic fibrosis, haemoglobin disorders, and dynamic mutations (**Table 1**; Kuliev *et al.*, 2007). Indications are expanding to include the risk for common diseases with genetic predisposition and such non-genetic conditions, as HLA typing with the purpose of stem cell therapy of the affected siblings in the family (Verlinsky and Kuliev, 2006).

Appendix A.6

Preferential amplification and allele-drop out-(ADO) are in the range of not lower than 10%, being the main potential cause of PGD misdiagnoses. With a sufficient number of linked markers tested together with the causative gene, a risk for misdiagnosis may be substantially reduced or practically eliminated. The protocol involves a multiplex nested PCR analysis, with the initial first round PCR reaction containing all the pairs of outside primers, followed by amplification of separate aliquots of the resulting PCR product with the inside primers specific for each site. Following the nested amplification, PCR products are analysed either by restriction digestion, real time PCR, direct fragment size analysis, or minisequencing.

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Received 2 November 2007; accepted 7 November 2007.