

ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'

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Among the many educational materials produced by the European Society of Human Reproduction and Embryology (ESHRE) are guidelines. ESHRE guidelines may be developed for many reasons but their intent is always to promote best quality practices in reproductive medicine. In an era in which preimplantation genetic diagnosis (PGD) has become a reality, we must strive to maintain its efficacy and credibility by offering the safest and most effective treatment available. The dominant motivators for the development of current comprehensive guidelines for best PGD practice were (i) the absence of guidelines and/or regulation for PGD in many countries and (ii) the observation that no consensus exists on many of the clinical and technical aspects of PGD. As a consequence, the ESHRE PGD Consortium undertook to draw up guidelines aimed at giving information, support and guidance to potential, fledgling and established PGD centres. The success of a PGD treatment cycle is the result of great attention to detail. We have strived to provide a similar level of detail in this document and hope that it will assist staff in achieving the best clinical outcome for their patients.

Key words: ESHRE guidelines/preimplantation genetic diagnosis/preimplantation genetic screening

Introduction

Why have guidelines?

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) are treatment options that are relatively unregulated and lack standardization compared with other forms of diagnostic testing. This is illogical considering the comparative difficulty in achieving the highest levels of accuracy and reliability with single cells as part of PGD/PGS versus more routine genetic testing. Many regulations, laws and voluntary networks exist in the mainstream diagnostic community to maintain the highest quality in diagnostic testing. For example, the European Quality Molecular Network has attempted to improve and standardize molecular diagnostic testing across Europe (Dequeker *et al.*, 2001). One step towards higher quality overall and standardization for PGD is to build consensus opinion on best practices within the

PGD/PGS community; a component of the mission of the ESHRE PGD Consortium (hereafter referred to as the Consortium) (ESHRE PGD Consortium Steering Committee, 2002).

PGD falls into two categories. High risk PGD (hereafter referred to as 'PGD') is carried out for patients at high risk of transmitting a genetic or chromosomal abnormality to their children, which includes single gene defects (autosomal recessive, autosomal dominant and X-linked disorders) as well as chromosomal abnormalities (translocations, structural aberrations, etc.). Low risk PGD (hereafter referred to as 'PGS') is carried out for infertile patients undergoing IVF with the aim of increasing the IVF pregnancy rates. Current examples of indications for PGS include women of advanced maternal age, couples with repeated IVF failure and couples with normal karyotypes who have experienced repeated miscarriages.

The Consortium recognizes that owing to variations in local or national regulations and specific laboratory practices, there will remain differences in the ways in which PGD/PGS are practised (from initial referral through IVF treatment, single cell analysis to follow-up of pregnancies, births and children). However, this does not preclude a series of consensus opinions on best practice based upon available evidence. Indeed, the American Society for Reproductive Medicine published a practice committee report for PGD in 2001 (American Society of Reproductive Medicine and Society for Assisted Reproductive Technology. Practice Committee Report, 2001) essentially reviewing PGD practice in the USA. The newly established PGD International Society (PGDIS) has also drafted 'guidelines' and, although more 'in-depth' than the ASRM report, these 'guidelines' are concise (Preimplantation Genetic Diagnosis International Society, 2004). The guidelines provided in this document not only reflect current use of PGD/PGS but also offer consensus-based specific guidance regarding how best to practise clinical PGD/PGS based upon clinical experience, and data, both published and unpublished.

The Consortium hopes that a minimum standard might be achieved across all centres actively providing clinical PGD/PGS. Achieving this goal could ultimately have the net effect that patients receive the best care possible regardless of the centre at which they are treated. Rather than drift towards the lowest common denominator, established and fledgling centres alike can learn from global experiences and be guided by consensus opinion.

These guidelines are not intended as rules or fixed protocols that must be followed, nor are they legally binding. The unique needs of individual patients may justify deviation from these guidelines, and they must be applied according to individual patient needs using professional judgement. However, guidelines may be incorporated into laws and regulations, and practitioners should check the status of clinical practice guidelines in their own countries to determine the status of this document.

How do the guidelines work in practice?

The Consortium guidelines rate practices in the following categories:

Recommended (sufficient experience and published data exist to suggest that the practice in question is safe and effective)

or **Not recommended** (sufficient experience and published data exist to suggest that the practice in question may not be safe and/or effective)

For example, these guidelines **recommend** ICSI as the method for insemination when performing a PCR-based diagnostic test on single embryonic cells since the risk of contamination from extraneous cells or DNA has greater consequences for the accuracy of the test when using PCR compared with fluorescence *in situ* hybridization (FISH). Conversely, for PCR testing, conventional insemination would be classed as **not recommended**.

When discrepancies exist in the literature, insufficient evidence exists for a particular method or equally valid methods

exist, it may be difficult to build consensus and the guidelines may be more ambiguous, e.g. the choice of lysis buffers for use in single cell PCR. In this scenario, the use of either alkaline lysis buffer or proteinase K for the lysis of single cells (as published in the literature) would be considered **acceptable**.

The remainder of this document provides a framework for identifying and classifying procedures that are fundamental to the practice of PGD/PGS. The framework is listed in chronological order from the perspective of a prospective patient/couple requesting PGD/PGS.

Organization of PGD/PGS centres

A PGD programme requires the involvement of a clinical genetics unit, an IVF unit and diagnostic testing laboratories. Timely, clear and comprehensive communications between these different units is imperative and must be kept under strict quality control (Geraedts *et al.*, 2001). All patients entering a PGD/PGS programme need to receive counselling appropriate to their circumstances (see below). Written consent to perform the treatment is **recommended**. The counselling, consenting and diagnostic procedures differ for PGD and PGS.

In the PGD procedure, the following steps are **recommended** for all couples at high genetic risk due to structural chromosome abnormalities or monogenic diseases: a clinical geneticist or genetic counsellor first counsels potential parents referred for PGD, to discuss the use of PGD. Thereafter, a clinical fertility specialist, as for routine IVF, evaluates the couple.

In the PGS procedure, the intake of the patients is predominantly via the IVF unit. Counselling by either the fertility clinician or a clinical geneticist is **recommended**, provided extra attention is paid to the couple's reproductive history.

The IVF laboratory should be organized according to the guidelines for good practice in IVF laboratory prepared by the ESHRE Embryology Special Interest Group (SIG) (Gianaroli *et al.*, 2000). All laboratory procedures and protocols should be collected in a manual that is kept updated by the laboratory director. Current procedures should be available for consultation to every member of staff to enable 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 (see relevant sections below).

Counselling, informed consent and approval

- It is **recommended** that all counselling be carried out under the responsibility of a qualified physician.
- Counselling of common problems by appropriately trained non-physician health care providers or non-genetic specialist physicians is **acceptable**.
- Counselling in the couple's own language is **recommended** or, alternatively, interpreters should be used.
- It is **recommended** that centres provide appropriate and sufficient information to allow patients to give informed

consent to PGD treatment. Informed consent should be documented.

● It is **recommended** that the patient has non-directive counselling with a suitably qualified professional(s) that includes the following issues.

Genetic counselling

- Genetic risk assessment and recurrence risk
- Explanation of nature and severity of inherited disorder
- Reproductive options and alternatives to PGD such as prenatal diagnosis, gamete donation, remain childless, accept risk without further examination, adoption, benefits and limitations of PGD compared with other alternatives to have healthy offspring
- Discussion about the risk of spontaneously conceiving a child affected by the genetic disorder if no contraception is used
- Testing only for genetic disorders previously characterized for that couple and for which testing is available
- Scope of the test, e.g. rejection of normal and affected males after sexing for X-linked disorders
- Number of embryos expected to be affected according to Mendelian ratios
- Information on specific laboratory tests to be used and their limitations
- Realistic time line for test availability
- Reliability of PGD diagnosis, chance of misdiagnosis or adverse outcome
- Decision making about disposition of affected embryos or undiagnosed embryos
- Decision making about transfer of carrier embryos (for autosomal recessive and X-linked recessive disorders)
- Decision making about mutation detection or FISH sexing (for X-linked disorders)
- Decision making about prenatal diagnosis if woman gets pregnant
- Discussion about possible increased risk of premature birth, low birth weight, perinatal mortality, congenital anomalies and/or developmental delay in children following IVF/ICSI/PGD treatment (Lambert, 2003), uncertainty about long-term adverse effects for children born after assisted reproduction with or without PGD and the importance of follow-up for children born after PGD.

Treatment-related counselling

- Description of and details regarding IVF/ICSI procedure
- Risk of medical complications during ovarian stimulation or oocyte retrieval
- Additional short- or long-term medical risks of the procedures and pregnancy for women affected with autosomal dominant or X-linked genetic disorder (e.g. risk of haemorrhage during oocyte retrieval and parturition for haemophilia carriers; thrombosis in women with clotting disorders such as factor V Leiden mutation)
- Uncertainty about future fertility and health of women after PGD treatment (Winston and Hardy, 2002)
- Specifics of PGD treatment compared with regular IVF/ICSI:

- The number of oocytes to be retrieved needs to be maximized with regard to safe medical practice
- Likelihood of transferring unaffected embryos
- Possibility that all embryos are affected
- Some embryos may be unsuitable for biopsy
- Some embryos may not survive the biopsy
- A diagnosis may not be possible for all biopsied embryos

- Number of embryos to be transferred
- Fate of non-diagnosed or non transferable embryos (see 'Embryo transfer')
- Chance of (ongoing) pregnancy/live birth per cycle started and per transfer
- Risk of multiple pregnancy
- Risk of miscarriage
- Maximum number of PGD treatments to be offered (if applicable)
- Time line for initial treatment
- Costs
- Cycle cancellation policy if pre-existing fertility problems or requirement for IVF exists irrespective of need for PGD.

In addition, for HLA testing

- If only HLA typing is performed, an average of 25% of embryos will be suitable for transfer
- If HLA typing is combined with specific PGD diagnosis for an autosomal recessive disorder, an average of only three out of 16 (18.8%) embryos will be suitable for transfer
- If HLA typing is combined with sexing for X-linked disorder, an average of only one in eight (12.5%) embryos will be suitable for transfer
- Couples should be referred to a centre for stem cell transplantation to obtain full information on the chances of success of stem cell transplantation of cord blood, available alternative treatments, possible complications of stem cell/bone marrow transplantation and optimal timing of stem cell transplantation
- Fate of unaffected, non HLA-matched embryos should be discussed.

Patients should be given information leaflets explaining all information on IVF/ICSI/PGD, possibly supplemented by a written report about special issues in their situation and they should be provided with a contact person if they need further information.

Psychological evaluation

Recommend psychological evaluation for:

- Patients with a history of reproductive failure
- Patients with a history of traumatic experiences
- Couples for whom the geneticist, gynaecologist or other member of the IVF/PGD team has doubts regarding welfare of existing/future children or psychological well-being/mental capacity of future parents
- Couples who actively ask for psychological intervention
- Couples in whom one of the future parents is the carrier of an autosomal dominant disorder and may have signs and symptoms of this disorder as determined by the appropriate specialist physician (e.g. neurodegenerative/psychiatric)

disorders such as Huntington's disease diagnosed by a neurologist).

Approvals

The following **recommendations** are made:

- Centres in which laboratory tests are performed should be approved by the State or by a competent authority in the State for that purpose

- The IVF and diagnostic laboratory should obtain local ethical approval to perform PGD procedures (including embryo biopsy and single blastomere diagnosis).

It is **acceptable** to make use of so-called 'transport PGD' or 'satellite PGD' testing laboratories (defined as the process whereby biopsied samples from an IVF centre are transported to an administratively separate diagnostic facility; see 'Quality control and quality assurance').

Patient inclusion/exclusion criteria

Inclusion and exclusion criteria for PGD/PGS treatment will vary from clinic to clinic (as do routine IVF inclusion criteria), but the following **recommendations** apply:

General inclusion criteria for PGD/PGS

Genetics

- Diagnosis is technically possible in principle
- Reliability of diagnosis is high (>90%).

Fertility

- Patients have a fertility problem likely to be overcome by IVF/ICSI.

General exclusion criteria for PGD/PGS

Genetics

- Diagnosis is not feasible with current techniques.

Fertility

- Maternal age above 40–45 years (exact age to be determined by each centre)
- Basal FSH >15 IU/l (exact value to be determined by each centre)
- Contra-indications for IVF/ICSI
- Body mass index (BMI) >30 kg/m² (exact value to be determined by each centre).

Inclusion criteria specific to PGD

Genetics

- Genetic diagnosis is certain or almost certain
- High recurrence risk exists at conception for a specific genetic disorder (e.g. >10% for chromosomal rearrangements, 25–50% for monogenic disorders) or recurrent miscarriage related to parental structural chromosome abnormality

- As a consequence of this genetic disorder, serious health problems are expected

In addition, for HLA typing

- Affected previous child of these parents has malignant disorder or genetic disorder, and the child is likely to be cured or life expectancy is substantially prolonged by stem

cell transplantation with cord blood from an HLA-identical sibling (after all other clinical options have been exhausted).

- It is technically possible to combine HLA typing with mutation detection in the case of a recurrence risk of autosomal recessive or X-linked recessive disorder or with FISH sexing in the case of an X-linked disorder.

Exclusion criteria specific to PGD

Genetics

- Genetic diagnosis is uncertain, e.g. due to genetic/molecular heterogeneity or uncertain mode of inheritance

- Low recurrence risk (e.g. <10%)

- PGD may be inappropriate if an affected spouse has serious mental/psychological/psychiatric problems due to genetic neurological/neurodegenerative/psychiatric disorder for which PGD is requested.

In addition, for HLA typing

- For cases, in which the affected child has an acute medical condition prohibiting safe stem cell transplantation or has extremely low life expectancy, consideration should be given to the time required for the PGD test to be developed, applied and an HLA-matched sibling born.

Fertility

- The woman has severe signs and symptoms of an autosomal dominant or X-linked disorder (for which PGD is requested), which could introduce medical complications during ovarian stimulation, oocyte retrieval or pregnancy.

Inclusion criteria for PGS

Genetics

- Recurrent miscarriage (>2 miscarriages; exact number to be determined by each centre).

It should be noted that patients with a history of recurrent miscarriage have a high chance of successfully conceiving naturally (Brigham *et al.*, 1999; Carp *et al.*, 2001).

Fertility

- Repeated implantation failure (e.g. >3 embryo transfers with high quality embryos or the transfer of ≥10 embryos in multiple transfers; exact numbers to be determined by each centre). Implantation failure is defined as the absence of a gestational sac on ultrasound at ≥5 weeks post-embryo transfer

- Advanced maternal age (>36 completed years; exact age to be determined by each centre).

Exclusion criteria for PGS

Fertility

- Low total antral follicle count: e.g. <7 (thresholds to be determined by each centre)

- Poor embryo quality.

Referrals

Relevant documentation

It is **recommended** that the following documents (where applicable) should be made available to the IVF/PGD centre to avoid duplication of consultations and consistency of care.

- Genetic counselling report
- Original results of DNA testing or other specific testing of affected child, future parents or other family members (when appropriate)
- Full pedigree and family history data (at least 3 generations).
- Data on health problems of female and male partners, and specialist consultations which may impact genetic diagnosis or IVF success and pregnancy (when appropriate)
- Female reproductive history, gynaecological and fertility status
- Male reproductive history, andrological history, fertility status, results of sperm analysis (especially if genetic disorder for which PGD is desired has effects on sperm parameters, e.g. chromosomal translocations or single gene disorders such as myotonic dystrophy and cystic fibrosis/congenital bilateral absence of the vas deferens).

In addition, for HLA typing, medical report of affected child, current situation, prognosis, options for treatment other than PGD, suitability for stem cell transplantation, results of previous HLA typing (serological and/or DNA markers) in affected child, parents and siblings.

Pre-cycle work-up on individual couples

FISH-based tests

• Sexing: if using a probe set previously shown to have a very low polymorphism rate (e.g. aneuvysion XY,18) it is **acceptable** to forego any pre-cycle work-up. If using DYZ1 (Yq12), pre-cycle testing of the male partner's interphase nuclei is **recommended** due to the relatively common occurrence of polymorphism in this region or the Y chromosome (Hsu *et al.*, 1987).

• Structural chromosome abnormalities: it is **recommended** that lymphocytes from each couple be tested with the specific probe set for clinical use. It is **acceptable** to perform testing on blastomeres from embryos donated to research prior to clinical PGD testing. It is **acceptable** to carry out FISH testing on sperm from male translocation carriers to predict the efficacy of PGD for these cases. (Escudero *et al.*, 2003).

• Aneuploidy screening (PGS): it is **recommended** that testing with the D15Z1 probe is performed on lymphocytes from both reproductive partners before treatment since it cross-hybridizes to chromosome 14 in ~15% of cases (Shim *et al.*, 2003). This recommendation can be generalized to any probe that is found to cross-hybridize at an appreciable frequency.

PCR-based tests

The following measures are **recommended**:

- Confirmatory testing of the mutation on DNA (using standard tests) *or* review of the documented testing at another institution by a suitably qualified professional
- Confirmatory testing of the clinical PGD assay on parental and proband blood DNA since the PGD assay could be non-informative owing to PCR failure as a result of polymorphisms or DNA sequence variations present in the population (Thornhill *et al.*, 2002).

Clinical IVF protocol

Guidelines for routine IVF are available from the ESHRE SIG Embryology IVF guidelines (Gianaroli *et al.*, 2000).

Special considerations for PGD patients

The following **recommendations** are made:

- Use of the oral contraceptive pill (OCP) to reduce significantly the risk of a spontaneously conceived child affected by the genetic disorder in PGD patients awaiting treatment
- Instruct patients to abstain from unprotected sex during a PGD cycle in case any uncollected oocytes fertilize *in vivo*
- Aim to increase the yield of high quality oocytes (Vandervorst *et al.*, 1998)
- Take precautions for patients with specific genetic disorders that might affect the safety of the IVF procedure (e.g. during oocyte retrieval: administration of desmopressin to prevent bleeding in haemophilia carriers and cardiopulmonary monitoring for women with myotonic dystrophy).

The following measures are **acceptable**:

- Consider pre-cycle antral follicle count to predict mature oocyte yield (Dumesic *et al.*, 2001) or poor ovarian response to stimulation (Bancsi *et al.*, 2004)
- Owing to a lack of consensus and clinic-specific cancellation policies, individual centres should determine their own cut-off numbers of follicles, oocytes or embryos for cycle cancellation.
- In the case of diagnostic test failure prior to a clinical cycle, the following options are **acceptable** (according to centre policy and specific circumstances):
 - Cancel cycle prior to stimulation with gonadotrophins
 - Coast patient on GnRH agonist for a limited time (Ulug *et al.*, 2004) until the diagnostic laboratory is ready to accept samples
 - Freeze all of the embryos at the pronucleate or cleavage stage for future thaw, biopsy and transfer.

Embryo culture and biopsy

Until the time of biopsy, routine IVF culture conditions apply (Gianaroli *et al.*, 2000). Embryo biopsy can be performed at three stages, polar body, cleavage stage and blastocyst, although in a recent survey of practices among 50 centres performing PGD, the majority perform only cleavage stage biopsy on day 3 of development (A.R.Thornhill, unpublished data).

There are several methods of cleavage stage biopsy, but the majority of clinics use zona drilling with acid Tyrodes or laser and aspiration of the blastomeres into $\text{Ca}^{2+}\text{Mg}^{2+}$ -free medium (Dumoulin *et al.*, 1998) for embryo biopsy (ESHRE PGD Consortium 1999, 2000, 2002; Harper and Thornhill, 2001; De Vos and Van Steirteghem, 2001; Harper and Doshi, 2003). Some centres perform polar body biopsy as a means to avoid removing embryonic cells (Verlinsky *et al.*, 1990, 1997), while others use this strategy exclusively, owing to regulations that prohibit embryo

biopsy in their region or country (e.g. Germany; Kupker *et al.*, 2001).

- It is strongly **recommended** that the embryo and blastomere identity is checked throughout the procedure so the diagnostic results can be unequivocally linked to specific embryos

- It is strongly **recommended** that all cumulus cells are removed before biopsy as these cells can contaminate both FISH and PCR diagnosis.

Insemination

ICSI is **recommended** for all PCR cases to reduce the chance of paternal contamination from extraneous sperm attached to the zona pellucida or non-decondensed sperm within blastomeres (ESHRE PGD Consortium 1999, 2000, 2002). ICSI or conventional insemination is **acceptable** for FISH cases.

Embryo culture

Standard IVF culture conditions are **acceptable** until the day of biopsy but, following biopsy, the following **recommendations** are made:

- Appropriate culture media for culture of embryos from day 3 onwards should be used
- Embryos must be cultured singly in individual drops or dishes with a clear identification system to ensure tracking of blastomeres removed and easy identification of embryos post-diagnosis
- Embryos are rinsed post-biopsy to remove traces of acid or biopsy medium as applicable.

Timing of biopsy

Polar body. Acceptable: the first polar body can be removed from the oocyte on the day of the oocyte collection between 36 and 42 h post-HCG injection (Verlinsky *et al.*, 1990). The second polar body can be removed from the zygote between 18 and 22 h post-insemination. The first and second polar body can be removed simultaneously (Verlinsky *et al.*, 1997), but the first polar body may have degenerated by day 1. Sequential removal of the polar bodies where the first polar body is removed on day 0 and the second polar body on day 1 is also **acceptable** (Strom *et al.*, 1998). Note that simultaneous biopsy of the two polar bodies is **acceptable** for FISH analysis since they can provide distinguishable results (Verlinsky *et al.*, 1998). However, sequential biopsy of polar bodies is **recommended** for PCR analysis to determine recombination events between the first and second polar body. In some cases, cleavage stage biopsy may be required to confirm the polar body diagnosis. The limitations of polar body biopsy have been documented previously (Harper and Thornhill, 2001; De Vos and Van Steirteghem, 2001; Harper and Doshi, 2003).

Cleavage stage biopsy. Biopsy on the morning on day 3 post-insemination is **recommended** but the exact timing varies according to timings of procedures in different laboratories. It is **acceptable** to exclude very poor quality embryos from the embryo biopsy procedure, but no specific criteria

are recommended here. However, it is **recommended** to set criteria prior to performing clinical PGD and adhere to them.

Blastocyst biopsy. Blastocyst biopsy on the morning of day 5 or 6 post-insemination is **acceptable**. However, experience is limited and clinical application has only been published after peer review by one centre (De Boer *et al.*, 2002). The limitations and strengths of this approach have been described elsewhere (De Vos and Van Steirteghem, 2001; Harper and Thornhill, 2001; Harper and Doshi, 2003).

Biopsy procedure

The following **recommendations** are made for preparations prior to any biopsy procedure on human embryos:

- Ensure all micromanipulation equipment is installed correctly, calibrated and maintained per written procedures
- Ensure the appropriate reagents and micromanipulation tools are available, sterile and within their expiration date
- Ensure that biopsy is performed by a suitably qualified person who is trained to a written procedure and adheres to that procedure (Human Fertilisation and Embryology Authority, 2003)
- Embryo biopsy dishes should be made up before the procedure, and clearly labelled with the patient name and embryo numbers
- Embryo biopsy dishes should contain a drop of biopsy medium of sufficient size to maintain pH, osmolality and temperature during the procedure
- Sufficient rinse drops comprising culture medium should be available to rinse embryos after the biopsy procedure
- Acidified Tyrodes solution (if applicable) should also be readily available to allow pipette priming between embryos.

Zona breaching

Laser or mechanical zona breaching is **acceptable** for polar body biopsy, but acid Tyrodes is **not recommended** as it may adversely affect the spindle (Malter and Cohen, 1989). **Acceptable** methods for zona breaching during cleavage stage or blastocyst biopsy include acidified Tyrodes solution, laser or mechanical methods.

Cell removal

- The following methods of removal are **recommended**:
- Removal of polar bodies by aspiration (Verlinsky *et al.*, 1990)
- Removal of cleavage stage blastomeres by aspiration (Hardy *et al.*, 1990)

Removal of trophoctoderm cells during blastocyst biopsy by herniation followed by laser (De Boer *et al.*, 2002) or mechanical excision (Dokras *et al.*, 1990).

The following methods of removal are **acceptable** (i.e. more clinical data are required for these methods to have proven efficacy):

- Removal of cleavage stage blastomeres by extrusion (Fallon *et al.*, 1999) or displacement techniques (Pierce *et al.*, 1997)

- Removal of trophectoderm cells during blastocyst biopsy by mechanical stitch and pull method (Muggleton-Harris *et al.*, 1993).

Number of cells to remove safely (minimum and maximum). There is no consensus on the number of blastomeres that can be safely removed during cleavage stage embryo biopsy. The decision to remove one or two cells is based on many factors including the embryo cell number and the accuracy and reliability of the diagnostic test used. If removal of two cells is considered, it is **recommended** to be undertaken only on embryos with six or more cells (Van de Velde *et al.*, 2000).

Rebiopsy of embryos. This practice is **acceptable** in the case of lost or anucleate blastomeres and failed diagnosis, but the embryo cell number and timing of rebiopsy should be considered. Use of the original zona breach site is **recommended**.

Selection of cells for removal. When possible the removal of mononucleate cells only is **recommended** (Munné and Cohen, 1993).

Biopsy medium

The use of standard IVF culture medium during biopsy is **acceptable** but its effectiveness may be highly dependent upon the developmental stage of the embryo biopsied. Use of commercial or 'home-brew' calcium, magnesium-free biopsy medium is **recommended** (Dumoulin *et al.*, 1998).

Time out of incubator

No specific recommendations for maximum time out of the incubator can be given, but ideally biopsy should be performed as quickly as possible to ensure pH, temperature and osmolality are maintained. A documented record for biopsy timings is **recommended** for quality control/quality assurance purposes.

FISH-based diagnosis

Sexing for X-linked disease

A probe set containing at least one probe specific for each centromere region of the X and Y chromosomes, and one autosome, is **recommended** (Staessen *et al.*, 1999; Harper and Wilton, 2001). Diagnosis on a single mononucleate cell is **acceptable** for sexing (Kuo *et al.*, 1998).

Chromosome rearrangements

It is **recommended** that the probe set should at least contain probes sufficient to detect all the expected forms of the rearrangement with chromosome imbalance. However, where suitable probes are not available, it is **acceptable** to use probe mixes that cannot detect some unbalanced forms of a rearrangement providing they have been assessed to be non-viable in a recognizable pregnancy or to have a very low prevalence (Scriven *et al.*, 1998; Delhanty and Conn, 2001; Munné, 2002; Ogur *et al.*, 2002; Scriven, 2003; Simopoulou *et al.*, 2004). In the latter case, patients should be counselled to this effect.

Diagnosis based on a mononucleate single cell is **acceptable** for chromosome rearrangements providing there are at least two informative probes for the chromosome imbalance associated with unbalanced forms of the rearrangement that are considered likely to be prevalent or viable in a recognizable pregnancy. Diagnosis based on concordant results from two mononucleate cells is **recommended** where there is only one informative probe available.

PGS (aneuploidy screening)

For aneuploidy screening, a probe set of at least five chromosome pairs from 13, 14, 15, 16, 18, 21, 22, X and Y is **recommended** (Munné *et al.*, 1999; Magli *et al.*, 2001; Wilton, 2002) Diagnosis on a single mononucleate cell is **acceptable** for PGS (Munné *et al.*, 1993).

Rehybridization procedures on single blastomeres are **acceptable** with the appropriate validation and written procedures (Munné *et al.*, 1998; Harrison *et al.*, 2000; Magli *et al.*, 2001).

Commercial versus home-made probes

The use of commercial probes is **recommended** since they generally come with quality control (QC) and validation. The use of home-made probes is **acceptable** with appropriate QC/quality assurance (QA) and validation.

Quality assurance/validation with probes/batches

It is **recommended** that all probe vials should be tested before clinical application, to confirm that they contain the correct chromosome-specific probe labelled with the correct fluorochrome or hapten, and to assess that signal specificity, brightness and discreteness are within acceptable parameters per predetermined individual laboratory criteria (as documented in written procedures).

Probe selection

For each test, it is **recommended** that only appropriately qualified personnel select probes with the appropriate chromosome specificity and labelling.

Assay validation

Preliminary work on lymphocyte or fibroblast cells is **recommended** for each different probe set, and should include analysis of both metaphase spreads and interphase nuclei. It is **recommended** that at least 10 metaphase spreads should be examined: (i) to ensure that the probes are specific for the correct chromosomes; (ii) to assess chromosome polymorphism and signal cross-hybridization; and (iii) with respect to a chromosome rearrangement carrier, to ensure that the probes hybridize as expected to the different segments of the rearrangement. It is **recommended** that at least 100 interphase nuclei should be scored using appropriate scoring criteria that should include an assessment of signal specificity, brightness and discreteness.

It is **recommended** that for probe mixes containing subtelomeric probes and locus-specific probes with known polymorphism and cross-hybridization, preliminary work should be done using cells from both reproductive partners.

Since the analytical performance in blastomeres frequently approaches that of lymphocytes, it is **acceptable** to test each new probe set on a limited series of blastomeres.

It is **recommended** that scoring criteria should be determined ahead of time (published or 'in-house') and should be adhered to per written procedure. No probe should be passed for clinical use unless it meets the individual laboratory's predetermined and documented minimum score for intensity, specificity and minimum background.

Use of intra-assay controls

The use of positive and negative controls for PGD is contentious since suitable positive controls are not readily available for most chromosome rearrangements, and negative controls only serve to confirm that the correct probes are in the mix. It is **acceptable** to perform such controls where possible, but their limitations must be understood.

Fixation and FISH protocols

It is **recommended** that cumulus cells be removed prior to biopsy as these can contaminate the slide with maternal cells and lead to inaccurate results.

Three methods of spreading and fixing single blastomeres have been described; all are **acceptable**: methanol/acetic acid (Tarkowski, 1966; Munné *et al.*, 1993) Tween/HCl (Coonen *et al.*, 1994; Harper *et al.*, 1994), and combined Tween/HCl–methanol/acetic acid (Dozortsev and McGinnis, 2001; Baart *et al.*, 2004). The use of hypotonic treatment of cells prior to spreading is **acceptable**.

FISH procedures

Many variations in FISH methods have been published and all appropriately validated methods are **acceptable** (Delhanty and Conn, 2001; Harper and Wilton, 2001). If using pre-hybridization steps such as pepsin and paraformaldehyde, it is **recommended** that steps should be taken to ensure appropriate quality control for these solutions (including expiration date and possible cellular contamination). Mounting medium containing antifade is **recommended** to allow visualization and maintenance of fluorescent signals.

It is **recommended** that prior to each FISH procedure, denaturation, hybridization and wash temperatures are verified. Temperature ranges should be validated in individual laboratories and instruments calibrated periodically to ensure accuracy.

Scoring clinical FISH results

The following **recommendations** are made:

- Signal scoring criteria should be established in the written protocol and adhered to for the interpretation of signals
- The fluorescence microscope should be equipped with and optimized for the appropriate filter sets for the probes being used
- Signals should be analysed by two independent observers and discrepancies adjudicated by a third observer (where possible). If no resolution is reached, the embryo should not be transferred, i.e. should be given the diagnosis of uninterpretable or inconclusive

- All single cell images should be captured and recorded for QC purposes and records
- Slides should be retained and stored at 4°C
- Results should be reviewed and signed by a suitably qualified person (this includes sexing and aneuploidy screening)
- A written report should be given to the IVF team to ensure transfer of the correct embryos.

It is **acceptable** to score signals from probes labelled with fluorochromes not detectable to the human eye using an image capture system.

PCR-based diagnosis

General comments

PCR can be used for the diagnosis of single gene defects at the DNA level (Sermon, 2002; Thornhill and Snow, 2002). This includes specific diagnosis for X-linked disease. Due to the risk of contamination and allele drop-out (ADO), it is **recommended** that PCR protocols include the use of linked or unlinked markers in addition to the disease locus (Verlinsky and Kuliev, 2000; Sermon, 2002; Thornhill and Snow, 2002).

When sexing only is being performed for X-linked diseases, it is **recommended** that FISH is used as FISH identifies chromosomal abnormalities of the sex chromosomes and is not influenced by contamination (ESHRE PGD Consortium Steering Committee 1999, 2000, 2002).

Validation using DNA samples

The following **recommendations** are made:

- To ensure that the test yields the expected results on different genotypes at the same locus, assay validation should be performed on appropriate DNA samples [including affected (autosomal dominant), carrier (autosomal recessive, X-linked diseases), and unaffected samples for the mutation to be tested, or heterozygous samples if markers only are to be used] before moving onto validation in single cells. This should be followed by assessment of single cells from affected, carrier (autosomal recessive, X-linked diseases) and unaffected individuals (Sermon, 2002; Thornhill and Snow, 2002).
- It should be noted that due to stochastic variation in pipetting, it may not be possible to get the test to work on 6 pg of DNA equivalent to one cell. One hundred picograms (~10–15 cell equivalents) is an **acceptable** alternative to single cells (with the exception of determining ADO rates).

Choice of acceptable cell types

It is **recommended** that single lymphocytes, lymphoblastoid cell lines, fibroblasts or buccal cells (or any somatic cell type with appropriate validation) are used to validate the assay at the single cell level. Testing of blastomeres would be beneficial, as these are the target cells, but this will depend on availability. Note that the cell type used may influence the amplification efficiency and ADO rate (Rechitsky *et al.*, 1998).

Minimum number of cells for validation

It is **recommended** that a minimum of 50 heterozygous cells in runs of 10–20 is used to assess amplification efficiency and the ADO rate. The same number of cell wash blanks to assess contamination should be included. If blastomeres are analysed, a minimum of 10 is **recommended** and, when possible, several blastomeres from the same embryo should be tested to determine the expected genotype, especially when using markers.

Use of intra-assay controls during a clinical PGD

It is **recommended** that for single gene defect cases, each clinical cycle should include the appropriate positive control samples (100 pg of DNA and/or control single cells). These should include for recessive diseases, one heterozygous and one homozygous affected; for autosomal dominant diseases, one heterozygous affected and one homozygous normal; and for linked markers, the two parents and if possible the genotype of an affected child. For each blastomere analysed, there should be one cell wash blank. The use of at least three cell-free wash negative controls and one reagent control (no DNA, no wash) is **recommended**. Use of the appropriate molecular weight marker ladders for agarose gels, and internal markers present in each positive sample for automated DNA analyzers is **recommended**.

Assessing amplification efficiency

An amplification efficiency of at least 90% is **recommended**.

Assessing allelic drop-out (ADO)

It is **recommended** that ADO rates should be determined using either cells carrying the mutation of interest, or wild-type heterozygous cells if polymorphic markers are analysed. ADO rates should be as low as possible (preferably < 10%). It should be noted that a higher ADO rate can be tolerated when dealing with autosomal recessive disease than autosomal dominant disease or compound heterozygous disease.

Methods to reduce ADO

Steps taken during PCR or design of the assay to minimize ADO are **recommended**. The following measures are **acceptable** for this purpose: increased denaturation temperatures (Ray and Handyside, 1996; Piyamongkol *et al.*, 2003), use and choice of lysis buffers (Gitlin *et al.*, 1996; El-Hashemite and Delhanty, 1997; Thornhill *et al.*, 2001), use and choice of DNA polymerases, and modified design of primer sequence and product size (Piyamongkol *et al.*, 2003).

Methods to better identify ADO

The incorporation of linked markers is **recommended** when setting up any new test, particularly to identify ADO (Verlinsky and Kuliev, 2000). Ideally the marker should be intragenic and could be a microsatellite or single nucleotide polymorphism (SNP). When no linked markers are available, or the couple is not informative for available markers, or the set-up of a reliable duplex PCR proves to be too difficult, the biopsy of two cells is an **acceptable** alternative. Their

subsequent independent analysis will help in identifying ADO, which will be seen as a discrepancy in genotype between the cells.

Assessing contamination

The following **recommendations** are made:

- For each cell assessed (either in the validation phase or during clinical PGD cases), one cell wash blank should also be tested
- The contamination rate should be < 5% (preferably zero)
- A polymorphic marker (linked or unlinked) should be included in every assay to allow the identification of contamination (Pickering *et al.*, 1994).

It is **acceptable** to run a large series of blanks periodically to assess baseline levels of contamination.

Minimal requirements for family members/gametes when linked markers are used

The same minimum standards exist for PGD testing as for routine prenatal diagnosis. A molecular biologist experienced in pedigree and linkage analysis should determine what samples are needed for reliable and accurate diagnosis.

Reagents

The following **recommendations** are made:

- All batches of reagents should be recorded so that they may be linked back to specific assays
- All new and old batches of reagents overlap and are subject to strict quality control
- Wherever possible, all solutions should be purchased ready to use and should be of ‘molecular biology’ grade or equivalent
- All solutions for PCR should be split into single use aliquots
- All plastic ware used in PCR should be certified DNA, DNase free.

The following measures are **acceptable**:

- All solutions made ‘in-house’ may be autoclaved (using a dedicated autoclave) or, if this is not possible, 0.2 µm filtered
- PCR master mix may be pre-treated either by UV irradiation or by restriction enzyme digestion for decontamination purposes.

Work practice controls

The following **recommendations** are made (Sermon, 2002; Thornhill and Snow, 2002):

- All personnel undertaking PCR-based diagnosis should be suitably trained to and follow written standard operating procedures
- Where accreditation exists, personnel should possess or be working towards that accreditation
- Training for single cell diagnostic assays should be at least to the standard required for routine testing in a clinical diagnostic laboratory
- Protective clothing for single cell PCR work should include full surgical gown (clean, not sterile, and changed after each case), hair cover/hat and face mask (covering nose

and mouth) and shoe covers. Gloves should be worn at all times and changed frequently. These should be well fitting, e.g. latex or nitrile, but not vinyl examination gloves

- Workflow design should incorporate the following:

1. Physical separation of pre-PCR, PCR and post-PCR laboratories and the biopsy laboratory

2. Performance of PCR in a positive pressure room or class II hood to provide sample protection from extraneous DNA contamination

3. Physical separation of the area for setting up the second round of PCR and both the primary PCR set up area and the area where secondary products are analysed when performing two rounds of PCR

4. Restriction of reagents and consumables for use to specific work areas

5. An attempt to perform unidirectional workflow by personnel to avoid contamination

- All PCR racks should be either one-use only or autoclaved/cleaned in 20% bleach after each use if they are to be re-used for PGD

- Only filter tips certified to be DNA, DNase free should be used for pre-PCR and PCR steps

- Work surfaces, equipment, etc. should be cleaned with DNA decontamination solutions or 20% bleach prior to each case

- Dedicated PCR equipment and consumables are preferred.

Protocols

Washing cells and placing them in tubes. It is **recommended** that blastomeres should be washed at least twice using a sterile transfer pipette before transfer into PCR tubes. It is **acceptable** to transfer blastomeres to tubes with or without microscopic visualization.

PCR protocols. A range of different techniques is available for both the PCR amplification and the analysis.

- Nested PCR is **acceptable** as long as it is reliable and accurate (Stern *et al.*, 2002).

- Fluorescent PCR is **recommended** over conventional PCR since its level of sensitivity is several orders of magnitude higher and one round of fluorescent PCR can reduce the likelihood of contamination and misdiagnosis due to errors in tube transfer.

- Multiplex PCR is **recommended** since it allows simultaneous amplification of both the mutation and linked or unlinked markers, allowing better identification of ADO and revealing the presence of contamination. Multiplex PCR can be performed using either non-fluorescently or fluorescently labelled primers. When extragenic linked markers are used, the risk of recombination needs to be considered.

Analysis of PCR results

The following **recommendations** are made:

- Individual diagnostic laboratories should determine thresholds for contamination, amplification and ADO prior to clinical application of an assay

- Prior to start of analysis, each PCR sample should be clearly identified and loaded in a predetermined written order, with DNA controls and molecular weight ladders always placed in the same order

- Results of PCR analysis should be made available on hard copy for review, sign off and QC records

- Results should be analysed by two independent observers and discrepancies adjudicated by a third observer (where possible). If no agreement is reached, the embryo should not be transferred, i.e. should be given the diagnosis of uninterpretable or inconclusive

- Results should be reviewed and signed by a suitably qualified person

- A written report should be given to the IVF team to ensure transfer of the correct embryos.

Embryo transfer

Method of embryo transfer

The Consortium refers to the IVF Guidelines set up by the SIG for Embryology (Gianaroli *et al.*, 2000). Local practice in selection of transfer medium is **acceptable**. Local practice in catheter selection is **acceptable**; physician experience with different catheter types is **recommended** (Wood *et al.*, 2000). There is increasing evidence to support the use of ultrasound guidance during embryo transfer to improve pregnancy rates and possibly reduce the number of difficult embryo transfers (Coroleu *et al.*, 2000; Matorras *et al.*, 2002). Ultrasound guidance for embryo transfer is recommended in difficult cases or when a problematic transfer can be expected.

Day of transfer

Embryo transfer on a day appropriate to the diagnostic test turn around time is **recommended**. Embryo transfer on either day 3, 4 (Grifo *et al.*, 1998), 5 or 6 (Gardner and Lane, 2003) is **acceptable** as in routine clinical IVF practice. Delay of transfer to day 4, 5 or 6 also allows more time for the diagnosis to be made, relieving pressure on scientific staff and facilitating the transfer within normal clinic hours. Since extended embryo culture is warranted by PGD diagnostic procedures, it is **recommended** that the culture and transfer procedures should be familiar to the IVF laboratory.

Number of embryos to transfer

It is **recommended** that the physician and patient should agree the number of embryos to be transferred with documented informed consent prior to the transfer procedure. The number should be decided upon using such factors as embryo quality, female partner's age, medical risks for the female partner associated with possible multiple gestation, and the number of previous transfers. Recent proposals from professional societies in Europe and the USA suggest that to avoid multiple gestations in routine IVF, a maximum of two embryos should be transferred in favourable prognosis patients. Such patients are defined slightly differently by the two societies, i.e. < 38 years of age, with normal ovarian response and good fertilization rate (ESHRE Task Force on

Ethics and Law, 2003) and <37 years of age, first cycle of IVF, high embryo quality, previous IVF success and surplus embryos to cryopreserve (American Society of Reproductive Medicine, 2004). In all circumstances, the couple's reproductive autonomy and liberty need to be balanced against concerns for the welfare of any child born as a result of the treatment. In addition, local regulations should be followed.

Embryo selection

The following post-biopsy criteria are **recommended** to facilitate embryo selection: diagnosis of unaffected status, cell number pre- and post-biopsy, evidence of active cell division post-biopsy and embryo morphology pre- and post-biopsy. It is **recommended** that selection criteria are based primarily on unaffected diagnosis and secondarily favourable embryo morphology.

Transfer of carrier embryos (of an autosomal recessive disorder) or possible carrier female embryos (of an X-linked disorder) is **acceptable** since adverse health consequences to the resulting child are unlikely (although for some assays there may be an empirically higher likelihood that the embryo is affected as a result of technical artefact). In addition, occasionally patients carrying genetic conditions (especially X-linked disorders) may manifest milder forms of the disease. Each situation needs careful evaluation and fully informed discussion with the couple.

No embryos for transfer

Following PGD or PGS, no unaffected embryos may be available for transfer. In such cases, transfer of affected embryos is **not recommended**. Couples electing to have PGD do so generally to avoid the chance of having an affected pregnancy and having to undergo invasive prenatal diagnosis. However, when faced with the fact that no unaffected embryos are available, couples may change their mind in this regard.

Occasionally the diagnostic laboratory will be unable to diagnose the genetic status of all embryos and there may be no unaffected embryos to transfer. Transfer of undiagnosed embryos is **not recommended** for PGD for monogenic disorders. In contrast, transfer of undiagnosed embryos is **acceptable** for PGS and PGD for chromosome rearrangements giving rise to non-viable conceptions. If couples choose to have undiagnosed embryos transferred, they should be offered the option of prenatal diagnosis if pregnancy occurs. It is **acceptable** for health care providers to object conscientiously to transferring embryos that are likely to result in the birth of an affected child. In such cases, providers should consider referring the couple to colleagues who are prepared to offer transfer of such embryos.

Fate of unaffected 'normal' transferable embryos

Cryopreservation of biopsied cleavage stage embryos. It is **acceptable** to cryopreserve supernumerary unaffected embryos post-biopsy since the possibility of cryopreserving surplus viable transferable embryos is an attractive option to maximize pregnancy rates from the same oocyte retrieval.

Most published experience has been negative, with poor post-thaw survival (Joris *et al.*, 1999; Magli *et al.*, 1999; Ciotti *et al.*, 2000), although recent modifications to the cleavage stage protocol appear to improve post-thaw survival of biopsied embryos (Lee and Munné, 2000; Voullaire *et al.*, 2002; Jericho *et al.*, 2003).

Extended culture to blastocyst stage prior to cryopreservation. Although there is little or no published experience with cryopreservation of biopsied embryos grown to the blastocyst stage, this practice is **acceptable**.

Fate of affected embryos

It is **recommended** that confirmatory diagnosis is performed on affected, untransferred embryos as part of QC/QA procedures. This is usually performed on whole embryos for FISH and on either whole embryos or single blastomeres for PCR.

Fate of undiagnosed embryos

It is **recommended** that confirmatory diagnosis is performed on non-transferred undiagnosed embryos as part of the QC/QA procedures unless couples wish to transfer such embryos (e.g. after aneuploidy screening).

Quality control and quality assurance

Proficiency testing/external Quality assessment

Currently, there is no formal mechanism in place for internal/external proficiency testing or external quality assessment for PGD/PGS diagnostic laboratories. It is **recommended** that a voluntary system be implemented that would solve this problem, with proficiency testing/assessment performed at least annually. Internal QC/QA should be an ongoing process.

Competency assessment of testing personnel

The following **recommendations** are made:

- All personnel involved in clinical testing must follow an approved documented training regimen that includes theoretical work and hands-on training in all aspects of single cell work
- Personnel should be trained to current procedures by a senior technologist and should demonstrate competency before being allowed to perform clinical work without supervision
- Competency assessment prior to, during clinical testing and periodically thereafter will require evaluation of skills in each of the laboratory tasks using criteria developed within the laboratory.

Protocols

Existence of and adherence to clinical testing protocol. The following **recommendations** are made:

- The clinical testing protocol should include explicit instructions regarding which embryos are acceptable for biopsy, how many cells will be removed from each embryo, summarized results from the validated assay, scoring criteria,

and reporting procedures, as well as a framework for counselling patients in the presence of diagnostic results

- Deviations from procedures should be recorded
- If frequent deviations occur, the laboratory should consider changing the procedure accordingly.

Confirmation of diagnosis

The following **recommendations** are made (ESHRE PGD Consortium Steering Committee 1999, 2000, 2002).

- Confirmation of the diagnosis should be performed on all embryos not transferred or cryopreserved following diagnosis to provide QA as well as accurate and up to date misdiagnosis rates to prospective PGD/PGS patients
- Clinics should make special efforts to follow-up with the parents following prenatal testing or birth, especially if confirmatory testing is not possible
- Follow-up of pregnancies (including multiple pregnancy rate and outcome), deliveries, the health of children at birth and beyond should be attempted and maintained along with the cycle data. These data should be used both for internal QC/QA purposes and sent to the Consortium or other data collecting entity, for use in comparing clinics and methods of diagnosis.

Baseline IVF centre pregnancy rates for PGD

Setting appropriate baseline pregnancy rates should be left up to the individual clinics. However, it is **recommended** that each IVF laboratory should compare PGD/PGS pregnancy rates and matched non-PGD/PGS (routine IVF) pregnancy rates within that IVF centre.

Satellite PGD/PGS

Satellite PGD is the term used to represent IVF services supplied at one centre and diagnostic services specific to PGD/PGS performed elsewhere and by unrelated entities. Satellite PGD is **acceptable** for aneuploidy screening and translocation testing. This requires transportation of slides containing fixed cells from the IVF centre to the diagnostic centre by courier or special expedited delivery. Satellite PGD for PCR assays involving single gene defects is **acceptable** as long as additional markers (to detect contamination) are included for each gene tested. This requires transportation of microcentrifuge tubes containing lysed or unlysed cells from the IVF centre to the diagnostic centre by courier or special expedited delivery.

The following **recommendations** are made:

- The IVF centre and the Diagnostic centre should agree on a set of clinical/laboratory protocols prior to shipment of any clinical samples
- The satellite diagnostic centre should validate the shipment protocols being used to ensure that transport of cells does not compromise amplification efficiency or FISH hybridization
- Satellite PGD/PGS should require stricter QC/QA than in-house operations, with appropriate documentation including written procedures for shipping samples, result reporting, etc.
- Centres sending out PCR tubes and/or cells fixed onto microscope slides should be trained in the biopsy, placing

single cells in tubes or fixation procedures according to the diagnostic centre's procedures. If this is not possible, the satellite centre should arrange to have a suitably qualified and trained embryologist to perform the biopsy and cell preparation.

Appropriate indications for specific tests

Specific indications for PGD/PGS should remain within the purview of individual clinics.

Misdiagnosis rates

It is **recommended** that misdiagnosis rates should be calculated for each type of assay (PCR or FISH) and for all assays from a particular clinic (Lewis *et al.*, 2001). Such rates include those clinical cases in which affected pregnancies arose and post-transfer confirmation of diagnosis assays that were discordant with the biopsy result. It is **recommended** that these misdiagnosis rates along with pregnancy rates should be made available to all prospective patients upon request.

Concluding remarks

PGD and PGS are treatment options that are relatively unregulated and lack standardization compared with other forms of diagnostic testing. The guidelines above reflect current use of PGD/PGS and offer consensus-based specific guidance regarding how best to practise clinical PGD/PGS based upon clinical experience and data. It is hoped that such guidelines, if reviewed and revised periodically, will help to ensure that patients receive the best care possible regardless of the centre at which they are treated.

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