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ESHRE PAGES

# ESHRE PGD consortium best practice guidelines for fluorescence *in situ* hybridization-based PGD†

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**ABSTRACT:** In 2005, the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium published a set of Guidelines for Best Practice PGD to give information, support and guidance to potential, existing and fledgling PGD programmes. The subsequent years have seen the introduction of new technologies as well as evolution of current techniques. Additionally, in light of recent advice from ESHRE on how practice guidelines should be written and formulated, the Consortium believed it was timely to revise and update the PGD guidelines. Rather than one document that covers all of PGD, the new guidelines are separated into four new documents that apply to different aspects of a PGD programme, i.e. organization of a PGD centre, fluorescence *in situ* hybridization (FISH)-based testing, amplification-based testing and polar body and embryo biopsy for PGD/preimplantation genetic screening (PGS). Here, we have updated the sections that pertain to FISH-based PGD. PGS has become a highly controversial technique. Opinions of laboratory specialists and clinicians interested in PGD and PGS have been taken into account here. Whereas some believe that PGS does not have a place in clinical medicine, others disagree; therefore, PGS has been included. This document should assist everyone interested in PGD/PGS in developing the best laboratory and clinical practice possible. Topics covered in this guideline include inclusion/exclusion criteria for FISH-based PGD testing, referrals and genetic counselling, preclinical validation of tests, FISH-based testing methods, spreading of cells for analysis, set-up of local IVF centre and transport PGD centres, quality control/ quality assurance and diagnostic confirmation of untransferred embryos.

**Key words:** European Society for Human Reproduction and Embryology / PGD / fluorescence *in situ* hybridization / preimplantation genetic screening / misdiagnosis

## Introduction

The rapidly changing nature of PGD/preimplantation genetic screening (PGS), specifically the technologies associated with its use and increasing patient access, has necessitated review and revision of the original the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium guidelines (Thornhill *et al.*, 2005). As a result, ESHRE PGD Consortium (hereafter referred to as the Consortium) has prepared four guidelines: one relating to the organization of the PGD centre and three relating to the methods used: amplification-based, fluorescence *in situ* hybridization (FISH)-based and biopsy/ embryology (Harton *et al.*, 2010a,b,c). The method guidelines should be read in conjunction with the organization of the PGD centre guidelines which contains information on personnel, inclusion/exclusion criteria, genetic counselling and informed consent, setting up an IVF centre, transport PGD, quality assurance/quality control (QA/QC) and accreditation (which is also further discussed in the paper by Harper *et al.*, 2010). In this document, the laboratory performing the diagnosis will be referred to as the PGD/PGS centre and the centre performing the IVF as the IVF centre. Topics covered in this guideline include general uses of FISH, laboratory issues relating to FISH, pre-examination validation, examination process and post-examination process.

PGD for chromosome rearrangements has become an accepted and routine procedure in most PGD centres performing FISH-based

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testing. PGD for chromosome rearrangements has been developed for patients at high risk of pregnancy loss, inability to achieve pregnancy and abnormal live born births resulting from inheritance of unbalanced products of the rearrangement.

PGS, called 'low-risk PGD' in the original guidelines, has been carried out for infertile patients undergoing IVF with the aim of increasing the IVF pregnancy and delivery rates. Current examples of indications for PGS include advanced maternal age (AMA), repeated implantation failure (RIF), severe male infertility and couples with normal karyotypes who have experienced recurrent miscarriages (RM). To date, eleven RCTs have been performed looking at PGS for various indications which have failed to show an improvement in delivery rates for poor prognosis (Staessen *et al*., 2004; Stevens *et al*., 2004; Mastenbroek *et al*., 2007; Blockeel *et al*., 2008; Debrock *et al*., 2008; Hardarson *et al*., 2008; Schoolcraft *et al*., 2009) and good prognosis patients (Jansen *et al*., 2008; Mersereau *et al*. 2008; Staessen *et al*., 2008; Meyer *et al*., 2009). These publications have led to an open discussion of PGS and its role in IVF (Fritz, 2008; Harper *et al*., 2008a; Simpson, 2008; The Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society for Reproductive Medicine, 2008). The general consensus is that since 10 of the RCTs have shown no benefit of cleavage-stage biopsy/PGS (probably owing to the high levels of mosaicism at cleavage stages and the limitations of FISH), further PGS RCTs should concentrate either on polar body or trophectoderm biopsy and a full chromosome count. The ESHRE PGS task force is running a multi-centred RCT to determine whether PGS truly improves the IVF outcome in patients of AMA using polar body biopsy and array comparative genomic hybridization (Geraedts *et al*., in press). As PGS is still being practised by some IVF and PGD centres, the Consortium felt it was important to set forth our opinion on the best practices that should be followed in a PGS laboratory as well as those for PGD.

PGD/PGS is still relatively unregulated and lacks standardization compared with other forms of diagnostic testing, however, more federal, state and local governments are beginning to take an interest in PGD and some have begun accrediting laboratories that offer PGD (Harper *et al*., 2010). This is a logical step 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/PGS is to build a consensus opinion on best practices within the PGD/PGS community; a component of the mission of the Consortium (ESHRE PGD Consortium Steering Committee, 1999, 2000, 2002; Harper *et al*., 2006, 2008b; Sermon *et al*., 2007; Goossens *et al*., 2008, 2009).

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 experience and available evidence. Indeed, the American Society for Reproductive Medicine (ASRM) published a practice committee report for PGD in 2008 (American Society for Reproductive Medicine and Society for Assisted Reproductive Technology. Practice Committee Report, 2008) essentially reviewing PGD practice in the USA. The PGD International Society (PGDIS) has also drafted guidelines, which were recently updated and although more in-depth than the ASRM report, these guidelines are concise and remain so in the recent revised edition (Preimplantation Genetic Diagnosis International Society, 2004, 2008). The consensus opinions provided in this document and the accompanying guidelines, not only reflect current use of PGD but also offer consensus-based specific guidance regarding how best to practise clinical PGD 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. 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 a drift towards the lowest common denominator, established and fledgling centres alike can learn from global experiences and be guided by a consensus opinion.

These opinions 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 opinions, and they must be applied according to individual patient needs using professional judgement. However, guidelines and opinions 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.

# 1. General uses of FISH

1.1. FISH can be used for embryo sexing for X-linked diseases or social reasons (gender selection/family balancing), inherited chromosome rearrangements and aneuploidy screening (PGS).

### 1.1.1. Sexing for X-linked diseases and social reasons

1.1.1.1. A probe set containing at least probes specific for the centromere regions of the X and Y chromosomes, and one autosome, is **recommended** (Staessen *et al*., 1999; Harper and Wilton, 2001).

1.1.1.2. Diagnosis on a single mononucleate cell is **acceptable** for sexing (Kuo *et al*., 1998).

1.1.1.3. It should be noted that FISH-based PGD for sexing to exclude transmission of X-linked diseases can be less advantageous when compared with PCR-based diagnosis of the disease-associated mutation along with gender (Renwick *et al*., 2006; Renwick and Ogilvie, 2007). A PCR-based diagnosis allows for transfer of unaffected males as well as the ability to select against carrier females.

1.1.1.4. Autosomal probes can be included to check for aneuploidy but if multiple rounds of FISH are being used, the X and Y probes should be included in the first round (Wilton *et al*., 2009).

### 1.1.2. Chromosome rearrangements

1.1.2.1. It is **recommended** that the probe set should at least contain sufficient probes to detect all expected unbalanced forms of the chromosomal rearrangement.

1.1.2.2. Where suitable probes are not available, it is **acceptable** to use probe mixes that cannot detect some unbalanced forms of a rearrangement, provided that 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; Scriven, 2003).

1.1.2.3. In the case of 1.1.2.2, patients should be counselled to this effect. A cytogeneticist or suitably qualified person should determine which probe combination to use.

1.1.2.4. Diagnosis on a single mononucleate cell is **acceptable** for chromosome rearrangements, provided that 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.

1.1.2.5. Diagnosis based on concordant results from two mononucleate cells is **recommended** where there is only one informative probe available.

1.1.2.6. The use of additional probes to screen for aneuploidies of chromosomes not involved in the rearrangement is **acceptable**.

1.1.2.7. For translocation detection, the PGD-FISH report should clearly state that the analysis cannot discriminate between embryos with a normal or a balanced translocation karyotype.

### 1.1.3. Aneuploidy Screening (PGS)

1.1.3.1. For aneuploidy screening, a probe set of at least eight chromosome pairs, including chromosomes 13, 16, 18, 21, 22, X and Y, is **recommended** (Munné *et al.*, 1999; Magli *et al.*, 2001; Wilton, 2002). The addition of other probes is **acceptable**.

1.1.3.2. Diagnosis on a single mononucleate cell is **recommended** for PGS (Cohen *et al.*, 2007).

1.1.3.3. Rehybridization procedures on single blastomeres (Munné *et al.*, 1998; Bahçe *et al.*, 2000; Magli *et al.*, 2001; Wilton 2002) are **acceptable** with appropriate validation and written procedures.

1.1.3.4. Whereas PGS remains controversial in clinical practice, the following indications for its use have been reported on:

1.1.3.4.1. RM [for example: >2 miscarriages (Harper *et al.*, 2006)—exact number to be determined by each centre].

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

1.1.3.4.2. RIF [for example: >3 embryo transfers with high quality embryos or the transfer of ≥10 embryos in multiple transfers (Harper *et al.*, 2006)—exact numbers to be determined by each centre].

1.1.3.4.2.1. Implantation failure is defined as the absence of a gestational sac on ultrasound at five or more weeks post-embryo transfer.

1.1.3.4.3. AMA (for example: >36 completed years (Harper *et al.*, 2006)—exact age to be determined by each centre).

# 2. Laboratory issues relating to FISH

## 2.1. Laboratory Materials

2.1.1. All clinical equipment should meet the criteria set for the intended application, be appropriately maintained and serviced, with all aspects supported by written standard operating procedures.

2.1.2. All batch numbers of reagents should be recorded so that they may be traceable to specific assays.

2.1.3. Whenever possible, all solutions should be purchased 'ready to use' and should be of 'molecular biology' grade or equivalent.

## 2.2. Work practice controls the following recommendations are made

2.2.1. It is essential that an adequate labelling system is used to match the cell diagnostic result with the embryo from which that cell was biopsied.

2.2.2. Labelling and sample identification should be confirmed for critical and high-risk steps. It is **recommended** that the unique patient identifier and embryo/cell number should be witnessed and signed by two scientists at the following steps:

(a) immediately after biopsy to confirm that the embryo and cell number match.

(b) at fixation or spreading to confirm that the cell identification matches the labelling on the relevant slide.

(c) when diagnostic FISH results are recorded to ensure accuracy and correlation with the correct cell and/or embryo identification.

2.2.3. All personnel undertaking FISH diagnosis should be adequately trained and should follow written standard operating procedures.

2.2.4. Training for FISH personnel should be documented. It is **recommended** that at least 50 blastomeres are successfully spread or fixed and subjected to FISH by each trainee prior to working on clinical specimens.

2.2.5. Deviations to standard operating procedures (SOPs) and protocols should be documented.

2.2.6. Training for FISH should be at least to the standard required for routine testing in a clinical cytogenetic laboratory.

## 2.3. Fixation protocols

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

2.3.2. The following three methods of spreading and/or fixing single blastomeres have been described, all of which are **acceptable**:

2.3.2.1. methanol/acetic acid (Tarkowski, 1966; Griffin *et al.*, 1992).

2.3.2.2. Tween/HCl (Coonen *et al.*, 1994; Harper *et al.*, 1994).

2.3.2.3. Combined Tween/HCl-methanol/acetic acid (Dozortsev and McGinnis, 2001; Baart *et al.*, 2004).

2.3.2.4. Each of these methods has pros and cons and all work effectively when performed well. The methanol/acetic acid method is rapid but the toxic nature of the solutions involved precludes its use in an embryology laboratory. The Tween/HCl method uses non-toxic solutions and can be performed in an

embryology laboratory. Some PGD scientists find that the methanol/acetic acid method produces nuclei that are overspread resulting in stretched FISH signals that are difficult to interpret. Others find that the Tween/HCl method results in nuclei that are too small and condensed, thus increasing the risk signal overlap. Each laboratory should determine which technique produces the best outcomes under their conditions.

2.3.3. The use of hypotonic treatment of cells prior to spreading is **acceptable**.

2.3.4. Spreading and/or fixing of one blastomere per slide is **recommended**.

Spreading and/or fixing of multiple blastomeres on a slide is **acceptable**, provided that sufficient measures are taken to ensure the correct labelling and identification of each blastomere. Please also refer to the Guidelines for organization of a PGD centre (Harton *et al.*, 2010a).

# 3. Pre-examination validation

## Probe selection

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

3.2. 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 that they are informative for the intended PGD couple, and to assess that signal specificity, brightness and discreteness are within acceptable parameters per predetermined individual laboratory criteria (as documented in written procedures).

3.3. For each test, it is **recommended** that only appropriately qualified personnel (as documented in written competency lists) authorizes selection of probes with the appropriate chromosome specificity and labelling.

3.4. For chromosome rearrangement cases, preliminary work on peripheral blood lymphocytes from both reproductive partners is **recommended** for each different probe and combined probe set, and should include both metaphase spread and interphase nuclei analysis. It is **acceptable** to study case-unrelated fibroblast or lymphocyte cells; however, use of these unrelated cell types will leave the lab unaware of signal polymorphism(s) in the reproductive partners and thus in the generated embryos.

3.5. It is **recommended** that at least 10 metaphase spreads should be examined: (a) to ensure that the probes are specific for the correct chromosomes, (b) to assess chromosome polymorphism and signal cross-hybridization and (c) with respect to carriers of a chromosome rearrangement, to ensure that the probes hybridize as expected to the different segments of the rearrangement (in other words, that they are informative for the intended couple).

3.6. 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 (see also: scoring clinical FISH results).

3.7. It is **recommended** that for probe mixes containing subtelomeric probes and/or locus-specific probes with known polymorphism and cross-hybridization, preliminary work should be carried out using (diploid) cells from both reproductive partners.

3.8. Since the analytical performance in blastomeres approaches that of peripheral blood lymphocytes and fibroblasts, it is **acceptable** not to test a new probe set on a limited series of blastomeres.

3.9. It is **recommended** that scoring criteria should be determined ahead of time (published or 'in-house') and should be adhered to as per written procedure.

3.10. No probe or combined probe set should be passed for clinical use unless it meets the individual laboratory's predetermined and documented minimum score for intensity, specificity and minimum background.

3.11. It is **recommended** that in every round of FISH each probe must be labelled with a different fluorochrome or combination of fluorochromes so that the colour of different probe signals can be distinguished from each other.

## Use of intra-assay controls

3.12. The use of positive and negative controls for FISH-based PGD assays is contentious.

3.12.1. Suitable positive controls are not readily available for FISH-based tests (i.e. single human blastomeres or other cell types to represent human blastomeres).

3.12.2. Normal human lymphocyte controls can serve to assess that the correct probes are in the mix and that they localize to the appropriately sized chromosome.

3.12.3. It is **recommended** that the individual laboratory's protocol includes checking procedures that are sufficient to ensure that the correct probes are in the mix.

## Assessing FISH efficiency

3.13. Each laboratory should validate and use a control for each FISH hybridization and the control slide should pass QC for each clinical run. Acceptable ranges of FISH hybridization efficiency should be determined in each laboratory for each FISH probe and combined probe set.

## Pre-cycle work-up on individual couples

3.14. **Sexing**: When 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 peripheral blood lymphocytes from both reproductive partners is **recommended** owing to the relatively common occurrence of polymorphism (Hsu *et al.*, 1987). If multiple rounds of FISH are being performed, the X and Y probes should be used in the first round (Wilton *et al.*, 2009).

3.15. **Structural chromosome abnormalities**: it is **recommended** that peripheral blood lymphocytes from both reproductive partners be tested with the specific probe set for clinical use. It is **acceptable** to perform the testing only on the partner who carries the rearrangement. It is a**cceptable** 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 in an attempt to predict the efficacy of PGD for these cases (Escudero *et al.*, 2003).

3.16. **Aneuploidy screening (PGS)**: it is **recommended** that testing with the D15Z1 probe (15p11.2) is performed on peripheral

blood lymphocytes from both reproductive partners before treatment since it cross-hybridizes to the short arm of other acrocentric chromosomes in around 15% of normal individuals (Shim *et al.*, 2003; Cockwell *et al.*, 2007).

### Reporting pre-examination validation

3.17. A report should be written detailing the protocol and validation steps of the PGD work-up (Harper *et al.*, 2010).

## 4. Examination process

### FISH protocols

4.1.1. Many variations in FISH methods have been published and all appropriately validated methods are **acceptable** (Delhanty and Conn, 2001; Harper and Wilton, 2001). The method used should have been previously implemented, tested and validated in the PGD centre.

4.1.2. When using prehybridization steps, such as pepsin and paraformaldehyde, it is **recommended** that steps should be taken to ensure appropriate QC for these solutions. Creation dates for solutions should be recorded and solutions checked prior to use for possible cellular contamination.

4.1.3. Mounting medium containing antifade is **recommended** to allow maintenance of fluorescent signals.

4.1.4. It is **recommended** that prior to each FISH procedure, denaturation, hybridization and wash temperatures are validated and that the temperature of the solution is verified prior to use.

4.1.5. Temperature ranges should be validated in individual PGD centres, and instruments serviced and calibrated regularly to ensure accuracy.

4.1.6. All critical steps in the FISH laboratory should be witnessed by an independent observer, preferably one who is trained in FISH. Critical steps include confirmation that the labelling of the biopsied cell and the slide onto which it is spread match, and use of the correct FISH probes for the case.

4.1.7. The following **recommendations** are made about the physical laboratory space needed for FISH-based PGD:

4.1.7.1. The FISH laboratory should be well-ventilated to minimize the effect of any noxious fumes. This is particularly important if cells are fixed using methanol and acetic acid

4.1.7.2. FISH outcomes, including cell spreading and fixation, are dependent on humidity. The humidity in the FISH laboratory should be controlled and stable. FISH protocols should be optimized in these conditions.

4.1.7.3. FISH signals may be bleached or weakened in bright light. It is **recommended** that the FISH laboratory be fitted with variable intensity incandescent lighting. Fluorescent lighting is **acceptable**.

### Existence of and adherence to clinical testing protocol

The following **recommendations** are made (Thornhill *et al.* 2005; Harper *et al.*, 2010):

4.1.8. Clinical testing protocols should include explicit instructions, including a summary of results from the validation steps of assay development, scoring criteria and reporting procedures as well as a framework for counselling patients in the presence of diagnostic results.

4.1.9. SOPs are required for all protocols, all equipment and all processes that take place in the PGD centre and should include selection and validation of examination procedures, clinical relevance, purpose of examination, specimen requirements and means of identification; equipment and special supplies, reagents, standards or calibrations and internal control materials; instructions for performance of the examination, limitations of the examinations, recording and calculation of results; internal QC procedures and criteria against which examination processes are judged, reporting reference limits and responsibilities of personnel in authorizing and reporting and monitoring reports, hazards and safety precautions assuring the quality of examinations.

4.1.10. Risk assessments are required for every stage of the PGD process. These assessments should be integrated to the SOPs. Laboratory staff should understand the SOPs clearly as these are the fundamental backbone of the service.

4.1.11. Deviations from protocol should be recorded. If frequent deviations occur, there should be a mechanism in place to change procedures accordingly.

4.1.12. Well-structured laboratory forms to report the work-up, PGD cycle and analysis of untransferred embryo results should be used.

4.1.13. In all of the critical stages of the PGD process witnessing and signing is **recommended** (see 2.2.2.).

### Scoring clinical FISH results

4.1.14. The following **recommendations** are made:

4.1.14.1. Signal scoring criteria should be established in a written protocol and adhered to for the interpretation of signals.

4.1.14.2. The fluorescence microscope should be equipped with and optimized for the appropriate filter sets for the probes being used.

4.1.14.3. 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 recommended for transfer, i.e. should be given the diagnosis of uninterpretable or inconclusive.

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

4.1.14.5. All single cell images should be captured and recorded for QC purposes and records.

4.1.14.6. Different locations around the world have specific laws or guidelines on what should be stored from an individual clinical case and for how long. Local accreditation schemes may also have recommendations along these lines. It is **recommended** that laboratories should follow local law or guidelines on storage of clinical samples and patient records.

4.1.14.7. If no local law or guideline exists, it is **recommended** that:

4.1.14.7.1. If there has been an embryo transferred or frozen, all slides from the case should be retained and appropriately stored. This can be at 4°C or dehydrated at room temperature.

4.1.14.7.2. If there is no embryo transfer or cryostorage, it is not necessary to keep FISH slides.

4.1.14.7.3. If no pregnancy results from fresh or frozen embryos, slides can be discarded.

4.1.14.7.4. If a pregnancy results, slides should be retained until the outcome of the pregnancy is known and, where possible, the FISH result confirmed.

4.1.14.8. Results should be reviewed and signed by a suitably qualified person.

4.1.14.9. A written or online electronic report should be given to the IVF centre to ensure transfer of the correct embryos. Results should not be transmitted verbally.

4.1.14.10. Reporting of clinical results to the IVF centre must follow local guidelines or law, or if nothing local exists, the guidelines in ISO15189 (Harper *et al.*, 2010).

# 5. Post-examination process

5.1. The following **recommendations** are made (ESHRE PGD Consortium Steering Committee, 1999, 2000, 2002; Sermon *et al.*, 2005, 2007; Harper *et al.*, 2006, 2008b; Goossens *et al.*, 2008, 2009; Preimplantation Genetic Diagnosis International Society, 2008).

5.1.1.1. Confirmation of the diagnosis should be performed on 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. It is **recommended** that this is performed on as many embryos as is practicable. It is **acceptable** to perform this periodically.

5.1.1.2. PGD and IVF centres should make special efforts to follow-up with the parents following prenatal testing or birth, especially if confirmatory testing is not possible.

5.1.1.3. Follow-up of pregnancies (including multiple pregnancy rate and outcome), deliveries, and 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 ESHRE PGD Consortium during annual data collections.

5.1.1.4. A paediatric follow-up working group has been formed by the Consortium for the follow-up of children born after PGD/PGS. PGD centres are encouraged to take part in this project.

### 5.2. Baseline IVF pregnancy rates for PGD

5.2.1. Setting appropriate baseline pregnancy rates should be left up to the individual centres. However, it is **recommended** that each IVF centre should compare PGD/PGS pregnancy rates and matched non-PGD/PGS (routine IVF) pregnancy rates within that IVF centre (Thornhill *et al.*, 2005).

5.2.2. Comparison of pregnancy rates with those reported by the annual data collections of the Consortium can also be carried out to set benchmarks for continual improvement of the PGD centre.

### 5.3. Appropriate indications for specific tests

5.3.1. It is **recommended** that specific indications for PGD/PGS should remain within the purview of individual clinics (Thornhill *et al.*, 2005).

### 5.4. Misdiagnosis rate

5.4.1. The Consortium makes the following **recommendations**: (Thornhill *et al.*, 2005; Preimplantation Genetic Diagnosis International Society, 2008; Thornhill and Repping, 2008, Wilton *et al.*, 2009).

5.4.1.1. It is **recommended** that misdiagnosis rates should be calculated for each type of assay and for all assays from a particular centre (Lewis *et al.*, 2001). Misdiagnosis rates include those clinical cases in which affected pregnancies arose and post-transfer confirmation of diagnosis assays that were discordant with the biopsy result.

5.4.1.2. It is **recommended** that confirmatory testing should be performed at least periodically as a QA. The Consortium is currently preparing a study on the follow-up of untransferred embryos by FISH. The Consortium member centres are encouraged to take part in this study.

5.4.1.3. It is **recommended** that the published and in-house estimates of misdiagnosis rates should be available on request to prospective patients along with pregnancy rates to allow informed consent for PGD.

5.4.1.4. Following a misdiagnosis, the PGD centre should investigate the possible causes of the misdiagnosis and make changes to protocols to eliminate the risk in the future (Wilton *et al.*, 2009).

5.4.1.5. Misdiagnosis should be reported to the Consortium each year during routine data collection (Thornhill *et al.*, 2005).

5.4.1.6. Many of the causes of misdiagnosis are avoidable by taking preventative action and following the principles of quality management present in modern accredited diagnostic testing laboratories. The Consortium has recently published an article reviewing the possible causes and adverse outcomes of misdiagnosis (Wilton *et al.*, 2009). It is **recommended** that the suggestions made in this paper for the prevention of specific misdiagnosis causes should be taken into consideration by the PGD centre to eliminate the possible causes of misdiagnosis.

# 6. Transport PGD

6.1. For general recommendations on Transport PGD see the Organization of a PGD centre guidelines (Harton *et al.*, 2010a).

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