QC standards in PGD and PGS.

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Abstract

The aim of PGD is to test the preimplantation embryo for specific conditions for couples at risk of transmitting that genetic abnormality to their offspring. The couple need to go through IVF procedures to generate embryos *in vitro*. The embryos can be biopsied at either the zygote, cleavage or blastocyst stage. PGS uses the same technology to screen for chromosome abnormalities in embryos from patients going through IVF procedures as a method of embryo selection. Chromosome analysis was originally performed using fluorescent in situ hybridisation (FISH) which has now been replaced by array comparative genomic hybridisation or next generation sequencing (NGS) For the diagnosis of single gene defects, the polymerase chain reaction (PCR) has been used and has become highly developed over the years. More recently, SNP arrays and karyomapping have been introduced.

PGD/PGS require partnership between IVF laboratories and diagnostic centres. As diagnosis can be performed using a variety of strategies with different technologies, accreditation of PGD diagnostic laboratories is important. Accreditation gives IVF centres an assurance that the diagnostic tests conform to specified standards. ISO 15189 is an international laboratory standard specific for medical laboratories. A requirement for accreditation is to participate in external quality assessment schemes

Key words

Quality control, Preimplantation genetic diagnosis

The current status of PGD and PGS

Initial clinical application of PGD

PGD was first performed in 1989 and since this time, genetic testing has seen major advances. PGD was developed as an alternative to prenatal diagnosis, for couples at risk of transmitting a genetic abnormality to their children. Couples have to go through IVF procedures to generate embryos in vitro, even though many of the couples that go through PGD are fertile. The embryos can be biopsied by the embryologists at the zygote stage (removal of the first and second polar body), cleavage stage (removal of 1-2 blastomeres from the 6-8 cell embryo) and blastocyst stage (removal of some trophectoderm cells)(Harton et al., 2011a). Up until recently, almost all PGD cycles were performed on blastomeres after cleavage stage biopsy (Harper et al, 2012, Moutou et al, 2014), but numerous studies have found that cleavage stage embryos exhibit high levels of chromosomal mosaicism, which means that biopsied cells may not be representative of the rest of the embryo (Harper et al, 1995, Munne et al, 1995, Fragouli et al, 2011, Taylor et al, 2014a). This is especially important when trying to perform PGD for a chromosome abnormality. Polar body biopsy is rarely used as it only gives genetic information on the maternal genome. In recent years, the IVF community have seen an increase in the use of blastocyst transfer (Glujovsky et al, 2012) and this has been reflected in the increased use of blastocyst biopsy for PGD (Moutou et al, 2014).

The genetic testing should be performed by a specialised genetic testing laboratory. The very first cases of PGD were performed using PCR to detect a

Y chromosome sequence for sexing for X-linked disease (Handyside et al, 1990).

Testing by FISH

FISH replaced PCR as the method of choice for embryo sexing (Griffin et al, 1994, Munne et al, 1995) and for chromosome analysis for patients carrying a Robertsonian or reciprocal translocation (Conn et al 1998, Fridstrom et al, 2001, Mackie Ogilvie and Scriven, 2002). FISH was limited as specific probe combinations needed to be used for a particular translocation and so individual tests had to be validated for each couple. Also FISH is not a very efficient technique to use at the single cell level (Ruangvutilert et al, 2000). At this time, some groups decided that PGD technology using FISH to analyse as many chromosomes as possible might be useful as an embryo selection method for patients of advanced maternal age, repeated implantation failure or repeated miscarriage (when the chromosomes in the parents were normal) (Munné et al, 1995, Verlinsky *et al.* 1995). This technique is usually referred to as preimplantation genetic screening (PGS) and should be differentiated from PGD as it is for a different group of patients and for a different reason.

Testing by PCR

PGD for couples at risk of a single gene disorder is usually performed using PCR (Harper and SenGupta, 2011). This technique has become highly sophisticated over the years, with one of the most important developments being multiplex PCR which allows the analysis of the mutation and also a contamination check (Harton et al, 2011b). Contamination can occur due to a number of reasons, such as cumulus cell contamination or from people

handling the cells. (SenGupta and Delhanty 2012). Molecular based analysis for PGD can either be performed by direct PCR amplification of the biopsied embryonic sample or following whole genome amplification (WGA). For direct PCR analysis inclusion of two informative short tandem repeat (STR) linked markers (within 1cM / 1MB) flanking each side of the mutation site, minimises the risk of misdiagnosis due to allele dropout at any one locus or due to contamination. Flanking markers allow the detection of cross over events in the region and assessment of the reliability of linkage analysis in these circumstances. The haplotype of the STR markers in phase with the mutation can be determined by identification of the shared haplotype between family members of known disease status. The limitation of direct PCR analysis is that an individual test has to be developed for each couple, which is time consuming and expensive. Each test has to be validated before being applied clinically.

The mutation site can be included for amplification in the multiplex reaction. Minisequencing is a commonly used method for mutation detection (Fiorentino et al, 2006). For *de novo* mutations in the male partners the haplotype in phase with the germline mutation can be determined by the analysis of single sperm. Similarly for *de novo* mutations in female partner's polar bodies can be used but these must be biopsied sequentially. Alternatively, phasing of alleles can be carried out from the analysis of embryos during the PGD treatment cycle however problems can arise when there are only a few embryos available for analysis. If all the embryos do not show the mutation and have the same haplotype, it is difficult to be certain

that the mutation was not present or if allele drop out had occurred at the mutation site in all the embryos. In such cases rebiopsy may be an option or cryopreservation of embryos that are blastocysts with analysis of whole embryos that arrest to confirm the STR phasing with mutational analysis.

Whole genome approaches

The introduction of whole genome amplification (WGA) methods have enabled the utilization of new, high throughput technologies which have increased the amount and type of information that can be obtained from an embryo biopsy sample (reviewed by Hughes *et al.,* 2005). Coupled with this is a reduction in work up time and the need of patient specific protocols. Techniques using WGA products are being applied clinically such as preimplantation haplotyping (PGH) which allows genotyping of multiple STR markers by PCR, or karyomapping, (SNP genotyping using an array) to perform PGD by linkage analysis (Renwick et al 2010, Handyside et al., 2012, Thornhill et al., 2015). The haplotypes obtained using these methods can also identify monsomies and trisomies of meiotic origin and can potentially be used to identify imbalances in embryos from translocation carriers and also distinguish between normal and balanced chromosome complements. Array comparative genome hybridisation identifies chromosomal imbalance in a WGA product and has been used in both PGD for chromosomal rearrangements and for PGS. Next generation sequencing (NGS) has also been applied for PGS (Wells et al 2014, Tan et al, 2014). It is expected that NGS will become the method that is primarily used for the detection of chromosomal imbalance and mutation analysis either as separate

tests or combined together in one analysis (Tan et al, 2014, Treff et al, 2013). Currently these whole genome approaches rely on whole genome amplification. The type of amplification used determines the artefacts that may be introduced into the sample and thereby affect the accuracy of the diagnostic test. Thus extensive validation of WGA in the context of the method of analysis (PGH, aCGH, karyomapping or NGS) with the indication for testing (chromosomal abnormality, single gene disorder) is required (Bergen et al 2005, Glentis et al 2009).

All direct multiplex PCR protocols require optimisation such that the efficiency of amplification at each locus approaches 95% and allele drop out is less than 5%. Optimisation needs to be carried out using isolated single cells from each partner whereby false positive and false negative rates for the overall protocol can be determined. When whole genome amplification is applied prior to PCR then optimisation at a single level is no longer necessary. The WGA however introduces artefacts into the sample and therefore more informative markers are required to ensure that the protocol is robust enough to overcome any resulting bias in the sample.

Causes of misdiagnosis in PGD and PGS

It is key that PGD is performed using tests that have been validated and optimised for the couple, as several reports of misdiagnosis have been reported (Wilton et al., 2009, Amagwula et al., 2012). The causes of misdiagnosis include contamination, allele dropout, mosaicism of the embryo, transfer of the wrong embryo and confusion of the embryo and test numbers.

Because of these risks, it is essential for the PGD laboratory to be adequately insured against the risk of misdiagnosis. Patients should be informed of the limitations of PGD when taking their consent to treatment and witnessing of appropriate stages during the procedure should be carried out and documented.

Developments in ART

Besides the need for individual tests for the majority of PGD cycles performed to date, the diagnosis is constrained as there is a time limit for the results of the diagnosis. The latest stage that embryos are normally transferred in IVF is day 6, as current culture conditions cannot sustain embryos for longer and embryos would normally be implanting around this stage, so they need to be returned to the uterus. Cleavage stage biopsy allows 2-3 days for the diagnosis, but blastocyst biopsy gives just 24 hours. This means that PGD labs have to run a 7 day a week service as the diagnosis has be performed within 24 hours. Also, running a single test for an array or NGS is expensive, therefore batching samples for these high throughput technologies makes it cost effective.

For many years, the freezing technique used in IVF did not support cryopreservation of biopsy embryos (slow freezing) but recent developments of the vitrification technique have allowed highly successful cryopreservation of biopsied embryos (Simopoulou et al.,2014, Chang et al., 2014). The use of vitrification is being tested to determine if freezing all embryos in routine IVF

cycles gives a higher success rate, as it allows for optimization of the endometrium (Roque et al., 2015). These freeze-all cycles, open up the opportunity to freeze all embryos post biopsy for PGD (Schoolcraft et al., 2011, Taylor et al., 2014b). This relaxes the time constraints on the PGD team to perform the diagnosis and allows batching of samples, both of which make the test significantly cheaper and improves the quality of the test results.

Accreditation of a PGD lab

Accreditation is a laborious process to establish and even harder to maintain but it ensures good quality management which is essential in today's diagnostic laboratories. PGD diagnostic laboratories need to conform to ISO 15189 (international laboratory standard specific for medical laboratories - Medical laboratories — Particular requirements for quality and competence)(Harper et al, 2010). The ISO has two parts: management and technical requirements. The ESHRE PGD Consortium have recommended that PGD is only carried out in an accredited laboratory (Harton et al., 2011c) and the HFEA in the UK have made this mandatory (HFEA, 2009). Similarly, the Organisation for Economic Co-operation and Development (OECD) "Guidelines for quality assurance in molecular genetic testing" (OECD, 2007) state that "All laboratories reporting molecular genetic testing results for clinical care purposes should be accredited or hold an equivalent recognition". PGD is slightly more complicated than some genetic testing as it requires a dialogue between the IVF centre and the PGD laboratory and it can be constrained by a short time period required for the test result.

Under personal requirements, ISO 15189 states that the PGD laboratory shall be *directed* “by a person or persons having executive responsibility and the competence to assume responsibility for the services provided” (5.1.3).

The management requirements address quality management including the quality policy and manual, document control, non-conformities and corrective actions, continual improvement, auditing, management review, contracts, referrals and resolution of complaints. Technical requirements include personnel competence (both technical and medical), equipment, accommodation and environment, and pre-analytical, analytical and post-analytical processes. Emphasis is placed on the particular requirements of patient care: notably sample identification and traceability, test validation, and interpretation and reporting of results. Quality indicators must be developed to monitor contributions to patient care and continual improvement.

The reports required

In PGD the protocol will need to be validated for the specific patient prior to the start of the treatment cycle. In PGS each methodology (FISH, array CGH, NGS) requires a separate validation prior to clinical application. This validation should be recorded and authorised for clinical application within a work up report. For validation appropriate control samples must be available. Equipment used must be in working order supported by documented evidence that it has been serviced and calibrated to the appropriate level for application

of the PGD protocol. Quality control measures need to be specified to ensure that the diagnostic results are within the acceptable limits of the reagent kits and equipment that was used. Staff training records need to be readily available to ensure that those performing the diagnostic tests are sufficiently trained to do so. The final protocol should be validated with the appropriate level of sensitivity for its intended use (polar body, single blastomere or 5-6 trophectoderm cells. Trend analysis of internal quality controls (diagnosis rates, contamination rates, equipment performance, staff training and root cause analysis of errors identified through audit) enables labs to identify good practise and potential problems. Acting on this findings leads to prevention of causes of misdiagnosis and improvement in the overall service.

For all cases there must be an authorised report for every PGD cycle, which in the PGD setup is sent to the IVF unit. The report must have the name and addresses of the PGD unit providing the results and IVF unit to which the results are being reported. For document control purposes the report should have a document name, number, the date of issue and the version number. The individual page numbers and the total number of pages of the report should be stated. There should be at least two identifiers for each partner (e.g., name, date of birth, hospital number/ unique patient number) along with the date of oocyte retrieval. For single gene disorders, the report should have the disorder name, the gene involved and their respective MIM numbers. The mutation being tested for should be written using the latest Human Genome Variation Society (HGVS) mutation nomenclature along with the reference sequence used to identify the mutation. For chromosomal disorders the

chromosomal rearrangements should be described using the latest International System for Human Cytogenetic Nomenclature (ISCN).

The date and time the samples are received in the PGD lab and the date and time the results report are issued are important as they can help keep track of the turn around times.

The results are best presented in a tabulated form and should include interpretative comments to indicate which embryos are suitable for transfer. An explanation of the results in the form of extra notes can be included which describe the minimum criteria used to provide a result including the expected error rates, which were determined during the work up of the protocol.

The report should be signed by the persons involved in carrying out the diagnosis and authorised by an appropriately qualified senior member of staff.

External quality assessment

A requirement for accreditation is to participate in an external quality assessment scheme, if available. EQA enables labs to compare their diagnostic workflow with that from other laboratories. All parts of the process can be assessed including the strategy for diagnosis, accuracy of results, the information given in the reports and turnaround times. Wet EQA schemes give labs the opportunity to test validated samples. Good practise and errors identified by the scheme assessors are summarised and circulated back to all participating labs in final scheme reports. EQA has the potential to identify

both major and minor problems in staff training, and equipment maintenance as well as ensure that the lab is performing tests that conform to current published guidelines (Harton et al 2011 b and d). EQA is an excellent tool for labs to objectively assess and improve the quality of their diagnostic service.

Participating labs are marked according to specific criteria resulting in a satisfactory performance or poor performance score. Individual feedback is given to each lab and labs can appeal poor performance scores. A panel of PGD experts (Specialist advisory group) review the appeal and they may uphold or revoke it. A lab with poor performance is required to complete a root cause analysis of the reason(s) that led to poor performance. Unlike actual clinical work, identification of poor performance in EQA does not only arise due to misdiagnosis but is often due to a weak protocol that is vulnerable to misdiagnosis, or poor or over - interpretation of the actual results obtained. A review of the first three years of the molecular PGD scheme run by NEQAS showed a marked improvement in the diagnostic reports submitted by all labs as the scheme summary reports gave clear feedback as to what needed to be included in them (Deans et al 2013). User meetings for the schemes provide a forum for participants to meet and discuss any difficulties experienced in performing the EQA. This process allows the EQA schemes to evolve and be appropriate to changes in clinical practice of the participating labs such as the introduction of new technologies.

PGD EQA for FISH based diagnosis

The EQA for FISH based diagnosis was started in 2008 as a collaboration between the Cytogenetics European Quality Assessment scheme (CEQAS) and the ESHRE PGD Consortium. Since this time it has run annually as an online system. This scheme is in two parts. In part I labs are sent two case scenarios and asked to select suitable probes for a pre-implantation genetic diagnosis work-up. Labs report the theoretical unbalanced and balanced products of the chromosomal rearrangement. A prediction of viable or frequently unbalanced products arising from the rearrangement enables labs to determine the appropriateness of their probe selection by assessing the limitations and their overall protocol and the risk of misdiagnosis due to probe efficiency or number of rounds of hybridisation. In part II, labs receive images of interphase nuclei from blastomeres with specified probe combinations for the case senarios presented in part I. Labs report their scoring of the FISH signals and interpretation of the results. In addition to the two case scenarios labs can also attempt an educational case which is marked but not scored.

PGD EQA for molecular based diagnosis

The molecular EQA was also started in 2008 as a collaboration between the United Kingdom National External Quality Assessment Service (UKNEQAS) and the ESHRE PGD Consortium. It has run annually as a wet method for analysis of blastomeres and trophectoderm. The scheme has two parts. In part 1 labs are sent a mock PGD referral with genetics reports of parental mutations and appropriate relatives together with genomic DNA from these

individuals. Labs design an appropriate molecular protocol for the germline mutation(s). When the scheme first started almost all labs performed direct multiplex PCR for diagnosis. More recently PGH and karyomapping are approaches that are being applied. Labs are required to submit a workup report with evidence, such as the haplotypes of the DNA samples to show that the strategy taken conforms to current guidelines (Harton et al 2011b). The strategy should ensure that allele dropout (heterozygous locus appearing to be homozygous due to failure of amplification of one allele) or contamination (maternal or external) can be detected. In Part 2 labs are sent single cells as mock embryos from the couple described in the referral. Labs perform diagnosis on these samples and then submit their lab reports together with the haplotypes in a proforma. Labs could report on one or two cells from each 'embryo sample' depending upon their normal practise. In recent years labs have been offered the option of having 4-5 cells together as a mock trophectoderm biopsy sample to reflect changing practice of participating labs.

This scheme has been run for cystic fibrosis, fragile X, Huntington disease and myotonic dystrophy type I.

EQA for the detection of aneuploidy or chromosomal imbalance by molecular methods

In 2013 two pilots were offered for arrays as a collaboration between UKNEQAS and CEQAS**.** Analysis for PB1 and PB2, blastomere and trophectoderm have been offered as wet schemes. Amplified DNA from PBI

and 2 samples and single cells or groups of cells were sent to labs for testing. Labs reported chromosomal imbalances detected. Labs have performed analysis by array CGH and more recently using NGS. These schemes are still being run as pilots to ensure that appropriate samples are provided to labs that give consistent results across a number of CGH or NGS platforms.

Follow up of untransferred embryos

One of the numerous difficulties in developing and validating PGD methods is that potentially affected embryonic cells are only available when the couple go through fertility treatment. Therefore it is essential to make use of the untransferred embryos from a PGD cycle to confirm the diagnosis. An ESHRE study of follow up on 940 untransferred embryos from PGD of monogenic disorders found that 93.7% of were correctly diagnosed in the treatment cycle. Diagnostic embryos accuracy was statistically significantly higher when two cells were tested compared to one cell (P=0.001). Sensitivity was significantly higher when multiplex protocols were used compared to singleplex protocols (P=0.005) however multiplex PCR-based methods on one cell, were as suitable as protocols involving two cells when the false negative rate was considered (Dreesen et al 2013).

The shift to methodologies requiring whole genome amplified products has the advantage of enabling the robust validation of new technologies. This is because the same whole genome amplified product can be tested on different platforms (Fiorentino et al 2014) and the impact on the results of altered conditions of analysis (for example hybridization time on arrays) can be

verified. The shift to trophectoderm biopsy at blastocyst stage has meant the effect of mosaicism in the sample needs to be validated for each new platform (Mamas et al 2012).

Internal controls

Biopsy, Tubing, labelling, or spreading of cells

Biopsy of cell(s) from an egg or an embryo is required for all PGD or PGS applications. The tubing and spreading of the biopsied is the most crucial part of the PGD/PGS process. If the cell(s) do not get in the PCR tube or the spreading is not carried out optimally the analysis of the DNA cannot be carried out leading to a no result or an inconclusive result. Oocytes and embryos that are donated for research and training can be used as controls by the embryologists to practice their tubing and spreading skills. For additional controls, DNA from the embryologists/scientists performing the biopsy and tubing should be included in the molecular tests being performed to check for contamination. A log of this training should be kept and used to determine the competency of the staff performing these tasks.

Clear FISH signals can be used as IQC for optimally spread blastomeres. Consistency in successful amplification for samples from separate oocytes and embryos, without maternal or other contamination for molecular analysis is a good indicator of competency in tubing the biopsied cells.

FISH

The main purpose of the FISH technique in PGD is to test for chromosomal imbalances. Before a FISH protocol can be applied clinically it needs to be optimised on lymphocytes using the appropriate probe combination that will detect the chromosomal imbalance. Lymphocyte preparations from an unaffected partner and partner with chromosome abnormality can be used as controls to test probe strategy and to confirm reported chromosomal abnormality. Efficiency of optimised FISH protocol determined in workup control samples can be used as a calibrator of the test and the acceptable values for the control samples during testing in the treatment cycle that were determined at workup can be used as internal quality control. FISH is not an efficient technique to perform for PGS as often not all chromosomes are tested. When numerous chromosomes are tested, FISH is challenging. For the majority of PGD cases the resolution of whole genome approaches such as array CGH and NGS is sufficient for the detection for predicted imbalances that can arise in embryos from patients with chromosomal rearrangements without the need for patient specific protocols. Whole genome approaches also allow for the option of checking aneuploidy in other chromosomes not involved in the parental chromosomal rearrangement.

Whole Genome Amplification (WGA)

DNA from single cells or a clump of cells is whole genome amplified and used in different platforms to detect chromosomal imbalances and single gene disorders mainly by linkage analysis. The reagents required for whole genome amplification are generally provided as a kit by manufacturers. Due to artefacts or bias that may arise during WGA it is important to validate which

type of WGA kit is suitable for each work flow with consideration of the following parameters: the starting material (PB, blastomere or trophectoderm), the indication for testing (single gene disorder, chromosomal rearrangement or PGS), the number of hours of WGA and the method of subsequent analysis using the WGA product (PCR, array or NGS). A positive control DNA sample of known concentration and a negative (no DNA) control can be processed and analysed with the test samples. The quality of the amplification can be assessed by visualising the size range and intensity of the amplified product on agarose gel electrophoresis or the double stranded DNA concentration of the product can be measured using an appropriate method such as Qubit. Recording these parameters of the control sample post WGA serves as an internal quality control by calibrating the efficiency of amplification.

Array CGH

Different aCGH platforms are used in PGD for the purposes of chromosomal imbalance detection. When available, samples with imbalance arising due to the chromosomal abnormality (affected family member, CVS, etc) can be used as controls. For internal quality control, QC measures as specified by array manufacturer for labelling, hybridisation and scanning could be used. The quality and accuracy of the profile and results from reference male and female DNA samples should also be checked as an internal quality control.

Multiplex PCR

Multiplex PCR is used in PGD to detect single gene disorders including

dominant de novo mutations known to be present in an affected parent. The PCR protocols need to optimised on single cells for each family to include a mutation detection method along with linked microsatellite markers. Single cells from known normal, carrier and affected individuals (as appropriate and available) from the family are used during the workup and during the treatment cycle as controls. Efficiency and ADO rates of all loci included in the final optimised multiplex PCR protocol developed in case work up are calculated that can be used as calibrators for the PGD cycle. Similarly false negative and false positive values for single cells of known mutational status are calculated and referred to during the PGD diagnosis.

The linked STR markers included in the protocol provide IQC measures to detect successful amplification, external and maternal contamination as well as any crossover events that might occur.

PGH

PGH is utilised in PGD for the detection of single gene disorders where linkage analysis can be carried out. DNA samples from family members who have had genetic testing and have been found to carry the familial mutation are used as controls.

Suitable informativity of markers linked to the mutation site is required to use as calibrators. Sufficient amplification of linked informative markers to distinguish haplotypes in phase with familial mutation or the normal allele, together with contamination and crossover detection provide the IQC

measures for PGH.

Karyomapping

Similarly to PGH, DNA samples from family members with known disease status are used as controls and suitable informativity of the SNPs linked to the mutation site is required. Sufficient amplification of linked informative markers to distinguish haplotypes in phase with familial mutation or the normal allele, together with contamination and crossover detection provide the IQC measures for karyomapping.

NGS

NGS has been applied to PGS. Reagent kits and software are available for different NGS platforms. For internal quality control, QC measures as specified by the manufacturer of the NGS reagent kit and platform should be followed. The quality and accuracy of the profile and results as well as the depth of the sequence read, of a WGA product from a known euploid genomic DNA sample can be used as a calibrator and an internal quality control.

Audit

Audits are an important part of quality control as they provide a means to monitor the service at regular intervals.

There are three types of audits that can be carried out, namely vertical, horizontal and examination audits. The aim of a vertical audit is to check the

whole of the management system and processes, for example by following a PGD case from the referral stage all the way through to the point when the final PGD report is issued. This could include several parameters such as referral system, document control, standard operating procedures, equipment maintenance, health and safety issues and staff training.

A horizontal audit examines one component of a process on more than one item. For example, a number of reagents could be checked for logging in process, storage conditions and COSHH assessment.

An examination audit, where an examination process is witnessed while being carried out, not only audits the accuracy of the SOP and its related documents, but also provides an opportunity to assess the competency of the person carrying out the procedure.

Any non-compliances from any of the audits should be recorded and have a root cause analysis carried out. The outcomes of the audits can be used to rectify the shortfalls of the management system and provide more staff training if necessary and lead to quality improvements.

Key quality Indicators

Key quality indicators are required so the lab can monitor its overall performance and capacity. Service improvements should be reflected in the key quality indicators which can be used to formulate future plans for the lab,

implement new technologies and change future practice. Key quality indicators could include turn around times, diagnosis rates, contamination rates, EQA performance and staff competency testing.

Conclusion

Quality control minimises the risk of misdiagnosis. All parts of the workup and diagnostic procedure from the initial referral to the delivery of the final report can be monitored with suitable controls and calibrators. Regular audit of these parameters enables the lab to assess the performance of their service and objectively measure improvements.

References

Amagwula, T., Chang, P.L., Hossain, A., Tyner, J., Rivers, A.L., Phelps J.Y. 2012. Preimplantation genetic diagnosis: a systematic review of litigation in the face of new technology. Fertil Steril.;98(5):1277-82.

Bergen, A.W., Haque, K.A., Qi, Y., Beerman, M.B., Garcia-Closas, M., Rothman, N,, Chanock, S.J. 2005. Comparison of yield and genotyping performance of multiple displacement amplification and OmniPlex whole genome amplified DNA generated from multiple DNA sources. Hum Mutat.;26(3):262-70.

Chang, L.J., Huang, C.C., Tsai, Y.Y., Hung, C.C., Fang, M.Y., Lin, Y.C., Su, Y.N., Chen, S.U., Yang, Y.S. 2013. Blastocyst biopsy and **vitrification** are

effective for preimplantation genetic diagnosis of monogenic diseases. Hum Reprod.;28(5):1435-44.

Conn CM, Harper JC, Winston RM, Delhanty JD. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. Hum Genet. 1998 Jan;102(1):117-23.

Deans, Z., Fiorentino, F., Biricik, A., Traeger-Synodinos, J., Moutou, C., De Rycke, M., Renwick, P., Sengupta, S., Goossens, V., Harton, G. 2013. The experience of 3 years of external quality assessment of preimplantation genetic diagnosis for cystic fibrosis. Eur J Hum Genet.;21(8):800-6.

Dreesen, J., Destouni, A., Kourlaba, G., Degn, B., Mette, W.C., Carvalho. F., Moutou, C., Sengupta, S., Dhanjal, S., Renwick, P., Davies, S., Kanavakis, E., Harton, G., Traeger-Synodinos, J. 2014. Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. Eur J Hum Genet.;22(8):1012-8.

Fiorentino, F., Biricik, A., Nuccitelli, A., De Palma, R., Kahraman, S., Iacobelli, M., Trengia, V., Caserta, D., Bonu, M.A., Borini, A., Baldi, M. 2006. Strategies and clinical outcome of 250 cycles of Preimplantation Genetic Diagnosis for single gene disorders. Hum Reprod.; 21(3):670-84

Fiorentino, F., Biricik, A., Bono, S., Spizzichino, L., Cotroneo, E., Cottone, G.,

Kokocinski, .F, Michel, C.E. 2014 Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril.;101(5):1375-82.

Fragouli, E., Alfarawati, S., Daphnis, D.D., Goodall, N.N., Mania, A., Griffiths, T., Gordon, A., Wells, D. 2011. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. Hum Reprod.;26(2):480-90.

Fridstrom, M., Ahrlund-Richter, L., Iwarsson, Malmgren, H., Inzunza, J., Rosenlund, B., Sjöblom, P., Nordenskjöld, M., Blennow, E., Hovatta, O. 2001. Clinical outcome of treatment cycles using preimplantation genetic diagnosis for structural chromosomal abnormalities. Prenat Diagn.;21(9):781-7.

Glentis, G., SenGupta, S., Thornhill, A., Wang, R., Craft, I., Harper, J.C. 2009. Molecular comparison of single cell MDA products derived from different cell types. Reprod Biomed Online.;19(1):89-98.

Glujovsky, D., Blake, D., Farquhar, C., Bardach, A. 2012. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev.;7:CD002118.

Griffin, D.K., Handyside, A.H., Harper, J.C., Wilton, L.J., Atkinson, G., Soussis, I., Wells, D., Kontogianni, E., Tarin, J., Geber, S., et al. 1994.

Clinical experience with preimplantation diagnosis of sex by dual fluorescent in situ hybridization. J Assist Reprod Genet.;11(3):132-43.

Handyside, A.H., Kontogianni, E.H., Hardy, K., Winston, R.M. 1990. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature.;344(6268):768-70.

Handyside, A.H., Harton, G.L., Mariani, B., Thornhill, A.R., Affara, N., Shaw, M.A., Griffin, D.K.. 2010. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet.;47(10):651-8.

Harper, J.C., Coonen, E., Handyside, A.H., Winston, R.M.L., Hopman, A.H.N., and Delhanty, J.D.A. 1995. Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic, preimplantation human embryos. Prenatal Diagnosis.;15: 41-49.

Harper, J.C., Sengupta, S., Vesela, K., Thornhill, A., Dequeker, E., Coonen, E., Morris, M.A. 2010 Accreditation of the PGD laboratory. Hum Reprod.;25(4):1051-65.

Harper, J.C., Wilton, L., Traeger-Synodinos, J., Goossens, V., Moutou, C., SenGupta, S.B., Pehlivan Budak, T., Renwick, P., De Rycke, M., Geraedts, J.P., Harton, G. 2012. The ESHRE PGD Consortium: 10 years of data

collection. Hum Reprod Update.;18(3):234-47.

Harton, G.L., Magli, M.C., Lundin, K., Montag, M., Lemmen, J., Harper, J.C.2011a. European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium/Embryology Special Interest Group. ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Hum Reprod.;26(1):41-6.

Harton, G.L., De Rycke, M., Fiorentino, F., Moutou, C., SenGupta, S., Traeger-Synodinos, J., Harper, J.C. 2011b. European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium. ESHRE PGD consortium best practice guidelines for amplification-based PGD. Hum Reprod.;26(1):33-40.

Harton, G., Braude, P., Lashwood, A., Schmutzler, A., Traeger-Synodinos, J., Wilton, L., Harper, J.C. 2011c European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium. ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. Hum Reprod.;26(1):14-24.

Harton, G.L., Harper, J.C., Coonen, E., Pehlivan, T., Vesela. K., Wilton, L. 2011d European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium. ESHRE PGD consortium best practice guidelines for

fluorescence in situ hybridization-based PGD. Hum Reprod.;26(1):25-32

Hughes, S., Arneson, N., Done, S., Squire, J. 2005. The use of whole genome amplification in the study of human disease. Prog Biophys Mol Biol. 88(1):173-89.

Human Fertility and Embryology Authority (HFEA). Code of Practice, 8th Edition (Updated 2015). (Available online at: www.hfea.gov.uk)

ISO 15189 (3rd edition) Medical laboratories -- Requirements for quality and competence. Geneva; International Organization of Standards, 2012

Mackie Ogilvie, C. and Scriven, P.N. 2002. Meiotic outcomes in reciprocal translocation carriers ascertained in 3-day human embryos. European Journal of Human Genetics.; 10(12): 801–6.

Mamas, T., Gordon, A., Brown, A., Harper, J., Sengupta, S. 2012. Detection of aneuploidy by array comparative genomic hybridization using cell lines to mimic a mosaic trophectoderm biopsy. Fertil Steril.;97(4):943-7.

Moutou, C., Goossens, V., Coonen, E., De Rycke, M., Kokkali, G., Renwick, P., SenGupta, S.B., Vesela, K., Traeger-Synodinos, J. 2009. ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010.Hum Reprod.;29(5):880-903.

Munné, S., Sultan, K.M., Weier, H.U., et al. 1995. Assessment of numeric abnormalities of X, Y, 18, and 16 chromosomes in preimplantation human embryos before transfer. Am J Obstet Gynecol. 172(4 Pt 1):1191-9

OECD. OECD guidelines for quality assurance in molecular genetic testing. 2007. (Available online at: www.oecd.org/dataoecd/43/6/38839788.pdf)

Renwick, P., Trussler, J., Lashwood, A., Braude, P., Ogilvie, C.M. 2010. Preimplantation genetic haplotyping: 127 diagnostic cycles demonstrating a robust, efficient alternative to direct mutation testing on single cells. Reprod Biomed Online.;20(4):470-6.

Roque, M., Valle, M., Guimarães, F., Sampaio, M., Geber, S. 2015. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil Steril. pii: S0015-0282(15)00092-8. doi: 10.1016/j.fertnstert.2015.01.045. [Epub ahead of print]

Ruangvutilert, P., Delhanty, J.D.A., Rodeck., C. and Harper, J.C. 2000 Relative efficiency of FISH on metaphase and interphase nuclei from non-mosaic trisomic or triploid fibroblast cultures. Prenatal Diagnosis. 20, 159-162

Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. Fertil Steril. 2011 Sep;96(3):638-40.

SenGupta. S.B., and Delhanty J.D. 2012.Preimplantation genetic diagnosis: recent triumphs and remaining challenges. Expert Rev Mol Diagn. 2012 Jul;12(6):585-92.

Simopoulou, M., Asimakopoulos, B., Bakas, P., Boyadjiev, N., Tzanakaki, D., Creatsas, G. 2014. Oocyte and embryo vitrification in the IVF laboratory: a comprehensive review. Folia Med (Plovdiv).;56(3):161-9.

Tan, Y., Yin, X., Zhang, S., Jiang, H., Tan, K., Li, J., Xiong, B., Gong, F., Zhang, C., Pan, X., Chen, F., Chen, S., Gong, C., Lu, C., Luo, K., Gu, Y., Zhang, X., Wang, W., Xu, X., Vajta, G., Bolund, L., Yang, H., Lu, G., Du, Y., Lin, G. 2104. Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience.;3(1):30

Taylor, T.H., Gitlin, S.A., Patrick, J.L., Crain, J.L., Wilson, J.M., Griffin, D.K. 2014a. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update.;20(4):571-81.

Taylor TH, Patrick JL, Gitlin SA, Michael Wilson J, Crain JL, Griffin DK. Outcomes of blastocysts biopsied and vitrified once versus those cryopreserved twice for euploid blastocyst transfer. Reprod Biomed Online. 2014b Jul;29(1):59-64

Thornhill, A.R., Handyside, A.H., Ottolini, C., Natesan, S.A., Taylor, J., Sage,

K., Harton, G., Cliffe, K., Affara, N., Konstantinidis, M., Wells. D., Griffin. D.K. 2015. Karyomapping-a comprehensive means of simultaneous monogenic and cytogenetic PGD: comparison with standard approaches in real time for Marfan syndrome. J Assist Reprod Genet.;32(3):347-56.

Treff ,N.R., Fedick, A., Tao, X., Devkota, B., Taylor, D., Scott, R.T. Jr. 2013 Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. Fertil Steril.;99(5):1377-1384.

Verlinsky, Y., Cieslak, J., Freidine, M., Ivakhnenko, V., Wolf, G., Kovalinskaya, L., White, M., Lifchez, A., Kaplan, B., Moise, J., Valle, J., Ginsberg, N., Strom, C., and Kuliev, A. 1995. Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescent in situ hybridisation. Mol. Hum Reprod.; 10, 1923-1927.

Wells, D., Kaur, K., Grifo, J., Glassner, M., Taylor, J.C., Fragouli, E., Munne, S. 2014. Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. J Med Genet.;51(8):553-62.

Wilton, L., Thornhill, A., Traeger-Synodinos, J., Sermon, K.D., Harper, J.C. 2009. The causes of misdiagnosis and adverse outcomes in PGD. Hum Reprod.;24(5):1221-8.