Stakeholder review report

Last updated 21/10/19

RECOMMENDATIONS:	Preimplantation Genetic Testing
Review period:	10 June 2019 – 10 July 2019

The ESHRE Recommendations for good practice in Preimplantation Genetic Testing were open for stakeholder review between 10 June and 10 July 2019.

The project consisted of 4 papers:

- ESHRE PGT Consortium good practice recommendations for the organisation of preimplantation genetic testing (*short title: Organisation of PGT (PGT-ORG))*
- ESHRE PGT Consortium and SIG-Embryology good practice recommendations for polar body and embryo biopsy for preimplantation genetic testing *(short title: Polar body and embryo biopsy for PGT (PGT-BIOPSY))*
- ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders (*short title: Detection of monogenic disorders (PGT-M))*
- ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations *(short title: Detection of structural and numerical chromosomal aberrations (PGT-SR/PGT-A))*

The drafts of the documents were published on the ESHRE website. Stakeholders were invited to submit comments through mailings, advertisements during the ESHRE annual meeting in Vienna, and on social media.

Results:

Overall, 39 reviewers, representing 17 countries and 7 (inter)national societies, submitted a total of 645 comments. Details per paper are outlined in the table below.

Paper	Nr of reviewers	Nr of comments
PGT-ORG	24	159
PGT-BIOPSY	25	155
PGT-M	15	160
PGT-SR/PGT-A	20	171

All comments were assessed by the working group members, and, if relevant, changes were made to the papers:

- 5 comments (0,7%) did not require any action from the working group (including positive feedback, unclear or redundant comments)
- 135 comments (20,9%) requested language corrections or adaptations to the layout and format of the papers
- 505 comments (78,3%) focussed on the content of the papers, requesting corrections, modifications, or the addition of additional information. Of these, 354 comments were judged as being relevant and resulted in changes to the recommendations papers. The remaining 151 comments were not incorporated in the paper. For these comments, the working group formulated a reply to the reviewer.

Figure 1. Summary of the results of the stakeholder review for the 4 Recommendations for good practice in Preimplantation Genetic Testing papers

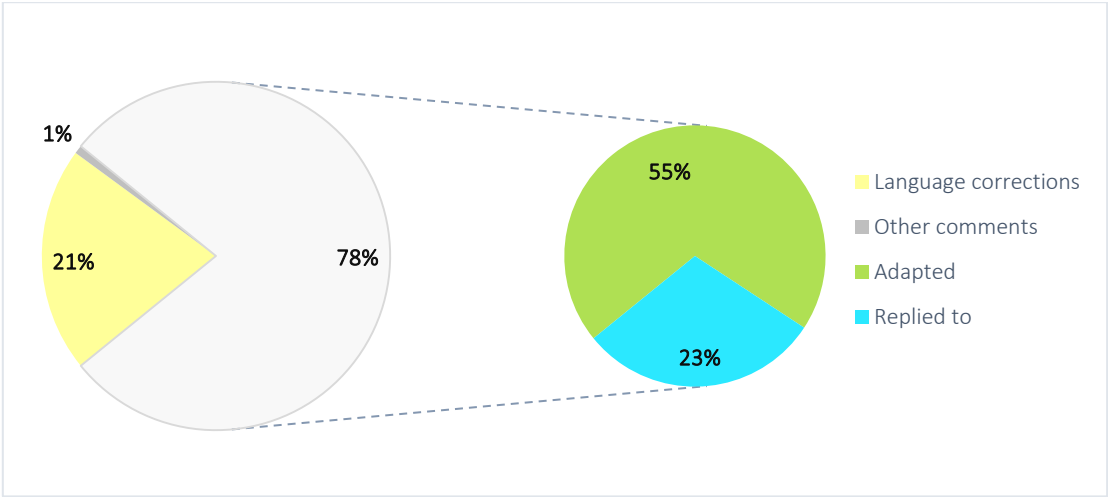
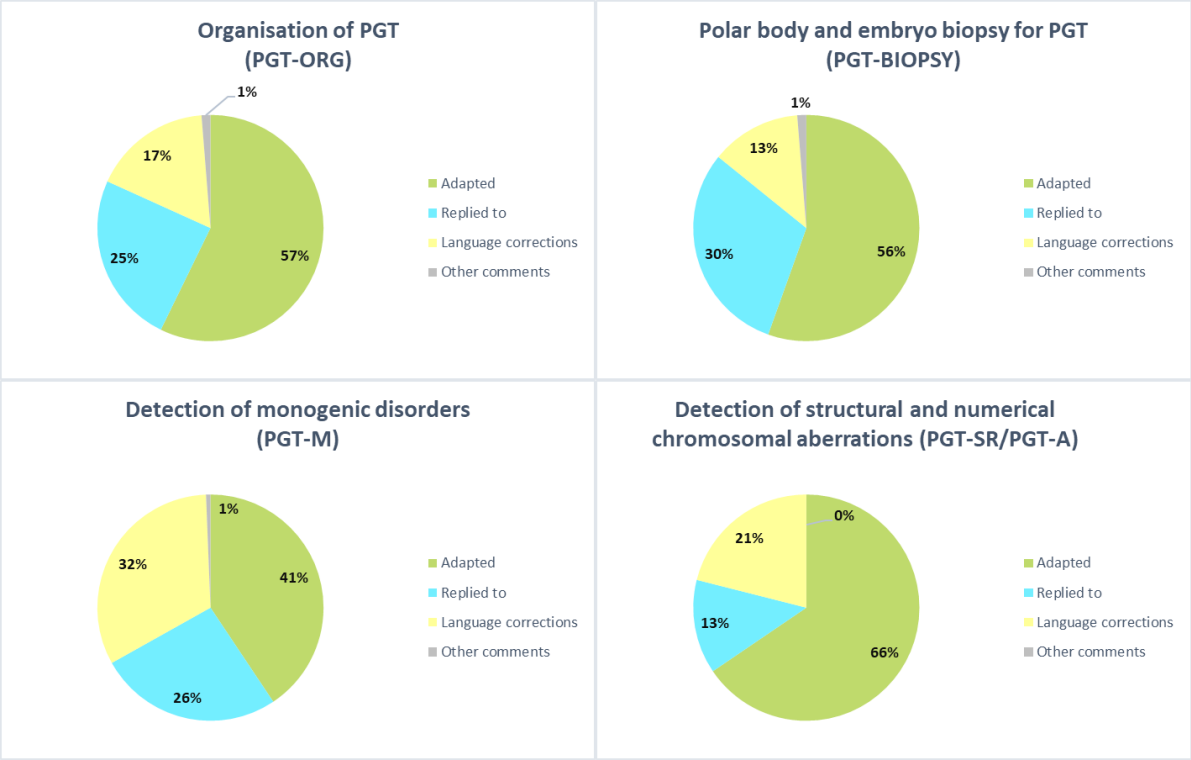


Figure 2. Summary of the results of the stakeholder review for the 4 Recommendations for good practice in Preimplantation Genetic Testing papers individually



List of reviewers

Reviewer Name	Organisation	Comments to			
		PGT-ORG	PGT-BIOPSY	PGT-M	PGT-SR / PGT-A
Sandi Deans and Farrah Khawaja	GenQA			√	
Ros Hastings, Katrina Rack, PGT Assessors from GenQA	GenQA	√	√		√
Italian Society of Human Genetics	Italian Society of Human Genetics	√		√	
Mariana Moura-Ramos	ESHRE Special Interest Group in Psychology and Counseling Steering Committee	√			
Philippe Gosset	SFDPI (Société Française de Diagnostic PréImplantatoire)				√
Marie-Laure Maurin	SFDPI (Société Française de Diagnostic PréImplantatoire)				√
Celine Moutou	SFDPI (Société Française de Diagnostic PréImplantatoire)		√	√	

Reviewer Name	Country	Comments to			
		PGT-ORG	PGT-BIOPSY	PGT-M	PGT-SR / PGT-A
M. Cristina Magli. Luca Gianaroli	Italy	√	√	√	√
Alan H Handyside	UK	√	√	√	√
Frank Broekmans	The Netherlands	√		√	√
Pamela Renwick	UK		√	√	
Emmanuelle Kieffer	France	√	√	√	
Laura Corti	Italy	√	√		
Joshua Blazek PhD; Elizabeth Cameron MS LCGC; Inger Britt Carlsson PhD; David Chrimes PhD; Tony Gordon PhD; Mike Large PhD; Colleen Lynch MSc; Beki Sanderson PhD; Kristine McWilliams MD, PhD	USA (5) and UK (2)	√	√	√	√
Susan Bint	UK				√
Alessandra Alteri	Italy	√	√		
Joanne Traeger-Synodinos, Thalia Mamas, Christina Vrettou	Greece	√		√	
Carlos Encinas	Bolivia		√		
Lauren Walters-Sen, Swaroop Aradhya, Michelle Strecker, Neha Kumar	United States	√	√		√
Tina Buchholz	Germany		√		√
Andreas Schmutzler	Germany	√			
Elena Zakharova	Russian Federation				√
Caio Graco Bruzaca	Brazil	√			√
Ahmet Berkız TURP	TURKEY	√	√	√	
Cristina Albanese	Italy		√		
Päivi Forsblom	Germany	√	√	√	√
Karen Sermon	Belgium	√	√	√	√

Christian Liebst Frisk Toft	Sweden				√
Raul Piña-Aguilar	USA, Mexico, UK	√	√	√	√
Susanne Knebel	Germany		√		√
Christina Hnida	Denmark		√		
Kersti Lundin	Sweden	√	√		
Hans Jakob Ingerslev	Denmark	√	√		
Véronique Cottin	Switzerland	√	√		
Inge Liebaers	Belgium	√			
Kelly Tilleman	Belgium		√		
Alexia Chatziparasidou	Greece	√	√		
Sandrine Chamayou	Italy	√	√	√	√
Servi J Stevens	Netherlands				√
Paul Scriven	UK				√

List of comments and replies (per paper)

Organisation of PGT (PGT-ORG)

GENERAL COMMENTS

Reviewer	Page	Line	Comment	REPLY
Inge Liebaers	20	738	Check reference please	This was corrected in the paper
GenQA	General Comment		These guidelines are extremely well written and comprehensive. One thing that is missing is detail on what should go in the genomic report.	We have adapted the section on the genomic report according to this and other comments.
GenQA	3	85	'until consensus' change to 'until consensus was reached'. Otherwise it is an incomplete sentence.	This was corrected in the paper
Ahmet Berkız TURP	General Comment		The paper is well organised and categorized. I want to add that in the subgroups of PGT-A ,PGT-M,PGT-SR can be individual testing and it can be combined also. I prefer you to add a sentence in the paper that some of the tests can be combined like Single gene disorder PGT-M or PGT-SR can be combined. This part can be explained in the first part. (It has mentioned in the PGT-M table) .If the patient is advanced for maternal age this has to be recommended to patient to test both single gene , HLA typing and also for trisomies. For blastomer that is day 3 can be done but it is discouraged, However for this situation day 5 TE biopsy can be best for there will be enough cells to work with all genetic testings. This increases the cost of PGT for patients. Patient has to be informed. This is mentioned in line 530 but it is very important to inform the patient before starting PGT cycle.	We have added the following sentence to the genetic counselling part: for PGT-M or PGT-SR combined with PGT-A, the policy for embryo (ranking and) transfer should be discussed.
Joshua Blazek and colleagues	General Comment		• In general, the recommendations read as being very lab focused, from the perspective of what is and is not technically feasible. Consider softening language such that consideration can be extended to families who do not meet these very black/white guidelines. o Specific examples include: § VUS classified by reference laboratory as category 3, when geneticist/specialist feels confident that this variant is causative of disease in the family § Consider possible addition of HLA typing when couple is presenting for PGT-M to avoid single gene disorder and already have an affected child at home who is not in current need of a transplant, but who may benefit from transplant in the future § Considering additional information specification/definition of phenotype severity	Indeed, three of the four guidelines are technical recommendations for laboratories. The organisation paper can be extended to families. It is not possible to 'soften' general statements because then they lose their meaning. For example, if the general statement would be that a VUS of class 3 can be included, when one is confident, then soon the general practice would be that class 3, 4 and 5 are included. If the general statement is that class 4 and 5 are included, a geneticist being confident with a class 3 can make an exception to the general statement. PGT with HLA typing and exclusion of a monogenic disorder concurrently is part of the paper, see paragraph starting at line 168. It is not possible to consider phenotype severity because of differences between countries and laws/regulations.
Joshua Blazek and colleagues	General Comment/ CHECK PAPER		• Incidental findings should be discussed during counselling, so patients/clinics are aware of what information will/will not be disclosed o Any guidelines ESHRE is able to provide as it pertains to incidental findings discovered during PGT that should and should not be reported back to clinicians would likewise be of great value	We have added the following sentence to the counselling section: - the reporting of results and the centre's policy on incidental findings.

GENERAL INTRODUCTION

Reviewer	Page	Line	Comment	REPLY
M. Cristina Magli. Luca Gianaroli	1	24	“patient group” change to “patients”.	This was corrected in the paper
M. Cristina Magli. Luca Gianaroli	2	Fig. 1	The steps related to FISH are missing.	We adapted the figure.
Inge Liebaers	1	31	Ref Harper et al. on behalf of ESJH and ESHRE, 2018 in the EJHG and in HRopen could be added	We added the reference for Harper JC, et al. Recent developments in genetics and medically assisted reproduction: from research to clinical applications. Eur J Hum Genet. 2018 Jan;26(1):12-33.
Raul Piña-Aguilar	1	16	"high risk genetic risk" is not defined in the glossary and is not a standard clinical genetics term. "increased risk of maternal aneuploidy" is a better term and it is the standard term in a maternal fetal medicine context	Within the numerical chromosomal aberration indication group, we want to distinguish between low risk PGT-A (former PGS) and high risk PGT-A (patients seeking PGT for numerical aberrations such as Klinefelter and other sex chromosome abnormalities).
Raul Piña-Aguilar	1	19	This is not correct; the PCR report in human embryos was for sex selection: Handyside et al. Nature. 1990;344(6268):768-70. The first direct study of a monogenic disease, CFTR and cystic fibrosis, was in 1992: Handyside et al. N Engl J Med. 1992;327(13):905-9.	We have rephrased and corrected the sentence
Raul Piña-Aguilar	2	Fig 1	"genetic testing for numerical aberrations": It is more precise to use "chromosomal aberrations". Partial aneuploidies are not numerical aberrations	Within the chromosomal aberration group, we want to distinguish between structural and numerical aberrations. Within the numerical chromosomal aberration indication group, we want to distinguish between low risk PGT-A (former PGS) and high risk PGT-A (patients seeking PGT for numerical aberrations such as Klinefelter and other sex chromosome abnormalities). It was decided that applying the ICMART definitions was not necessarily appropriate and correct. This has been explained in the introduction of the paper.
Karen Sermon	1	29	The value of PGT-A as such is still under debate, not just which patient groups would be suitable	The paper states that the value of PGT-A for all patients remains an ongoing discussion, and in addition the patient groups are mentioned. We have checked the sentence but assume this is sufficiently covered.
Frank Broekmans	1	29	If the INDICATIONS for PGT-A is outside of the scope, IT SHOULD BE considered to inform on WHERE this part of GOOD Practice recommendations is then discussed WITHIN the scope: is there any Guideline that addresses this. If NOT: this is REALLY an urgent matter for ESHRE	The value of PGT-A as such is still under debate, and if of value, the indications are unclear. As the current series focused on technical aspects, it was not relevant to include it here. Future ESHRE recommendations on PGT-A are an option.
Karen Sermon	1	-	It should be made clear what the difference is between guidelines and recommendations, and why these are recommendations not guidelines https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Guideline-development-process	We added a sentence on this in the methodology section.
Frank Broekmans	2	64	Please create conformity in terminology in the 6th column: Adhere to PGT-A, PGT-M, and PGT-SR.	It was decided not to use the abbreviations in the figure, as the figure is more clear as it is now.

1. PATIENT INCLUSION/EXCLUSION CRITERIA

Reviewer	Page	Line	Comment	REPLY

Mariana Moura-Ramos	3	94	The first comment refers to the professionals included in the multidisciplinary team involved in the decision of accepting/declining patients to Preimplantation genetic testing (PGT) services. Considering that one of the criteria is the presence of psychological problems [Doc 1, Line 114: "PGT may also be inappropriate if one of the partners has serious physical or psychological problems"] we would suggest that a mental health professional could be included as a member of the team.	We added "mental health professional" in the brackets.
M. Cristina Magli. Luca Gianaroli	3	94-100	The situation is different for PGT-A and should be specified.	We have discussed this comment and checked the first paragraph. It is formulated very broadly and therefore applicable to PGT-A. The only difference to PGT-M / PGT-SR would be that a smaller number of HC professionals would need to be involved. It was decided not to adapt the paragraph.
Frank Broekmans	3	94	I would strongly suggest adding here the necessity of indicating any of the PGT procedures according to evidence-based medicine rules. This is the first step in quality management. In spite of the absence of consensus or guideline…!	We have added a sentence in the PGT-A inclusion/exclusion, stating that the indication should be applied according to clinical studies and EB guidelines.
Hans Jakob Ingerslev	3	97	"PGT requests should be considered…" Not in all countries should cases be considered by local ethics board. In Denmark, we are working within the framework of our legislation.	The sentence was rephrased to reflect that request should either be considered by an ethics board or compliant with national legislation.
Italian Society of Human Genetics	3	97	In the document on the organization of PGT, at page 3, lane 97, the document reports that "PGT requests should also be considered by local ethic boards…". While we agree that local ethic boards should be involved during the process of the authorization of a lab to perform PGT, in some countries the submission of each single PGT request to a local ethic board would be time consuming and would induce delays in the entire procedure. We would suggest to take in account this problem in the document and change the sentence in "PGT requests COULD also be considered by local ethic boards…".	This statement was already changed based on another reviewer's comment. It states now that it should be compliant with national legislation, and where needed, also be considered by a local ethics board.
Joshua Blazek and colleagues	3	101,201	Currently, there is a lack of distinction between responsibility that falls with ordering clinician / local care team and responsibility that falls with the PGT lab (examples: section 1.1, 2.1). Consider revising to allow for increased clarification on responsibilities/roles of these two groups	In rewriting the paper, this statement was deemed no longer relevant. As it is stated now, any decision on patient inclusion/exclusion is to be taken by the team, and therefore it is irrelevant to define the responsibilities of the clinicians/lab.
Inge Liebaers	3	104	Overall error risk 1-3%: add ref or explain how figures were obtained?	This is based on the expertise of the working group members and the data from the PGT consortium. We added the reference to the paper,
Hans Jakob Ingerslev	3	104	"…error rates of 1 to 3 %". Maybe add that accordingly that PGT is a risk reducing procedure and not eliminating risk.	After assessing the comment, we decided not to modify the sentence in the paper.
Joanne Traeger-Synodinos and colleagues	3	104	We think that a definition on "error-rates" is essential. Do they only refer to misdiagnosis or to inconclusive results as well?	We added a clarification stating that these error rates can result in misdiagnosis. Inconclusive results are more related to efficiency, rather than error rates.
Kersti Lundin	3	107	"chance of success", of what? Finding the right probe, being able to get oocytes, getting pregnant, getting a "disease-free" embryo? Perhaps define?	Chance of success refers to genetic testing being feasible and reliable, which was covered in the first paragraph. We have removed "chances of success" later in the section.
Italian Society of Human Genetics	3	107	In the same document, at page 3, lane 107, it is reported that "When considering PGT, the following criteria should be considered: chance of success….body mass index (BMI) and other contraindication for IVF".	The sentence was slightly rephrased based on another comment, as it intended to list general contraindications for IVF and thus for PGT.

			Although the document with this sentence likely indicates the increased BMI of the female partner of the couple, it should be stressed that in the last years several reports have described the presence of epigenetic alterations in the sperm DNA of obese men which could represent a risk factor for the outcome of IVF. Thus, it would be better to specify if the mention of BMI is referred to the female partner only or to both partners.	Epigenetic factors were not considered in this sentence. We assume the rephrasing of the sentence clarifies this.
Kersti Lundin	3-4	107-109	This sentence is a bit strange, it lists criteria and contraindication in the same sentence. It starts by saying chance of success, health issues etc. and ends by saying “and other contraindications” Would suggest to split up in one sentence of criteria and one with possible contraindications.	We have rephrased the sentence to make is clearer and more deleted "chances of success" as a contraindication
Caio Graco Bruzaca	4	113-114	Maybe this is the main objective of the PGT. For example a man with Holt-Oram Syndrome or achondroplasia..	This comment was discussed, but it was unclear what the reviewer would want to change in the text. Therefore, no adaptations were made.
Raul Piña-Aguilar	4	113	sentence starting with "PGT may" : This is confusing, i guess this paragraph refers to problems outside the tested disease. However, in Huntington disease patient may have severe psychiatric manifestations when they use PGT.	We added a clarification to the sentence reading "either linked to the tested disease, or due to other conditions"
Karen Sermon	4	113	What is meant by “put a child born at risk of harm”? Do you mean you would deny couples to be parents because you think they could not take care of them? This is a wider ethical issue in IVF that should not be mentioned lightly.	We agree with this comment. To improve/correct the sentence we have replaced the "put a child at risk of harm" to "medical risk at birth".
Hans Jakob Ingerslev	4	114	Already mentioned above (p.3,l. 97)	This was indeed a repetition of the sentence above, and it was corrected
M. Cristina Magli. Luca Gianaroli	4	113-114	“serious physical or psychological problems”. Who decides how serious these problems are? This is an IVF center decision, nothing related to PGT.	After discussion, the group still supports the message that the centre (after multidisciplinary discussion) can decide not to perform genetic testing for some patients (fi with serious physical or psychological problems). However, it was decided to slightly rephrase the sentence and make it less strong. The sentence now reads that PGT should be carefully considered in these cases.
M. Cristina Magli. Luca Gianaroli	4	116	This is an ethical statement, not for a recommendation paper.	Although the working group still supports the statement on social sexing, we agree that this is a technical paper, and therefore this ethical statement was removed.
Karen Sermon	4	116	As much as I agree with the statement on social sexing, I don’t think it has a place here.	Although the working group still supports the statement on social sexing, we agree that this is a technical paper, and therefore this ethical statement was removed.
Lauren Walters and colleagues	4	116	If a family has a healthy male and female embryo and a son at home and now want a daughter, it is not permissible for them to have the option to choose? Or is this targeted to desire to transfer a lower grade or mosaic/abnormal of the preferred sex over a higher grade euploid embryo of the non-preferred sex?	Although the working group still supports the statement on social sexing, we agree that this is a technical paper, and therefore this ethical statement was removed.
Joanne Traeger-Synodinos and colleagues	4	116	Shouldn’t it be mentioned that sexing is allowed for X-linked or gender dependent disorders?	Although the working group still supports the statement on social sexing, we agree that this is a technical paper, and therefore this ethical statement was removed.
Karen Sermon	4	121	Exceptional as in different from the rest, or as in not often applied?	We have adapted the sentence replacing "exceptional" with "different (no mutation detection)"
GenQA	4	124	It has been strongly recommended in the genomics community NOT to use the term ‘mutation’ . Please can you use ‘variant’ or ‘pathogenic variant’	We have adapted "mutation" to "pathogenic variant” throughout the paper

Inge Liebaers	4	126	Genetic variants other than class 4 or 5	By removing the (class 4-5) in the sentence above, it is no longer relevant to add here "other than class 4 or 5"
M. Cristina Magli. Luca Gianaroli	4	130	"in case of low recurrence risk (e.g. <10%)". Is this figure arbitrary? Also in this case, I think that it is a patients' decision. This is not an ethical paper, but a technical paper. I would delete the sentence.	The sentence was adapted leaving out the recurrence risk and focussing on technical aspects only.
Karen Sermon	4	134	Please specify known X-linked recessive single gene disorders with a clear unequivocal clinical diagnosis. Many diseases are clearly X-linked when looking at the pedigree, which does not mean that we know where on the X-chromosome they are located.	We clarified this sentence as suggested by the reviewer. The sentence now reads "Similarly, it is acceptable to offer PGT for known X-linked recessive single gene disorders with a clear unequivocal clinical diagnosis where no pathogenic variant mutation was found in the proband, but low- and high-risk haplotypes can be identified based on the family history. "
Caio Graco Bruzaca	4	134	I disagree, it is not acceptable if the parents don't have any mutation for x-linked diseases even if it's described in the pedigree.	We clarified this sentence based on the comments from another reviewer. The sentence now reads "Similarly, it is acceptable to offer PGT for known X-linked recessive single gene disorders with a clear unequivocal clinical diagnosis where no pathogenic variant mutation was found in the proband, but low- and high-risk haplotypes can be identified based on the family history. "
M. Cristina Magli. Luca Gianaroli	4	137-140	This is a couple's decision. Line 139 delete "is not recommended as it". The drawbacks of exclusion testing should also be indicated.	We have rephrased the sentence slightly to highlight the differences between exclusion and non-disclosure testing, and we eliminated the comment on moral issues.
Caio Graco Bruzaca	4	139	Why PGT with non-disclosure is not recommended? In clinical genetics, many families have such late onset diseases, in those cases they really don't want to know the results.	We have slightly rephrased the sentence, now stating that exclusion testing is preferred over non-disclosure testing.
Karen Sermon	4	153	Arrested embryos are usually not at the blastocyst stage, so how can they tell something about the mtDNA load at that stage?	We deleted the sentence on arrested embryos in reply to this comment
Raul Piña-Aguilar	5	154	homoplasmy where? Testing for mtDNA occurs in leukocytes, but there are disorders in which testing occurs in other tissues i.e. muscle for CPEO, Kearns-Sayre syndrome, MELAS or liver and urine. Therefore, it is required to specify the tissue or the conditions for "homoplasmy".	We have assessed this comment but explaining this in further detail is outside the scope of the current paper.
Karen Sermon	4	155	This is not clear: usually women are less affected by mtDNA (e.g. Leber opticus atrophy) so female embryos would be transferred, not male ones.	We have assessed this comment, and in the end decided that the sentence was possibly confusing and deleted it.
M. Cristina Magli. Luca Gianaroli	5	170-171	"….or an extremely low life expectancy.." this is a couple's decision.	We have kept the sentence, as the couple must be aware and advised. However, we changed the strength stating now that these cases should be carefully considered rather than excluded.
M. Cristina Magli. Luca Gianaroli	5	181	Too many parentheses after NGS.	The brackets were checked and are correct.
Inge Liebaers	5	181	Delete brackets after (NGS),	The brackets were checked and are correct.
Joshua Blazek and colleagues	5	186	• Section 1.4 – recommendation for both couples to undergo chromosome analysis prior to PGT-A testing seems a bit strong; consider changing to be 'should be considered'	This comment was discussed, and it was decided to keep the statement recommending a previous karyotype of both partners
Frank Broekmans	5	187	This again a moment to stress the correct application of EBM	We added a reference to the ESHRE RPL Guideline from 2017, but to our knowledge, there are no evidence-based guidelines on the use of PGT-A for other indications

Caio Graco Bruzaca	6	191	The RPL ESHRE consortium doesn't recommend PGT for recurrent miscarriage, maybe have a confusion between the two documents.	The ESHRE RPL Guideline states the following: "all RPL couples with results of an abnormal fetal or parental karyotype may be informed about the possible treatment options available, including their advantages and disadvantages" The guideline did not make a recommendation on PGT for unexplained RPL.
Inge Liebaers	6	192	Ref? f.i. CliffordKetal.HR,997,12,387	We added a reference to the ESHRE RPL Guideline from 2017 to support this statement.
Raul Piña-Aguilar	6	197	Why couple karyotype is required in recurrent implantation failure. Segregatio of chromosomal rearregments in one partner may led to preimplantation lethality but this is extremely rare. RIF is not an evidence based indication for karyotype or supported by professional guidelines.	This comment was discussed, and it was decided to keep the statement recommending a previous karyotype of both partners, also for RIF. Furthermore, we do not state any evidence-based indications for PGT-A, but which indications have been reported. In addition, we have stated upfront (in the introduction) that the value for IVF patients (or selected groups) remains heavily debated.
Lauren Walters and colleagues	4	123-124	It is more clear to specifically state the type of variant (pathogenic or likely pathogenic) using the system of the reference cited, than it is to refer to "class 4-5" variants, which is not language anywhere to be found in Richards et al. 2015. (but is taken from https://doi.org/10.1002/humu.22956, in which it has been adapted by a 2008 publication by Plon et al.)	We have adapted "mutation" to "pathogenic variant" throughout the paper
Päivi Forsblom	4	124 and throughout the paper	The use of the term mutatio, even though defined as a variant of class 4-5 is not adequate anymore. Already since 2015 this term has been replaced with the term varaint and the corresponding variant class. See Richards 2015, Genet.Med. 17:405-424 and Human Gene Variation Society website: http://varnomen,hgvs,org/bg-material/basics/ The use of the term mutation just because it has been conventional in the field of PGT and/or because of the ease of use cannot be justified especially as the recommendations currently being reviewed are going to be the 'handbook' for PGT providers for several years to come. The terminology used in the ESHRE recommendations is likely to be adopted to PGT report. It is thus important to use the concurrent terminology in the recommendations. A fundamental discrepancy of terminology exists if the molecular genetic reports state generic alterations to be a variants of class 4 or 5 and the following PGT-M reports describe the same variants with the term mutation.	We have adapted "mutation" to "pathogenic variant" throughout the paper
Raul Piña-Aguilar	4	139-140	This is statement is not supported by current practices in diagnostic genetic laboratories. For example, many gene panels test the exome and only report the panel, laboratories will not report pathogenic mutations in other genes if they were not ordered. Testing direct mutation may help the PGT laboratory to increase the accuracy of the test even if they keep as exclusion testing.	We have slightly rephrased the sentence, now stating that exclusion testing is preferred over non-disclosure testing. We decided not to further expand on this.
Andreas Schmutzler	5	186-200	As a co-author of the two previous "guidelines" in this regards, i. e. for the organization paper, and as a clinician working in the field, I would like to enter, with due respect, some points for the chapter about PGT-A. I think the organisation paper covers nearly all important points. But PGT-A looks a little bit neglected, even shorter as before (see my comparison below). This is in contrast to its vast global clinical application, the scientific	The Working group would like to thank the reviewer for the detailed comment, based on which the section on PGT-A was re-discussed and slightly adapted. (1) The reviewer suggests clarifying the aims of PGT-A testing. The working group clarified that the application of PGT-A is heavily debated, including the relevance and aims for testing. It was therefore decided not to expand on any aims of PGT-A. (2) The reviewer

			development (“PGD 2.0”) and its broad discussion in the literature. As I participated frequently in this, worldwide in congresses in public controversial scientific discussions and recently in a review (see enclosed), please allow me a suggestion which I find improves the paper. As ESHRE obviously tends to broaden its geographical scope I also think we should cover the subject by giving it a broader recognition. I think, PGT-A must be seen critically, with strict indications. But the paper until now is treating inclusion and exclusion criteria only superficially. I tried my best to keep it short but to name the critical points, as you did in the rest of the paper. I. Harton et al., ESHRE Guidelines Organization PGD Center, Hum Reprod 2011 “Inclusion criteria specific to PGS Although PGS remains controversial in clinical practice (see Abstract and Introduction), the following indications for its use have been reported: • 2.17. AMA (>36 completed years—exact age to be determined by each centre). • 2.18. RIF (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 or more weeks post-embryo transfer. • 2.19. RM (≥3 miscarriages—exact number to be determined by each centre). 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). Special considerations for PGS patients The following recommendations are made: There is a three step decision-making process by the gynaecologist in cooperation with the embryologist and the geneticist, after consultation with the patients: • 2.20. Before start of controlled ovarian stimulation, there should be discussion about whether PGS is appropriate for the couple. • 2.21. After OR, there should be discussion about whether PGS of oocytes or embryos should be performed and/or after review of fertilization and embryo developmental progress whether PGS of embryos should be performed. • 2.22. There should be discussion after review of the genetic results as to which oocytes or embryos should be selected for culture and transfer.” (232 words) II. PGT Consortium et al., ESHRE Recommendations Organisation PGT, Hum Reprod 2019 (draft) “1.4 PGT-A: inclusion/exclusion Although PGT-A remains controversial in clinical practice, the following indications for its use have been reported:	suggest including a 3-step decision-making schedule. The working group wants to clarify that the aim of the papers is to advise on technical aspects. Patient inclusion/exclusion and general information on patient counseling is considered part of papers, but further details on decision-making is not within the scope, A general statement was included in the introduction of the section, reading "The decision to accept or decline patients in PGT services should be undertaken by a team of dedicated healthcare professionals, based on well-defined inclusion/exclusion criteria." (3) The reviewer suggests a last paragraph comparing the outcomes of PGS with non-PGS cycles. The working group has attempted to write the section on inclusion/exclusion as objective as possible and decided not to expand or give their opinion on the relevance of PGT-A.

			- Advanced female/maternal age (AMA) - Recurrent implantation failure (RIF) - Recurrent Miscarriage (RM). It should be noted that couples with a history of RM have a high chance of successfully conceiving naturally. - Severe male factor (SMF) The exact definition (e.g. age limit, number of losses) of these factors should be determined by each centre. International definitions are provided in the glossary (See Supplementary data 1. Glossary). For all, but in particular for RIF, RM and SMF couples, a previous karyotype of both partners is recommended, since there is a higher chance of structural rearrangements for these indications. If an abnormal karyotype is identified, the technology for the detection of unbalances can differ from the regular PGT-A." (137 words) III. Andreas Schmutzler, Suggestion for a new paragraph 1.4 for the 2019 version "1.4 PGT-A: inclusion/exclusion The use of PGT-A remains controversial. The treating gynecologist / specialist in reproductive medicine has to inform the patient about the possibilities of PGT-A and evaluate the patients' perspectives and aims which might compete which each other. • In clinical practice there should be a three-step decision-making process by the gynaecologist in cooperation with the embryologist and the geneticist, after consultation with the patients: - Before start of controlled ovarian stimulation, there should be discussion about whether PGS is appropriate for the couple. - After OR, there should be discussion again about whether PGT-A of oocytes or embryos should be performed and/or after review of fertilization and embryo developmental progress whether PGS of embryos should be performed. - Finally, there should be discussion after review of the genetic results as to which oocytes or embryos should be selected for culture and transfer. For this process one has to distinguish between aims and indications. • For clinical practice, the following indications have been reported: o Advanced female/maternal age (AMA) o Recurrent implantation failure (RIF) o Recurrent Miscarriage (RM). It should be noted that couples with a history of RM have a high chance of successfully conceiving naturally. o Severe male factor (SMF). The exact definition (e.g. age limit, number of losses) of these factors	

			should be determined by each centre. International definitions are provided in the glossary (See Supplementary data 1. Glossary). • More importantly the predominant aim of the patients must be evaluated: o To increase the chance of pregnancy o To reduce the risk of miscarriage o To reduce the risk of multiples o To reduce the risk of malformation o To reduce the risk of pointless ART treatments. To improve the pregnancy rate, PGS makes no sense if a stochastic selection advantage is not to be expected. Furthermore, patients may have to choose between the chance of rapid success with a first fresh embryo transfer of blastocysts and a possibly higher overall cumulative chance of pregnancy from fresh and thawed transfers of four- to eight-cell embryos. Finally, the patients, dependent on their views, perspectives and experiences, even may have to choose between a potential decrease of the chance of pregnancy vs. a potential reduction of the risk of miscarriage, multiples, malformation and pointless ART treatments. For all, but in particular for RIF, RM and SMF couples, a previous karyotype of both partners is recommended, since there is a higher chance of structural rearrangements for these indications. If an abnormal karyotype is identified, the technology for the detection of unbalances can differ from the regular PGT-A." (423 words) Reference Schmutzler AG. Theory and practice of preimplantation genetic screening (PGS). Eur J Med Genet. 2019 May 25:103670. doi: 10.1016/j.ejmg.2019.103670. [Epub ahead of print]	

2. COUNSELLING AND INFORMED CHOICE

Reviewer	Page	Line	Comment	REPLY
M. Cristina Magli. Luca Gianaroli	6	223	The case of PGT-A is different from -M and –SR, as indicated in lines 258-259. It should be better clarified.	The section was formulated to fit to PGT-M and PGT-SR, as well as PGT-A, the working group has checked the section and still feels the items are acceptable for all PGT.
Kersti Lundin	7	227	Since PGT could also be performed for single women the "As" should be replaced by "When" (PGT involves a couple…). Or perhaps "If"..	This was corrected in the paper.
Inge Liebaers	7	239	Additional counselling for HLA typing	We have checked this comment, but decided not to specify or clarify this further as additional counselling may be needed for any indication
Sandrine Chamayou	7	242	The patients should sign a written consent for all procedures	We added this sentence to the text
Joshua Blazek and colleagues	7	243	· Section 2.3 HLA section, very important to discuss the risk of a unique crossover in the proband, leading to very low likelihood of identifying a transferable embryo	We added this sentence to the text at the end of section 2.3

Frank Broekmans	7	243	Should we consider to provide a CHECKLIST card for use in practice?	The working group invites practitioners to copy-paste the list in the paper and use it as a checklist. After finalisation of the documents, the working group will discuss the relevance of complementing the paper with checklists and other tools.
Véronique Cottin	7	258	transfer is not acceptable. An exception can be made for PGT-A but requires patients' fully Tipping error	We have checked the sentence, but we think it is correct
Raul Piña-Aguilar	7	266	placental or fetal tissue, i.e. CVS is a valid follow-up technique.	This was corrected in the paper
M. Cristina Magli. Luca Gianaroli	8	272	Add "according to local regulations"	This was added in the paper, as suggested by the reviewer
Hans Jakob Ingerslev	8	281	See above	unclear comment
Karen Sermon	8	303	This partially contradicts 116: you are allowed to reveal the sex, but not to act upon it, ie to give patients the possibility to choose between two transferable embryos of opposite sex	Based on another comment, the sentence on social sexing was deleted. The current sentence, in our opinion not contradicting the sentence on social sexing, is about information, rather than choice. This might be important during pregnancy when confirmation of PGT is discussed.
Raul Piña-Aguilar	8	305	"repeat instability" is a more accurate term. Dynamic mutations is not a widely use term in clinical genetics, other type of changing or moving mutations are transponsons and those are not related with repeats and susceptible of anticipation or expansion/contraction phenomena.	We have adapted this to "dynamic pathogenic variants with repeat instability"
Hans Jakob Ingerslev	9	325	Psychological support is not readily available in Danish Centers	We added "When available in the centre," to the sentence to address this comment
Joshua Blazek and colleagues	8	293,551,637	• Line 293 vs. line 551 vs. line 637 - option for prenatal testing, vs. need for prenatal testing, vs. should be offered to all women following PGT	Prenatal testing is discussed at several points in the paper, and we have tried to formulate all consistently. Prenatal testing should be offered to all couples pregnant after PGT. For some PGT cases, Prenatal testing is recommended (in the report). In counselling, both options should be discussed. By adapting this sentence, the recommendations on prenatal testing are now more consistent.
Mariana Moura-Ramos	9	326_340	The second comment refers to the section of Psychological Support and Evaluation (Doc1., Section 2.4., Lines 325-340). We agree with the PGT Consortium that psychological support should be offered to every couple before, during and after PGT, including in unsuccessful cycles (lines 326-327). In addition to this, and considering the different aims of psychological evaluation and psychological support and intervention, we suggested some changes in order to clarify the differences between psychological evaluation and psychological support. Therefore, we included some small edits (in italic in the following text) to the proposal of the ESHRE PGT consortium, section 2.4.: "Psychological support should be offered to every couple before, during and after PGT, including unsuccessful cycles. Psychological evaluation should be considered for the following patients: - Couples for whom the geneticist, gynaecologist or other member of the IVF/PGT team has doubts regarding the welfare of existing or future children or the psychological physical wellbeing or mental capacity of future parents	We have checked this comment and modified the section to address the difference between psychological support and evaluation.

			- Couples in whom one of the future parents is the carrier of an autosomal dominant disorder and may have signs and/or symptoms of this disorder as determined by the appropriate specialist physician (e.g. neurodegenerative/psychiatric diseases) - Couples who are undergoing PGT HLA-typing to evaluate their 'child wish' and the extent to which the new child is welcomed, not only as a donor but also as a full family member, appreciated for whom s/he is Psychological support and intervention should be recommended to: - Couples with a history of reproductive failure - Patients with past traumatic experiences - Patients with current salient psychological distress - Couples who actively request psychological intervention" With these edits we aimed to clarify the specificities of the work of the psychologist/counsellor (Psychological evaluation and psychological support intervention) with patients undergoing PGT.	

3. Basic requirements of an IVF/PGT centre

Reviewer	Page	Line	Comment	REPLY
GenQA	10	353	For English grammar please change 'for single and/or few cell processing' to 'for processing single and/or few cells'. Few cells is plural.	We have corrected this in the paper.
Joanne Traeger-Synodinos and colleagues	10	372	The use of multichannel pipettes, especially in the first steps of amplification, may increase the risk of contamination	We agree with this comment and have clarified that the multichannel pipette can be used in secondary amplification steps.
M. Cristina Magli. Luca Gianaroli	10	372	Add "in the PGT laboratory"	This was added to the paper, as suggested.
M. Cristina Magli. Luca Gianaroli	10	375	Add "in the IVF laboratory"	The sentence already includes "for DNA work" and 'in the IVF centre"
GenQA	10	375	For English grammar please change 'for single and/or few cell DNA amplification work should' to 'for DNA amplification of a single and/or few cells should'. Few cells is plural.	We have corrected this in the paper.
GenQA	10	388	Change to 'According to internal quality standards'	We have corrected this in the paper.
GenQA	11	400	Add 'Specific issues for handling of reaction tubes to reduce cross – contamination:	We have corrected this in the paper.
Joanne Traeger-Synodinos and colleagues	11	404	When using a multichannel pipette the tubes are left open longer than when using single-channel pipettes.	We have deleted the second part of the sentence (and open only one tube at a time) to allow the use of multichannel pipettes
Joshua Blazek and colleagues	11	413	• Section 3.2, consider adding reference(s) to discuss personnel requirements in further detail	As the current document was not supported by a literature review (as these as recommendations for good practice), it was decided upfront to only refer to other guidance documents. Therefore, adding references to this section would not be appropriate.
GenQA	11	415	Change 'appropriate person' to appropriately trained person'	We have corrected this in the paper.
Inge Liebaers	11	415	'appropriate' to be specified	This was corrected to "an appropriately trained" person, as suggested in another comment.

Joanne Traeger-Synodinos and colleagues	11	418	Where do these training programs exist? Do you refer to postgraduate degrees?	We specified that training programs refers to workshops, hands-on courses, one-to-one training. Also, we rephrased the sentence, now reading "Joining specific training programs (workshops, hands-on training, one-to-one training) for embryology and PGT procedures is recommended."
GenQA	11	430	Change 'and' to 'plus' so it is clear you need two unique patient identifiers plus the embryo/cell number	We have corrected this in the paper.
Inge Liebaers	12	435	Inedible??	Thank you for indicating this error. We have corrected this to "uneditable"
Karen Sermon	12	435	Inedible: not fit or suitable for eating. Change to indelible.	Thank you for indicating this error. We have corrected this to "uneditable"
Lauren Walters and colleagues	12	435	inedible?	Thank you for indicating this error. We have corrected this to "uneditable"
GenQA	12	445	Figure legend:	We have corrected this in the paper.
Emmanuelle Kieffer	12	446	It should be acceptable that the tubing process is not witnessed if only one embryo sample is treated at a time. In this case, the sample collection tubes are not labelled until the sample is brought to the tubing laboratory.	Thank you for this comment, it is valuable. However, we have kept the statement that in general witnessing is recommended for tubing.
Ahmet Berkız TURP	10	362-367	You have referenced ESHRE Guideline 2015-2016. However just add a sentence that these guidelines can be updated. Or the last updated guideline can be referenced. May be this guideline can be changed so the PGT guideline can be seen as a dynamic and everchanging.	Ideally, we should refer to the last version of any guidelines, but in practice, this may be complicated.
Sandrine Chamayou	11	419-421	'the number of…and communication'. This sentence is important for the organization of an IVF lab but not especially for PGT procedures. Otherwise it should be written also for the PGT lab in the genetic part.	This statement definitely refers to the ART centre, but it is also important for the genetic lab. We have adapted it slightly to clarify.
GenQA	12	442 & 443	Capital letter at the start of a sentence 'Outline' and a full stop at the end of this sentence is needed.	We have corrected this in the paper.
GenQA	12	450 & 452	The 'and' is in the wrong place. Delete from row 450 and between 'correct embryo diagnostic result, and (7) At the'	We have corrected this in the paper.
Sandrine Chamayou	12	Figure 2	Particular attention should be taken when writing the genetic report in the PGT lab. Witnessing should be applied when filling the final report in correspondence with samples.	We have slightly adapted the explanation for step 5 to address this comment.
Joanne Traeger-Synodinos and colleagues	12	Figure 2	There are several steps during the PGT procedure, between "sample in tube" and "diagnostic report" where witnessing is needed. For example, when positive controls are added (relative DNA, crucial for haplotyping) and transfer of samples for downstream reactions.	The issues specific to different techniques (FISH, ArrayCGH and NGS) are included in the specific technical papers (PGT-A/SR paper). This was clarified in the heading
M. Cristina Magli. Luca Gianaroli	12	Fig. 2	"sample in tube" add " / on slide".	This was corrected in the paper

4. PRECLINICAL WORK-UP, EXAMINATION AND POST-EXAMINATION PROCESS

Reviewer	Page	Line	Comment	REPLY
GenQA	12	455	Change to 'PRECLINICAL WORK-UP REPORT'	We have added "report" in the paper, as suggested,
GenQA	12	456	Change to 'Preclinical work-up Report'	We have added "report" in the paper, as suggested,
Joshua Blazek and colleagues	12	456	• Section 4.1, very important to provide guidance regarding the extent to which family member results should be present on the referred patient's	A sentence on this was added to the section reading "samples and genetic status of relevant family members can be mentioned only with

			PGT-M report. Local privacy laws and specific reference lab accreditation must be considered prior to reporting of family member medical/genetic information	their informed consent and should be in accordance with GDPR and/or local privacy regulations"
GenQA	13	476	Add 'Type of required testing, the referral reason, parental karyotypes/genomes'	We have added this information, as suggested.
GenQA	13	482	After 'a clear summary of the result' add below and additional bullet point '-clear description and interpretation of results' As an EQA provider we see many PGT reports and many do not have sufficient information for a clinical geneticist/clinician to counsel the patient.	We have added this information, as suggested.
Karen Sermon	13	485	Wouldn't haplotyping actually reveal sample mix-up, or non-paternity?	The reporting is based on haplotyping, but should not necessarily present these haplotypes. It was decided not to adapt this in the paper
Karen Sermon	14	504	No control by a second person? See 436 on label identification	This sentence is on the report and states that it should be reviewed (by a second person). Further details on scoring of the results is included for instance in the PGT-M paper 4,1 where it is mentioned 'It is recommended that results are analysed by two independent observers and discrepancies adjudicated by a third observer (where possible)'.
GenQA	14	517	Change 'embryo' to 'oocyte'. You cannot extrapolate for the embryo as Polar body 1 & 2 do not contain the paternal genome set.	We have corrected this in the paper.
Inge Liebaers	15	530	It is crucial to agree: who has to agree???	We have adapted the sentence now stating that the centre should have a policy.
Joanne Traeger-Synodinos and colleagues	13	536	We assume that "timing of the IVF Centre" is "day and time of embryo transfer" and we think that this should be specified	We have adapted the sentence to make it clearer to the reader.
GenQA	14	540	As an EQA provider we see many PGT reports and many do not have sufficient information for a clinical geneticist/clinician to counsel the patient. The following must be included: the size of the abnormality in MB (array & NGS); a summary of the results for each embryo in ISCN/HGVS {indicating the chromosome involved, chromosome band/nucleotides, quantifying the gain or loss (e.g. x3)}; a brief written description of the result (in case the nomenclature is unclear) including when applicable the type of pathogenic variant e.g. missense, truncated.	The EQA provider sees a lot of reports, but most reports are on identification of aberrations, in which case it is compulsory to provide detailed information f ex whether the mutation identified is a missense mutation or a truncated mutation. However, in a PGT cycle report, we assess whether an a priori known mutation is present in the embryos, we do not see the added value of reporting per embryo the missense mutation using HGVS. It is applicable for new findings of chromosomal aberrations. We added a sentence, in a slightly modified version to ensure the addition did not contradict with the other sentences.
Joshua Blazek and colleagues	15	555	• Section 4.3, Misdiagnosis rate, specify that misdiagnosis rates include those clinical cases in which affected pregnancies arose and cases for which re-analysis results were discordant with the biopsy result after verifying concordance between the sample tested and the pregnancy that ensued.	In the glossary, misdiagnosis is defined as: "When a technical procedure has failed, is inaccurate or has been incorrectly interpreted. Misdiagnoses may be sample- or technique-specific. " It was decided not repeat this definition in the text.
Frank Broekmans	15	557	Is this confirmation process really feasible: what are the costs necessary to install this Quality Control. It is not the moment to put this in the GP document without having considered financial, patient consent and organisational issues	The idea is to perform this on a subset of embryos; without indicating the exact number, the WG considers it feasible to re-analyse a minimum of embryos, not used for the patient (this should be part of their IC) as it is part of quality control and accreditation, rephrased the sentence by adding 'a subset of'.
Laura Corti	15	557 - 561	In case of the PGT diagnosis confirmation, it's mandatory to consider the TE mosaicism (in PGT-A)	This should indeed be considered, but it was outside the scope to elaborate on mosaicism.
GenQA	15	559	Change to 'internal quality assurance'	We have corrected this in the paper.
M. Cristina Magli. Luca Gianaroli	15	568-570	Rephrase. "If no local regulations or guidelines exists on storage of clinical samples and patient records. , it is recommended that "	We have added " on storage of clinical samples and patient records" to the sentence

M. Cristina Magli. Luca Gianaroli	15	576-579	This is according to the centre policy.	The sentence provides a recommendation on how to proceed when no local regulations or guidelines exist. Off course, the centre can still have a different policy.
Joanne Traeger-Synodinos and colleagues	15	578	Surely lab accreditation requires storage of samples for a longer period of time.	The sentence provides a recommendation on how to proceed when no local regulations or guidelines exist. In the previous sentence it is stated to follow local regulations or accreditation schemes,
Lauren Walters and colleagues	16	580-96	It remains challenging for laboratories to calculate misdiagnosis rates due to lack of follow-up data.	We agree, but this is why we recommend centres to initiate various follow-up studies such as confirmatory testing of a subset of embryos not destined for the patient or sending out questionnaires on the health of the PGT babies etc.
Alan H Handyside	16	583	Surely this will be difficult if not possible for individual centres. Is this not monitored by the Consortium? Is it not possible to recommend what should be quoted for different approaches?	It can be monitored by the Consortium but each PGT centre should take responsibility in calculating their misdiagnosis rate. Therefore we recommend centres to run minimum follow-up studies such as confirmatory testing per method on a subset of embryos not destined for the patient, or initiate follow-up studies of babies born.
Laura Corti	16	585-586	In case of the misdiagnosis, it's mandatory to consider the TE mosaicism (in PGT-A)	This should indeed be considered, but it was outside the scope to elaborate on mosaicism.
Inge Liebaers	16	598	OK for pregnancy rates but more important are live birth rates or live delivery rates!!!!	Live birth rates are indeed better then pregnancy rates, we have adapted this.

5. TRANSPORT PGT

Reviewer	Page	Line	Comment	REPLY
Alexia Chatziparasidou	16	615	Minimal Criteria for transport companies for certifying their suitability to responsible transport the biopsy material.	We added one recommendation on the transport company reading "Transportation companies entitled to transport biopsied material should certify their suitability to transport the biopsied material, provide the likelihood of a sample loss or sample delivery delay and provide actions taken against these risks." to address this and other comments.
Alexia Chatziparasidou	16		The patients should be informed for the transportation procedure required and consent to take the risks involved during transportation	We added one recommendation on the transport company reading "Transportation companies entitled to transport biopsied material should certify their suitability to transport the biopsied material, provide the likelihood of a sample loss or sample delivery delay and provide actions taken against these risks." to address this and other comments.
Alexia Chatziparasidou	16		Transportation companies entitled to transport biopsied material should provide the likelihood of a sample –loss or sample-delivery delay	We added one recommendation on the transport company reading "Transportation companies entitled to transport biopsied material should certify their suitability to transport the biopsied material, provide the likelihood of a sample loss or sample delivery delay and provide actions taken against these risks." to address this and other comments.
Alexia Chatziparasidou	16		Transportation companies should provide the actions taken against these risks	We added one recommendation on the transport company reading "Transportation companies entitled to transport biopsied material should certify their suitability to transport the biopsied material, provide the likelihood of a sample loss or sample delivery delay and provide actions taken against these risks." to address this and other comments.
Alexia Chatziparasidou	16		Transportation companies should provide the relevant consent forms for the patients	We added one recommendation on the transport company reading "Transportation companies entitled to transport biopsied material

				should certify their suitability to transport the biopsied material, provide the likelihood of a sample loss or sample delivery delay and provide actions taken against these risks." to address this and other comments.
GenQA	17	631 & 632	Change to (compliant with GDPR)	We have corrected this in the paper.
Joanne Traeger-Synodinos and colleagues	16	606-609	The wording of this paragraph is not very clear and does not make clear sense.	We have split the sentence and rephrased it to make it clearer for the reader
Alan H Handyside	16	606	'Transport' PGT is now the standard of practice. Surely using the phrase 'is acceptable' is out of date	We slightly rephrased the sentence, now stating that transport PGT is an option, rather than "can be an option"

6. FOLLOW-UP OF PGT PREGNANCIES AND CHILDREN

Reviewer	Page	Line	Comment	REPLY
Joshua Blazek and colleagues	17	636	• Section 6.1, in discussing prenatal diagnosis, consider adding language about difficulty of locating laboratory to perform prenatal testing via exclusion (as this is typically recommended by the PGT lab but oftentimes difficult to carry out in clinical practice)	We have assessed this comment, but decided it not to be necessary to add this information
Alan H Handyside	17	636	The recommendations here should include NIPT as follow up for PGT-A and possibly NIPGT for common monogenic disease. Of course, the problem is that NIPT following PGT-A can cause anxiety through false positives.	We have added a clarification on this issue in the sentence on prenatal diagnosis.
Karen Sermon	17	639	This reads as if US and NIPT are invasive. Please rephrase.	We have clarified the sentence on prenatal diagnosis to address this (and other) comments
Sandrine Chamayou	17	640	Please be more precise of which prenatal diagnosis (CVS, amniocentesis, NITP) is recommended according to PGT type (M, SR, A…)	We have clarified the sentence on prenatal diagnosis but decided not to specify which test is to be used in function of the genetic test.
GenQA	17	640	This sentence needs more clarity. There are two types of non-invasive test. One NIPD (monogenic disorders and sexing) is a diagnostic test and the other, NIPT for aneuploidies is a screening test.	We have added a clarification on this issue in the sentence on prenatal diagnosis.
Raul Piña-Aguilar	17	640	I recommend to remove non-invasive prenatal test, such fetal free-DNA from this section. Based on: a) Fetal-free DNA tests are not diagnostic. ffDNA are screening tests that require confirmation by a diagnostic test (CVS, amnio). Keep this statement in PGT guideline is a risky confusion for patients, clinicians and stakeholders to think that another screening test can be used at the same level than diagnostics tests (CVS or amniocentesis). b) The majority of clinical ffDNA tests are restricted to a small number of chromosomes, therefore it is misleading to say that can be used as follow-up test of PGT-A of 23 chromosomes. Illumina is working in NIPT Veriseq version 2 release with 23 chromosomes level, but it is not available yet on in clinical use. c) ffDNA test will delay diagnostic test sand professional societies as ACOG/SMFM states that all women with a positive-cell free DNA test result should have a diagnostic procedure before any irreversible action such as pregnancy termination is taken (Obstet Gynecol. 2016;127(5):979-81) d) ACMG clearly does not recommend NIPS to screen for genome-wide CNVs. If this level of information is desired, then diagnostic testing (e.g.,	Thank you for your comment, but it was decided to keep NIPT in the paper. Based on other comments, the sentence was rephrased and clarified.

			chorionic villous sampling or amniocentesis) followed by CMA is recommended (Genet Med. 2016;18(10):1056-65.)	
M. Cristina Magli. Luca Gianaroli	17	642-643	This is a couple's decision.	In many countries, testing of minors for non-actionable conditions is described in the legislation. We have adapted the sentence clarifying this.
Kersti Lundin	17	646	Also many concerns about cryopreservation/vitrification, which is part of the PGT procedure. Perhaps add here?	We added "and cryopreservation/vitrification to the sentence.
Véronique Cottin	17	647	Neonatal outcomes of live births after blastocyst biopsy in preimplantation genetic testing cycles: a follow-up of 1,721 children published July 2019 in fertility sterility. Of course more follow up are needed, it's a first step in direction of safety of TE Biopsies at least in follow up of children just after birth.	The suggestion is consistent with the sentence "So far there is no indication that embryo biopsy causes an increased risk for adverse neonatal outcome." We decided not to specify this further.
Alessandra Alteri	17	647	The following statement needs to be added: "In relation to the blastocyst biopsy, more evidence is needed on obstetric outcomes."	Discussion of the different options for biopsy and their strength and limitations are included in the biopsy paper, and we decided not to repeat it here.

7. ACCREDITATION AND QUALITY MANAGEMENT

Reviewer	Page	Line	Comment	REPLY
GenQA	18	665	Change 'Accreditation, with' to 'Accreditation, together'	We have corrected this in the paper.
M. Cristina Magli. Luca Gianaroli	19	712	Add as author "Preimplantation Genetic Diagnosis International Society (PGDIS)".	We have corrected this in the paper.
M. Cristina Magli. Luca Gianaroli	20	738-739	Correct and update the reference.	We have corrected this in the paper.

SUPPLEMENTARY DATA 1. GLOSSARY

Reviewer	Page	Line	Comment	REPLY
Kersti Lundin	21	Glossary: AMA	I (strongly) disagree that AMA is defined as between 36-40 years. It is true that you can find this age group in Verpoest et al 2018, but it is NOT stated as a definition of AMA, it is just the study group that they have used. There are many articles to be found where AMA is defined as 35+. (eg. Debrock 2010, Lean et al 2017 metanalysis). I would recommend to use that here and skip the upper limit (otherwise, what are those 40+? Extremely advanced? EAMA?) There are also definitions from 36+ and 37+ of course….	We have modified the AMA definition to maternal age above 35 years, as indeed an upper limit is not relevant.
GenQA	22		Change: Genome coverage. The	We have corrected this in the paper.
GenQA	23		Monosomy. If a fetus is XY then it is not correct to state absence of one of the homologues. Add 'of one of the two homologous chromosomes or sex chromosomes in embryos' Polar Bodies (PBs). Change to 'telomere I and normally only contains chromosomes each with 2 chromatids (2c)'. Change to 'activation and normally only contains chromosomes each comprising of a single chromatid (1c).	We have adapted the definition in the paper based on the suggestions of the reviewer.

GenQA	24		Recurrent miscarriage: I do not know why this definition and reference has been given. Miscarriage occurs in <1 in10 pregnancies so 1 in 100 people will have had 2 miscarriages. Assessment for rec. misc. is done when there are 3 or more miscarriages (i.e. 1 in 1000 pregnancies) otherwise you are screening too many people. UPD: Change to 'and no copies from the other parent' to be grammatically correct.	This definition is copied from the ESHRE Guideline on Recurrent Pregnancy Loss. The guideline group felt a less strict definition would allow patients to access care sooner, however they recommend some tests only after a third pregnancy loss. (UPD, this language correction was also addressed)
Raul Piña-Aguilar	glossary	homoplasmy	Tissue specification is required as I mentioned in my comments before	This was discussed, but this is considered too much detail, and outside the scope of the current paper.
Karen Sermon	20	738	Are you sure this reference is correct? See also glossary.	This was corrected in the paper
M. Cristina Magli. Luca Gianaroli	21	Glossary	"Advanced maternal age". The reported age interval comes from the inclusion criteria in the ESTEEM study. Therefore, it is not a definition applicable in this guideline. According to the literature, AMA starts at 36 years or 38. Obviously, there is no superior limit as far as women with own oocytes are considered.	We have modified the AMA definition to maternal age above 35 years, as indeed an upper limit is not relevant.
M. Cristina Magli. Luca Gianaroli	21	Glossary	"Allele drop in". If I understood correctly, it refers to unrelated DNA contamination. Is it correct to define it false positive? False positive to me normally indicate a non-transferrable embryo.	Allele drop in refers to an artefact, not to contamination. We agree with the comment on false positives and therefore removed the part of the sentence "like a false positive".
M. Cristina Magli. Luca Gianaroli	22	Glossary	I do not agree with this definition because competence implies much more than development to blastocysts. In addition, a lot depends on the blastocyst quality.	We have removed "developmental competence" from the biopsy paper and from the glossary
M. Cristina Magli. Luca Gianaroli	22	Glossary	"Embryo biopsy" Delete "oocyte".	This was adapted in the paper
M. Cristina Magli. Luca Gianaroli	22	Glossary	"Diploidy/euploidy". Delete "Adapted from".	This was adapted in the paper
M. Cristina Magli. Luca Gianaroli	22	Glossary	"Exclusion testing". The provided link doesn't work (404 page not found)	This was checked and corrected
M. Cristina Magli. Luca Gianaroli	22	Glossary	"Freeze-all cycle". Delete "Adapted from".	This was checked and corrected
M. Cristina Magli. Luca Gianaroli	23	Glossary	"Polyploidy". Add "Adapted from".	This was checked and corrected
M. Cristina Magli. Luca Gianaroli	24	Glossary	"Recurrent miscarriage (RM) / Recurrent pregnancy loss". Correct the given ref. It is not RPL, but "The ESHRE guideline group on RPL…"	This was checked and corrected
M. Cristina Magli. Luca Gianaroli		Glossary	Some definitions are not listed in alphabetical order: Sequencing read depth, Diploidy/euploidy, Chromosomal Mosaicism.	This was checked and corrected

Polar body and embryo biopsy for PGT (PGT-BIOPSY)

GENERAL COMMENTS

Reviewer	Page	Line	Comment	REPLY
Véronique Cottin	general		Very good guidelines! Congratulation for this great work	Thank you for this comment.
GenQA	general		These guidelines are extremely well written and comprehensive. One thing that is missing is detail on what should go in the work-up report and the final genomic report.	The issue of the genomic report is covered in the paper on the organisation of PGT.
Tina Buchholz	General		My concern in general is, that the specific characteristics of polar bodies (pbs) compared to embryonic cells are not reflected enough in the papers. Sometimes the polar bodies are mentioned, sometimes in a specific section, but then they intermixed at specific points, where it is not correct.	We have checked the paper and corrected this throughout.
GenQA	1	17	'until consensus' change to 'until consensus was reached'. Otherwise it is an incomplete sentence.	This was corrected.
Kelly Tilleman	17	509	Very few references are used in this document which is a pity. There is definitely information out there to support recommendations on e.g hatching on day ¾ or day 5. It would be very interesting if the group would include more scientific evidence.	As these are technical papers, evidence for most recommendations would not be relevant anyway. Therefore, there was an upfront decision not to use reference, except when referring to other guidance documents
M. Cristina Magli. Luca Gianaroli	17		It seems to me strange that a recommendation document is supported by 3 references only.	We added a sentence explaining the methodology and the lack of references in the methodology section of the 4 papers.

Introduction to biopsy and sample collection

Reviewer	Page	Line	Comment	REPLY
Hans Jakob Ingerslev	2	38	"the exposure…." It might be helpful with suggestions as to time (pulse) and watt	As there are different types of laser systems, we may not suggest time and watt.
Carlos Encinas	2	38	There are 3 main types of laser systems available in the market, it is crucial to carefully follow the manufacturer's specifications to attain the best results.	We have addressed this comment in the paper.
Celine Moutou	2	41	"too small, to allow embryo hatching at the blastocyst stage" Is this relevant ?hatching occurs naturally even without previous opening of the ZP.	Thank you for the comment. We removed the statement on an opening that is too small.
Christina Hnida	2	42	Zona opening size: The blastocyst should manage to hatch by its self, so the size needed should be only depending of the biopsy procedure a quite a is a small opening the exposure…." It might be helpful with suggestions as to time (pulse) and watt	Thank you for the comment. We removed the statement on an opening that is too small. As there are different types of laser systems, we may not suggest time and watt.
Tina Buchholz	2	46	"pbs" under the heading: embryonic cell removal (they are no embryonic cells)	The title was corrected to "Sample (PB or Embryonic cell) removal"
Christina Hnida	2	47	The method where you pull the aspirated cells away from the embryo could be explained a bit more. There is a high risk for TE-biopsy to pull the blastocyst out of the zona	More information on this is given in the section on the blastocyst biopsy procedure in this paper. We decided not to add such detail to this introduction
Tina Buchholz	2	53	"removal of pbs" under: stages of embryo biopsy !!	The title was corrected to "Time of biopsy"

Tina Buchholz	2	59	pb analysis can also performed for structural rearrangements	This was added to the sentence which now reads: "when only maternal mutations, structural rearrangements or aneuploidies are investigated"
Sandrine Chamayou	2	41-42	'but neither too small, to allow embryo hatching at the blastocyst stage'. Where did you find this conclusion? I never read something similar. When a hole is made in ZP for blastomere biopsy, it is always large and should not be so small to prevent hatching.	Thank you for the comment. We removed the statement on an opening that is too small.
Pamela Renwick	3	74	Rebiopsy will result in more than 10 TE cells in total so the caveat on line 290 applies as the impact on further embryo development remains an area of investigation.	We have added a statement on the impact on embryo development in the text

1. Laboratory issues related to biopsy

Reviewer	Page	Line	Comment	REPLY
Christina Hnida	3	79	The dot after decontaminated has to be deleted	This was corrected.
Christina Hnida	3	79	The use of UV-light should also be mentioned here.	The section is on biopsy, and therefore UV light is not required (if the tubing is performed in a separate room or area)
Karen Sermon	3	79	Delete superfluous "." .	This was corrected.
Ahmet Berkız TURP	3	79	The point is wrong (style error).' decontaminated. with disinfectants '	This was corrected.
Karen Sermon	3	82	The gloves, do they pertain to the tubing only or also to the ICSI and biopsy? It reads like that, but I assume you do not recommend that people do ICSI or micromanipulation with gloves on.	We added "During PGT-related procedures," at the start of the paragraph
Päivi Forsblom	3	85	according to several personal communications as well as an oral presentation by Lynch C et al at PGDIS 2019 (Geneva) single sperm cannot be amplified by the standard WGA method (PicoPlex/SurePlex) for PGT-A. Thus ICSI is not necessary to avoid paternal contamination. Several large clinics and the corresponding PGT centres currently perform IVF and not ICSI for PGT-A. This adjustment makes PGT-A more widely available for patients as the costs of the procedure are lower. Denudation of oocytes is of course absolutely necessary to avoid contamination through cumulus cells.	We changed the sentence, it now reads "ICSI is preferable" and clarified the need for rinsing of zygotes.
Lauren Walters and colleagues	3	85	Guidelines indicate that ICSI is mandatory. This goes against the findings of Feldman et al. (PMID: 28612309) in which they state "... contamination with paternal DNA, through contamination with sperm cells, was negligible. Not one single case of misdiagnosis was encountered during the study period."	We changed the sentence, it now reads "ICSI is preferable" and clarified the need for rinsing of zygotes.
Joshua Blazek and colleagues	3	85	Line 85: Do not agree that ICSI is mandatory	We changed the sentence, it now reads "ICSI is preferable" and clarified the need for rinsing of zygotes.
Alan H Handyside	3	85	Cooper Genomics no longer request ICSI based on experiments that demonstrate that sperm do not amplify (alkaline lysis is normally required). As universal use of ICSI for IVF is questioned it may be good to reconsider this advice.	We changed the sentence, it now reads "ICSI is preferable" and clarified the need for rinsing of zygotes.
Véronique Cottin	3-4	85-87	https://www.ncbi.nlm.nih.gov/pubmed/28612309 in this large publication they conclude that ICSI should be done only in case of male infertility. J Assist Reprod Genet. 2017 Sep;34(9):1179-1183. doi: 10.1007/s10815-017-0966-7. Epub 2017 Jun 13.Pre-implantation genetic diagnosis-should	We changed the sentence, it now reads "ICSI is preferable" and clarified the need for rinsing of zygotes.

			we use ICSI for all? Feldman B1,2,3, Aizer A1, Brengauz M1, Dotan K2, Levron J1,3, Schiff E1,3, Orvieto R4,5,6. As ICSI seems to impact the live birth chances in non male infertility, maybe IVF could be preferred in the cases of PGT if ICSI is not necessary and male dna contamination not of high risk…	
Celine Moutou	3	Figure 2	Text is very small, there is enough room to enlarge it	We will take this comment into consideration when preparing the figures for publication
Karen Sermon	4	87	What is meant by “non-decondensed sperm within blastomeres”? Does that often happen? I have tried to follow the thread of this statement back to its origin but got to a dead end, ie a reference that does not mention non-decondensed sperm at all (https://academic.oup.com/humrep/article/26/1/25/704530). See also https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5581785/	We removed "non-decondensed sperm within blastomeres”
Hans Jakob Ingerslev	4	106	Suggestions for maximum might be helpful	Because of different equipment that can be used and as this also depends on the expertise of the practitioner, it is not possible to define minimum and maximum duration for the procedure. It was decided to keep the sentence as it is, recommending minimising the duration of the procedure.
Christina Hnida	4	106	Optimally, the biopsy procedure should not last for longer than 5 min.	Because of different equipment that can be used and as this also depends on the expertise of the practitioner, it is not possible to define minimum and maximum duration for the procedure. It was decided to keep the sentence as it is, recommending minimising the duration of the procedure.
Christina Hnida	4	107	The Laser pulse and hole diameter of the laser should get adjusted assisting the different procedure steps, e.g. opening the zona, making a channel, separate the cells from blastocyst	It was decided not to add these details as it depends on the laser.
Celine Moutou	4	114	What does patient name means? female partner only ? or male and female partner ? This should be specified	Changed as "Ensure that biopsy dishes are prepared, equilibrated and clearly labelled with at least the patient name and surname (female partner only or both female and male partners, according to each laboratory policy), and oocyte/embryo number."
Alessandra Alteri	4	115	“osmolality” is more appropriate than “osmolarity” in this context	We included both osmolality and osmolarity in the sentence
Tina Buchholz	4	117	chapter 1.3. only speaks about labelling the embryo … not about the oocyte ……	This was corrected throughout section 1.3
Sandrine Chamayou	4	126	Witnessing is mandatory when the straw with the tested embryo to transfer is taken from the cryogenic bank.	This recommendation is included in the organisation paper. The witnessing section in the Biopsy paper was limited to the biopsy steps before acquiring the genetic report, but we have now included it.
Christina Hnida	4	106-107	You could add: “Laser pulses of 0,4 to 0,8 ms is recommended.	We are aware of settings where 0,2 ms is used. We decided not to specify this in the text.
Kersti Lundin	4	91-92	I would remove the sentence “The exposure of the embryo to sub-optimal environmental conditions should be limited, whenever possible”. It is superfluous and sounds a bit patronising. In addition, it is already covered by the previous sentence about culture conditions.	We removed the sentence as suggested.

Kersti Lundin	4	92-93	Having “time-lapse” does not necessarily mean that the exposure is limited. It is the “closed” culture systems that are used in most (not all) time lapse systems that is reducing the exposure. The sentence should be revised to indicate that.	This was adapted as suggested,
Kersti Lundin	4	94-95	Perhaps: Following biopsy, oocytes and embryos should be thoroughly rinsed from the biopsy medium before culture or cryopreservation	This was adapted as suggested,
Karen Sermon	5	127	Repetition of 96	The sentence was deleted in line 96-98. Thank you for pointing this out.
Joshua Blazek and colleagues	5	137	Line 137: “Since biopsy is invasive, it could damage cells and DNA. Therefore, the impact of the laser on the integrity of biopsy samples should be validated before clinical application.” Not sure this would actually be a validation? But we should have data or references to supply to users.	This sentence was rewritten. It now reads; “Since biopsy is invasive, it could damage cells and DNA. Therefore, information about the integrity of biopsy samples (cell lysis, degeneration, degradation, …) should be noted and shared with the genetic laboratory"
Hans Jakob Ingerslev	5	138	…the integrity… How should it be validated? Suggestions?	This sentence was rewritten. It now reads; “Since biopsy is invasive, it could damage cells and DNA. Therefore, information about the integrity of biopsy samples (cell lysis, degeneration, degradation, …) should be noted and shared with the genetic laboratory"
Celine Moutou	5	127-129	Redundant with line 96-98	The sentence was deleted in line 96-98. Thank you for pointing this out.

2. Biopsy laboratory infrastructure, equipment and materials

Reviewer	Page	Line	Comment	REPLY
Sandrine Chamayou	5	148	When it is not possible to perform embryo-biopsy in a separate area what do you suggest? To perform embryo-biopsy in a separate moment that routine IVF?	This issue was discussed in depth with the working group, and it was decided to advise a "dedicated area". If a separate dedicated area may not be available then timings of embryo biopsy should differ from the routine IVF work, according to the SOPs. However, the recommendation is to move forward to dedicated areas for biopsy.
Joshua Blazek and colleagues	5	158	Line 158: Re laser usage “The lowest amount of heat is recommended to avoid any embryo damage risk. A CE mark would be an advantage.” I find this slightly misleading as this isn’t a technical way in which we normally talk about lasers – we discuss power, pulse length/width, and hole size. A high-powered laser fired for a short time, will make a larger hole than a low powered laser fired for the same time. The Saturn laser is the most powerful commercially available laser which therefore allows you to apply less total energy to create a specific sized breach. I think the text in the paper could easily be misconstrued as to low power lasers being better. Also, don’t know if the Saturn laser is CE marked	We have discussed this comment, but we cannot advise one laser in particular. There are several different lasers on the market and labs should test their own parameters for their own laser. Regarding the parameters, these can change from one laser to another, depending on the provider/supplier, and again specifying details would not be relevant. We removed the sentence "The lowest amount of heat is recommended to avoid any embryo damage risk. "
GenQA	5	159	For clarity- specify on which piece of equipment a CE mark is advantageous	The sentence on CE mark was clarified in the text, now reading " a CE mark is recommended for all equipment, taking into consideration local legislation"
Sandrine Chamayou	5	160	Equipment: please suggest the laser to use for biopsy (diode 1.48um).	To ensure the paper is future-proof, it was decided not to add specifications on the laser.
Karen Sermon	5	145 and 147	Are these two different references? It read as if it is the same one. But they have two different publication dates.	These are the same - the date in the title differs from the actual publication date of the paper in HR. I adapted the title to avoid further confusion
Kersti Lundin	5	152-153	I would remove the sentence “This equipment can be used either for ICS or for any stage of oocyte/embryo/biopsy”. Seems out of place/ unnecessary.	We have adapted the paragraph, merging the 2 sentences.

			As an option, the preceeding sentence could be given the addition: "…., placed on antivibration pads, equivalent to a setup for ICSI procedures".	
M. Cristina Magli. Luca Gianaroli	5	141	The first sentence contradicts what written in lines 152-153 (if the micromanipulator can be used for ICSI it is not in a dedicated area for biopsy). To be deleted.	We have rephrased the sentence in line 152 to correct the contradiction.

3. Training for biopsy

Reviewer	Page	Line	Comment	REPLY
M. Cristina Magli. Luca Gianaroli	6	173	Biopsy is an embryologist's matter. Delete "and preferable also qualifications in medical genetics".	We have changed the word "qualifications" to "basic knowledge of medical genetics", as s/he will also be responsible for interpreting the PGT report.
Celine Moutou	6	173	Expertise in clinical embryology: agree. Qualification in medical genetics: do not agree if you mean a diploma I would skip this.	We have changed the word "qualifications" to "basic knowledge of medical genetics", as s/he will also be responsible for interpreting the PGT report.
M. Cristina Magli. Luca Gianaroli	6	173	Biopsy is an embryologist's matter. Delete "and preferable also qualifications in medical genetics".	We have changed the word "qualifications" to "basic knowledge of medical genetics", as s/he will also be responsible for interpreting the PGT report.
Karen Sermon	6	177	Deviation from, not deviation to SOPs	This was corrected
Sandrine Chamayou	6	182	Please write '50 oocytes for PB biopsy, 50 embryos for embryo biopsy at the same stage'.	We clarified 50 oocytes or 50 embryos but decided not to specify the stage.
Joshua Blazek and colleagues	6	182	Line 182: No of embryos for training has been reduced from 100 to 50.	We have assessed the comment, but we stay with the current recommendation of 50 oocytes or embryos
Kelly Tilleman	6	192	All parameters should be comparable to the standards of the lab and the PGT consortium. Please elaborate what these parameters of the PGT consortium are or at least give a reference to the indicators published by the PGT consortium	We added the reference to the last PGT consortium report but decided not to add further information.
Kelly Tilleman	6	193	To state the biopsy should be supervised by an embryologist with a recognition or ESHRE certification is not a scientific recommendation. The ESHRE certificate does not specifically entails a criterion for the correct performance of embryo biopsy. Although I do realize that the ESHRE recommendations will support their certification program, stating that the biopsy program should be supervised by an ESHRE certified embryologist is just not right. There are many biopsy programs running just fine by embryologists not having the ESHRE certification. I would change the sentence to - Biopsy must be supervised by an experienced clinical embryologist. Additionally, relevant certification for their own country or the ESHRE certification for clinical embryologists might be appropriate.	We have revised the sentence, now reading "• Biopsy should be supervised by a clinical embryologist, preferably holding the relevant certification for their own country, and/or the ESHRE certification for clinical embryology. "
Alessandra Alteri	6	193	Although it is true that biopsy should be supervised by a clinical embryology, the statement on certification is not correct for the present situation in the various countries. Importantly, in the logbook, the ESHRE certification does not consider the biopsy.	We have revised the sentence, now reading "• Biopsy should be supervised by a clinical embryologist, preferably holding the relevant certification for their own country, and/or the ESHRE certification for clinical embryology. "
Sandrine Chamayou	6	194	When there is no relevant certification in the country, I do not think that ESHRE certification is mandatory. It is proposed…	We have revised the sentence, now reading "• Biopsy should be supervised by a clinical embryologist, preferably holding the relevant

				certification for their own country, and/or the ESHRE certification for clinical embryology. "

4. Biopsy stage and procedure - PB

Reviewer	Page	Line	Comment	REPLY
Kersti Lundin	6 and 10	198-199 - 318 resp	Give examples of how PB1 and PB2 can be distinguished?	To keep the papers concise, we decided not to expand on this by providing more details, except for stating that they are distinguished based on size, shape and position within the PVS.
Raul Piña-Aguilar	7	208	mechanically? at the beginning in page 2, line 35 you said acid drilling is not anymore in used. Do you refer to needle opening? Your should clarify the method.	In the introduction, we indeed mention that acid drilling is not recommended, but we do not recommend against mechanical ZP opening using a micropipette, As mechanical ZP opening is explained in the introduction, we decided not to adapt it here
Susanne Knebel	7	218	It is recommended to analyze polar bodies 1 and 2 separately, since in the amplification of pooled polar bodies one cannot be sure that there is an asymmetric amplification of partial DNA from both polar bodies or a loss of one polar body during tubing.	We agree with this comment, and therefore we have suggested to discriminate and report PB1 and PB2. We feel this is sufficiently covered in the paper.
Tina Buchholz	7	216 and 318	pbs I and II can also be pulled both in one tube and analysed as one sample, giving rise to the corresponding secondary oocyte. There is not always a need to distinguish them reliably.	Pooled analysis may be possible, but we still recommended to analyse polar bodies 1 and 2 separately, since in the amplification of pooled polar bodies one cannot be sure that there is an asymmetric amplification of partial DNA from both polar bodies or a loss of one polar body during tubing.

4. Biopsy stage and procedure - DAY3

Reviewer	Page	Line	Comment	REPLY
Joshua Blazek and colleagues	7	237	Line 237: “Embryos that did not reach the 6-cell stage on the time of biopsy may be included to help establish haplotypes, for instance in de novo mutation cases (see also the paper on detection of monogenic disorders (refer PGT-M paper), but they should not be transferred”. Not directly relevant to our practice but an interesting statement to make without any references. I would historically have biopsied embryos from 5-cell stage and had at least one baby from a 4-cell stage biopsy (although that was not routine practice). Mentioning the lack of referencing here – present with many other statements – as I am guessing they may cite lack of evidence for safety of using IVF for insemination.	In the section, it is stated that it is recommended to biopsy embryos at 6 or more cell stage on day 3 with acceptable grade. Based on the comments of the reviewers, it was decided to delete the paragraph explaining what to do with embryos that did not reach the six-cell stage. We also slightly modified the above sentence by deleting "only" at the 6-cell stage.
Christina Hnida	7	233-235	Direct cleavages might also be mentioned here leading to embryos with lower chance of implantation and with genetic impact.	We wrote "an acceptable grade (fragmentation limited to 25%) and according to the laboratory policy", hereby assuming that the laboratory policy states that direct cleavage embryos should preferentially not be used for transfer or biopsy. It was decided not to add these details to the paper.
M. Cristina Magli. Luca Gianaroli	7	235	“(misdiagnosis, failed diagnosis)”. Any evidence for this statement?	This statement is based on experience from the group. Unfortunately, there are no references to support this.
Kersti Lundin	7	237-239	I find it too hard a recommendation that embryos below 6-cell stage should never be transferred. If there are no other (better) embryos available, and if	In the section, it is stated that it is recommended to biopsy embryos at 6 or more cell stage an day 3 with acceptable grade. Based on the

			the patient is informed that these embryos will have a lower chance of pregnancy, why not transfer? Especially since you even include embryos with up to 50% fragmentation. Would you give this advice also for PGT-A? I would propose to remove or revise the sentence.	comments of the reviewers, it was decided to delete the sentence explaining what to do with embryos that did not reach the six-cell stage. We also slightly modified the above sentence by deleting "only" at the 6-cell stage.
Carlos Encinas	8	244	The opening diameter should be approximately 10-15 µm	The diameter of the biopsy pipette was mentioned earlier in the paper and specified according to the biopsy stage ("30-35µm for cleavage stage biopsy"). We here wrote that the opening diameter should be "up to diameter of the biopsy pipette" and decided not to explain it further in this section.
Joshua Blazek and colleagues	8	245	Line 245: "If the blastomere lyses, it is recommended to change the biopsy pipette." The have removed suggestion to change biopsy pipette between each embryo at cleavage stage.	It is recommended to change pipette between each blastocyst for TE biopsy, but not between each blastomere at cleavage stage biopsy if there is no lysis. The working group decided not to change their recommendation based on this comment
Celine Moutou	8	245	Binucleated cells are avoided mostly for FISH analysis. In our experience, results are correct for amplification based PGT	We decided to change the sentence, stating now that it is "binucleated cells should be avoided for FISH analysis"
M. Cristina Magli. Luca Gianaroli	8	247	Change the sentence "The biopsied embryo should be rinsed in culture medium at least once before continuing culture" to "The biopsied embryo should be thoroughly and gently rinsed in culture medium before continuing culture"	This was adapted in the paper.
M. Cristina Magli. Luca Gianaroli	8	252-253	According to Zhang et al., Reprod Biomed online 2009, it was preferable to vitrify at the blast stage. Any evidence for the written sentence?	We have adapted the sentence as suggested.

4. Biopsy stage and procedure – DAY5

Reviewer	Page	Line	Comment	REPLY
Joshua Blazek and colleagues	8	263	Line 263: Point of interest – state biopsy performed D5-7. Many of our European customers are still dubious about day 7.	We understand that Day7 biopsy is not universal practice but decided not to modify this in the paper.
Christina Hnida	8	264	„Advantageously" instead for „Alternatively"	Alternatively, is the correct word here. We decided not to modify it.
Alexia Chatziparasidou	8	268	TE biopsy – Currently the biopsy technique is not standardized- There are numerous variations among operators and PGT labs.	We added a sentence reading "Furthermore, there are some variations among operators and laboratories. " to the paper
Alessandra Alteri	8	271	It should be better to replace "HEPES-buffered medium" with "zwitterionic buffers" or "buffered media"	HEPES and MOPS are indeed zwitterionic buffers but so are others that have different pH range. We adapted the sentence to "buffered media"
Alexia Chatziparasidou	8		Define/address the parameters that need to be standardize so all operators should record them in order all these data to be accumulated and their impact assessed. Potential parameters: Time of ZP opening/Method of opening/stage of blastocyst during biopsy (non-herniated/herniated/fully hatched)/ method of biopsy (pulling/fliking/combination/Time of biopsy/ number of cells/day of biopsy (5-7)/ Time to vitrification/ survival post warming/ Quality of blastocyst (before biopsy-after warming) Pooling of all these data in relation with LBR may give us critical information and data to standardize and optimize blastocyst biopsy technique	We have assessed this comment, but decided that defining such parameters is outside the scope of the current paper
Christina Hnida	9	282	The ICM should be positioned at 7 OR 11 o'clock, but NOT directly at 9 o'clock where the ICM could get compromized by the suction of the holding pipette.	We added a sentence reading "avoiding the ICM by the suction by the holding pipette " to the paper

Carlos Encinas	9	286	Try to fire as few laser shots as possible to remove the TE cells.	We added a sentence reading "It is recommended to fire as few laser shots as possible“ to the paper
Sandrine Chamayou	9	289	The biopsy of 5-10 cells is requested for PGT-A and mosaicism valuation, not for PGT-M. For PGT-M, less cells can be removed and tested.	The decision on recommending 5-10 cells was made in collaboration with the working groups of the genetic testing papers and acceptable by all. It was decided not to add a sentence on this, as often PGT-M is combined with PGT-A.
Joshua Blazek and colleagues	9	289	Line 289: Point of interest – recommend removal of 5-10 cells	The decision on recommending 5-10 cells was made in collaboration with the working groups of the genetic testing papers and acceptable by all. It is unclear whether the reviewer agrees or suggests a modification
Sandrine Chamayou	9	292	Ca2+/Mg2+ -free medium must not be used, instead of ‘should’	Should and must have the same value, and in the current sentence, “should” is probably more correct English
Karen Sermon	9	292	Mg not MG	This was corrected
Joshua Blazek and colleagues	9	293	Line 293: “To avoid cross contamination during biopsy, it is recommended to change the biopsy pipette for each blastocyst. Alternatively, it is acceptable to thoroughly rinse the biopsy pipette, but it should be verified in the laboratory that this suffices to avoid cross-contamination.” We should look to have a procedure by which labs can verify this as part of our procedures for setting up the service as very few people change pipette unless lysis observed.	Although we agree with the comment of the reviewer, there is (for now) not a standardized method for that. Therefore, it was decided not to add further information
Celine Moutou	9	293	Nothing about holding pipette. Could it be mentioned that there is no need for changing the holding pipette or is it obvious ?	The group has assessed this comment, and judged this is obvious
Karen Sermon	9	296	Add “the” in front of “blastocyst”	This was corrected in the text.
Joshua Blazek and colleagues	9	299	Line 299: “Embryo transfer can be performed in a fresh cycle if genetic testing results are available in a short time and embryos are not in an advanced stage” Referring specifically to blastocyst biopsy. Interesting as I would have thought the consensus now was that a frozen cycle was better than transfer on day 6	Although many labs have moved to biopsy and vitrification, this does not mean that fresh transfer should never be applied, especially for Monogenic disorders where test results may be available within 7 hours. We reduced the strength of the sentence, now stating fresh transfer is acceptable,
Celine Moutou	9	299	Is there a limitation in the day (5?) according to implantation capacity of the endometrium	This sentence in the paper is about the potential impact of extensive culture on the biopsied blastocyst per se (if by the time of biopsy, it was in an advanced stage already). Regarding the endometrium, on Day5 in almost all cases the implantation window is open. For Day 6 more data need to be collected.
Joshua Blazek and colleagues	10	304	Line 304: “Time between blastocyst biopsy and cryopreservation is very important; it is recommended to cryopreserve them as soon as possible before re-expansion, particularly in those cases where blastocysts are totally hatched.” Would like to see references for this. Not something most labs I know of are strict about. Also, many labs don’t realise they need to alter vitrification timings for collapsed blastocysts.	As this paper was not supported by an extensive literature search, we refrain from adding random references. We feel the current statement is clear and in line with the comment of the reviewer
Karen Sermon	10	310	What is the cryopreservation recommendation after rebiopsy?	A sentence was added to the section stating that "following rebiopsy, it is recommended to proceed immediately to cryopreservation"

5. General strengths and limitations

Reviewer	Page	Line	Comment	REPLY
Karen Sermon	10	319	Delete “latter”	This was corrected

M. Cristina Magli. Luca Gianaroli	10	319	Add “especially in the case of PB fragmentation”	This was added as suggested.
M. Cristina Magli. Luca Gianaroli	10	325	Change the sentence “such as fragmentation or degeneration” to “such as PB fragmentation or degeneration”	We added a clarification; the sentence refers to PB fragmentation/ degeneration
Alan H Handyside	10	324	Is this polar body or embryo fragmentation/degeneration?	We added a clarification; the sentence refers to PB fragmentation/ degeneration
Tina Buchholz	10	328	ff do not apply to pb bx!! under the heading 5.1. polar body biopsy	The working group discussed this comment but could not understand what the reviewer wanted to change.
Alan H Handyside	10	329	Our experience is that PB biopsy and SNP analysis is highly reliable (100% in a recent publication with 51 embryos). What is this 10% based on?	The technology (karyomapping or genetic testing) is highly reliable, but inconclusive results can also be due to no nucleus included, or no good quality DNA (limited, or damaged). Therefore, it was decided not to modify the sentence.
Alan H Handyside	10	332	There is an early publication from Verlinsky though an RCT has never been done. It has also been used routinely by RGI in Chicago and Germany for many years. I do not think the treatment of this polar body section is therefore very balanced.	We removed the statement as it could be misinterpreted
M. Cristina Magli. Luca Gianaroli	10	332	The only available data – to my knowledge – are from ESTEEM (table VII reports the live-births for controls vs. embryos without a diagnosis). If there is nothing else, I would report this information.	We removed the statement as it could be misinterpreted, and actually removed all statements on reproductive competence as they did not add much to the paper and could be misinterpreted
Susanne Knebel	10	325+	An advantage of polar body diagnostics is the absence of mosaic findings.	We agree, but we decided not to add this, as also in the other sections "mosaic" findings are not specifically addressed
Sandrine Chamayou	10		Part 5: you should give an advice of at which stage should be performed biopsy to know what. Since the paper of Mastenbroek and the impact of cleavage biopsy on implantation rate, Fragouli 2011 or Vanneste 2009 and the percentage aneuploidy at cleavage stage, it should be clearly said that cleavage stage is not recommended, in particular for PGT-A. The bad practice should be discouraged.	Thank you for your comment, but the aim of the current paper was to provide technical recommendations for best practice in the biopsy techniques.
Karen Sermon	11	345	Insert “the” before “genetic result”	This was corrected
Karen Sermon	11	352	Replace “origin” with “rise”	This was corrected
Karen Sermon	11	356	Insert “(Figure 3)” after “alternative biopsy approaches”	We added a reference to figure 3, as suggested
Celine Moutou	11	370	Could “platform” be replaced with “technic” since genome wide technologies are not systematically applied after TE biopsy	This was modified in the paper
Joshua Blazek and colleagues	11	373	Line 373: “the estimated rate of inconclusive diagnosis is expected to be lower than 5%”. Point of interest, in reference to blastocyst biopsy.	Polar body and cleavage stage are less accurate than blastocysts. We have assessed this comment, but don't think it requires any changes.
M. Cristina Magli. Luca Gianaroli	11	374-376	This is applicable also to PB and blastomere biopsy	We agree that running multiple analysis is also possible after PB or cleavage-stage, but the analysis is probably more efficient with blastocyst biopsy. We have slightly adapted the sentence, but decided not to repeat it in the PB or cleavage-stage biopsy sections,
Celine Moutou	11	375	Only if WGA is applied. Should be specified	This was added to the sentence.
Christina Hnida	11	376	”Neither for NORMAL BLASTOCYST BIOPSY nor for blastocyst rebiopsy transfer can be performed within the timing to allow fresh embryo transfer”	This is correct, and it is mentioned higher up in the text that with D5 biopsy, cryopreservation is mostly mandatory. We deleted the sentence: Rebiopsy cannot be performed within the timing to allow fresh embryo transfer.

Christina Hnida	11	341	Table 1 says „mostly mandatory“ for cryopreservating after blastocyst biopsy. The same is true for re-biopsy. It is NOT within the timing for fresh embryo transfer.	This is correct, and it is mentioned higher up in the text that with D5 biopsy, cryopreservation is mostly mandatory. We deleted the sentence: Rebiopsy cannot be performed within the timing to allow fresh embryo transfer.
Kersti Lundin	11	351 + 373 + more places	I have a bit of a problem with the word “fragment” in this context, since it is so widely used in another context in embryology. Is there another possible word? Piece? Portion? Section? Or maybe not…. (just a thought)	We replaced "fragment" with the word “section”.
Kersti Lundin	11	366-367	Saying that laboratories “should” have in place an efficient cryoprogram is not enough. I would replace by “need to” or “is necessary to” even “must”.	We changed the sentence as suggested to 'laboratories must have in place an efficient cryo-program"
Karen Sermon	12	377	Rephrase, it reads as if there are no data on blastocyst biopsy available. Rather, the data do not report negative impact.	We rephrased the sentence to clarify that indeed the current data show no impact
Karen Sermon	12	382	Replace “components” with “members”.	This was corrected.
Kersti Lundin	12	Table 1	For embryo developmental competence it says “Unpredictable at this stage” both for PB biopsy and Cleavage stage biopsy. I would like to have a bit more distinction, clearly it is at least more predictable on day 3 than on day 0. Perhaps something like “Only cleaved embryos of a certain morphological quality are biopsied”, in line with the statement for TE biopsy.	We adapted the statement for cleavage stage biopsy, as suggested
Karen Sermon	12	Table 1	Replace “not” with “no”	This was corrected in the table
Karen Sermon	12	Table 1	Under “impact on embryo”: the same sentence is written under PB and TE: “Not reported, but more data are still required”. However, from the text it can be concluded that no studies have been conducted for PB, while for TE there is evidence that it is not harmful.	This was corrected in the table, as it was indeed inconsistent with the text above
Raul Piña-Aguilar	12	table 1 - cleavage - mitotic errors assessed?	Why not? an abnormal blastomere can come from a mitotic error. If more than on blastomere is biopsy is possible to see the mosaic error than presumably if is real is a mitotic error.	Although an abnormal blastomere may arise from a mitotic error, to assess this origin will require the implementation of at least 2 cell biopsy, which is something that is not advised.
Raul Piña-Aguilar	12	table 1 - inconclusive diagnosis	Inconclusive diagnoses based on what? Embryo biopsy is just one component, a non-diagnostic can come from unproper tubing, WGA amplification failure, library and PCR related failures.	This refers to inconclusive diagnoses based on the amount of DNA analysed. We decided not to add this in the paper
M. Cristina Magli. Luca Gianaroli	12	Table 1	“Embryo developmental competence”. I do not agree with the definition given in the glossary as competence is not synonymous of development to blastocyst. In addition, it doesn’t take into consideration the blastocyst quality I would call it “Development to good quality expanded blastocysts”	We have clarified that this is on embryo development (in the table) and have removed the word "competence"
M. Cristina Magli. Luca Gianaroli	12	Table 1	“laboratory workload”. Clearly the work of biopsy increases the lab workload, but the point here is to compare the different approaches to biopsy. “Day 3 hatching-based strategy: Moderate to high”. It cannot be classified as HIGH as PB. At most it will be MODERATE as for the cleavage stage biopsy. “Morula hatching-based strategy: Moderate to high”. It cannot be classified as HIGH as PB. It will be MODERATE. “Same day hatching-based strategy: Moderate to high”. It cannot be	We have revised the statements and adapted them; PB: very high to high, Cleavage stage: high to moderate, Day 3 hatching high to moderate, Morula stage moderate, Same day hatching based strategy moderate and Simultaneous ZP and TE biopsy strategy moderate to low.

			classified as HIGH as PB. It will be MODERATE to LOW. "Simultaneous ZP opening and TE cells retrieval strategy: Moderate". It cannot be classified as MODERATE as for the cleavage stage. It should be LOW.	
M. Cristina Magli. Luca Gianaroli	12	Table 1	"Impact on embryo reproductive competence". Under PB, it has no sense what is written. Either you write "Not reported" deleting the rest of the sentence, or you write "Reported but more data are still required" if you consider to include the reference to ESTEEM.	After further discussion, it was decided to remove all statements on reproductive competence as they did not add much to the paper and could be misinterpreted

6. Tubing of cells

Reviewer	Page	Line	Comment	REPLY
Raul Piña-Aguilar	13	383	"Tubing" should be included in the glosary of organization paper. Because tubing is not an standard English world.	Tubing was added to the glossary
Tina Buchholz	13	385	"embryonic cells" – not mentioned oocytes	We changed "embryonic cells" to "the sample" to be applicable also to PB biopsy.
Joshua Blazek and colleagues	13	392	Line 392: "Gloves should be worn at all times and changed frequently. These should be well-fitting (e.g. nitrile, but not vinyl examination gloves)." Does not state requirement for sterile gloves, which I would.	We corrected this to "sterile gloves"
Laura Corti	13	394	Bleach is not recommended in the IVF-lab	We added a sentence stating that the use of bleach is not recommended in the embryology lab.
Christina Hnida	13	395	Exposure to UV-light should be mentioned here	We added a sentence on UV-C irradiation, as suggested.
Carlos Encinas	13	397	Read and follow the reference genetics laboratory instructions (if PGT cases are carried out in external facilities)	We added a sentence in the paper stating that materials can also be prepared "by the staff of the IVF centre according to the instructions of the reference genetic laboratory"
Celine Moutou	13	397	Maybe this is done by the staff of the IVF centre. I would not specify and keep only "The material and reagents for tubing should be prepared in advance"	We added a sentence in the paper stating that materials can also be prepared "by the staff of the IVF centre according to the instructions of the reference genetic laboratory"
Joshua Blazek and colleagues	13	406	Line 406: "The tubing area should be in a DNA-free environment (pre-amplification area). DNA decontamination measures required for the tubing area are mostly incompatible with IVF good laboratory practices." Point of interest – this is not realistically achievable for most labs I know. Also, pre-amplification area is also used to refer to the lab where the first stages of WGA are performed. I would not have thought it would be recommended to tube cells in this area?	We removed the phrase "pre-amplification area" to avoid any confusion.
Joshua Blazek and colleagues	13	411	Line 411: "contamination, the preparation of materials and reagents, and the tubing of biopsied cells should be performed in a dedicated class-II laminar flow hood, which is irradiated with UV-C light for DNA decontamination prior to each use" This is interesting as these UV lights are pretty useless and I would like to see evidence that they are any use whatsoever in terms of DNA decontamination in this situation.	These are guidelines for good laboratory practice. Therefore, to support the effectiveness of every single procedure described here based on published evidence is not within the aims of the present document.
M. Cristina Magli. Luca Gianaroli	13	411-412	"tubing of biopsied cells 412 should be performed in a dedicated class-II laminar flow hood". Is there any evidence to support this statement?	We have removed the specification that it needs to be a class-II laminar flow hood, but consider a "dedicated" flow hood good laboratory practice and a precaution to avoid DNA contamination.

Kelly Tilleman	13	412	To work in a DNA free manner is not the same as to work in a sterile manner. In fact, you do not need a Class II cabinet to work DNA free. Most DNA work in other sectors, like DNA fingerprinting labs is performed in a specific cabinet without air flow. And also here, DNA is sometimes extracted from very few cells, even single cells. Air flow is a risk for contaminating items with DNA. Because of the EUTCD, we do need to work in a GMP environment A, and this can also be reached in a class I cabinet. Stating that It is needed to work in a class II cabinet in order to minimize DNA contamination is just not correct. The irradiation is fine and necessary. Bleach is also mentioned several times, this is not preferred in a IVF lab. There are also other possible specific decontaminating agents to remove DNA.	We have reformulated the paragraph based on this comment.
Celine Moutou	13	414	Or an inverted microscope	This was added in the paper.
Joshua Blazek and colleagues	13	416	Line 416: “All reagents (purchased and in-house) should be tested (for efficiency and contamination)”. Impacts in biopsy kits	We assume the reviewer comments on testing biopsy kits as well, but in our opinion, this is no different as testing reagents. We decided not to address this in the paper.
Kelly Tilleman	14	425	To autoclave reagents: this is very old school and this is for the purpose of sterilisation, not for the purpose of working DNA free. The best to do is to put your solution through a sterile filter and then to irradiated with UV-C.	We added a sentence on UV-C irradiation, as suggested.
Alessandra Alteri	14	442	The following statement needs to be added: “Alternatively, dishes with numbered drops of washing buffers should be prepared immediately before the tubing procedure without using mineral oil”.	This was added in the paper, as suggested.
Joshua Blazek and colleagues	14	442	Line 442: States that wash buffer should be under mineral oil which is in contradiction with our recommendations.	We added a sentence (“Alternatively, dishes with numbered drops of washing buffers should be prepared immediately before the tubing procedure without using mineral oil”.) based also on another comment.
Emmanuelle Kieffer	14	443	“Similarly” : = prior to biopsy? Labelling should be done only when the biopsied sample is ready to be tubed (embryos aimed to be biopsied may not all be successfully biopsied in the end, and their order may change).	The word "similarly" was deleted to avoid confusion.
Pamela Renwick	14	444	Validation can be performed to assess the effect of reducing the washing stages on the efficacy of the test to be performed. As each wash introduces a risk of losing cells.	A sentence was added: "However, care should be taken to avoid losing genetic material between consecutive washing steps."
Ahmet Berkız TURP	14	447	It(capital)	This was adapted in the paper
Sandrine Chamayou	14	453	Remove ‘with or’	We stated that it is acceptable not to visualise the cells after significant consideration within the working group and we decided to not modify this recommendation.
Carlos Encinas	14	453	Visualising the cells going into the tube every time is highly recommended.	We stated that it is acceptable not to visualise the cells after significant consideration within the working group and we decided to not modify this recommendation.
Christina Hnida	14	454	”tubing can be performed in PBS, BIOPSY MEDIA, or directly in lysis buffer…..”	We have checked but decided not to add "Biopsy medium" to the list of media for tubing (assuming this was suggested by the reviewer)
Joshua Blazek and colleagues	14	457	Line 457: “A minimum of one negative control per buffer (sample collection buffer, biopsy media, or washing media, depending on the protocols of the PGT centre) is recommended to control for contamination during each	We believe the paragraph more than adequately highlights the importance of using blanks. It is up to the individual practitioners to decide the exact number of blanks they wish to use. This also depends

			procedure of cell sample collection (i.e. the IVF laboratory negative control).” Contradicts our current recommendations.	on proven lab efficiency and previous documentation of a lack of contamination with exogenous DNA.
Alan H Handyside	14	457	Cooper Genomics no longer require ANY blanks. Our experience with PGT-A is that blanks often come back positive and if a medium blank is used that undermines all of the results in that cycle. We were never able to work out the source of the contamination which was often aneuploid but unrelated to the samples. For that reason we also decided not to use blanks.	Although usefulness may be limited, collecting blanks is still considered good practice and widely performed by many laboratories.
Cristina Albanese	15	463	Biopsied cells can be stored for future use at -80°C	Storage at -80°C is not the only storage option, and actually it is rather uncommon. We added the following sentence on storage 'After tubing, the samples can be kept at room temperature, cooled or frozen, depending on the duration of storage, the laboratory conditions and recommendations of the genetic laboratory.'
Sandrine Chamayou	15	464	Please precise which cells, lysed cells or DNA should be transported at room temperature or cooled or frozen. What at which temperature.	The sentence specifies the transport of cells (not DNA). The storage temperature can impact in the integrity of the cells and different temperatures are acceptable depending on the lab conditions and genetic lab recommendations according to different protocols.
Cristina Albanese	15	468	For shipment of biopsied cells at room temperature it is recommended to cover them with oil to preserve the materials	A sentence on covering the cells with oil was added in the paper (The buffer containing the biopsied material within the reaction tube may be covered with mineral oil during transport.)
Celine Moutou	14-15	457-463	Not clear enough. How many “blank controls” with washing media are recommended after single cell (on per cell? per embryo ? or less) and after multiple cell biopsy (not needed, one per embryo, one per biopsy ?) Is the recommendation different if WGA or if targeted amplification is performed after biopsy?	We believe the paragraph more than adequately highlights the importance of using blanks. It is up to the individual practitioners to decide the exact number of blanks they wish to use. This also depends on proven lab efficiency and previous documentation of a lack of contamination with exogenous DNA.

7. Cryopreservation of biopsied embryos

Reviewer	Page	Line	Comment	REPLY
Tina Buchholz	15	469	chapter 7. cryopreservation of biopsied embryos – but also “oocytes”	This was adapted in the paper. The heading is now consistent with the text below.
Sandrine Chamayou	15	480	Change ‘should be vitrified individually’ by ‘must be vitrified individually’’	This was adapted in the paper
Karen Sermon	15	481	In a cryo-support, not on a cryo-support	This was adapted in the paper

8. Alternative sampling methods

Reviewer	Page	Line	Comment	REPLY
Raul Piña-Aguilar	15	487	This part is inadequacy and misleading for policy makers and bioethicists. It is essential if this part if is included is renamed to: ALTERNATIVE EXPERIMENTAL SAMPLING METHODS. There is no evidence that these methods are solid for clinical use. In my opinion should not be included in the standards of PGT that these guidelines will be when are published. PGT is full of unsupported claims and this section is supporting methods that there is no evidence that can be used or representative of inner cells mass. This harms the field of PGT.	We have split the section between alternative biopsy methods and alternative sampling methods. With each of the described techniques, we clearly state that more research or optimisation is needed. We do not feel we are supporting these methods but remain confident they could be mentioned.

Karen Sermon	16	498	There is nothing controversial about blastocoel biopsy, it's just not efficient or reliable enough at the moment	This was adapted in the paper

Detection of monogenic disorders (PGT-M)

GENERAL COMMENTS

Reviewer	Page	Line	Comment	REPLY
Päivi Forsblom	1	26 and throughout the paper	The use of the word mutation is not appropriate. Please see the comment on paper 1,	We changed the word "mutation" to "pathogenic variant(s)" all over the text, including in Figure 2.
Sandi Deans and Farrah Khawaja	1	15	Change “deducted” to “created”	This was adapted in the paper
Sandi Deans and Farrah Khawaja	1	35	Figure 2 should be figure 1	The 4 papers will share the introduction and figure 1 in the final version. Figure 2 is correct here.
Sandi Deans and Farrah Khawaja	2	41	Throughout the document, the term “mutant” has been used. It is recommended to use “pathogenic variant” instead.	We have modified the term "mutant" to "pathogenic variant" throughout the papers.
Sandi Deans and Farrah Khawaja	3	68	Need an outcome – suggest change to “negative controls should be included to ensure no contamination is present”	The sentence includes an outcome, i.e. to monitor contamination. It was decided not to adapt this.
Sandi Deans and Farrah Khawaja	3	72	Same as above (p3, line 68)	The sentence includes an outcome, i.e. to monitor contamination. It was decided not to adapt this.
Sandi Deans and Farrah Khawaja	3	87/89	Inconsistent spelling of “workup” and “work-up” throughout document.	The documents have been sent for proofreading and this will be corrected in the final version.

INTRODUCTION

Reviewer	Page	Line	Comment	REPLY
Joanne Traeger-Synodinos and colleagues	2	47	“Remains” instead of “becomes”	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	3	66	and the whole of the document: “Few-cell” instead of “few cell”	This was corrected throughout the paper
Emmanuelle Kieffer	3	70	30 to 50 samples per trainee represent a lot of WGA reactions for training. Maybe it would be better to recommend that each trainee practices 2 to 3 WGA series (without a number of samples)?	After discussion, it was decided not to modify the sentence as it reflects what the group would recommend, being 30 to 50 samples in multiple testing rounds. If we would recommend 2 or 3 testing rounds, the numbers of samples per round would also need to be defined, with the same total number.

1. Single or few cell methods

Reviewer	Page	Line	Comment	REPLY
Sandrine Chamayou	8		Add a paragraph on NGS since PGT-M can also be performed using it.	NGS is considered in section 1.4 together with WGA methods, and more technical details are described in the PGT-A/SR paper
Joanne Traeger-Synodinos and colleagues	3	91	“…based on comparing the results…”. Also, the authors mention here that in cases of de novo mutations the high-risk haplotype can be determined during the clinical cycle. However, in section 3.1 other methods of establishing phase are, correctly, presented (ie use of sperm). We think this should be added here as well.	We agree with this comment, but the current section is meant to be a general introduction, and as the reviewer states, further explanation and details have been covered in the specific sections.

Joanne Traeger-Synodinos and colleagues	4	97	"variants themselves" instead of "loci"	This was adapted as suggested by the reviewer
Joanne Traeger-Synodinos and colleagues	4	105	Change the sentence to: "A fully informative STR marker will have different sizes for each of the four parental alleles, allowing to discriminate all possible embryo…"	This was added in the paper
Karen Sermon	4	118	This statement is only valid for AD diseases when the affected parent is heterozygous. Things are completely different in AR diseases where preferably both parents are heterozygous and the affected proband child is homozygous.	We agree with this comment and have consequently adapted the sentence to make it more general.
Joanne Traeger-Synodinos and colleagues	4	113-114	Non-informative markers will not be helpful in any protocol. "potentially supports the protocol" instead of "can be helpful"	We added "(partially) informative" to the sentence to address this comment
Joanne Traeger-Synodinos and colleagues	4	123-125	"detect the monogenic disorder…contamination)" change to "to evaluate the status of the embryo relative to the monogenic disorder, as well as other parameters such as occurrence of ADO, monosomy, trisomy, contamination etc."	This was corrected in the paper
Alan H Handyside	4	115	Here and later, it is stated that 3 SNPs are the equivalent of 1 STR marker. I can think of reasons for claiming this but I think this needs clarification.	This comment was discussed within the working group. It is clear that a SNP has a lower information content, and more markers are needed. The expert opinion on the equivalence of 3 SNPs to 1 STR can be supported by a paper from María E. Fernández et al. Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred Angus herd. Genet Mol Biol. 2013 Jul; 36(2): 185–191.
Ahmet Berkız TURP	5	127	Table 1 the alleles named 120-122,126…some hard to orientate like table 2 . Can you name or explain this that anyone who reads for the first time may be explanatory. Adding a pedigree scheme may be helpful?	We have re-assessed the table and decided that it is sufficiently explained. Adding a pedigree will probably not be very helpful because haplotypes change with the example.
Sandi Deans and Farrah Khawaja	5	Table 1	Inconsistent spelling of "wildtype" and "wild-type" in table.	The documents have been sent for proofreading and this will be corrected in the final version.
Joanne Traeger-Synodinos and colleagues	7	132	"Basic" instead of "basics"	This was corrected in the paper
Päivi Forsblom	7	133	No fluorescent primers are used in the mini-sequencing method	This is correct, but this is a general introduction for the different techniques described in the section,
Joanne Traeger-Synodinos and colleagues	7	134	"Subsequently" instead of "afterwards"	This was not adapted in the paper, as the initial version was acceptable to the proof-reader and "subsequently" does also not fit the sentence
Sandi Deans and Farrah Khawaja	7	135	D-ARMS should be defined as first use.	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	7	136	"in some cases" – this whole paragraph is very general and maybe confusing. Is this about mini-sequence? What else?	We agree that these statements are very general, but this is an introduction prior to the specific techniques described below. DNA purification can be done before mini-sequencing, of course, but also before restriction enzyme analysis.
Joanne Traeger-Synodinos and colleagues	7	141	Modify sentence as "…migration patterns of fluorescently labeled DNA molecules…."	This was added in the paper
Joanne Traeger-Synodinos and colleagues	7	148	"Differing by 1bp"	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	7	150	Add "but specificity is challenging when amplifying from limited initial DNA"	We do not think it is necessary to add this information. What we really want to say is that if using other methods different from fragment

				length analysis, post-PCR reaction will be needed. That amplification from minute amounts of DNA is challenging has been already said at the beginning of the paper.
Joanne Traeger-Synodinos and colleagues	7	163	“complete restriction digestion” instead of restriction digest completion	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	7	167	During scoring of the restriction enzyme digestion procedure, what is important to bear in mind is that in autosomal recessive disorders mutation homozygosity is rare. In addition, in autosomal dominant conditions a mutation always coexists with a normal allele. It is therefore preferable to ensure that the product of digestion is the mutant allele, considering that a normal allele is always expected. ADO and preferential amplification should also be considered. The current strategy presented is the opposite.	The WG does not agree with this statement. Wild type should be the one to be digested and the mutant one the undigested. If mutant is present you will always see it, it does not depend on the enzyme digestion (in absence of ADO and PA). In case of failed or incomplete digestion of the normal allele, result is compatible with the presence of the mutation, so (although if this happens you will lose a healthy embryo) you will never transfer an affected embryo.
Emmanuelle Kieffer	7	167	I think you should recommend that a second restriction site should be present in the amplified fragment (as an internal positive control for digestion).	We agree that it would be nice if this happened, but unfortunately, it won't happen very often, so we can't recommend it. The “problem" of limited digestion has been addressed in the “principle of the test" section, and we decided not to add more information.
Joanne Traeger-Synodinos and colleagues	8	171	Note: We are concerned about the specificity of D-ARMS at the single cell level	Similar methods like minisequencing, TAQMAN probes, … are being used with reliable results. We can agree that D-ARMS, would not be the first choice, but it has been used by a number of labs. The working group decided to describe the methods regardless of the number of laboratories using them.
Karen Sermon	8	173	Delete “to” in front of amplification	This was corrected in the paper
Karen Sermon	8	175	Move “primer” to after “labelled”: a common fluorescently labelled primer	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	8	179	Start the sentence with: “For ARMS primers”	This was corrected in the paper
Emmanuelle Kieffer	8	179	I think “four or five nucleotides” may be replaced by “in the 3’ part of the primer” or “between 3 and 5 nucleotides” to be less restrictive	This was modified in the paper
Joanne Traeger-Synodinos and colleagues	8	203	Sentence beginning “the size of the fragments obtained is altered….” Please clarify- consider changing the word “altered”	We adapted the paragraph to make it more clear. The mini-sequencing reaction requires purified PCR products as template, together with a specific unlabelled mini-sequencing primer (forward and/or reverse), designed to anneal adjacent to the target site. The mini-sequencing primer is extended with a single dideoxy nucleotide, complementary to the target site. Each dideoxy nucleotide is labelled with a different fluorochrome, allowing alleles to be distinguished on an automated sequencer.
Joanne Traeger-Synodinos and colleagues	9	213	“mandatory” instead of “a requisite”	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	9	214	“Practices” instead of “attitude”	This was corrected in the paper
Sandi Deans and Farrah Khawaja	9	214	Suggest changing “working attitude” to “good laboratory practice”.	This was adapted in the paper based on another comment.
Pamela Renwick	9	230	Please specify what is meant by a simple cabinet.	We added an explanation to the paper
Sandi Deans and Farrah Khawaja	9	230	Secondary reactions should be performed in the post-PCR room (not a pre-PCR room).	We have modified the sentence to "• Secondary amplification reactions can be performed in the post-amplification area in a simple cabinet like

				a PCR workstation or dedicated area in which one has a constricted area to process the samples."
Joanne Traeger-Synodinos and colleagues	9	233	"Backflow" instead of "backfire"	This was corrected in the paper
Sandi Deans and Farrah Khawaja	9	233	Change "backfire" to "transfer of"	"backfire" was changed to "backflow" based on another comment,
Emmanuelle Kieffer	10	265	It is not clear how many of each negative control should be included in the reaction: - for trophectoderm samples (with or without WGA): 2 sample collection buffer (OR washing buffer? or both?) negative controls + 1 amplification mixture only ? - for single cell samples: more negative controls: which ones? how many? It is not described if the "washing buffer" should either be directly pipetted in the tube from the primary stock tube or should be tubed with the same embryonic cells-transfer pipette than the one that was used for embryonic cells tubing (to check for contamination in the medium that was in contact with the tubed cell)?	On the number of each negative control to take, we do not want to be too strict. However, we recommend that one should include negative controls in the test, at least one per sample collection and amplification series. Therefore, the working group gave minimum criteria. Centres are free to include more negative controls. For the pipetting of the washing buffer, it is important that negative controls are included in the biopsy and sample collection procedure. Whether one takes the negative controls in the biopsy procedure from the washing drop or upfront from the stock tube, is less relevant.
Joanne Traeger-Synodinos and colleagues	10	284	"Confirm" instead of "reconfirm"	This was corrected in the paper
Frank Broekmans	10	284	Storing WGA products: needs this to be obligatory?? Is there any sense in organising this for Quality control??	We adapted the sentence, now reading that they should be stored, according to the local quality system or legislation.
Joanne Traeger-Synodinos and colleagues	10	289	"Within the context" instead of "in function"	We have changed "in function of" to "with respect to" throughout the paper
Joanne Traeger-Synodinos and colleagues	11	291	"PGT-M protocols" instead of "haplotyping"	We adapted the sentence according to this comment.
Joanne Traeger-Synodinos and colleagues	11	295	Title: Additional laboratory infrastructure, equipment and materials, specifically for WGA	We added the suggested sentence in the text, rather than the title
Pamela Renwick	11	299	No distinction has been made between MDA (1 stage) and Picoplex/Sureplex (2 stage) amplification. There should be adequate separation of the 2 amplification stages	We agree with this comment, but the paper provides general recommendations, rather than recommendations for every specific application or WGA amplification separately.
Joanne Traeger-Synodinos and colleagues	5, 6	Tables 1 +2	"Affected male partner" and "unaffected female partner"	This was corrected in the paper

2. Pre-examination process

Reviewer	Page	Line	Comment	REPLY
Joanne Traeger-Synodinos and colleagues	12	324	Comment: What about confirming disease-associated genotypes? If not done in house, maybe recommend that results are only acceptable from expert centres/accredited labs.	A recommendation on confirming the pathogenic variant(s) was included at the end of the section, and is now moved up,
Emmanuelle Kieffer	12	341	I don't understand ", but a proven carrier would be recommended" => in my sense all tested DNA must have a proven genetic status.	The sentence was rephrased to make it clear for the reader.
Joanne Traeger-Synodinos and colleagues	12	346	We think that this should be at the beginning of the section. See comment above.	We moved the sentence to the beginning of the section, as suggested
Joanne Traeger-Synodinos and colleagues	12	357	"When there is" instead of "which leaves"	This was not corrected as the proof-reader judged 'which leaves" to be appropriate and "when there is" has another meaning

Joanne Traeger-Synodinos and colleagues	12	363	“at” instead of “to”	This was corrected in the paper
Joshua Blazek and colleagues	12	334-343	Section 2.1 (Lines 334-343) Additional guidance regarding PGT-M phase establishment in the setting of no available suitable family member for phasing is recommended. Should such a family situation be considered an exclusion criterion for PGT-M, or are alternative phasing strategies appropriate?	We added a statement on analysis when no suitable family members are available
M. Cristina Magli. Luca Gianaroli	12	337-343	What happens in cases where no affected individuals are available?	We added a statement on analysis when no suitable family members are available
Joanne Traeger-Synodinos and colleagues	12	339-341	This is not clear	The sentence was rephrased to make it clear for the reader.
Sandi Deans and Farrah Khawaja	12	345	“reference sequence” should change to “appropriate gene reference sequence are obtained from an appropriately accredited laboratory”	This was adapted to “appropriate gene reference sequence". The group did not add "appropriate" to accredited as either one is accredited or not.
Sandrine Chamayou	12	357-358	Remove the sentence ‘Targeted amplification and fresh embryo transfer’.	We removed the sentence, as suggested
Joanne Traeger-Synodinos and colleagues	12	360+362	Specify which is the first and second strategy. It is easier for the reader.	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	13	367	And remaining of document: “SNP arrays” instead of “SNP array”	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	13	368	Change the sentence to “…allow genome-wide haplotyping, as well as copy-number typing.”	This was corrected in the paper
Joanne Traeger-Synodinos and colleagues	13	374	“WGA based” instead of “second and third”. Is day 7 biopsy something that is being done?	We changed "Day 5-7 biopsy" to TE biopsy to make it more correct.
Joanne Traeger-Synodinos and colleagues	13	389	Add “the selected, most suitable amplicons”	This was added in the paper
Joanne Traeger-Synodinos and colleagues	13	403	Change the sentence to “reduce any confounding ambivalence due to the phenomenon..”	This was added in the paper
Joshua Blazek and colleagues	13	378, 473	Sections 2.2.1, 2.2.3 Additional guidance regarding acceptable test phase performance for PGT-M based on targeted amplification would be useful. For example, when combined with mutation analysis, only one flanking marker on each side of the gene is required. Should one marker unexpectedly drop out, does the test remain adequate to produce a diagnosis if only based on one flanking marker plus sequencing?	We have assessed this comment and decided not to insert further specifications in the text regarding the adequacy of the PGT test based on target amplification. We have already given many specifications regarding the selection of markers in the tables. The adequacy of the test in case of dropout of a marker should be established by the single laboratory that knows how strong the amplification of the other marker and the specific mutation is.
Sandi Deans and Farrah Khawaja	14	420	Include recommend flanking markers	It was decided not to include "flanking markers" in this (introductory) sentence. We feel this is sufficiently covered further down in the text and does not have to be repeated.
Karen Sermon	14	404	“wide range of alleles” is confusing, see also line 419. Rather, use “a very wide range of allele size”.	This was adapted in the paper
Karen Sermon	14	421	Specify that 1 Mb = approximately 1cM	This was added in the paper
Joanne Traeger-Synodinos and colleagues	14	424	With markers within 2Mb distance the risk of recombination is higher and this should be factored in when evaluating work-up test results	This was addressed by adding 'but not advisable"' in the paper
Karen Sermon	14	426	Please consider adding: “Careful selection of markers flanking the mutation of interest will reduce the risk of misdiagnosis due to recombination.	This was added in the paper

Alan H Handyside	14	427	It could be helpful to include a list of the most important recombination hotspots	We have slightly modified the sentence, but we did not include a list as this is considered outside the scope of the current paper. In addition, a list could be considered exhaustive.
Alan H Handyside	14	436	With targeted approaches more markers does not necessarily result in more robust/accurate results. One of the problems with this approach is that AF or ADO can result in ambiguous results. Multiple markers are only more reliable if they are all concordant. Otherwise a majority interpretation has to be applied.	The group has discussed this comment in depth, and they do not fully agree with the comment. Off course a validation is needed, but in general, more markers give more robustness to the test. We consider that the advantages and disadvantages of adding more markers are sufficiently discussed in the paper.
Raul Piña-Aguilar	14	428	few cells	This was corrected as suggested by the proof-reader
Joanne Traeger-Synodinos and colleagues	14	432	“further optimized” instead of “improved”	This was corrected in the paper
Karen Sermon	14	436	Add: two loci closely linked to and flanking the gene	This was added in the paper
Emmanuelle Kieffer	14	439	It should be acceptable to perform a “Genetic Marker only” for a familial case of a “frequent” disease. If not, we should develop a new test for every single couple needing a PGT-M with a not previously –developed mutation, when working with multiplex PCR without WGA…	We added that indeed genetic markers only can be used for linkage analysis in general.
Joanne Traeger-Synodinos and colleagues	14	440	Haplotype-only analysis	This was added in the paper
Joanne Traeger-Synodinos and colleagues	14	441	“locus/genomic region”	This was added in the paper
Joanne Traeger-Synodinos and colleagues	14	443	“feasible” instead of “successful”	This was adapted in the paper
Celine Moutou	14	440-446	add indirect diagnosis for linkage analysis in general (to avoid to develop a test including the mutation).	This was added to the sentence as point vii
Joshua Blazek and colleagues	14	455-457)	Sections 2.2.1, 2.2.3 (Lines 455-457) Language clarification whether marker only based PGT-M for genes near centromeres and telomeres is not recommended would be appreciated. Additionally, in these regions, it is not uncommon for flanking markers to be present on one side of the gene, but not the other. Guidance regarding treatment of these cases would be appreciated (and relates to the above comment regarding unexpected ADO of expected flanking markers). Additional description of the haploblocking and phasing strategy to be employed in the absence of flanking markers and mutation analysis would be appreciated. For example, should markers at distances beyond 2MB be employed and, if so, is an anticipated probability of crossover relative to genetic distance of the established informative markers recommended? Should availability of prenatal testing for the familial mutation be confirmed before validation of the test for use?	We have added some details to the sentence in accordance with this comment.
Karen Sermon	15	462	…And following. Do you really want to include a test that is used by only a handful of centres and which is inferior to the ones mentioned below?	It was decided to describe the methods in use regardless of the number of laboratories that use them.
Joanne Traeger-Synodinos and colleagues	15	470	Add “by this strategy” before the end of the sentence	This was added in the paper
Joanne Traeger-Synodinos and colleagues	15	483	PCR-based haplotyping	We considered adding "PCR-based", but feel the statement is also relevant for SNP arrays. It was decided to keep it more general, and therefore not add "PCR-based" to the sentence

Emmanuelle Kieffer	16	487	I agree that the WGA protocol has to be validated, but it loses its benefits if every single downstream test has to be validated as well. WGA is used to increase the number of markers we can test and also to avoid multiplex development, because we can perform as many reactions as we need. I agree that the AF/ADO and preferential amplification is relevant to be tested for direct mutation testing on patients lymphocytes (if it is a new direct test), but I don't think we should validate all STR of all indications. If every single marker/PCR has to be validated by the same manner than the single cell (without WGA) tests, validation of the tests will be very hard to perform. Maybe it would be more interesting to validate the overall WGA process (by assessing a sufficient number of STR from a sufficient number of samples amplified by WGA) and to limit the use of WGA to trophectoderm samples to reduce ADO rates.	We agree that the validation should concern the overall WGA process. Our statement here does not refer to every single marker, but to the test/approach.
Joanne Traeger-Synodinos and colleagues	16	488	We do not understand what "in function" means	We checked with the proof-reader and "in function of" is a valid expression. However, we changed it to 'in respect to" throughout the paper to make it more clear.
Joanne Traeger-Synodinos and colleagues	16	491	Add "after WGA" in the title	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	16	496	Add "than for single cells" before the end of the sentence	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	16	504	Add "after WGA" in the title	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	16	520	"Various" instead of "different"	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	16	522	Add "…and therefore the position and number of SNPs…"	This was added in the paper
Joanne Traeger-Synodinos and colleagues	17	528	Add "compared to other WGA methods" at the end of the sentence	This was added in the paper
Joanne Traeger-Synodinos and colleagues	17	532	"a" instead of "function of the". The validation does not need to be done on biopsied cells but any type of cells. "No-call rates" instead of "no call-rates"	We have changed "in function of" to "with respect to" throughout the paper
Karen Sermon	17	551	Replace "can be happening" with "can take place"	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	17	551	"happens" instead of "can be happening"	This was adapted to 'can take place" based on another comment
Pamela Renwick	17	562	Should say 'in respect to the number of biopsied cells'	This sentence was adapted in the paper
Joshua Blazek and colleagues	17	527-528	Section 2.2.4 (Lines 527-528) Is a citation available? This would be particularly helpful given PGT-M via these arrays were not available at the time of the last ESHRE PGT consortium review. It is unclear from where the evidence for this claim is drawn.	The citation of scientific papers is beyond the scope of this manuscript, the statements are based on commonly accepted scientific knowledge. The readers are encouraged to perform a literature review for related publications. Additionally, lower genotyping error rates are due to the specific nature of the enzyme used for MDA-based WGA (phi29: proof-reading activity).
Karen Sermon	17	544, 584	At least one close relative: what is meant here? One affected relative? One member of the couple? Relative to whom?	We clarified in the paper that this is a first degree relative of the partner carrying the mutation.
Joanne Traeger-Synodinos and colleagues	17	544+584	Need to specify 1st degree relatives	We clarified in the paper that this is a first degree relative of the partner carrying the mutation.

Joanne Traeger-Synodinos and colleagues	18	573	Remove the “with respect to the upcoming sample combination”	This was adapted in the paper
Sandi Deans and Farrah Khawaja	18	583	Document refers to long range sequencing but the error rate is still high so should state it is not suitable for use yet	This comment was discussed, and the group disagrees with the statement of higher error rates. They want to clarify that the long-read sequencing technologies require a different approach to analysis. It was decided not to modify the sentence.
Joanne Traeger-Synodinos and colleagues	18	587	Change the sentence to: “In the absence of a ready software support of a skilled…”	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	18	589	“and the software will require further validation”	This was adapted in the paper
Karen Sermon	18	601	This statement seems superfluous: why should PGT-A be performed if the result is not taken into account upon transfer? Perhaps it should be recommended that if the centre policy is not to take into account PGT-A results, then PGT-A should not be carried out.	There are different policies regarding the transfer policies when it comes to PGT-A. Some centres do transfer aneuploidy embryos, others not. We recommend that centres that transfer aneuploidy embryos give priority to euploid embryos (if any), based on the PGT-A result.
Alan H Handyside	18	601	In general, I would of course support the recommendation to transfer euploid embryos as a priority. However, currently with some labs performing NGS based copy number analysis in parallel to PGT-M, there is a problem with mosaic and/or ‘complex aneuploid’ outcomes in unaffected embryos. These results need to be challenged because in most cases the embryos are euploid. In fact, in our practise we prefer not to have copy number analysis for this reason.	This recommendation is made given that a thorough validation of the method used for PGT-A has been performed to ensure minimization of false positive results, both for full as well as for mosaic aneuploidies. Determination of the detection limits per lab and technique is a prerequisite. Detection of real aneuploidies that are absent from the rest of the embryo due to biological reasons (mitotic aneuploidies, mosaicism) is also possible and in such cases, prioritization is also of importance.
M. Cristina Magli. Luca Gianaroli	18	604-607	Out of place considering the title of the session (line 590).	We added PGT-SR to the title of the section as there was indeed a discrepancy between the title and the content
Joanne Traeger-Synodinos and colleagues	19	608	Start the sentence with “For PGT-SR”	We are not necessarily referring to PGT-SR. Detection of segmental aneuploidies in the context of PGT-A is also possible.
Karen Sermon	19	616	It is not clear why phasing is necessary for PGT-A.	As indicated in the title, we are referring to methods combining PGT-M and PGT-A. Such methods/algorithms require the presence of phasing references. If PGT-M and PGT-A are performed independently, no phasing reference is required for PGT-A. The sentence was clarified.
M. Cristina Magli. Luca Gianaroli	19	616-617	Sentence not clear to me.	As indicated in the title, we are referring to methods combining PGT-M and PGT-A. Such methods/algorithms require the presence of phasing references. If PGT-M and PGT-A are performed independently, no phasing reference is required for PGT-A. The sentence was clarified.
Pamela Renwick	19	622	Haplotyping approaches CAN detect meiotic trisomy	We did not modify the statement as meiotic and meiotic errors cannot be distinguished in 100% of the cases (e.g. in the absence of homologous recombinations).
Joanne Traeger-Synodinos and colleagues	19	623	Change the sentence to: “Defining the threshold of mosaicism detection is recommended”	This was adapted in the paper
Joshua Blazek and colleagues	19	624	Section 2.3 Additional guidance regarding validation criteria for direct mutation analysis is requested	The criteria listed for targeted STR-based testing actually also apply to variant analysis. This was corrected, and thereby resolves this comment.
Sandi Deans and Farrah Khawaja	19	630	Through the EQAs, many labs want clarification what “misdiagnosis rate should include”.	A definition of misdiagnosis rate is included in the glossary. It was decided not to repeat it in the papers.
Emmanuelle Kieffer	19	631	Criteria for validation should be distinct depending on the type of analysis. They seem ok for single/few-cells samples without WGA. I think the term	We have added "accuracy" to the glossary

			"accuracy" should be define somewhere (is it a conclusive result on the status of the cell, even with the presence of ADO/ADI etc?). For WGA (same as comment for page 16-line 487), if performed on trophectoderm biopsy sample: - if included in the test, mutation detection should be validated on patients lymphocytes (amplified by WGA as well) - WGA protocol should be validated on a number of samples for a number of markers, but not for each marker tested for every single PCR.	
Joanne Traeger-Synodinos and colleagues	19	633	We think that a definition of "accuracy" is needed	We have added "accuracy" to the glossary
Joshua Blazek and colleagues	20	671	Section 2.4 Addition of assessments regarding risks of incidental findings and risk of test failure (i.e. insufficient markers and/or sequencing to produce a diagnosis) is recommended, as well as establishment of protocols to mitigate such risks. Residual risks should be included in pre-test counselling and quantified, when possible	These additional points were added to the paper
Pamela Renwick	20	671	Unclear what the risk refers to. Better to name 'Misdiagnosis Risk Assessment' or 'Risk Assessment of Misdiagnosis'	The section does not only cover risk of misdiagnosis, but also fi risk of errors in sample tracking. Therefore, the title of the section was not adapted, but we clarified "misdiagnosis" in the text
Sandi Deans and Farrah Khawaja	20	674	Not just distance to gene of interest but to location of mutation. Particularly important if a large gene	We corrected this sentence, now stating 'has to take into account the genetic distance of the flanking markers towards the variant or gene of interest"
Pamela Renwick	20	683	Only applicable if providing a result where a recombination has occurred. Flanking SNPs will not require the distance of the informative SNPs, it will be double recombination risk	Indeed, applicable when a recombination has occurred as mentioned in the text. The residual risk refers to recombination events and this was clarified in the text,
Joshua Blazek and colleagues	20	693	Section 2.5 A legal and ethical assessment should be carried out by each lab regarding inclusion of sensitive family member information (i.e. personal identifiers and genetic status) on the patient's PGT-M work-up report. Additionally, guidance regarding residual risk calculation, especially in the setting of uncertain mutation population frequencies, would be helpful	Legal and ethical matters are covered by local and international GDPR regulations. We have considered this comment for the counselling section of the paper on the organisation of PGT and added a sentence.
Sandi Deans and Farrah Khawaja	21	698	Need to include testing methodologies performed	The text already mentioned 'specify the test strategy", which we feel covers this comment.
Sandi Deans and Farrah Khawaja	21	701	Gene reference sequencing	This was adapted in the paper.

3. Special cases

Reviewer	Page	Line	Comment	REPLY
Italian Society of Human Genetics	21	3,1 de novo	In the document on the detection of monogenic disorders, at page 21, chapter 3.1 (De novo mutations) may be it would be useful to remind the relevance of the exclusion of a post-zygotic origin of a de novo mutation in a previously affected child of the couple. In this case, of course, the recurrence risk is very low and the option a IVF treatment with PGT should be carefully evaluated.	A sentence on this was added in the paper, both for de novo mutation in the prospective parent, and for de novo mutation in an affected child.
Raul Piña-Aguilar	21	708	The risk of germinal mosaicism is low is the de novo variant has been determined in the trio. I don't think is a common condition encounter in the	It was already stated that if an affected offspring is available (trio), the case can be dealt as a usual PGT-M request.

			clinic or that public healthcare system can afford. Is there a threshold of germinal risk to recommend PGT in a family with an affected offspring with a de novo mutation? Is this ethical? Prenatal diagnosis is always an option in these cases.	- It is difficult to give a threshold since mosaicism cannot be clearly established in the germline cells (especially for women). - What a public health system can afford is not in the scope of this paper. - it is already stated that prenatal diagnosis is strongly recommended (line 753)
Emmanuelle Kieffer	21	712	It should be added that the same recommendations apply for familial cases with no DNA available from any affected family members (or when the mutated-prospective parent was adopted).	The sentence was modified to "If no DNA samples from affected offspring are available". We decided not to include the comment on adoption.
Joanne Traeger-Synodinos and colleagues	21	723	"long read sequencing by NGS"	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	22	728	"In the scenario when" instead of "in case"	This was adapted in the paper
Emmanuelle Kieffer	22	747	Maybe I misunderstood, but I think it should be the reverse: "Somatic mosaicism detected in the prospective parents can be indicator of germline mosaicism"??	Both are true, depending on what is detected first. The sentence was adapted by adding "and vice versa"
Joanne Traeger-Synodinos and colleagues	22	751	"….embryos who carry the wild-type allele for the mutation locus and the high-risk haplotype because of the ADO risk…"	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	22	752	"who also have a" instead of "but"	This sentence was adapted in the paper based on another comment
Raul Piña-Aguilar	22	755	Maybe is more adequate to called demonstrated germinal mosaicism in a parent	It was decided not to change this in the paper, since it is sometimes not possible to evaluate germline mosaicism in the parents and PGT is performed anyway
Joanne Traeger-Synodinos and colleagues	22	759	Change the end of the sentence to: "prior to initiating a PGT-M procedure"	This was adapted in the paper
Raul Piña-Aguilar	22	760-761	Are there European public healthcare systems paying for this?	European public healthcare systems are paying for this, so we decided not to adapt the paper
Joshua Blazek and colleagues	22	760-761	Section 3.1 (Lines 760-761) The general practice recommendations article presents recurrence risk of <10% as an exclusion criterion for PGT-M. This opinion should be cross-referenced here or an alternative exclusion recurrence risk stated.	The organisation paper states: "PGT testing is inappropriate in case of uncertain genetic diagnosis (f.ex. genetic/molecular heterogeneity), in case of uncertain mode of inheritance, and in case of low recurrence risk (e.g. <10%) This sentence refers to legal restrictions in some countries where no PGT is feasible if the mutation is not proven in at least one prospective parent. It was decided not to change the paper.
M. Cristina Magli. Luca Gianaroli	22	760-761	Any evidence? Otherwise delete.	The organisation paper states: "PGT testing is inappropriate in case of uncertain genetic diagnosis (f.ex. genetic/molecular heterogeneity), in case of uncertain mode of inheritance, and in case of low recurrence risk (e.g. <10%) This sentence refers to legal restrictions in some countries where no PGT is feasible if the mutation is not proven in at least one prospective parent. It was decided not to change the paper.
Joanne Traeger-Synodinos and colleagues	23	770	"families" instead of "relationships"	This was adapted in the paper
Joshua Blazek and colleagues	23	770	Section 3.2 Given the potential clinical risks of LOH in the patient and/or partner, a disclosure policy of incidentally-discovered LOH is recommended	A sentence on this was be added to the counseling section of the paper on the organisation of PGT

			and should be incorporated into pre-test counseling for consanguineous couples (as should anticipated failure risks or any expected reduction in test accuracy)	
Joanne Traeger-Synodinos and colleagues	23	775	“consanguinity” instead of “consanguineous relationship”	This was adapted in the paper
Joshua Blazek and colleagues	23	790	Section 3.3 Guidance on the ability for PGT-M testing methods to provide pre-test assessment of unique HLA crossover events in the proband would be helpful.	A paragraph was added on using the most comprehensive approaches to allow multiple genetic regions to be tested. This paragraph also resolves the current comment.
Joanne Traeger-Synodinos and colleagues	23	778+779	The diagnosis is based on the low-risk haplotype of both partners	This was adapted in the paper
Alan H Handyside	23	798	Genome-wide SNP haplotyping (karyomapping) is the ideal tool for these cases as HLA type can be combined with linkage based testing for the condition if desired. It has hundreds of SNP markers across the relevant region on chr 6p and most importantly allows recombination in the affected child to be detected which is an advantage over targeted approaches. Similarly at some point in these guidelines it should be pointed out that array or NGS based SNP haplotyping allows multiple conditions to be tested and is the most comprehensive test available.	A paragraph was added on using the most comprehensive approaches to allow multiple genetic regions to be tested.
M. Cristina Magli. Luca Gianaroli	24	824-835	The exclusion testing will discard all the embryos carrying the haplotype of the affected prospective grandparent, half of which will be normal for the tested mutation. This brings along moral and ethical issues probably more or similar to a non-disclosure approach. Each IVF center / PGT lab can decide which approach to offer, but I do not think that it is within the scope of the current paper to express an ethical or moral recommendation.	We have adapted the sentence based on another comment, now reading "Exclusion testing is preferred over PGT with non-disclosure of the direct test results to the couple as the latter raises even more practical and ethical issues. "
Joanne Traeger-Synodinos and colleagues	24	827	Change the sentence to: “….of the results as this imposes confidentiality issues on the PGT team, along with moral and ethical issues.”	We have adapted the sentence, now reading "Exclusion testing is preferred over PGT with non-disclosure of the direct test results to the couple as the latter raises even more practical and ethical issues. "
Joanne Traeger-Synodinos and colleagues	24	836	Paragraph 3.5	It was unclear to the working group what was meant with this comment.
Joanne Traeger-Synodinos and colleagues	24	839	Add: “PGT based on quantifying mutation load…”	This was added as suggested
Joanne Traeger-Synodinos and colleagues	24	845	It should be specified that heteroplasmy needs to be investigated in all available family members in order to find the threshold.	This comment was assessed by the working group and it was decided that this is not necessary. A general threshold is defined in a meta-analysis, and more information can be found in the Organisation paper, section PGT for Mitochondrial disorders.
Joanne Traeger-Synodinos and colleagues	24	847	Is restriction enzyme digestion after PCR the only recommended technique for quantitative analysis of all mtDNA mutations? If not, are mutagenesis primers designed? How is the analysis performed? Fragment analysis with capillary electrophoresis?	We decided not to add more details to this section, as we feel it is sufficiently detailed for people who perform and are acquainted to PGD for mitochondrial disorders.
Joanne Traeger-Synodinos and colleagues	25	852-854	All amplification products (samples+controls) have to be spiked. Please specify.	We slightly adapted the sentence to clarify that all amplification products have to be spiked
Joanne Traeger-Synodinos and colleagues	25	859-860	Does this refer to diluted genomic DNAs?	The reference to section 1,3 was clarified by adding "(diluted and/or undiluted genomic DNA, IVF and genetic laboratory negative control)"

4. Examination process

Reviewer	Page	Line	Comment	REPLY
Raul Piña-Aguilar	25	881	No result rescue, should be properly defined, because rescue can be performed at WGA level or biopsy level.	We have rewritten the paragraph on "no result rescue" to properly address this comment, as well as other comments, and further clarify the approach
Raul Piña-Aguilar	25	882	a second biopsy also can be performed at blastocyst stage	To clarify the sentence, we added “(at the blastocyst stage)”
Joanne Traeger-Synodinos and colleagues	25	872	Add the “preferred” requirements	We added "preferred" as suggested
Joanne Traeger-Synodinos and colleagues	25	873	“When a nucleus is not observed”	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	25	884	“and/or” instead of “as well as”	This was adapted in the paper
Joanne Traeger-Synodinos and colleagues	25	872-874	Shouldn’t quality of the cells and embryos always be noted?	A sentence on this has been included in the biopsy paper (• Since biopsy is invasive, it could damage cells and DNA. Therefore, information about the integrity of biopsy samples (cell lysis, degeneration, degradation, …) should be noted and shared with the genetic laboratory.) It was decided that this information should not be repeated in the current paper
M. Cristina Magli. Luca Gianaroli	26	901	It seems to me strange that a recommendation document is supported by 3 references only.	We added a sentence explaining the lack of references in the methodology section of the 4 papers.

Detection of structural and numerical chromosomal aberrations (PGT-SR/PGT-A)

GENERAL COMMENTS

Reviewer	Page	Line	Comment	REPLY
GenQA	1	16	'until consensus' change to 'until consensus was reached'. Otherwise it is an incomplete sentence.	This was corrected in the paper
GenQA	general		These guidelines are extremely well written and comprehensive. One thing that is missing is detail on what should go in the work-up report and the final genomic report.	The issue of the genomic report is covered in the paper on the organisation of PGT.
Karen Sermon	ALL		This part is the most difficult to read, with English that could be improved here and there. Will the guidelines be read by an English editor?	While under stakeholder review, the papers have also been sent for English proofreading. The corrections of the proof-reader will be incorporated in the final version of the papers
Tina Buchholz	general		In general, I think, pb bx and analysis should be taken out of those papers and handled completely separately! They are a different material, they need special considerations regarding handling and particularly interpretation of results. The experience with the EQA for nearly 10 years has proven, that the specific difference in interpretation of the result is the major burden for most of the labs. It is an indirect testing, compared to embryonic cell analysis. The specific characteristics is not at all clearly demonstrated here.	We have included specific comments on PB biopsy throughout this paper, based on other comments from this and other reviewers. Furthermore, we have referred to other paper for further details.
Karen Sermon	1	1	Mention that sex chromosome abnormalities (47,XXY, 45,X) are included? See also page 1 line 16 of the ORG paper.	The general introduction will be used for the 4 papers. As such, the comment on chromosomal numerical aberrations of high genetic risk will also be mentioned at the start PGT-A/SR paper
Karen Sermon	5	147	There is some difference in how the different papers are structured. For instance, the documentation in this paper is on page 5, while in the PGT-M paper it is only on page 21	There is indeed some variation in how the papers are structured. While the structure for the PGT-SR/A paper was logical, we were unable to structure the PGT-M paper in a similar fashion, i.e. per technique.
Karen Sermon	5	170	There is very little information on cell spreading in FISH.	Thank you for this comment. We have added a section on cell spreading/fixation in the biopsy paper (in the sample collection section)
M. Cristina Magli. Luca Gianaroli			In several parts of this document, as well as in the other three, the title is mostly summarized as PGT-A/SR. Nevertheless, the section of PGT-SR is described first followed by the PGT-A. I suggest consistency, but considering that, according to the data of the PGD consortium, PGT-A cases are much more numerous than those of PGT-SR, I would have started with the PGT-A section. This is for the simple reason that the readers interested in PGT-A have to continuously refer to the PGT-SR section due to the many technical parts in common. I understand the reason for which priority was given to PGT-SR, but I am sure that the number of potential readers won't like this order.	Although we understand the comment on that PGT-A is more often applied, it was a conscious decision to first discuss PGT-SR and this decision was not revised. We agree with the reviewer that there is an inconsistency and have modified the abbreviation "PGT-A/SR" to "PGT-SR/PGT-A" throughout the 4 papers.
M. Cristina Magli. Luca Gianaroli	27	1001	It seems to me strange that a recommendation document is supported by 3 references only.	We added a sentence explaining the lack of references in the methodology section of the 4 papers.

INTRODUCTION TO PGT-A/PGT-SR TECHNIQUES

Reviewer	Page	Line	Comment	REPLY

Paul Scriven	1	23-26	There is no specific new PGT terminology for sexing for sex-linked diseases using FISH, which seems to be included in these guidelines. It would seem to be more appropriate to include it as PGT-M rather than PGT-SR.	As the technical aspects of FISH are covered in the PGT-A/SR paper, it was decided to also add a paragraph on FISH for sexing in case of X-linked diseases. It was clarified in the paper that this is PGT-M (rather than PGT-A/SR) and a sentence was added to the PGT-M paper referring to this section for technical recommendations.
Christian Liebst Frisk Toft	1	24-26	The introduction should mention SNP array alongside FISH, aCGH and NGS	We added SNP Array to the list of techniques, and we added a sentence referring to the PGT-M paper for further technical recommendations.
M. Cristina Magli. Luca Gianaroli	1	31	After “authorized” add “person”.	We corrected the sentence in the paper
GenQA	2	57	Change to ‘or samples do not meet the internal requirements (e.g. lysed cells, nucleus not seen) for testing, this should’ for clarity	We corrected the sentence as suggested
GenQA	2	61	Change ‘purview’ to ‘scope’. Most not native speakers will not understand purview	We corrected the sentence as suggested
GenQA	2	33 & 38	‘cytogenetic’ change to ‘cytogenomic’ throughout the document	This was corrected in the paper
Paul Scriven	2	53-56	Testing is not expected to increase the risk of an affected pregnancy. An ideal test will enumerate the copy number of all the segments involved in a chromosome rearrangement and exclude from transfer all embryos with an unbalanced product of the rearrangement, which is expected to decrease the risks of an affected live born offspring, miscarriage and stillbirth compared to natural pregnancy. A test which cannot detect all the segments, and possibly some unbalanced products, will have a lower post-test probability that a normal test result is correct and may be less effective.	After discussion, the sentence was reformulated. It states now: 'a test which cannot detect all segments, and possibly some unbalanced products, may be less effective in decreasing the risk of a viable unbalanced offspring, first trimester miscarriage and stillbirth. This should be mentioned in the preclinical work-up report.
Lauren Walters and colleagues	2	42	[First instance, continues throughout document] Guidelines only refer to PGT-A by NGS following WGA (using a WGS-based approach). There are other methods beside WGS that are comparably sensitive and even offer additional clinical information through genotyping. We encourage ESHRE to accommodate more inclusive language that is agnostic to the specific method.	We have added SNP Array as a possible method, and added a sentence referring to the PGT-M paper for more technical details.
M. Cristina Magli. Luca Gianaroli	2	50-59	While the 3rd bullet point is general, the 1st two only apply to PGT-SR. It should be indicated.	We have rearranged the section to address this comment.

1. Preimplantation testing for structural chromosomal rearrangements (PGT-SR) - FISH

Reviewer	Page	Line	Comment	REPLY
Susan Bint	3	3	We can also test for unbalanced products from insertions so can the 3rd line read: translocations, deletions, duplications, inversions and insertions, all of which may be inheritable or occur de novo.	We agree with the comment, and we added "insertional translocations" to the text.
GenQA	3	70	Add ‘insertional translocations’ – these are different from reciprocal translocations and carry a high recurrence risk	We agree with the comment, and we added "insertional translocations" to the text.
Raul Piña-Aguilar	3	71	insertions and inversions	We agree with the comment, and we added "insertional translocations" to the text.
Susan Bint	3	76	Can the sentence on this line read: Several methods are applied to perform PGT-SR, these include FISH, aCGH and NGS.	Thank you for this language suggestion, we have checked this with the English proof-reader and … "these include" instead of "amongst which"

Tina Buchholz	3	79	The FISH section takes to much room here, because it is rather outdated. The potential additional non-disjunction in cases of SR is not mentioned. Particularly for pb analysis FISH is in the light of alternatives not longer acceptable, primarily due to the fact, that pbs are no intact nucleated cells.	In some countries FISH is necessary due to legal restrictions and should therefore be included in the paper as well. We tried to keep it as short as possible but had to include the most important facts.
Tina Buchholz	3	79	It is not always necessary to have both pb separately – you can also pull them together in one sample and evaluate indirectly the secondary oocyte content. However in structural rearrangements – it is mandatory to analyse pb I to catch mal-segregation in meiosis I following quadrivalent formation. For the SR exclusively, it is not mandatory to catch pb II, since this is only a reduction division. For the quadrivalents the premature chromatide separation is absolute rare. However additional aneuploidies are not included then in the analysis.	The paper is more method related than material related - therefore the polar body specificities were not explained in all detail. We have now added specific information on PB biopsy where relevant
Raul Piña-Aguilar	3	89	Why are small, presumably as compared to large, fragments acceptable for FISH? What is the definition of small?	We added (e.g.<10Mb)' at the end of the sentence, as suggested in another comment.
GenQA	3	90	Perhaps add ' (e.g.<10Mb)' at the end of the sentence.	We added (e.g.<10Mb)' at the end of the sentence, as suggested.
Paul Scriven	3	102-103	Not all chromosome rearrangements are translocations or form a quadrivalent, e.g. Robertsonian translocations form a trivalent; not all translocations are reciprocal and some with distal breakpoints will segregate 1:1:1;1. It would be more appropriate to state that the analysis of chromosome rearrangements should include an assessment of the plausible mechanism for chromosome pairing and the products of disjunction following the first and second meiotic divisions.	The working group agreed with the comment and modified the paragraph as suggested by the reviewer.
Tina Buchholz	3	74 - 77	ff analysing pb does not identify …. at stages of embryo development - "but oocyte integrity"	We added a sentence to the paragraph stating that "preconception testing of PBs provides a means to indirectly identify chromosomally unbalanced oocytes.
Paul Scriven	3	79-85	Consider a separate section: FISH-based PGT-M for sex-linked diseases	The paragraph on FISH for sexing was retained in the PGT-A/SR paper as the technology of FISH is discussed here. To clarify, we added to the heading that this is in fact PGT-M.
Sandrine Chamayou	3		FISH or array-based or NGS: please give an advice on which method should be used because 'gold standard' and which method should be abandoned.	The aim of the current papers was to give advice and recommendations on the technical aspects of the most commonly used techniques for PGT, including the strengths and limitations. Recommending a gold standard technique was not the aim.
GenQA	4	102	Segregation outcomes	This was corrected in the paper
Philippe Gosset	4	103	the quadrivalent scheme does not include "meiosis II nondisjunction"	This sentence was rephrased and corrected based on another comment, which also resolves the current comment.
Karen Sermon	4	104	Change "in correlation to" to "relative to"	This was corrected in the paper
Tina Buchholz	4	114	pbs are not a cell or cells with a nucleous – but they are not mentioned here.	We have clarified that this paragraph deals with cleavage-stage embryos and added a paragraph on PB.
Paul Scriven	4	118	I suggest "… only one informative probe available for the chromosome imbalance involved …"	This was adapted as suggested
GenQA	4	121	I cannot find the abbreviation 'TE' given in full earlier in the document.	This was corrected in the paper
Paul Scriven	4	104-106	According to ISCN reciprocal translocations include two-break, three-break, four break and more complex rearrangements. Three probes would not be appropriate for a three-way reciprocal translocation. I suggest "two-way reciprocal translocation" at line 106.	We added "for more common two-way reciprocal translocation" as suggested.

Paul Scriven	4	106-108	This needs to be expanded. For Robertsonian translocations and inversions two probes are acceptable; one for each chromosome arm for the former, and one for each of the non-inverted segments for the latter. For single chromosome deletions and duplications, locus-specific probes for the deleted or duplicated region should be used and a control probe to determine ploidy should be included in the diagnostic cycle.	We agree with the comment, but this information is already available in the materials and methods section (bullet nr 7) further down in the paper, and therefore we decided not to adapt the paper
Paul Scriven	4	114-116	I suggest “PGT diagnosis on a single mononucleated cell is acceptable for chromosome rearrangements provided that there are informative probes for at least two unbalanced segments for those products considered likely to be prevalent or viable in a recognisable pregnancy.”	This was adapted as suggested
GenQA	4	128 & 133	Change ‘should’ to ‘must’. Unless ‘should’ is being used to mean obligatory- in which case can you state this at the beginning of the document.	In this sentence and throughout the papers, "should" is being used to mean obligatory. We decided not to adapt the sentence
Paul Scriven	5	149	ISCN recognises haploid karyotypes of 550 and 850 bands per haploid set (not “800” and not “Mb”)	This was corrected in the paper
Raul Piña-Aguilar	5	149	550-800 bands, no Mb	This was corrected in the paper
Päivi Forsblom	5	149	What is a 550-800Mb karyotype? That must be 500-800 bands, not Mb	This was corrected in the paper
Philippe Gosset	5	149	“high resolution (550-800Mb) GTG-band-based parental karyotype preferable with FISH” “GTG” is superfluous as R-banding (RHG) can also give a good result. Resolution of a karyotype is rated by the number of discernible bands (550 to 800 Bands corresponds to medium to “high” resolution) So, I propose: ” high resolution (550-800 Bands) parental karyotype preferable with FISH”	We adapted the sentence as suggested, as it is the resolution that counts, not the banding method
GenQA	5	152	Change ‘cytogenetic analysis of previous’ to ‘cytogenomic analysis of any previous’	This was corrected in the paper
GenQA	5	168	What are ‘maps’? this doesn’t make sense in English	We changed this to "folders" in the paper.
Raul Piña-Aguilar	5	168	What is the meaning of “maps” in this sentence?	We changed this to "folders" in the paper.
Paul Scriven	5	170	“fluorescence microscope” not “fluorescent”	This was corrected in the paper
Frank Broekmans	6	210	Identification can be done by a written OR a barcode system: it may be important here to note which difference exists in the reliability of these two modes: can the computer improve the level of precision or accuracy of the human brain here? If so do we need to recommend use of a barcode system??	We discussed this comment and decided not to voice our opinion on written versus barcode labelling in the paper.
GenQA	6	222	An extra bullet point	This was removed in the paper
Raul Piña-Aguilar	7	252	What if there is poor signal strength for a probe in the normal partner? How would you know if you didn’t test all probes to be used in each partner? We once predicted by FISH a normal result in a fetus who was trisomy 18 and had ultrasound features of trisomy 18, only to find three 18’s in the metaphase analysis. We were using the centromeric 18 probe, and it turns out that one copy of 18 had a very minimal signal (from the Dad) that we did not see on interphase analysis of amniocytes. I know there have also been interphase FISH misdiagnoses of copies of the X chromosome from FISH due also to population variation in the number of centromeric repeats	The WG has reassessed this statement and decided to modify it stating that it is recommended to perform validation on both partners, but acceptable to perform validation only on the partner who carries the rearrangement.
Susan Bint	7	252	It is not clear that the validation of FISH probes should be carried out on both partners, not just the partner with the chromosome rearrangement.	The recommendation states that indeed, the validation can be performed only on the partner who carries the rearrangement. The WG

				has reassessed this statement and decided to modify it stating that it is recommended to perform validation on both partners, but acceptable to perform validation only on the partner who carries the rearrangement.
GenQA	7	260	Add 'In addition, it is recommended…' As some centres only do metaphases or interphases- not both. It needs to be clear both are needed.	We added "in addition" as suggested
Marie-Laure Maurin	7	227-228	metaphase lymphocytes of the carrier may serve as a control to ascertain that the probes in the hybridization mixture identify the chromosomal regions involved in the chromosomal rearrangement	We corrected this in the paper, now stating "normal or carrier human metaphase lymphocytes"
GenQA	8	274	Add 'latest version' of ISCN.	This was adapted in the paper
Karen Sermon	8	274	Reporting should rely on the ISCN	This was adapted in the paper
Karen Sermon	8	287	If a recombination leads to an unbalanced segregation, then the resulting embryo is also unbalanced and the diagnosis is correct rather than inconclusive or false?	We have discussed this comment and decided that the statement is correct. There is a risk of inconclusive or false results due to biological reasons like unbalanced segregation or chromosomal mosaicism.
Karen Sermon	8	292	How can a FISH result be "below the resolution of the test"?	This was indeed incorrect. We modified it to 'chromosomal imbalance that is unrelated to the test"
Sandrine Chamayou	8	304	Add 'impossibility to detect mosaicism'. Also FISH is a single cell detection method. It is not applicable from trophectoderm cells sample.	We added a statement on mosaicism. TE biopsy for FISH is covered sufficiently earlier in the FISH section, and we decided not to add this as a limitation

1. Preimplantation testing for structural chromosomal rearrangements (PGT-SR) - Array based

Reviewer	Page	Line	Comment	REPLY
Frank Broekmans	9	308	Is this method still available??	We agree that aCGH is been rapidly replaced by NGS. However, still some labs are using it for PGT-A and PGT-SR
Raul Piña-Aguilar	9	318	commercial	We added 'commercial" in the paper
Karen Sermon	9	320	"Oligonucleotides providing a resolution", not "oligonucleotides-providing a resolution"	This was corrected in the paper
Raul Piña-Aguilar	9	326	data are	We have checked and "data is" or "data are" are both correct
Karen Sermon	9	336	Is this correct? Imbalances should always be detected by aCGH, no matter what their origin is.	The paragraph was rewritten based on this and another comment.
Karen Sermon	9	341	Please fix the grammar of the sentence	The sentence was rewritten
Karen Sermon	9	347	Delete "-" between "hybridization" and "efficiency"	This was corrected in the paper
M. Cristina Magli. Luca Gianaroli	9	316-317	Sentence not needed.	We agree with the reviewer and have deleted the sentence
Philippe Gosset	9	318 - 321	This section, of minor importance, will be rapidly outdated.	We agree that aCGH is been rapidly replaced by NGS. However, still some labs are using it for PGT-A and PGT-SR
Joshua Blazek and colleagues	9	333-337	This should specify that polar bodies can be used in the case the structural rearrangement is of MATERNAL origin... this method will not help if the paternal contribution contains the SR	The paragraph was rewritten based on this and other comments.
M. Cristina Magli. Luca Gianaroli	9	333-337	If there is a high risk of misdiagnosis, PB biopsy should not be defined as acceptable.	We have adapted the sentence clarifying that the risk of misdiagnosis is higher. However, PB biopsy is not unacceptable.
M. Cristina Magli. Luca Gianaroli	9	333-340	Nothing is written about the TE biopsy. I understand that it has already been mentioned in the biopsy paper, but the same is true also for PB and blastomere biopsy.	A sentence on TE biopsy was added.

Susanne Knebel	9	335-337	As long as the fragments formed by uneven crossing over are not below the resolution of the array or NGS platform used, they are clearly detectable and the risk of misdiagnosis in polar bodies is not especially increased. Provided that both polar bodies can be analyzed, all unbalanced products of meiotic segregation can be detected so that it is possible to know the contents of the oocyte.	The paragraph was rewritten incorporating this comment
Raul Piña-Aguilar	9	341-342	Re-write: It is recommended to use a WGA protocol that is compatible with the specific aCGH platform on which it has been validated	This was corrected in the paper
Paul Scriven	10	350	550-850 bphs	This was corrected in the paper
Raul Piña-Aguilar	10	350	550 bands? The resolution in cytogenetics is used in bands, not in Mb.	This was corrected in the paper
Päivi Forsblom	10	350	see previous comment (bands instead of Mb)	This was corrected in the paper
Raul Piña-Aguilar	10	354	What about complexity at the breakpoint(s) of a balanced structural rearrangement that results in an incorrect karyotype? It is important considered that PGT platform being used have sufficient coverage to accommodate that. Some rearrangements that appear balanced and are due to chromothripis where there is involvement of a chromosome that is not even known to be present in the apparently balanced rearrangement. Inversions that might distort the cytogeneticist's call on the breakpoints. All of this "stuff" needs to be in a disclaimer from the testing laboratory in terms of limitations of the test.	This section is on documentation, and after assessing the comment and reviewing the section, it was decided to remove this information, as it was not relevant for documentation.
M. Cristina Magli. Luca Gianaroli	10	356	Not all genetic counselling reports specifically recommend PGT. Add "possibly".	We agree with the suggestion of the reviewer and added "possibly" to the sentence.
Karen Sermon	10	363	Delete "e.g. by a corridor"	This was removed in the paper
M. Cristina Magli. Luca Gianaroli	10	372	Delete "only" – redundant.	This was corrected in the paper
Karen Sermon	10	373	Environmental regulation is necessary for hybridisation, not for DNA labelling which is done in a PCR tube. Generally, as all steps are performed within instruments with their own environment, this constant regulation is less stringent than for FISH.	The sentence was rewritten to clarify that the regulation of environmental conditions applies to all steps, rather than the labelling of DNA
M. Cristina Magli. Luca Gianaroli	10	383	Delete the last word "and".	This was corrected in the paper
Karen Sermon	11	404	Change to "cyanine 5-UTP" and "cyanine 3-UTP"	We adapted this to "fluorophore-marked dUTP", based on another comment
Philippe Gosset	11	404	"fluorophore-marked dUTP" rather than "Cyanine-3-UTP and cyanine -5-UTP"?	We adapted this to "fluorophore-marked dUTP", as suggested
GenQA	11	411	Change 'and' to 'plus' so it is clear you need two unique patient identifiers plus the embryo/cell number	This was corrected in the paper
Karen Sermon	11	407 and 383 and 658 and 776	delete "and" – also all the "," at the end of a line in a bullet list	This was corrected in the paper
M. Cristina Magli. Luca Gianaroli	11	407	Delete the last word "and".	This was corrected in the paper
GenQA	12	442	This is 'Internal Quality Control (IQC)' – the guidelines need to clearly differentiate between IQC and External Quality Control (EQA).	This was corrected in the paper

M. Cristina Magli. Luca Gianaroli	13	477	Irrespective of the temperature? This is not our experience with storage at -80°C. Anyway, “extended period of time” should be quantified.	We have adapted the sentence, now reading "• Storage time and temperature have an impact on the integrity of cells, DNA and/or solutions and laboratories should validate that the conditions used in their protocols are fit for purpose. Furthermore, it is not recommended to use repeatedly frozen-thawed solutions containing DNA or enzymes."
Karen Sermon	13	481	Not Cy3?	We adapted this to “fluorophore-marked dUTP", based on another comment.
Paul Scriven	13	509	Not all chromosome rearrangements have four segments, therefore “ … that 3 out of 4 segments for two-way reciprocal translocations are detected …”	This was corrected as suggested by the reviewer, and consistent with changes in other sections.
Raul Piña-Aguilar	13	509	Is there some statistical analysis that supports this? or is it just a gut feeling that everything is likely to be okay if 3 of 4 segments are identified	This was corrected to "3 out of 4 segments for two-way reciprocal translocations" based on another comment.
Karen Sermon	13	528	See 9 336 : Is this correct? Imbalances should always be detected by aCGH, no matter what their origin is.	We have discussed this comment and decided that the statement is correct. There is a risk of inconclusive or false results due to biological reasons like unbalanced segregation, chromosomal mosaicism or embryos of poor morphology:
Paul Scriven	13	501-502	Karyotype reports should be obtained for both partners from an accredited/certified cytogenetics laboratory.	This sentence was added as suggested to the preclinical work-up section
M. Cristina Magli. Luca Gianaroli	14	522-523	Be more specific.	We have clarified that this is about tubing and washing,
Karen Sermon	13	524 and 350 and 603 and 774	If a translocation is visible on G-banding, and G-banding has a lower resolution than aCGH, than it follows that translocation segments visible on G-banding must be visible on aCGH. I therefore do not understand the statement “the potential risk that the actual translocation segments are smaller than expected”	Often the translocation breakpoints are defined based on GTG-banded chromosomes. As the resolution of this technique is quite low, there is a potential risk that the actual translocation segments are (much) smaller than expected, and hence the probability of detection of all unbalanced segregation products of the SR much lower. It was decided, for clarity, not to include this explanation in the text, but we added to the sentence a clarification "based on non-uniform reporting".
Sandrine Chamayou	14	549	A comment on array and mosaicism detection?	A sentence was added stating that aCGH is less sensitive than NGS to detect mosaicism
Raul Piña-Aguilar	14	552	..in DNA from the embryo biopsy	This was corrected in the paper
Philippe Gosset	14	545-549	“Prenatal diagnosis for UPD is strongly recommended in these cases.” I do not agree and it does not respect the conclusions of Kotzot, 2008: “(for) paternal UPD 6 and maternal UPD 7, prenatal UPD testing is questionable. Because of the highly variable phenotype for paternal UPD 11p, maternal UPD 14 and maternal UPD 16, prenatal testing should be discussed critically on an individual basis.”	This sentence was revised and now reads "Prenatal diagnosis for UPD is acceptable but should be assessed critically on an individual basis
Karen Sermon	15	556	“an” should read “one”	This was corrected in the paper
Paul Scriven	15	558	“rearrangements” not “translocations”	This was corrected in the paper
Paul Scriven	15	559	“rearrangements” not “translocations”	This was corrected in the paper

1. Preimplantation testing for structural chromosomal rearrangements (PGT-SR) - NGS

Reviewer	Page	Line	Comment	REPLY
M. Cristina Magli. Luca Gianaroli	15-16	551-563	Why i this section so short, while the one of FISH (poorly used nowadays) is so extended? No risk assessment, no limitations…..	We have extended the section on SNP Array and referred to the PGT-M paper for more information.
GenQA	15	580	includes	This was corrected in the paper

Sandrine Chamayou	16	595	Which resolution do you suggest, accept?	It is impossible to give absolute values for these metrics. Resolution and coverage required for accurate analysis should be defined with validation experiment (see pre-examination process section). This was added in the paper.
Raul Piña-Aguilar	16	595	if partial aneuploidies (deletion and duplications), the specific chromosomal region coverage is required.	We assessed and modified this paragraph, based on this and other comments
Christian Liebst Frisk Toft	16	595	The average read depth might also be a relevant metric.	This was added to the paper, as suggested
M. Cristina Magli. Luca Gianaroli	16	599-600	Same comment as above page 13, line 477. ie Irrespective of the temperature? This is not our experience with storage at -80°C. Anyway, "extended period of time" should be quantified.)	We have clarified short and long-term, and added -80°C
Paul Scriven	16	603	"Karyotype reports at 550-850 bphs resolution for both partners from an accredited/certified cytogenetics laboratory. Often, rearrangement breakpoints …"	This was adapted in the paper
Servi J Stevens	16	603	Line says "a patients' karyotype, preferably at high resolution (550-800Mb)". What is probably meant is "550-800 bands per haploid chromosome set.	This was corrected in the paper
Päivi Forsblom	16	603	see previous comment (bands instead of Mb)	This was corrected in the paper
Philippe Gosset	16	604	"high resolution (550-800Mb) GTG-band-based parental karyotype preferable with FISH" "GTG" is superfluous as R-banding (RHG) can also give a good result. Resolution of a karyotype is rated by the number of discernible bands (550 to 800 Bands corresponds to medium to "high" resolution) So, I propose: " high resolution (550-800 Bands) parental karyotype preferable with FISH"	We have modified the sentence to "- a patients' karyotype, preferably at high resolution (550-800 bands), if available with verified breakpoints from an accredited/certified cytogenetics laboratory"
M. Cristina Magli. Luca Gianaroli	16	609	Same comment as above page 10, line 356. (ie Not all genetic counselling reports specifically recommend PGT. Add "possibly".)	We have adapted the sentence as suggested.
M. Cristina Magli. Luca Gianaroli	17	658	Delete the last word "and".	This was corrected in the paper
Karen Sermon	18	692	For consistency, use the terms "IVF lab controls "and "genetic lab controls" as used in the other documents.	"(I.e. The ivf laboratory negative control)" and "(i.e. The genetic laboratory negative control)" were added to the sentence to make it consistent with the other papers
GenQA	18	695	This is 'Internal Quality Control (IQC)' – the guidelines need to clearly differentiate between IQC and External Quality Control (EQA).	This was corrected in the paper
Karen Sermon	18	708	Normal and abnormal what?	Normal and abnormal samples. This was corrected in the paper.
Karen Sermon	19	723	Gel electrophoresis is recommended (not would be)	This was adapted as suggested
Karen Sermon	19	725	Is it necessary to specify this?	The WG has assessed this comment and feels it is important to keep this statement
Karen Sermon	19	741	Insert a "-": a test-specific threshold	This was corrected in the paper
Raul Piña-Aguilar	19	748	versions	This was corrected in the paper
Karen Sermon	19	753	Delete "-": number of sequence reads	This was corrected in the paper
Paul Scriven	19	755	Not all chromosome rearrangements have four segments, therefore " … that 3 out of 4 segments for two-way reciprocal translocations are detected …"	This was corrected as suggested to "3 out of 4 segments for two-way reciprocal translocations"
M. Cristina Magli. Luca Gianaroli	20	770-771	Be more specific.	We have clarified that this is about tubing and washing,
M. Cristina Magli. Luca Gianaroli	20	776	Delete the last word "and".	This was corrected in the paper

Lauren Walters and colleagues	20	781	Guidelines state that NGS-based technologies cannot detect whole ploidy changes, which is true of current WGS-based approaches. However NGS-based targeted sequencing allow for sufficient depth to call SNPs, which in turn provides information about ploidy. We encourage ESHRE to accommodate more inclusive language that is agnostic to the specific method of NGS-based PGT.	We clarified that these limitations are related to the standard NGS protocols for PGT-SR without genotyping
Christian Liebst Frisk Toft	20	782	NGS is capable of differentiating between balanced and normal results if phasing.	We clarified that these limitations are related to the standard NGS protocols for PGT-SR without genotyping
Frank Broekmans	20	785	For the NGS method, the post processing is crucial: is there any possibility to describe the NGS information post processing? In fact this is briefly described in page 26, sufficient??	The WG has assessed this comment and feels this is sufficiently covered in the text.
Raul Piña-Aguilar	20	755-756	Is there some statistical analysis that supports this, or is it just a gut feeling that everything is likely to be okay if 3 of 4 segments are identified	This was corrected to "3 out of 4 segments for two-way reciprocal translocations" (based on another comment)
GenQA	21	755	Why is it acceptable to reliably detect 3 out of 4 translocation segments for NGS but all four for the other techniques? You may still miss a viable product if the rearrangement is small or involves an acrocentric	This was corrected to "3 out of 4 segments for two-way reciprocal translocations" (based on another comment)

2. Preimplantation testing for numerical aberrations

Reviewer	Page	Line	Comment	REPLY
Raul Piña-Aguilar	21	787	this is confusing, high risk of maternal of aneuploidy is not what is defined here. PGS was based in maternal related aneuploidy. Saying that high risk is for numerical aberrations such Klinefelter is not consistent with PGT organization paper. Who is in high risk of Klinefelter? Patients with Klinefelter and in high risk of sex chromosome aneuploidies, but it is not the right sense of advanced maternal age.	We have rewritten the paragraph avoiding the terms low-risk and high-risk as these are probably confusing.
Raul Piña-Aguilar	21	793	This is incorrect, why qPCR is limited to a small number of samples? qPCR can be run in 96 well or 384 machines that is enough for run more than one sample in multiplex reactions.	We agree with this statement and deleted the comment on the small number in the paper
Lauren Walters and colleagues	21	787-88	If using “high risk” and “low risk” this also involves oocyte’s age and not just whether a person has an SCA themselves. In fact, men with XXY who undergo ICSI are not thought to have a significantly higher risk of having a child with an SCA (PMID 22749222).	We have rewritten the paragraph avoiding the terms low-risk and high-risk as these are probably confusing.
Elena Zakharova	21	790-791	I would like you to consider my comments on this phrase, and here are the reasons why I am asking to revise it: Regardless of PGD, the FISH method has a long history and is an efficient tool for a wide range of examinations. This is considered to be a reliable cytogenetic method, which is applicability is now questioned neither in medicine nor in science (other than by ART specialists). Disadvantages of using FISH and PGT are not due to the method itself, but mostly to the quality of the biopsy material being examined (especially if FISH is performed on 1-2 cells). In this case, the reliability of the study is determined more by the quality and quantity of the material examined than	The WG discussed this extensively but they decided to keep the prior statement. The WG does not state that the other techniques are better in general, but specifically for PGT-A, techniques using 24-chromosome analysis are considered more appropriate. We acknowledge that in some countries FISH is still used frequently, but this is should not be recommended generally.

			by the method (in terms of molecular genetics, FISH is no less reliable than NGS or aCGH methods). The FISH method allows to see not all chromosomes, but only most common aneuploidies. PGT-A (as well as prenatal screening) is carried out to exclude most frequent aneuploidies, and the FISH method, when properly performed, absolutely meets this requirement. And the limited number of chromosomes studied cannot be considered as a reason to ban the FISH method, because of its availability and conservatism. There is a number of publications questioning the relevance of examining all chromosomes for PGT-A. There are the following arguments behind this view: - highly sensitive methods detect spontaneous chromosomal abnormalities occurred in the trophectoderm, an extraembryonic structure with a high mitotic index, which frequently causes a diagnostic error. - there is no consensus about which embryo is “normal” (for example, in the case of mosaicism). NGS and aCGH methods actually have no reliable reference for interpreting the results. -unreasonably high cost of 24 chromosomes testing. Thus, the question of the number of chromosomes examined and the method of might still be open. Although this view is not shared by the members of the PGT consortium, it cannot be ignored. As long as a verified and conservative FISH method is available, the modern methods cannot become the only one recommended. Examination using the NGS and aCGH method is not always available to IVF clinics for the following reasons: - The NGS and aCGH methods is performed in specialized molecular genetic laboratories (outside the IVF clinic), whereas the FISH method is available within the IVF clinic. Thus, it is too early to talk about its general availability. - There are countries where NGS or aCGH is still under examinations, whereas FISH is a certified conventional method. In some cases, this legislative status may play an important role and make us choose a method which is more conventional (FISH). - The cost of NGS is much higher than that of FISH. In countries where the government or the insurance systems is not paying for examination, and all the burden would fall on the shoulders of patients, FISH is an affordable alternative. As there are no strict indication for PGT-A, it might be performed only with the consent and / or if requested by patients themselves. Then the patient’s right to decide on which method to use for PGT-A should not be violated. The patient has the right to make a decision based on the following: - status of the method (under examination or certified conventional method), if determined by local legislation; - cost of examination (if the patient pays for himself); - volume of examination (which determines its cost);	

			- duration of examination (ET in the fresh cycle or using cryopreservation of embryos). Taking into consideration all these arguments, I would suggest to replace this phrase with the one more objectively reflecting the issue in its depth, as the following: "PGT-A can be performed using NGS and aCGH methods or the FISH method. NGS and aCGH methods allow us to determine the aneuploidy of all 24 chromosomes, whereas the FISH method determines only the most frequent aneuploidies. If choosing examination method is not limited by its cost, availability, laboratory policy or local laws, with other things being equal, the PGT consortium recommends to examine all 24 chromosomes using NGS and aCGH methods.“	
Karen Sermon	21		After page 21, the style of writing is different and much easier to read than before.	This should be corrected with proofreading, but we will check the final version
GenQA	22	828	This is ‘Internal Quality Control (IQC)’ – the guidelines need to clearly differentiate between IQC and External Quality Control (EQA).	This was corrected in the paper
Tina Buchholz	22	833	ff chapter quality control: only single cells are mentioned, not pb!	We have added a sentence on preclinical testing in polar bodies
Lauren Walters and colleagues	22	842	Given the inter- and intra-clinic (and even provider) variability in obtaining such samples, it is not always possible to achieve a clean result. Since some samples are very noisy, perhaps a range should be recommended, rather than the specific number of 20%?	The lower threshold is 20-30%, given inter and intra variability, and a good quality result. In case of a noisy experiment, the sample should be reanalysed for a proper diagnosis.
Tina Buchholz	22	843	– 100% is not mosaic	We corrected this to 90% in the paper
Raul Piña-Aguilar	23	866	what means high resolution? Reproseq kit from Thermo Scientific is a low-pass genome approach, with a mean genome coverage of 0.4X	High resolution was deleted in the paper
Susanne Knebel	23	897	`Intracytoplasmatic sperm injection is not necassary as a preventive measure against paternal cell contamination in preimplantation genetic testing´ Oral communication Lynch, C. et al. 18th PGDIS conference, Geneve April 2019 Conference transcript page 49	This comment is correct, and we removed "sperm cells" from the sentence
Karen Sermon	23	897	So you don’t recommend ICSI in the case of PGT-A?	We removed "sperm cells" from the sentence, as suggested in other comments.
Joshua Blazek and colleagues	23	897	Where is the data that show sperm can contaminate a PGT-A result...Generally sperm DNA is tightly packaged and we has been shown it is not amplifiable without extreme measures being taken that are not part of PGT-A protocols.	This comment is correct, and we removed "sperm cells" from the sentence
Tina Buchholz	23	900	chapter Limitations of the test: does not include pbs. If we consider that in one article, then we have to refer to the different tissues clearly!! For example mosaic is not an issue for pb. But we better state that!	It was clarified in the sentence that this refers to mosaicism inferred from a multi-cell TE biopsy, rather than post zygotic mosaicism can always lead to the embryo being different from the biopsied material.
Lauren Walters and colleagues	23	900	Methods that employ SNP-based algorithms can detect polyploidy and haploidy, including NGS-based targeted sequencing.	We clarified that these limitations are related to the standard NGS protocols for PGT-SR without genotyping
Joshua Blazek and colleagues	23	891-892	Cite scientific literature here that shows this impacts ploidy status in a PGT-A assay...	It was decided upfront not to include references other than references of other guidance paper.
GenQA	24	909	Could you please give an estimate of the level of mosaicism detected for aCGH in this sentence too.	This sentence was adapted and levels for mosaicism were added.
Lauren Walters and colleagues	24	909	Once again, given the different laboratories, methodologies and variety of external variables, a range for mosaicism detection 20% may be preferable.	This sentence was adapted and levels for mosaicism were added

Päivi Forsblom	24	914	Not all NGS platforms can be used to identify the nature and/or parent-of-origin of an aneuploidy even if phasing references are available. Suggestion: "whereas some NGS platforms can"	we changed the sentence to highlight that genotyping-based NGS can detect this
Alan H Handyside	21	788	Is there any good evidence that Klinefelters or other sex chr abnormalities are at increased risk of aneuploidy in embryos? Perhaps a review of recent papers is needed. What about men with extreme forms of oligospermia etc?	In this introductory sentence, we decided to stick to genetic indications and not further expand. The paragraph was slightly adapted based on other comments.
Alan H Handyside	21	797	Array CGH has almost disappeared after the withdrawal of 24Sure. Is this section still relevant? I realise there is an Agilent array so not attempting to be biased here. But in other places you have made a decision to focus the guidelines on the main methodologies.	We agree that aCGH is been rapidly replaced by NGS. However, still some labs are using it for PGT-A and PGT-SR

3. Strengths and limitations

Reviewer	Page	Line	Comment	REPLY
Joshua Blazek and colleagues	24	926	Believe this statement to be inaccurate. How does one define workflow? Would argue that running a SNP array and subsequental aCGH array from the same product (PGT-A + PGT-M) can be constituted as a "workflow" and that this is the most widely used workflow for this methodology in the world. Should remove this statement.	We changed the word "workflow" to "experiment"
Lauren Walters and colleagues	24	931	Using NGS-based FAST-SeqS, it is possible to utilize the SNP profile to identify uniparental isodisomy (but not heterodisomy) same as with a SNP-based array like Karyomapping.	We adapted this to "NGS (without genotyping)"
Tina Buchholz	24	920-931	ff chapter 3. Strengths and limitations: pbs not mentioned, neither are oocytes. The chapter deals with embryos, but that is not enough.	We added a few statements on pbs, where relevant
Joshua Blazek and colleagues	24	928-930	How do they define therapeutic here? Suppose a 30 yr old presenting with infertility does an IVF cycle with PGT-A, has 8 embryos and all come back aneuploid. Follow up parent of origin testing reveal significant aneuploidy of paternal origin. Based on this information the patient can now choose the best path forward (sperm donor etc.) that will allow her to conceive.	We removed the statement on the importance of knowing the parental origin.
Servi J Stevens	25	932	Table 1, row 8, risk of misdiagnosis in FISH: consider mentioning “incomplete nucleus, or presence of nuclear fragments”.	We adapted this as suggested
Christian Liebst Frisk Toft	25	932	Grant SNP array its own column. Since the techniques and possible results are different between aCGH and SNP arrays, they should be separated. As example, aCGH cannot detect UPD, which is possible using SNP array if phasing.	We have discussed this, but we feel SNP array is sufficiently covered in the PGT-M paper and sufficiently referred to in the PGT-A/SR paper.
Caio Graco Bruzaca	25	932	ABNORMALITIES NOT DIAGNOSED – the NGS technology may not be useful to identify the difference between normal and balanced embryos, according to the manuscript can be confound the reader, as well, the clinician may think that all NGS based could identify the difference.	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table
Caio Graco Bruzaca	25	932	UNIPARENTAL DISOMY (UPD) – how using NGS based can be used for identify uniparental disomy (UPD)? The way it was written could confound the reader.	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table
Raul Piña-Aguilar	25	NGS - UPD	Only if SNPs are analyzed and major commercials kits do not include SNPs	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table

Raul Piña-Aguilar	25	NGS- abn not diagnosed	This is not accurate, the two major commercial kits for from NGS (Veriseq from Illumina/Vitrolife, Reproseq from Thermo) do not include SNPs. Both analysis software BlueFuse (for embryos) and Ion Reporter cannot peform SNP analysis. Theoretically, phasing with parental samples if SNV coverage is good is possible but it is not in use and misguide the reader and non-geneticists professionals.	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table
Sandrine Chamayou	25	Ngs colomn	What do you mean by 'phasing reference' (abnormalities not diagnosed)	This term is explained in the glossary. However, based on other comments, the term 'phasing references" was removed from the table.
Raul Piña-Aguilar	25	NGS- origin of aneuploidy	This is currently only possible with SNP microarrays, and this table do not include them. Main NGS kits Veriseq and Reproseq cannot diagnose the origin, because they do not include SNPs. Actually the largest published data about origin from aneuploidy is from SNPs arrays (PLoS Genet. 2015;11(10):e1005601).	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table
Raul Piña-Aguilar	25	table 1	SNP-microarray is missing in Table 1. However it is included in Figure 2 of PGT-M paper	We have discussed this, but we feel SNP array is sufficiently covered in the PGT-M paper and sufficiently referred to in the PGT-A/SR paper.
Tina Buchholz	25	table 1	– same: does not include specific issues for pbs	We clarified in the table that the information present is based on cleavage-stage or blastocyst biopsy. It was decided not to expand further for PB in the table.
GenQA	15 & 25	563 & 932	As this a SNP array UPD should be detectable. This section does not state this nor does Table 1.	We adapted this to "NGS (without genotyping)" in the heading of the table to make it more clear for the reader, and removed referral to the more elaborate testing throughout the table

4. Examination process

Reviewer	Page	Line	Comment	REPLY
GenQA	27	982-996	As an EQA provider we see many PGT reports and many do not have sufficient information for a clinical geneticist/clinician to counsel the patient. The following must be included: Parental karyotype of both parents in pre-clinical and the PGT report; the size of the abnormality in MB (array & NGS); a summary of the results for each embryo in ISCN/HGVS {indicating the chromosome involved, chromosome band/nucleotides, quantifying the gain or loss (e.g. x3)}; a brief written description of the result (in case the nomenclature is unclear).	We have adapted the section on the report in the Organisation paper according to this and other comments
Tina Buchholz	26	961	this point is the only few lines to try to explain the specific characteristics of pbs. But this is not understandable, does not explain the major difference in the interpretation of results for pbs and embryo cells. The solid and clear differentiation between chromatids and chromosomes is not addressed.	We have rephrased the sentences to make it more clear to the reader.
Joshua Blazek and colleagues	26	969-972	Are we really recommending embryos for transfer. More so we are providing with a piece of information to help them make their transfer recommendation.	We removed the section on recommending for transfer. Furthermore, after reassessing the sentence, it was deleted as with aCGH and NGS, uninterpretable or inconclusive results due to lack of consensus are rare.
Lauren Walters and colleagues	27	995	Genetic counseling AND appropriate parental studies if indicated (such as karyotyping or metaphase FISH).	We corrected "monitoring" to "follow-up". It was decided not to further expand on this, as this is covered in the ORG paper. A reference to this paper was added

M. Cristina Magli. Luca Gianaroli	27	980	“can be recommended for transfer” change to “can be considered for transfer after discussion with the patients”.	We adapted the sentence as suggested