OMS in FISH-PGD procedures

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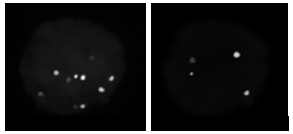
PGD - aneuploidy screening



Counseling

- **Qualified physician**
- **Inclusion criteria for PGS (indications)**
  - **Recurrent miscarriage**
  - **Repeated implantation failure**
  - **Advanced maternal age**

Aneuploidy screening

At least 5 probes
Standard 8 probes (13, 15, 16, 18, 21, 22, X, Y)

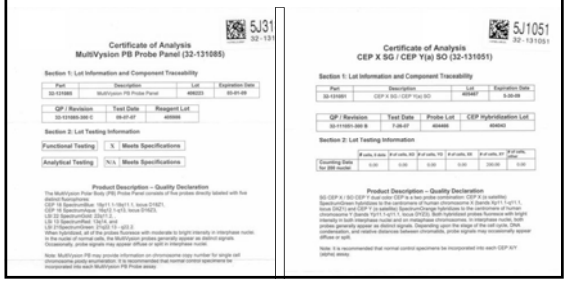
MultiVysion PGT (Abbott)
- **13 Spectrum Red**
- **18 Spectrum Aqua**
- **21 Spectrum Green**
- **X Spectrum Blue**
- **Y Spectrum Gold**

MultiVysion PB (Abbott)
- **13 Spectrum Red**
- **16 Spectrum Aqua**
- **18 Spectrum Blue**
- **21 Spectrum Green**
- **22 Spectrum Gold**

- **CEPX/Y**
- **CEP15**

PGS Probe testing

- **Commercial probes come with quality control and validation**


Certificate of Analysis
MultiVysion PB Probe Panel (32-131085)

Section 1: Lot Information and Component Traceability

Part	Description	Lot	Expiration Date
32-131085	MultiVysion PB Probe Panel	406223	03-01-09

QP / Revision	Test Date	Reagent Lot
33-131085-300 C	06-07-07	405566

Section 2: Lot Testing Information

Functional Testing	X	Meets Specifications
Analytical Testing	N/A	Meets Specifications

Product Description – Quality Declaration

Certificate of Analysis
CEP X SG / CEP Y(a) SO (32-131051)

Section 1: Lot Information and Component Traceability

Part	Description	Lot	Expiration Date
32-131051	CEP X SG / CEP Y(a) SO	405467	5-30-09

QP / Revision	Test Date	Probe Lot	CEP Hybridization Lot
32-111051-300 B	7-26-07	404469	404043

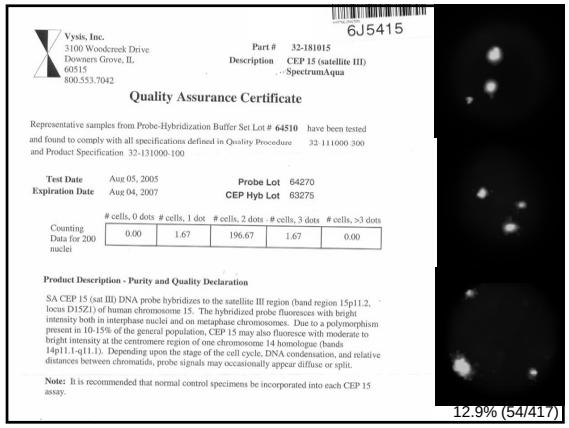
Section 2: Lot Testing Information

	# of cells, X dots	# of cells, XO	# of cells, YQ	# of cells, XX	# of cells, XY	# of cells, other
Counting Data for 200 nuclei	0.00	0.00	0.00	0.00	200.00	0.00

Product Description – Quality Declaration

PGS Probe testing

- **Pre-cycle work-up on individual couples**
  - **Probes with very low polymorphism rate are acceptable to use without testing**
  - **Probes with common occurrence of polymorphism**
    - **chromosome 15 satellite III probe (D15Z1)**
      - testing on uncultured lymphocytes from both reproductive partners
    - **chromosome Y satellite III probe (DYZ1, Yq12)**
      - testing on male partner's uncultured lymphocytes



6J5415

Vysis, Inc.
3100 Woodcreek Drive
Downers Grove, IL
60515
800.553.7042

Part # 32-181015
Description CEP 15 (satellite III)
SpectrumAqua

Quality Assurance Certificate

Representative samples from Probe-Hybridization Buffer Set Lot # **64510** have been tested and found to comply with all specifications defined in Quality Procedure 32-111000-300 and Product Specification 32-131000-100

Test Date Aug 05, 2005
Expiration Date Aug 04, 2007
Probe Lot 64270
CEP Hyb Lot 63275

	# cells, 0 dots	# cells, 1 dot	# cells, 2 dots	# cells, 3 dots	# cells, >3 dots
Counting Data for 200 nuclei	0.00	1.67	196.67	1.67	0.00

Product Description - Purity and Quality Declaration

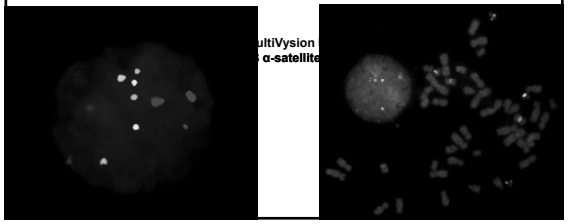
SA CEP 15 (sat III) DNA probe hybridizes to the satellite III region (band region 15p11.2, locus D15Z1) of human chromosome 15. The hybridized probe fluoresces with bright intensity both in interphase nuclei and on metaphase chromosomes. Due to a polymorphism present in 10-15% of the general population, CEP 15 may also fluoresce with moderate to bright intensity at the centromere region of one chromosome 14 homologue (bands 14p11.1-q11.1). Depending upon the stage of the cell cycle, DNA condensation, and relative distances between chromatids, probe signals may occasionally appear diffuse or split.

Note: It is recommended that normal control specimens be incorporated into each CEP 15 assay.

12.9% (54/417)

Suspected rare polymorphism

- **Reanalysis of blastomeres using telomeric probes**
- **Contemporaneous testing of polymorphism type on parents' lymphocytes**



Biopsy and fixation

- **Multinucleated or anucleated blastomeres are not suitable**
- **1-2 cell biopsy**
- **Fixation**
  - **acetic acid/methanol**
  - **HCl/tween**
  - **HCl/tween and acetic acid/methanol**

Localization of nuclei on slide

- **Recording positions in phase contrast**
- **Computer-controlled motorized stage (motion controller based on stepping motors)**

FISH

- **Many variations in FISH methods have been published and all appropriately validated methods are acceptable**
- **Better to avoid pretreatment (pepsin and paraformaldehyd)**
- **Verification of denaturation, hybridization and wash temperatures**
- **Instruments should be calibrated periodically**

Scoring

- **Scoring criteria should be established**
- **Two independent scorers**
- **All single cell images should be captured**
- **Multinucleated blastomeres are not suitable for PGD because the number of chromosomes in each nucleus varies greatly**
- **Binucleated blastomeres**
  When both nuclei are chromosomally normal the remainder of the embryo is probably also normal
- **"No result rescue" is recommended to reduce the number of cells with dubious results**
  - **reanalyzing with a probe binding to the same chromosome but to a different locus**

name; protocol No.:

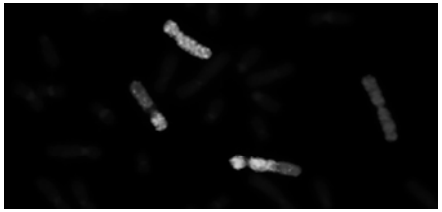
	chromosomes								
blastomere	13	22	21	16	18	15	X	Y	note
1a	2	2	2	2	1	2	1	1	
1b	2	2	2	2	1	2	1	1	
2a	2	2	2	2	2	2	2	0	
2b	2	2	2	2	2	2	2	0	
3a	2	2	2	1	2	1	1	1	
3b	2	2	2	2	2	1	1	1	
4a-1	2	2	1	2	1	1	1	0	
4a-2	0	0	1	0	1	1	1	0	
4b	2	2	2	2	2	2	2	0	
5a	2	2	2	2	2	2	2	0	
5b	2	2	2	2	2	2	2	0	
6									anucleated blastomere
7	1	2	1	1	2	2	2	0	
8	2	2	2	2	2	2	1	1	

Reanalysis of spare embryos

- **Total error rate <10%**

Interlaboratory tests

PGD for translocations



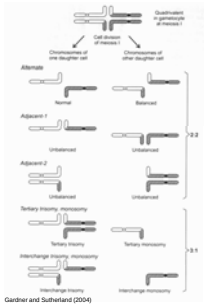
Translocation carriers

- **Neonatal popul.** **rcp t** **0.2%**
  rob t **0.1%**
- **Infertile couples** **0.6%**
- **IVF failure** **3.2%**
- **Recurrent abortions** **9.2%**

Reproductive risks

- **Spontaneous abortions**
- **Affected child**
- **Infertility**

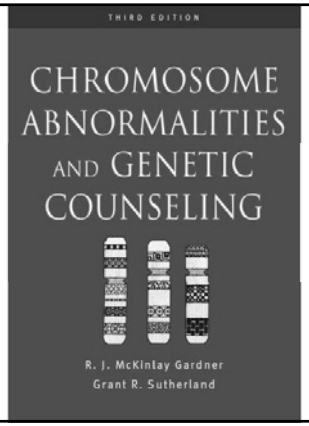
Gardner and Sutherland (2004)


Gardner and Sutherland (2004)

Formation of complexes of translocated chromosomes and malsegregation of these complexes

Counseling

- **Qualified physician**
- **Inclusion criteria for PGD**
  - **High recurrence risk at conception for a specific genetic disorder (e.g. >10% for chromosomal rearrangements)**
- **PGD for translocations can be offered as an alternative to prenatal diagnosis and pregnancy termination of unbalanced fetuses**



Oxford University Press 2004

Sperm analysis as a prognostic tool

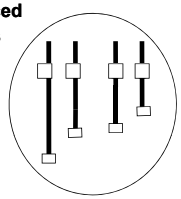
- **Pregnancy rate is inversely proportional to the number of abnormal gametes (embryos)**
- **Unbalanced sperm in carriers**
  Rcp t 19 - 81% (40 – 70% in our laboratory)
  Rob t 3 - 36% (4 – 24% in our laboratory)
- **Patients with 65% or fewer chromosomally abnormal spermatozoa have a good chance of conceiving.**
- **Patients with higher rates will have to produce 10 or more good quality embryos to have a reasonable chance of conception.**
- **More embryos needed than for aneuploidy screening**

Probe selection

- **Appropriately qualified personnel**
- **Breakpoint spanning probes**
  - **differentiate between unbalanced, balanced and normal**
  - **QC, QA, validation**
  - **time-consuming, expensive**
- **Commercial probes**
  (centromeric, subtelomeric, locus specific)

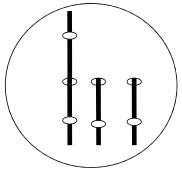
Probe selection
Reciprocal translocations

- **Proximal (centromeric, satellite)**
- **Distal (subtelomeric)**
  differentiate between unbalanced and balanced/normal embryos
  - **3 probes**
    - **detect all unbalanced embryos**
  - **4 probes**
    - **detect all unbalanced embryos**
    - **more robust design**



Probe selection
Robertsonian translocations

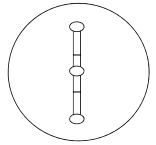
- **Chromosome enumeration probes**
  telomeric, locus specific, ...
  - **2 probes detect all aneuploid embryos**
  - **differentiate between unbalanced and balanced/normal embryos**
- **Combining with aneuploidy screening**
  - **aneuploidy screening panel: chr. 13, 15, 21, 22**
  - **telomeric probe: chr. 14**



Probe selection
Pericentric inversions

- **2 probes distal to the breakpoints (telomeric), preferably in combination with centromeric probe**

differentiate between unbalanced and balanced/normal embryos



Manufacturers

- Abbott (Vysis)
- CytoCell
- Kreatech Diagnostics

- It is possible to combine probes from different manufacturers

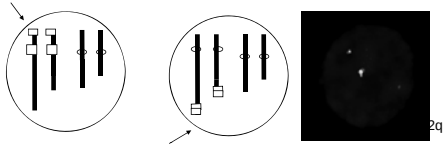
- fluorochromes
  - green
  - red (Cytocell, red filter)
  - red (Kreatech, Abbott, gold filter)
  - green

Probe testing

- It is necessary to assess each probe combination prior to clinical treatment (probe validation)
  - on metaphase chromosomes
    - informativeness (ability to detect unbalanced rearrangements)
  - on interphase cells
    - quantitative assessment of the assay and qualitative assessment of FISH signal intensity and discreteness
    - efficiency of 95% for individual probe
    - FISH efficiency is higher in blastomeres than in lymphocytes

No result rescue

- Subtelomeric probe from non-translocated segment to verify centromeric probe



- Subtelomere probe specific for different locus (from another manufacturer)

Prenatal diagnosis

- **Prenatal diagnosis testing to confirm the results of PGD is strongly encouraged because PGD have technical limitations that include the possibility for a false negative result**

rob(13;14)
uncultured
amniocytes
TEL13q TEL14q