A rapid and reliable chromosome analysis method for products of conception using interphase nuclei

InteGen LLC
Interphase Chromosome Profiling

Ramesh Babu, Ph. D[1]; Daniel L. Van Dyke, Ph. D[2]; Saurabh Bhattacharya, Ph. D[3]; Vaithilingam G. Dev, Ph. D[4]; Mingya Liu, MS[4]; Minjae Kwon, MS[4]; Guangyu Gu, M.D.[4]; Prasad Koduru, Ph. D[5]; Nagesh Rao, Ph. D[6]; Cynthia Williamson, BS[2]; Ernesto Fuentes, BS[1]; Sarah Fuentes, BS[1]; Stephen Papa, BS[1]; Srikanthi Kopuri, MS[1] and Vandana Lal, M.D.[7]

[1]Department of Research and Development, InteGen LLC, Orlando, FL 32819; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55902; [3]Department of Cytogenetics, Dr. Lal PathLabs Ltd., Rohini, New Delhi 110085 India; [4]Department of Clinical Cytogenetics, Genetics Associates Inc., Nashville, TN 37203; [5]Department of Pathology, UT Southwestern Medical Center, Dallas, TX 75390; [6]Department of Pathology and Laboratory Medicine, David Geffen UCLA School of Medicine, Los Angeles, CA 90024; [7]Department of Administration, Dr. Lal PathLabs Ltd., Rohini, New Delhi 110085 India

ABSTRACT

Background: Karyotype determination has a central role in the genetic workup of pregnancy loss, as aneuploidy and polyploidy are the main causes. Current methods of obtaining a karyotype using traditional cytogenetics, FISH with a limited number of probes, and CMA have limitations of culture failure, incomplete results, lower sensitivity, and longer turnaround time. **Methods:** A Standard Resolution Interphase Chromosome Profiling (ICP) probe set, a variation of a recently developed High Resolution probe set (Babu et al., in press), was developed that targets only the subtelomere and paracentromeric regions. Abnormalities commonly encountered in POC samples, such as trisomy and unbalanced translocations, can be easily detected with this design. To detect Robertsonian translocations, each acrocentric chromosome's paracentromeric area is targeted and a mixture of these five targets is used in a separate analysis. First, initial familiarization of signal patterns from the probe set was achieved, followed by validation of this method using 83 blind samples from miscarriages from three different laboratories. Finally, the clinical utility of the method was tested on 291 samples in two commercial reference laboratories on two different continents. A minimum of 20 interphase cells were analyzed for each chromosome to identify an abnormal clone. For detection of Robertsonian translocations, a normal cutoff was set at 20% for any two paracentromeric probes to co-localize by random chance or by satellite association. **Results**: All 374 samples had results by the ICP method and the average turnaround was less than 48 hours. This new molecular approach not only detected all the chromosomal changes observed by current methods, but also significantly improved the abnormality detection by characterizing derivative chromosomes and finding subtle subtelomeric deletions (Xq and 17q) and a balanced translocation involving 2q and 10q. All Robertsonian translocations were also detected. Abnormalities detected only by ICP included trisomy 4, 7, 8, 14, 15, 16, 19, 20, and 22. The abnormality rate was 54% on clinical samples from commercial lab 1 and 63% from lab 2. **Conclusion:** The attributes of this method make it an ideal choice for the genetic workup of miscarriages, namely 1) near 100% successful results, 2) greater sensitivity than conventional chromosome analysis or FISH panels, 3) rapid reporting time, and 4) favorable comparisons with chromosomal microarray.

ILLUSTRATIONS

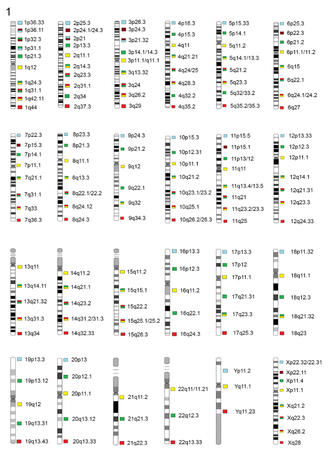

Fig 1. Illustration of the High Resolution ICP ideogram at approximately 600 band level showing each color band and its corresponding ISCN band designation.

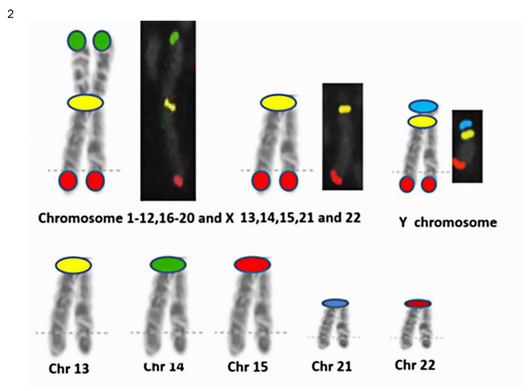

Fig 2. The top row illustrates a typical metacentric chromosome with green and red signals in the distal short and long arms, respectively, and a yellow pericentromeric signal. The Y chromosome (far right) short arm has an aqua signal, and the acrocentric chromosome has no short arm signal. The bottom row illustrates the distinct pericentromeric signal for each acrocentric chromosome, which is essential to identify a Robertsonian translocation. The chromosome 13 signal is yellow, 14 is green, 15 is red, 21 is aqua, and 22 is far red.

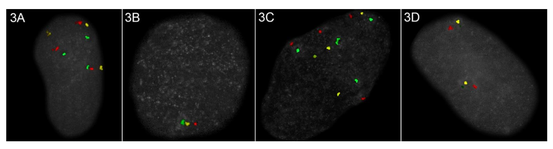

Fig 3. A) Trisomy with three green, yellow, and red signals; B) Monosomy with only one green, yellow, and red signal. C) Tetrasomy with four of each signal. A similar pattern for the other autosomes would indicate a tetraploid conceptus; D) A typical normal diploid signal pattern.

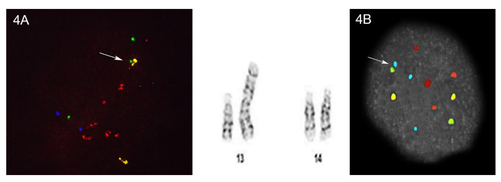

Fig 4. Using the Acrocentric ICP probe set (see Figure 2), image 4A has two red, two aqua and two far red signals indicating normal pairs of chromosomes 15, 21, and 22. There is one free yellow signal and two free green signals for chromosomes 13 and 14. There is also one consistently fused yellow and green signal, representing an unbalanced 13;14 translocation. Trisomy 14 is evident by the G-banded chromosomes displayed to the right. Shown in the upper left of image 4B is the consistent fusion of a green chromosome 14 signal and a third aqua chromosome 21 signal, indicating the translocation trisomy 21.

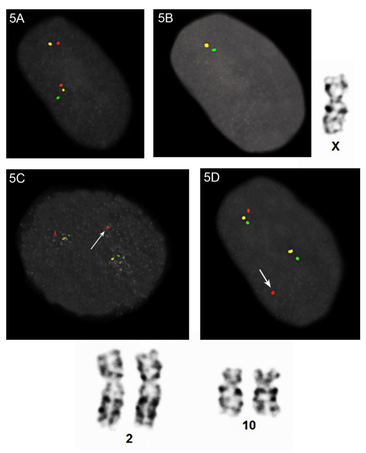

Fig 5. A) Short arm deletion of one chromosome 9 illustrated by the absence of green signal on the chromosome on the top (left panel); B) Deletion of the long arm telomere region on the only X chromosome by the absence of the red signal (middle panel); in the far right is a metaphase chromosome X from the same case presented in the middle panel. The deletion was not evident by G-banding; C) and D) A balanced translocation between chromosomes 2 and 10. Displacement of the red signals from the long arms of chromosomes 2 (C) and 10 (D) are indicated by the arrows. Partial karyotypes of chromosomes 2 and 10 from the same case in C and D are shown below. The translocation was not evident by G-banding.

ILLUSTRATIONS (continued)

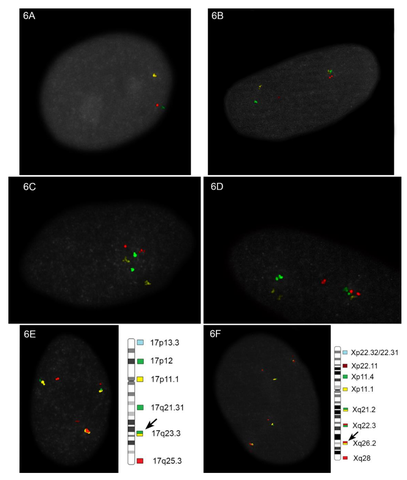

Fig 6. A) Monosomy X (compare with Figure 3B); B) 17q deletion with the constant dim red signal on one chromosome 17; C and D) Normal chromosome 17 pairs from a different case (C) and a different cell from the same case shown in A (D); E) Chromosome X and 17 hybridized together with unique tags on each chromosome showing monosomy X and 17q deletion. Green/yellow tag (at band 17q23.3) tracks chromosome 17 q telomere and red/yellow (at band Xq26.2) tag tracks X chromosome long arm telomere; F) A normal cell with same tags as in E. Tag locations are shown by solid arrows on the corresponding high-resolution chromosome ideograms.

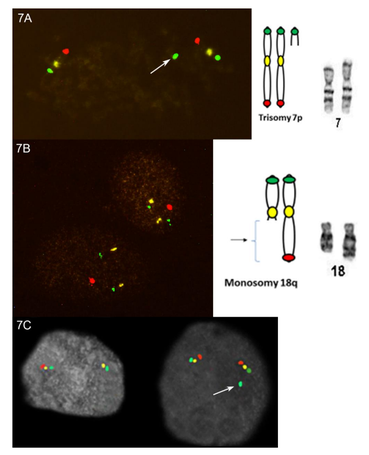

Fig 7. This case was originally described as an add(18). By using ICP, it was further defined as an unbalanced translocation between chromosomes 7 and 18 with duplication 7p and deletion 18q. A) Chromosome 7 showing the extra green band; B) Loss of one red signal on chromosome 18; C) An unbalanced translocation between chromosomes 1 and 11 showing loss of red signal on chromosome 11 (left) and gain of green signal from chromosome 1 indicated by arrow (right).

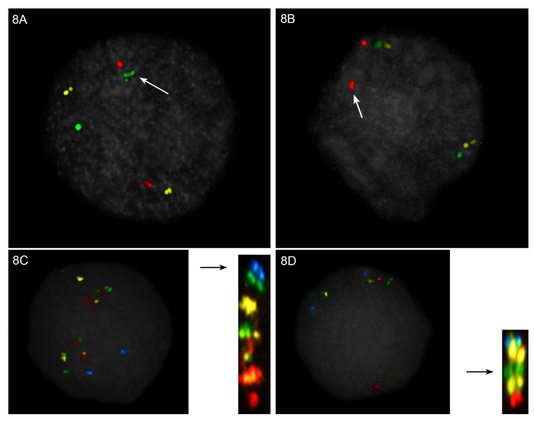

Fig 8. Precise determination of translocation breakpoints. A) Initial breakpoint assignment between centromere and the short arm telomere on chromosome 12; B) Initial breakpoint assignment between centromere and the long arm telomere on chromosome 18; C and D) Breakpoint clarification as 12p13 and 18q12, respectively, using the High Resolution ICP probe set. Arrows on the reference metaphase chromosomes in the middle point out the exact breakpoints.

MATERIALS AND METHODS

Fluorochromes: DEAC (aqua), Fluorescein-12 (green), Cyanine555 (yellow), Cyanine647 (far red), and CF594 (red)
Chromosomal targets: sub-telomere and centromere/pericentromeric regions
Number of interphase cells analyzed for each target: 20
Hybridization scheme: as per figure 2
Normal cut-off value: 20%
Total samples studied – initial validation: 83; clinical study: 291
Hybridization time: Overnight
Filter cube source: Semrock Inc.

RESULTS

Total clinical cases: 291
Total abnormalities: 175 [Numerical: 154; Structural: 21]
Trisomy detected only by ICP (48 cases): Chromosomes involved - 4, 7, 8, 14, 15, 16, 19, 20, and 22
Structural abnormalities: Deletions (7), Balanced translocations (4), Unbalanced rearrangements including "add" and "mar" (9), Robertsonian translocations (8)

CONCLUSION

Standard Resolution ICP appears to be an appropriate tool for first line or reflex testing in the genetic workup of POC samples. Results of this study have confirmed that ICP is 1) highly reliable, 2) more sensitive than the traditional FISH approach using a limited number of probes, 3) capable of detecting both numerical and gross structural aberrations including characterization of "add" material in the derivative chromosomes, and 4) does not require cell culture, which allows a faster reporting time. As with microarray, karyotype analysis, and FISH panels, results of Standard Resolution ICP studies will assist in genetic counseling for recurrence risks of aneuploidy, polyploidy, and balanced and unbalanced chromosome rearrangements.

REFERENCES & ACKNOWLEDGEMENTS

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Please contact us at rbabu@integenllc.com
www.integenllc.com

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