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ORIGINAL ARTICLE

BACs-on-Beads™ assay, a rapid aneuploidy test, improves the diagnostic yield of conventional karyotyping

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Abstract

BACs-on-Beads (BoBs™) assay is a rapid aneuploidy test (RAT) that detects numerical chromosomal aneuploidies and multiple microdeletion/microduplication syndromes. This study was conducted to appraise the usefulness of the BoB™ assay as a complementary diagnostic tool to conventional karyotyping for the rapid detection of chromosomal aneuploidies. A total of 485 prenatal (amniotic fluid and chorionic villi) and blood/products of conception samples were collected between July 2013 and August 2018, and analyzed by the BoBs™ assay and cytogenetic karyotyping and further validated by fluorescence in situ hybridization (FISH). Forty-three of 484 qualifying samples (8.9%) were identified as abnormal by the BoBs™ assay. The assay was comparable to karyotyping in the detection of common structural abnormalities (trisomy 21, trisomy 18, X, and Y), with a sensitivity of 96.0% and a specificity of 100%. BoBs™ assay detected 20 microdeletion and microduplication syndromes that were missed by karyotyping. BoBs™, however, missed 10 cases of polyploidies and chromosomal rearrangements which were identified by conventional karyotyping. Our findings suggest that BoBs™ is a reliable RAT which is suitable in combination with conventional karyotyping for the detection of common aneuploidies. The assay also improves the diagnostic yield by recognizing clinically relevant submicroscopic copy number gains and losses.

Keywords BACs-on-Beads™ · Karyotype · Aneuploidies · Microdeletions · Microduplications

Introduction

Fetal chromosomal analysis using G-banding karyotyping has traditionally been considered the gold standard detection method for aneuploidies and large chromosome rearrangements (~5 Mb), namely inversions, translocations, duplications and deletions [1–4]. Although characterized by very high accuracy and specificity [1, 2, 4], karyotyping is a time- and labor-consuming methodology that requires fetal cells to be cultured in vitro for about 2 weeks prior to analysis [2, 5, 6]. The technique also suffers a limited resolution capacity preventing the identification of chromosomal microdeletions and microduplications [5, 7].

Because timely diagnosis of fetal aneuploidies is highly desirable for a prompt medical decision making and for reducing couple anxiety, complementary rapid aneuploidy tests (RATs) have been largely explored [3, 8, 9]. RATs are often less costly alternatives to conventional karyotyping [10–12], and include quantitative fluorescence-polymerase chain reaction (QF-PCR), fluorescence in situ hybridization (FISH), and multiplex ligation-dependent probe amplification (MLPA) [3, 8, 10, 13]. These methods nonetheless allow the detection of whole chromosome aneuploidy for a limited number of chromosomes (13, 18, 21, X and Y). Expanding the range of chromosome detection may mean the introduction of additional molecular reactions and consequently could imply a substantial increase in cost [5, 8, 10, 11, 14]. To overcome the limitations of available RATs [8, 10, 11, 14], chromosomal microarray analysis (CMA) was introduced as a molecular tool for the genome-wide

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identification of submicroscopic abnormalities [15–18]. CMA nonetheless was deemed to be a costly method with doubtful outcome when copy number variations of unknown pathogenic significance are detected [15–18]. Accordingly, the technical limitations of conventional karyotyping and RATs, as well as the diagnostic uncertainties associated with CMA, have led to the development of the bacterial artificial chromosomes (BACs)-on-Beads (BoBs™) assay [7, 19–21].

BoBs™ is a newly developed molecular diagnostic technique consisting of a bead-based multiplex assay using microspheres with two distinct fluorochromes of variable concentrations to create an array of more than 100 different unique probes. Each probe is derived from DNA amplified from bacterial artificial chromosomes, allowing for the rapid detection of chromosomal abnormalities [19, 21, 22]. Each bead contains several copies of the same BAC adherent to its surface. Five independent BACs-on-Beads probes are included for chromosomes 13, 18, 21, X and Y. Targeted BoBs™ assay not only detects common aneuploidies of chromosomes 13, 18, 21, X and Y, but it also covers a broad set of microdeletions and microduplications in the regions causing the following syndromes: Wolf-Hirschhorn, Cri du Chat, Williams–Beuren, Langer–Giedion, Prader–Willi/Angelman, Miller-Dieker, Smith-Magenis, and Di-George [7, 19, 21]. Four to eight probes are available for each of the nine well-characterized microdeletion critical regions.

In this study, we compared the results of the BoBs™ assay for 485 samples with those obtained by conventional karyotyping, seeking to (i) calculate the parameters of diagnostic accuracy, (ii) estimate the additional diagnostic yield in prenatal and blood/products of conception (POC) samples, and (iii) evaluate the benefits and limitations of this technology with respect to conventional karyotyping.

Materials and methods

Study design and sample selection

Between July 2013 and August 2018, the Medical Genetics Laboratories at the American University of Beirut Medical Center performed genetic testing on 485 samples including amniotic fluid, chorionic villi, POC, and blood. Samples were collected and transported at room temperature. Each sample was then divided into two aliquots: The first was processed for DNA extraction either immediately after reception or after an overnight storage at 4 °C. Genomic DNA was extracted and purified from samples using QIAamp DNA mini kit (Qiagen, Inc., Germany) according to manufacturer's recommendations. The other was processed then cultured immediately for 10–14 days with 5% CO_2 at 37 °C under sterile conditions for conventional karyotyping.

Each sample underwent chromosomal analysis using two diagnostic modalities: targeted BoBs™ assay and conventional karyotyping. Chromosomal findings with BoBs™ were validated by conventional karyotyping for common chromosomal aneuploidies and FISH for submicroscopic structural abnormalities. In the case of prenatal samples, maternal cell contamination testing was performed utilizing multiplex-PCR based small tandem repeat (STR) genotyping.

BACs-on-Beads™ technique (PerkinElmer®, BoBsoft® 2.0)

Following extraction, genomic DNA was amplified with a primer solution, labeled by enzymatic incorporation of biotinylated nucleotides, and purified using a purification plate for biotinylated DNA. It was then hybridized to BACs-on-Beads probes by overnight incubation as per manufacturer's protocol, washed and bound to the reporter molecule and then washed again. Thereafter, the fluorescent signals were measured using a Luminex xMAP cytometric acquisition system (Luminex Corp., Austin, Texas) equipped with BoBsoft® 2.0 software technologies (PerkinElmer®, Wallac Oy, Turku, Finland) for fluorescence data analysis [19]. Experiments passing quality control had more than 100 beads/BACs analyzed alongside male and female reference DNAs that were obtained from normal patients tested in our lab. Test sample analyses were performed in singletons and reference samples were analyzed in duplicates. A sample was labelled as "duplicated" or "deleted" in a chromosome locus when single copy gains and losses generate fluorescence ratios ranging from 1.3 to 1.4 and from 0.6 to 0.8, respectively.

The targeted BoBs™ assay was designed for the detection of aneuploidies of chromosomes 13, 18, 21, X and Y, in addition to gains and losses of DNA in chromosomal regions associated with the following nine microdeletion syndromes: Di-George syndrome region (22q11.2, 10p14), Wolf-Hirschhorn syndrome (4p16.3), Cri du Chat syndrome region (5p15.3-p15.2), Williams–Beuren syndrome region (7q11.2), Langer–Giedion syndrome region (8q23-q24), Prader–Willi/Angelman syndrome region (15q11-q12), Miller-Dieker syndrome region (17p13.3), and Smith-Magenis syndrome region (17p11.2) (7, 19, 21). The turnaround time for the assay was 3–5 days.

Karyotyping, QF-PCR and FISH

Conventional karyotyping was performed using the standard G-banding method, and karyotype description was based on the International System for Human Cytogenetics Nomenclature [5]. QF-PCR was performed according to the manufacturer's protocol [22], and it was based on polymorphic

STR markers on chromosomes 13, 18, 21, X, and Y. FISH was performed to confirm the presence of microdeletion and microduplication syndromes using a fluorophore-labeled DNA probes used routinely for microduplications and microdeletions.

Data analysis

Data are reported as ratios and percentages. The results of the BoBs™ assay were compared with conventional karyotype patterns.

Results

Common chromosomal abnormalities detected by BACs-on-Beads™ assay

A total of 485 prenatal and blood/POC samples were analyzed by targeted BoBs™ genetic testing, obtaining conclusive results on 484 cases with an overall failure rate of 0.2%. The failed case was due to low DNA quantity and quality. There were 312 prenatal samples (amniotic fluid and chorionic villi) and 173 blood/POC samples. Indications for chromosomal analysis were abnormal antenatal aneuploidy screening (180; 37.1%), abnormal ultrasound morphological features (76; 15.7%), advanced maternal age (56; 11.5%), recurrent pregnancy loss (24; 4.9%), and neonatal dysmorphic features/developmental retardation (149; 30.7%). Significant maternal cell contamination was detected in 5 prenatal samples which were not retained (1.5%).

Following targeted BoBs™ testing, 43 of 484 qualifying samples were classified as abnormal on the basis of their allelic ratio, an overall detection rate of 1/11 (43/484, 8.9%) (Table 1). The remaining 441 samples were classified as normal. A representative BoB™ plot revealing a normal disomic pattern is shown in Fig. 1. In prenatal samples (amniotic fluid and chorionic villi), the aneuploidy detection rate was 1/14 (22/312, 7.0%). When considering the indication for prenatal diagnosis, the detection rate was 1 in 8 for abnormal ultrasound findings, 1 in 22 for abnormal prenatal maternal aneuploidy markers screening tests, and 1 in 14 for advanced maternal age. Overall, trisomy 21 (Fig. 2) was the most common finding (13/43) representing 30.2% of all abnormal results. Other numerical abnormalities were trisomy 18 (5/43, 11.6%), monosomy 21 (1/43, 2.3%), monosomy X (4/43, 9.4%) and Klinefelter syndrome (1/43, 2.3%).

With karyotype as the comparator, the diagnostic performance of the BoBs™ assay for common numerical aneuploidies (21, 18, X, and Y) was defined by a sensitivity of 96.0% and a specificity of 100%. No false-positive results were observed.

Submicroscopic structural chromosomal abnormalities detected by BACs-on-Beads™ assay

Among the 451 cases with normal karyotype patterns, the BoBs™ assay detected 16 cases of microdeletions and 4 cases of microduplications that were otherwise classified as normal by G-banding karyotyping. There were nine cases of deletion of the Di-George syndrome region (22q11.2; 2.0%) (Fig. 3), three cases of deletion of the Prader–Willi/Angelman syndrome region (15q11.2; 0.7%), two cases of deletion of the Williams–Beuren syndrome region (7q11.2; 4.6%), one case of deletion of the Cri du Chat syndrome region (5p15.3;p15.2; 2.3%), one case of deletion of the Wolf-Hirschhorn syndrome region (4p16.3; 2.3%), two cases of duplication of the Miller-Dieker region (17p13.3; 4.6%), and two cases of duplication of Di-George region (22q11.2; 4.6%). There were no false-positive results observed following FISH confirmation of abnormal findings.

In total, 5 and 15 submicroscopic copy number losses and gains were detected by BoBs™ assay in prenatal and blood/POC samples, respectively, thus providing an additional detection yield for prenatal diagnosis of 1/62 (5/312, 1.6%). All five prenatal cases were non-suspicious for submicroscopic imbalances as they presented for common indications of prenatal diagnosis (abnormal aneuploidy screening, advanced maternal age and abnormal ultrasound findings). The estimated additional diagnostic yield of BoBs™ for cryptic imbalances in blood samples from children with dysmorphic features and/or developmental delay was 1/12 (15/173, 8.7%).

Chromosomal abnormalities not detected by BACs-on-Beads™ assay

After conventional karyotyping, 10 cases of false-negative results (2.1%) missed by targeted BoBs™ assay were observed (Table 2). Two of them were cases of triploidy (69, XXY) which were initially misinterpreted as normal disomic males. Two were cases of mosaicism, one of which had a mosaic pattern involving chromosome 22 (mos 47,XX,+22[6]/46,XX[15]). Five cases of cytogenetically visible chromosomal rearrangements, translocations and inversions, involving chromosomes 9, 10, 13, 14, and 22 were also missed by BoBs™.

Discussion

In this study, we compared the diagnostic performance of targeted BoBs™ assay with gold standard karyotype. Our findings suggest that BoBs™ assay is a reliable method for the rapid detection of common aneuploidies in prenatal and blood/POC samples, with the added benefit of identifying

Table 1 Chromosomal abnormalities detected by BoBs™ assay

	Samples ($n=43$)	Karyotype	FISH	BoBs™ assay
Trisomy 21	4	47, XY +21	–	Trisomy 21
	1	47, XX +21	–	Trisomy 21
	1	46, XX, rob (21,21)(q10;q10)	–	Trisomy 21
	7	47, XY +21	–	Trisomy 21
Trisomy 18	1	47, XX +18	–	Trisomy 18
	1	47, XX +18	–	Trisomy 18
	1	47, XY +18	–	Trisomy 18
	2	47, XY +18	–	Trisomy 18
Monosomy 21	1	45, XY −21	–	Monosomy 21
Monosomy X	2	45, X	–	Monosomy X
	2	45, X	–	Monosomy X
Di-George syndrome	2	46, XX	46,XX.ish del(22)(q11.2q11.2) (D22S75−)	22q11.2 deletion
	1	46, XY	46,XY.ish del(22)(q11.2q11.2) (D22S75−)	22q11.2 deletion
	1	46, XX	46,XX.ish del(22)(q11.2q11.2) (D22S75−)	22q11.2 deletion
	4	46, XY	46,XY.ish del(22)(q11.2q11.2) (D22S75−)	22q11.2 deletion
Klinefelter syndrome and Di-George	1	47, XXY	47,XXY.ish del(22)(q11.2q11.2) (D22S75−)	22q11.2 deletion+XXY
Prader–Willi syndrome/Angelman syndrome	1	46, XY	46,XY.ish del(15)(q11.2q11.2) (SNRPN−,D15S10−)	15q11 deletion
	1	46, XX	46,XX.ish del(15)(q11.2q11.2) (SNRPN−,D15S10−)	15q11 deletion
	1	46, XX	46,XX.ish del(15)(q11.2q11.2) (SNRPN−,D15S10−)	15q11 deletion
Williams–Beuren syndrome	1	46, XX	46,XX.ish del(7)(q11.23q11.23) (ELN−)	7q11.2 deletion
	1	46, XY	46,XY.ish del(7)(q11.23q11.23) (ELN−)	7q11.2 deletion
Cri du Chat syndrome	1	46, XX	46,XX.ish del(5)(p15.2p15.3) (D5S23−, D5S721−)	5p15.3-p15.2 deletion
Wolf-Hirschhorn syndrome	1	46, XX	46,XX.ish del(4)(p16.3p16.3) (D4F26-,D4S96-)	4p16.3 deletion
Di-George region duplication	1	46, XY	46,XY.ish dup(22)(q11.2q11.2) (D22S75−)	22q11.2 duplication
	1	mos 47, XX, +22[6]/46, XX[15]	46,XX.ish dup(22)(q11.2q11.2) (D22S75−)	22q11.2 duplication
Miller-Dieker region duplication	1	46, XY	46,XY.ish dup(17)(p13.3p13.2) (MDCR+)	17p13.3 duplication
	1	46, XY	46,XY.ish dup(17)(p13.3p13.2) (MDCR+)	17p13.3 duplication

submicroscopic structural chromosomal abnormalities otherwise undetected by conventional karyotyping.

Our results confirmed the high diagnostic accuracy of the BoBs™ assay in detecting common chromosomal abnormalities (13, 18, 21, X and Y) in prenatal and blood/POC samples. A concordance rate of 100% with conventional karyotyping was found, which is in agreement with the findings of other studies [2, 5, 21, 23, 24]. With karyotype as the comparator, targeted BoBs™ assay demonstrated a sensitivity of 96.0% and a specificity of 100% in identifying common aneuploidies. Comparable diagnostic performance parameters were also reported by other investigators [2, 21, 23].

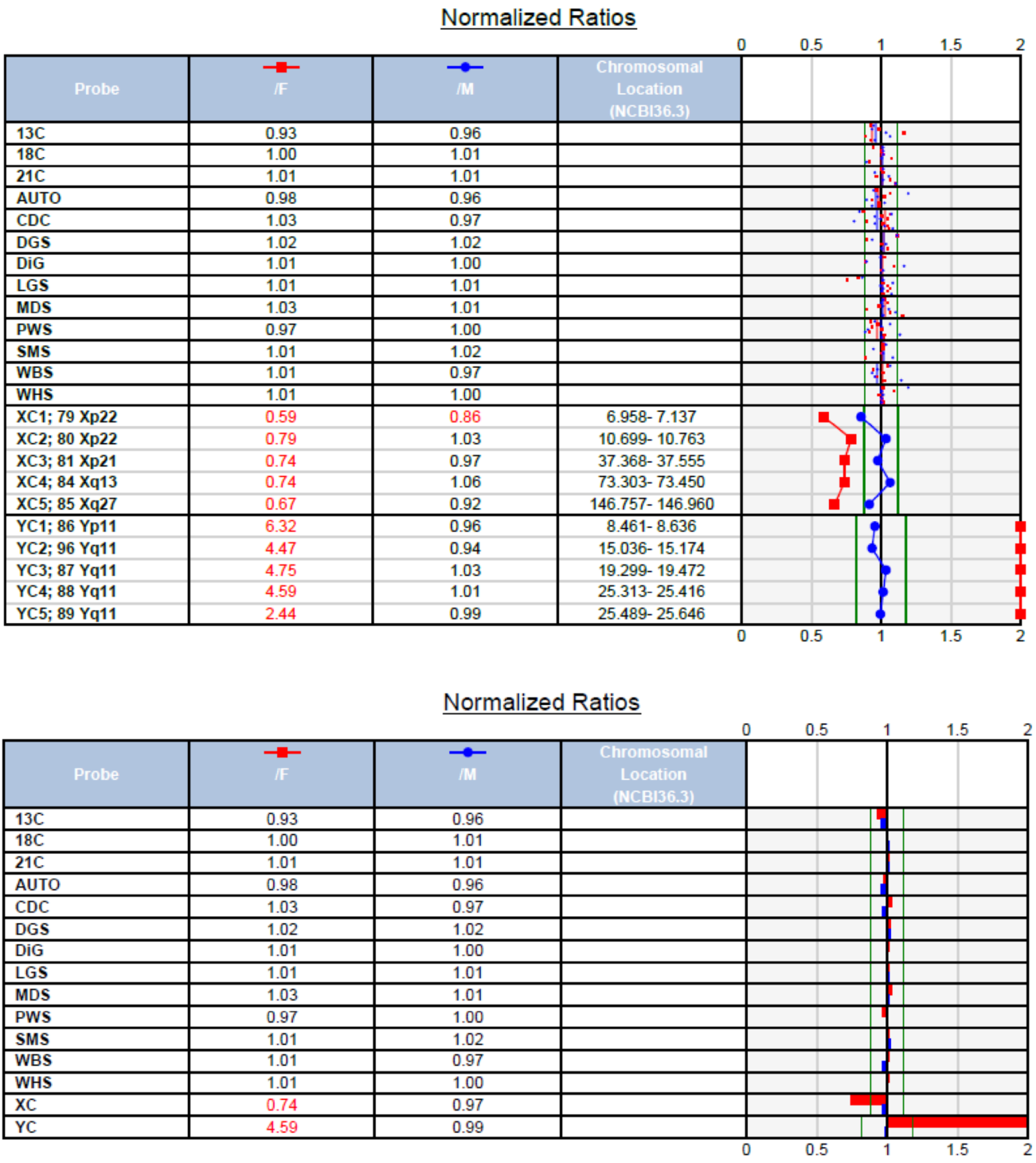
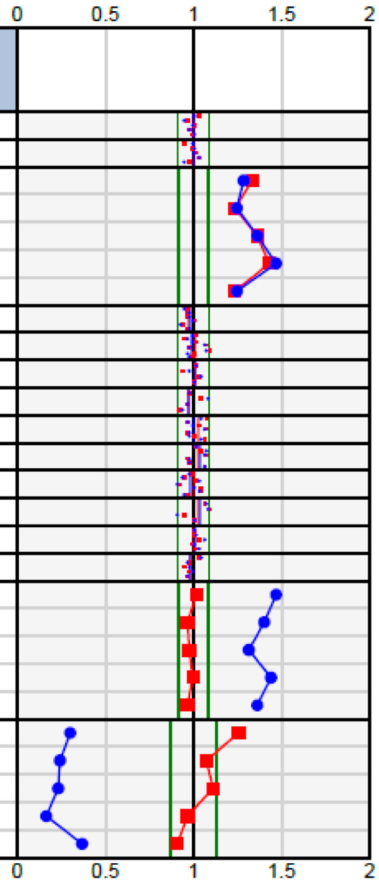


Fig. 1 Representative BoBs™ plot of a normal male (46, XY). Blue line represents a normal male reference DNA. Red line represents a normal female reference DNA. Green lines represent the normal range. (Color figure online)

Although RATs, including BoBs™, offer the significant benefit of early reporting, there is no agreement that anyone of these tests may be used as a stand-alone method to replace conventional karyotyping. It should be noted that RATs are often targeted assays designed specifically to detect common chromosomal aneuploidy. Abnormalities of chromosomes other than 13, 18, 21, X and Y remain therefore untargeted and invariably fall out of detection range. In prenatal diagnosis for example, it has been estimated that about 15–30% of aneuploidies detected by karyotyping are missed by RATs

Normalized Ratios

Probe	/F	/M	Chromosomal Location (NCBI36.3)
13C	1.00	1.00	
18C	1.00	1.00	
21C1; 74 21q22	1.33	1.29	32.208- 32.373
21C2; 75 21q22	1.24	1.25	34.661- 34.820
21C3; 76 21q22	1.36	1.36	35.229- 35.372
21C4; 77 21q22	1.43	1.47	43.556- 43.770
21C5; 78 21q22	1.24	1.25	44.146- 44.311
AUTO	0.97	0.98	
CDC	1.01	0.99	
DGS	1.01	1.01	
DiG	0.97	0.97	
LGS	1.03	1.00	
MDS	1.03	1.04	
PWS	1.00	0.98	
SMS	1.03	1.04	
WBS	1.00	1.01	
WHS	0.98	0.98	
XC1; 79 Xp22	1.01	1.47	6.958- 7.137
XC2; 80 Xp22	0.97	1.40	10.699- 10.763
XC3; 81 Xp21	0.98	1.31	37.368- 37.555
XC4; 84 Xq13	1.00	1.44	73.303- 73.450
XC5; 85 Xq27	0.97	1.36	146.757- 146.960
YC1; 86 Yp11	1.26	0.30	8.461- 8.636
YC2; 96 Yq11	1.07	0.24	15.036- 15.174
YC3; 87 Yq11	1.11	0.23	19.299- 19.472
YC4; 88 Yq11	0.97	0.16	25.313- 25.416
YC5; 89 Yq11	0.91	0.37	25.489- 25.646



Normalized Ratios

Probe	/F	/M	Chromosomal Location (NCBI36.3)
13C	1.00	1.00	
18C	1.00	1.00	
21C	1.33	1.29	
AUTO	0.97	0.98	
CDC	1.01	0.99	
DGS	1.01	1.01	
DiG	0.97	0.97	
LGS	1.03	1.00	
MDS	1.03	1.04	
PWS	1.00	0.98	
SMS	1.03	1.04	
WBS	1.00	1.01	
WHS	0.98	0.98	
XC	0.98	1.40	
YC	1.07	0.24	

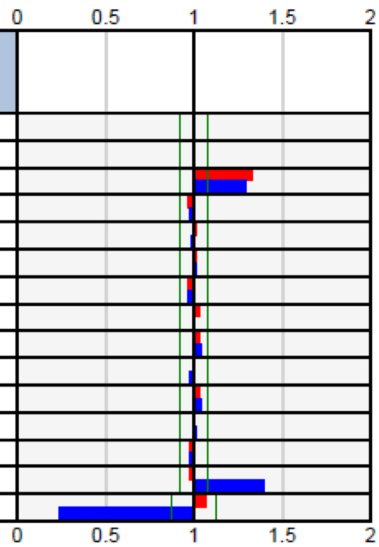


Fig. 2 Representative BoBs™ plot of Trisomy 21. Blue line represents a normal male reference DNA. Red line represents a normal female reference DNA. Green lines represent the normal range. (Color figure online)

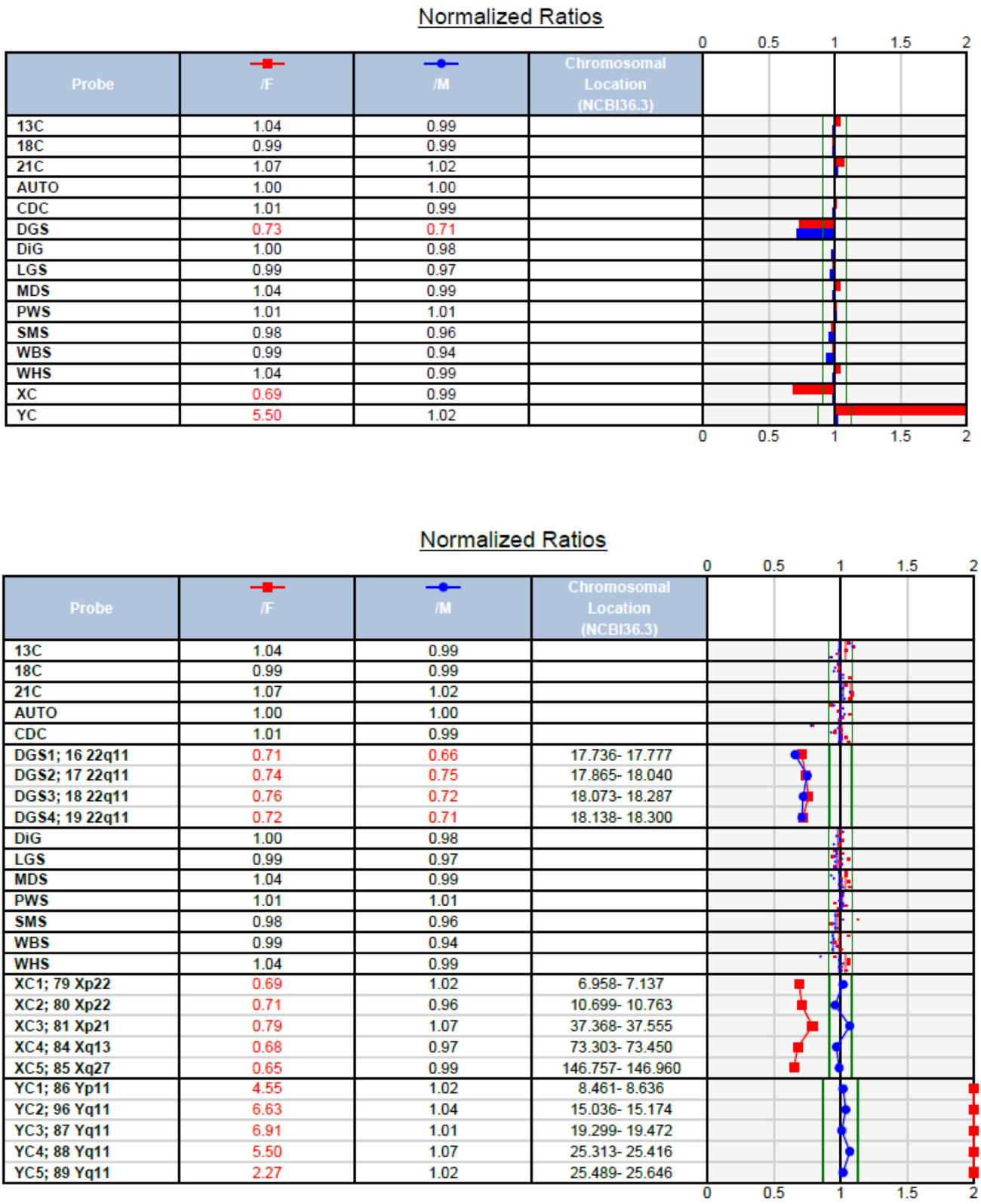


Fig. 3 Representative BoBs™ plot of Di-George microdeletion (22q11.2). Blue line represents a normal male reference DNA. Red line represents a normal female reference DNA. Green lines represent the normal range. (Color figure online)

Table 2 Chromosomal abnormalities missed by BoBs™ assay

Samples ($n=10$)	Karyotype	BoBs™ assay
1	45, XY, rob(13;14)(q10;q10)	Normal
1	46, XY, inv(9)(p11q13)	Normal
1	46, XX 9q+	Normal
1	mos 46, XY[47]/46, XX[33]	Normal
1	45, XY, rob(13:22) (q10,q10)	Normal
1	46, XY, t(14;15)(q32.2;q25)pat	Normal
1	Triploidy (69, XXY)	Normal
1	46, XX, inv(10)(p13q22.1)	Normal
1	Triploidy (69, XXY)	Normal
1	mos 47, XX,+22[6]/46, XX[15]	Dup(22q11.2)

[25], and that one clinically significant chromosomal abnormality for every 250 invasive prenatal samples may be undetected by these tests [26, 27]. The potential consequences of these diagnostic inaccuracies may be significant in terms of the serious medical, emotional and financial burden imposed by the birth of an affected child, unless used in conjunction with conventional karyotyping.

Like other rapid aneuploidy detection tests, targeted BoBs™ assay underperforms conventional karyotyping in the diagnosis of specific types of chromosomal abnormalities. In our study, the assay failed to recognize two cases of triploidy and five cases of chromosomal rearrangements which were misclassified as normal. These findings are in line with the study by Choy et al. [2], in which BoBs™ assay missed four cases of polyploidy. Vialard et al. [21] also reported six false-negative cases of triploidy and seven cases of cytogenetically visible unbalanced rearrangement. Furthermore, targeted BoBs™ has been shown to detect mosaicism ($\geq 20\%$) with a sensitivity of 57.1% [28]. It is therefore reasonable to believe that the combination of BoBs™ and conventional karyotyping would reduce false negatives and improve overall sensitivity of prenatal genetic testing.

In contrast to other rapid aneuploidy detection tests, targeted BoBs™ was found to increase the overall detection rate of conventional karyotyping by 1 in 24 in our study sample, taking into account all 20 cases of microdeletions and microduplications missed by cytogenetic analysis. When considering only amniotic fluid and chorionic villi samples submitted for prenatal diagnosis, the additional detection rate was 1 in 62. In these cases, targeted BoBs™ enabled the detection of submicroscopic copy number aberrations in the absence of pathognomonic ultrasound markers. For postnatal blood samples from children with dysmorphic features and/or developmental delay, the additional diagnostic yield of BoBs™ for cryptic imbalances was 1/12.

In this study, the 20 cases of microduplications and microdeletions identified by BoBs™ corresponded to nine well-characterized clinical syndromes covered by the assay, namely Di-George, Wolf-Hirschhorn, Cri du Chat, Williams–Beuren, Langer–Giedion, Prader–Willi, Angelman, Miller-Dieker, and Smith-Magenis. Other investigators [2, 5] confirmed this diagnostic advantage when they reported the detection of microdeletion and microduplication syndromes which were missed by karyotyping and other RATs. Karyotyping using routine banding resolutions (750-band level), lacks the diagnostic capability of detecting submicroscopic structural abnormalities of less than 5 Mb. This technical shortcoming explains the false-negative diagnoses associated with conventional karyotyping for specific microduplication and microdeletion syndromes. As a result of this unique diagnostic advantage, BoBs™ appears to be particularly suited to complement conventional karyotyping in prenatal and blood/POC genetic diagnosis.

In conclusion, the findings of this study suggest a high concordance between targeted BoBs™ assay and conventional karyotype for the detection of common chromosome aneuploidies in prenatal and blood/POC samples. Complementary BoBs™ also offers the added benefit of increasing the diagnostic yield by enabling the detection of microdeletion and microduplication syndromes with a relatively significantly shorter turnaround time. The main shortcoming of the technique remains its limitation to recognize polyploidies, low-level mosaicism, and chromosomal rearrangements.

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Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval The study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed consent was clearly documented. Ethical approval was obtained from the Institutional Review Board at the American University of Beirut.

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