



Study on the application value of BACs-on-Beads technology combined with chromosome karyotype analysis in prenatal diagnosis

Lin Chen, Jianming Du, Junlong Wang, Shuangling Chen, Wei Wang, Wei Yang, Yanying Zhang, Hui Zhang, Miao Zhang

Department of Laboratory Medicine, Harbin Red Cross Central Hospital, Harbin, China

Contributions: (I) Conception and design: L Chen, J Du, J Wang; (II) Administrative support: W Yang, Y Zhang, H Zhang; (III) Provision of study materials or patients: H Zhang, S Chen; (IV) Collection and assembly of data: M Zhang, L Chen, J Du; (V) Data analysis and interpretation: L Chen, W Yang, S Chen, Y Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Lin Chen. Department of Laboratory Medicine, Harbin Red Cross Central Hospital, 415 Xinyang Road, Harbin 150076, China. Email: clyx0451@126.com.

Background: Bacterial artificial chromosome (BAC) marker-microsphere identification/separation technique [BACs-on-Beads (BoBs)] not only has a high detection rate for major chromosomal changes, but also for the other 9 microdeletion syndromes. In this study, the application value of BoBs combined with karyotype detection in prenatal diagnosis was evaluated.

Methods: The amniotic fluid samples of 132 pregnant women with prenatal diagnosis indications in Harbin Red Cross Central Hospital from June 2018 to June 2019 were collected and subjected to the detection of BoBs and routine karyotyping.

Results: Among the 132 pregnant women's amniotic fluid samples, 30 cases were abnormal in BoBs detection, with a detection rate of 22.73%, and 29 cases were abnormal in chromosome karyotype analysis, with a detection rate of 21.97%. Among them, 1 case of DiGeorge Type I microdeletion syndrome BoBs was successfully detected. The karyotype analysis failed to detect the same syndrome; the total coincidence rate of two methods was 99.24%, the positive coincidence rate was 100.00%, and the negative coincidence rate was 99.03%; the sensitivity, specificity and positive predictive value (PPV), and negative predictive value (NPV) of the chromosome karyotype analysis was 96.67%, 100%, and 99.03%, respectively; the accuracy, specificity, and PPV/NPV of BoBs detection were 100%.

Conclusions: When BoBs technology is combined with chromosome karyotype analysis, it can increase the detection rate of fetal chromosomal abnormalities, which could provide a basis for clinical prevention and follow-up diagnosis and treatment.

Keywords: BACs-on-Beads technology (BoBs technology); chromosome karyotype; prenatal diagnosis; amniotic fluid; abnormal

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Introduction

There is a large population in China, which is facing the important public health problem of neonatal birth defects. Along with the rapidly increasing number of geriatric

pregnancies caused by the opening of the second- and third-child policy in China, neonatal birth defects are increasingly prevalent, and it is necessary to undertake predictive and diagnostic measures in the prenatal period to

control the birth rate of newborns with birth defects, reduce the neonatal mortality rate, as well as to reduce the burden on families and society. Chromosomal abnormalities are an important cause of birth defects, for which karyotyping could be considered the gold standard for detecting the number and structure of fetal chromosomes (1). However, the karyotyping method of detecting defects is complex and time-consuming (involving cell culture, slice making, and others processes), and the microdeletions, microduplications of <5 Mb, and other structural abnormalities cannot be detected. Chromosomal microarray analysis (CMA) identifies a genetic cause in an additional 12% to 15% of affected children, as compared with the current standard of karyotyping, leading to recommendations that microarray analysis become the first-tier test for such children (2). The bacterial artificial chromosome (BAC) marker-microsphere identification/separation technique [BACs-on-Beads (BoBs)] was rapidly promoted, which was one method of Chromosomal microarray. The BoBs technology is a molecular detection technique based on the principle of liquid-phase gene microarray with biotin-labeled samples, hybridized with reference DNA and solidified into fluorescence-encoded microspheres, which are detected by specific BACs DNA probes. Finally, the chromosome number and structure were detected by Luminex system for classification and signal detection (Luminex Corp., Austin, TX, USA). Studies have demonstrated (3) that the BoBs technique has a high detection rate for the major chromosomal changes (13, 18, 21, X, and Y) as well as 9 other microdeletion syndromes (Wolf-Hirschhorn, Cri-du-chat, DiGeorge Type I and II, and so on), which is also the reason for the superior feedback of BoBs technique over fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), and ultrasound during pregnancy (4).

Microarray analysis for prenatal diagnosis has been evaluated in some studies involving women whose fetus had a high probability of having chromosome abnormalities, such as those resulting in structural anomalies (5-9). These studies have shown the situation in other countries or regions, and are not being carried out in Northeastern Region, China, for more evidence-based clinical application of the technique, in this paper, the amniotic fluid BoBs test combined with karyotype analysis was performed in 132 pregnant women to observe the value of BoBs test application. We present the following article in accordance with the STARD reporting checklist (available at [\[tp.amegroups.com/article/view/10.21037/tp-22-16/rc\]\(https://tp.amegroups.com/article/view/10.21037/tp-22-16/rc\)\).](https://</p></div><div data-bbox=)

Methods

Participants

Amniotic fluid samples from 17–33 weeks gestation (mean, 22.36±2.03 weeks) were collected from June 2018 to June 2019 from 132 pregnant women aged 35–41 years old with a mean age of 37.03±1.20 years. The inclusion criteria for pregnant women stipulated that they must have at least 1 of the following indications for invasive prenatal diagnosis (10): (I) advanced age (age ≥35 years); (II) high risk for serologic screening; (III) high risk for noninvasive DNA prenatal testing; (IV) abnormal fetal ultrasound structure; (V) history of birth of chromosomally abnormal children; (VI) family history; and (VII) chromosomal structural abnormalities in 1 of the spouses. The exclusion criteria included pregnant women who did not receive two testing modalities or failed in one of the testing methods. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Harbin Red Cross Central Hospital [No.2021[14]]. All pregnant women provided their written informed consent.

Study methods

Specimen collection

The medical staff informed 132 pregnant women to empty their bladders as much as possible before the collection, and they maintained the supine position with assistance during the sample extraction. Afterwards, amniocentesis punctures were made through the abdomen to the amniotic cavity, and about 30 mL of clear amniotic fluid was extracted. Samples were divided into 20 and 10 mL for karyotype analysis and BoBs detection, respectively.

Karyotype analysis

For karyotype analysis, the amniotic fluid samples were centrifuged at 2,000 rpm for 10 min, the supernatant was discarded and incubated, and then it was harvested after 8–9 days according to the culture results. Each sample was counted and analyzed for 20–30 mitosis phases, and 5 karyograms were collected, observed, and analyzed, followed by photographing and preservation. The number of karyotype analysis was increased if any abnormalities were detected.

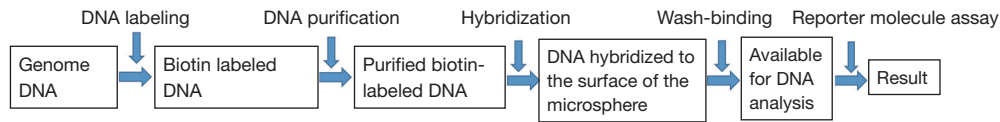


Figure 1 BoBs detection method. BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome.

Table 1 Content of BoBs detection

Target point	Chromosome region	Number of probes
Chromosome aneuploidy 13	13q.13.3–13q21.2	5
Chromosome aneuploidy 18	18p11.32–18q22.1	5
Chromosome aneuploidy 21	21q22.11–21q22.3	5
X chromosome aneuploidy	Xp22.31–Xq27.3	5
Y chromosome aneuploidy	Yp11.2–Yq11.23	5
Wolf-Hirschhorn syndrome	4p16.3	5
CriduChat syndrome	5p15.3–5p15.2	8
Williams-Beuren syndrome	7q11.2	5
Langer-Giedion syndrome	8q23–8q24	7
Prader-Willi/Angelman syndrome	15q11–15q12	7
Miller-Dieker syndrome	17p13.3	6
Smith-Magenis syndrome	17p11.2	4
DiGeorge Type I	22q11.2	4
DiGeorge Type II	10p14	4

BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome.

BoBs detection of amniotic fluid

DNA extraction of samples

Genomic DNA was extracted by column method by QIAamp DNA Blood Mini Kit, Qiagen (Germany) kit, and the concentration of DAN was determined by Hangzhou Allsheng Nano-100 microspectrophotometer after cell lysis and release, adsorption of DNA, removal of adhering proteins and ions, dissolution and other steps.

Sample BoBs detection

The genomic DNA of normal male and female controls, and samples was firstly labeled with enzymatic biotin nucleotides according to the instructions of Prenatal BoBsTM (Bacterial Artificial Chromosome Labeling-Microsphere Identification/Separation Method) (PerkinElmer, Waltham, MA, USA), and the labeled genomic DNA was subsequently purified. The purified labeled DNA and BoBs hybrid mesoporous microspheres

mixture was run in a single well for overnight hybridization. Hybrid microspheres were washed with washing buffer, following which the microspheres were incubated with a reporter molecule (streptavidin-phycoerythrin) to bind the reporter molecule to the biotin-labeled genomic DNA. The microspheres were washed again to keep them suspended. The DNA fluorescence values bound to the microspheres were detected with the Luminex 200TM Cellular Assay System, data were collected, and the ratio of the sample detection value to the reference detection value was analyzed by BoBsoft 2.0 software (PerkinElmer). The method and the detection contents are shown in *Figure 1* and *Table 1*.

Statistical methods

The statistical analysis of data was conducted using the

Table 2 Confirmation rate of BoBs and chromosome karyotype

Project	Number of cases	Confirmed BoBs, n (%)	Chromosome karyotype analysis, n (%)
Confirmation rate	132	30 (22.73)	29 (21.97)

BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome.

Table 3 Comparison of BoBs and chromosome karyotype analysis

Symptoms of pregnant women	Cases	BoBs diagnosis	BoBs cases	Chromosome karyotype analysis	Cases of chromosome karyotype analysis
Normal	102	BoBs diagnosis	102	46, XN	103
Trisomy 13	0	–	0	–	0
Trisomy 18	3	18-Trisomy	3	47, XN, +18	3
Trisomy 21	20	21-Trisomy	20	47, XN, +21	20
Super-female syndrome	1	XXX	1	XXX	1
Klinefelter syndrome	4	XXY	4	XXY	4
Rob translocation	1	21-Trisomy	1	46, XN, rob (21:21) (q10:q10) +21	1
DiGeorge Type I	1	22q11.2	1	46, XN	0

BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome; Rob, Robertsonian translocation.

software SPSS 23.0 (IBM Corp., Armonk, NY, USA), and the main outcome analysis was the consistency of any numerical, structural, or submicroscopic chromosomal abnormalities between BoBs and conventional karyotype analysis, for which the categorical data were expressed as numbers and rate (%).

Results

Comparison of the detection results of two methods

One hundred and thirty-two cases of amniotic fluid were detected successfully by BoBs and karyotype analysis, with a success rate of 100%. Among 132 amniotic fluid samples from pregnant women with prenatal indications detected by BoBs, there were 30 abnormal cases, with a detection rate of 22.73%; 29 abnormal cases of karyotype analysis, with a detection rate of 21.97%, as shown in *Table 2*; a total of 0 cases of Trisomy 13, 3 cases of Trisomy 18, 20 cases of Trisomy 21, and 5 cases of sex chromosome abnormalities (1 case of XXX, 4 cases of XXY) was detected by two methods. One case of Robertsonian translocation (Rob) was detected by karyotype analysis as well as BoBs. One case of DiGeorge Type I microdeletion syndrome was successfully detected by BoBs, however, it

was failed to be detected by karyotype analysis. Refer to *Table 3*.

Comparison of the compliance rates of two assays

Samples with normal results of two assays were included in the normal criteria, while the abnormal results of chromosome karyotype analysis or BoBs were included in the abnormal standard. Normal consistency was indicated by (–) and abnormal consistency was indicated by (+). The overall compliance rate, the positive compliance rate, and the negative compliance rate was 99.24%, 100.00%, and 99.03%, respectively (*Table 4*).

Comparison on sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of two assays

Specimens clearly diagnosed with chromosomal abnormalities were included as the “gold standard”, and the sensitivity of conventional karyotype analysis was 96.67%, the specificity and PPV were 100%, and the NPV was 99.03%. while the accuracy, specificity, and PPV/NPV of BoBs assay was 100%, as shown in *Tables 5,6*.

Discussion

With the continuous improvement of detection technology and the popularization of prenatal knowledge for pregnant women, the number of geriatric pregnant women receiving prenatal screening is gradually increasing and birth defects are gradually becoming a hotspot for research direction. Common karyotype analysis could detect chromosomal number abnormalities and structural abnormalities greater than 5 Mb. However, such analyses are time-consuming, have complicated operation, high culture limitation, and other defects. Moreover, some microdeletions/microduplications that can clearly lead to fetal malformations and abnormal intellectual development cannot be detected (11). For example, one case of DiGeorge Type I microdeletion syndrome in the study was missed in the detection of karyotype analysis (12).

The current detection techniques for routine detection of microdeletions/microduplications are mainly CMA, second-generation sequencing (NGS), and BoBs techniques (13). Among them, CMA and NGS techniques detect pathogenic chromosome copy number abnormalities along with some undefined abnormalities of chromosome copy number, which could increase the difficulty of result interpretation and genetic counseling (14,15).

The BoBs technique can compensate for these shortcomings. The basic principle is taken that a specific BAC can be hybridized with the genome of its target region, and the hybridized sample can be compared with the fluorescence intensity of the reference DNA and compared in fluorescence intensity, so that the changes of DNA copy number and structural defense could be determined and reflected. It has the advantages of no cell culture, high throughput, high speed, low quality of DNA requirement, clear results, easy interpretation, and others (16). In this study, 132 amniotic fluid samples of pregnant women with prenatal diagnostic indications were tested by BoBs combined with karyotype analysis, among which 103 cases were detected by karyotype analysis normally, 102 cases were detected by BoBs test normally, all detectable results could be yielded by BoBs tests (22.73%, 30/132). While 1 case of karyotype analysis was missed (21.97%, 29/132), the BoBs technique detected 29 fetal chromosomal number abnormalities and 1 chromosomal microdeletion without false positive results in the study, with higher success rate

Table 4 Comparison of compliance rate between BoBs and karyotype analysis

BoBs	Chromosome karyotype analysis		Coincidence rate
	+	-	
+	29	1	100.00% (29/29)
-	0	102	99.03% (102/103)
In total	29	103	99.24% (131/132)

BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome.

Table 5 Comparison of the consistency between karyotype analysis and gold standard

Chromosome karyotype analysis	Gold standard		Sensitivity	Specificity	PPV	NPV
	+	-				
+	29	0	96.67% (29/30)	100.00% (102/102)	100.00% (29/29)	99.03% (102/103)
-	1	102				
In total	30	102	-	-	-	-

PPV, positive predictive value; NPV, negative predictive value.

Table 6 Comparison of the consistency between BoBs and gold standard

BoBs	Gold standard		Sensitivity	Specificity	PPV	NPV
	+	-				
+	30	0	100.00% (30/30)	100.00% (102/102)	100.00% (30/30)	100.00% (102/102)
-	0	102				
In total	30	102	-	-	-	-

BoBs, BACs-on-Beads; BAC, bacterial artificial chromosome; PPV, positive predictive value; NPV, negative predictive value.

and wider detection range. In further analysis, BoBs and karyotype analysis were consistent in detecting numerical abnormalities in aneuploidy, with 28 abnormalities detected (excluding 1 case of Robertson translocation and DiGeorge Type I microdeletion, respectively), with a detection rate of 21.21%, with little difference compared with other studies (17,18), which indicated the reliability of two tests for detecting aneuploidy abnormalities.

It should be noted that both tests were successful in detecting 1 case of karyotype analysis for Robertson translocation. However, 1 case of DiGeorge Type I microdeletion syndrome was successfully detected by BoBs. Fetuses with DiGeorge syndrome are more likely to have precocious heart disease, hypoparathyroidism, and mental retardation, for which it is usually <5 Mb after major fetal chromosomal aberrations, caused by the missed karyotype analysis. The presence of chromosomal microdeletion abnormality was confirmed by high-throughput genomic sequencing analysis. Therefore, the overall compliance rate of the two combined detection methods was 99.24% in the study, with a positive compliance rate of 100.00% and a negative compliance rate of 99.03%.

Moreover, the sensitivity of the karyotype analysis was 96.67%, the specificity and PPV were 100%, and the NPV was 99.03%; while the accuracy, specificity, and PPV/NPV with BoBs detection was 100%, and the advantage of the assay data was significantly better, which was also caused by the missed detection of one microdeletion in karyotype analysis. Analysis suggested that it may be related to the fact that the BoBs technique reduces the detection steps for free of cultivation, and the chromosomal microdeletion syndrome often manifests as multiple malformations and growth retardation. The result fully demonstrates that the BoBs technique has certain advantages over karyotyping. Although the combination of both methods can improve the success rate of testing, it could help to shorten the detection cycle and reduce the decision-making time for pregnant women.

However, it should be noted that although BoBs assay can effectively compensate for the drawbacks of karyotype analysis and other assays, it also has some limitations, such as inability to detect ploidy changes, balanced rearrangements, point mutations, uniparental diploidy, methylation changes, chimerism, and others (19). The number of positive specimens was consistent with the number of positive cases. However, many studies have been reported in the literature, such as a study by Choy *et al.* (3) which reported that 7 cases of chimeric BoBs were

not detected, while karyotype analysis had been detected successfully. It was relatively small sample size included in this study, with only 1 case of chromosomal microdeletion, which may have caused some bias in the results. Therefore, a larger sample size and conduction of an in-depth study is required in the future.

It could be concluded that the combination of karyotyping and BoBs testing can significantly improve the detection rate and reduce the risk of underdiagnosis, which could provide a more reliable basis for clinical follow-up.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at <https://tp.amegroups.com/article/view/10.21037/tp-22-16/rc>

Data Sharing Statement: Available at <https://tp.amegroups.com/article/view/10.21037/tp-22-16/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tp.amegroups.com/article/view/10.21037/tp-22-16/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Harbin Red Cross Central Hospital [No.2021[14]]. All pregnant women provided their written informed consent.

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