Evaluation of interpretation methods to improve accuracy of the prenatal BACs-on-Beads™ assay in prenatal diagnosis

YU JIANG[1*], JIAN ZHANG[1*], LILI WU[2*], WENBO WANG[1] and QIWEI GUO[3]

[1]Prenatal Diagnosis Centre, [2]Department of Obstetrics and Gynecology, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, Fujian 361003; [3]Department of Experimental Medicine, United Diagnostic and Research Center for Clinical Genetics, School of Public Health, Xiamen University, Xiamen, Fujian 361102, P.R. China

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Abstract. Prenatal BACs-on-Beads™ (PNBoBs™) technology has been approved for use in routine clinical prenatal diagnosis in numerous countries. However, the influence of data interpretation on the accuracy of the results remains to be evaluated. The present study aimed to determine the accuracy of existing data interpretation approaches and develop an optimization method to improve the performance of the PNBoBs™ assay in prenatal diagnosis. A total of 2,289 prenatal cases with known karyotypes and raw ratio data from PNBoBs™ assays were recruited for the present study. Positive results, according to the data interpretation methods used for the PNBoBs™ test, were validated against current gold-standard approaches. Statistical analyses were then performed to evaluate the accuracy of existing methods in data interpretation to provide a basis for the optimization of a follow-up approach. Among the existing methods, the 'trimmed standard deviation threshold' approach had the highest sensitivity and false-positive rates, with 98.1 and 4.2%, respectively. The 'n-1 or greater probes' rule had the highest specificity (99.7%) and the second-highest false-negative rate (11.5%). The method optimized in the present study provided a reasonable balance between sensitivity (98.1%) and specificity (99.6%) with regards to the interpretation of the data obtained from the PNBoBs™ assay. The results indicated that the present optimization method outperforms existing approaches in data interpretation for the PNBoBs™ assay, and as a result, may reduce unnecessary verification turnaround time and cost in prenatal diagnosis.

Correspondence to: Mr. Yu Jiang, Prenatal Diagnosis Centre, Women and Children's Hospital, School of Medicine, Xiamen University, 10 Zhenhai Road, Xiamen, Fujian 361003, P.R. China
E-mail: jiangyu98@xmu.edu.cn

Professor Qiwei Guo, Department of Experimental Medicine, United Diagnostic and Research Center for Clinical Genetics, School of Public Health, Xiamen University, 4221 Xiangannan Road, Xiamen, Fujian 361102, P.R. China
E-mail: biozone@163.com

*Contributed equally

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Introduction

Molecular techniques, such as quantitative fluorescence PCR (QF-PCR) and the multiplex ligation-dependent probe amplification (MLPA), are regularly combined with karyotyping for rapid aneuploidy testing for the detection of common aneuploidies during invasive prenatal diagnosis (1,2). Recently, a bead-based multiplex technique, known as Prenatal BACs-on-Beads™ (PNBoBs™), has been widely used as an alternative method for the detection of common aneuploidies, as well as specific microdeletion syndromes, during prenatal diagnosis (3-20). Among these methods, the MLPA technique detects the sequence dosage differences in a semi-quantitative manner (21), whilst QF-PCR combines qualitative and semi-quantitative approaches for the interpretation of peak profiles of target chromosomes (22). However, to date, there has been little agreement on the criteria to be used for the interpretation of the data obtained from the PNBoBs™ assay. The instruction method, provided in the kit, denotes a positive result as one where three or more probes within a given target region exceed the threshold, set at ±2 trimmed standard deviations (SDs) of the autosomal probes. Similarly, Gross *et al* (3) proposed the 'n-1 or greater probes' rule as the criterion for denoting a result as positive in the PNBoBs™ test. In addition to these qualitative methods, Vialard *et al* (5) proposed a semi-quantitative approach to data interpretation, in which the mean normalized ratio (MNR) of a probe group is used as a metric for comparison with the threshold value. In practical terms, this method may be broadly divided into two types: The 'fixed threshold' and the 'trimmed threshold' rules (8,12), according to the choice of threshold. However, certain studies have not explicitly provided specific data interpretation criteria when using the PNBoBs™ technique for prenatal diagnosis.

In this context, it is difficult to compare the data across studies. As such, it is essential to evaluate the impact of

different data interpretation methods used for the results obtained from PNBoBs™ assays in the available studies. In the present study, retrospective data from >2,200 prenatal samples were utilized to evaluate the accuracy of existing methods based on different performance measures with the aim of proposing a versatile data interpretation approach for use in the PNBoBs™ assay for prenatal diagnosis.

Subjects and methods

Subjects. A total of 4,496 prenatal diagnoses were performed at Women and Children's Hospital, School of Medicine, Xiamen University (Xiamen, China) between January 2018 and June 2019. Among them, 2,291 cases who applied for karyotyping and PNBoBs™ testing simultaneously were recruited for the present study. Of these participants, two with failed karyotype analysis were excluded. Finally, a total of 2,289 cases, who had karyotypes and raw data of the PNBoBs™ assay were obtained for subsequent research. Cases interpreted as aneuploidy in targeted chromosomes by any of the data interpretation methods available for the PNBoBs™ assay were verified by karyotyping. Cases interpreted as microdeletion or microduplication in targeted regions by any of the data interpretation methods available for the PNBoBs™ assay were verified by chromosome microarray analysis (CMA). Ethics approval was obtained from the Ethical Review Committee of the Women and Children's Hospital Affiliated to Xiamen University (Xiamen, China). Each participant provided written informed consent in compliance with the Declaration of Helsinki prior to being included in the present study.

CMA. Prenatal diagnostic samples interpreted as copy number variation in targeted regions by PNBoBs™ assay were verified by CMA using a CytoScan® 750K Array Suite kit (cat. no. 901859; Affymetrix Inc.; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Genomic DNA was extracted from the amniotic fluid cells using the QIAamp DNA Mini Kit (cat. no. 51306; Qiagen, Inc.). The resulting DNA concentrations and purities were estimated by NanoDrop One Spectrophotometer (Thermo Fisher Scientific, Inc.). Data were analyzed using the Chromosome Analysis Suite 4.0 (r28959) software (Affymetrix Inc.; Thermo Fisher Scientific, Inc.).

Performance evaluation of data interpretation methods for the PNBoBs™ assay. A methodological review of the literature was performed with the search term of 'Prenatal BoBs™' or 'PNBoBs™' using the PubMed database, with the last publication date for inclusion being January 15th, 2020. All related literature that shared the same or similar positive call criteria of the PNBoBs™ assay were grouped. The performance of the data interpretation methods was evaluated based on the following statistical indicators: Sensitivity, specificity, false-positive rate (FPR) and false-negative rate (FNR).

Optimization targets of the new data interpretation method. The optimization approach was developed based on statistical results across existing positive call methods, using the following optimization targets: i) The accurate detection of fetal aneuploidy of chromosomes X, Y, 13, 18 and 21; ii) improvement in the detection sensitivity for microdeletion/microduplication syndrome; and iii) ensuring a relatively low rate of false positives in the detection of aneuploidy and microdeletion/microduplication syndrome.

Statistical methods. Statistical analysis was performed with the SPSS Statistics version 20 (IBM Corp.). For comparison of the MNR of the Y chromosome between male and female fetuses, the unpaired Student's t-test was used. One-way ANOVA was performed to compare the MNR of different copy number groups of non-Y chromosomes followed by the Student-Newman-Keuls test for post hoc analysis.

Results

Characteristics of the analyzed samples. The typical profiles of the true-positive and false-positive results of the PNBoBs™ assay are presented in Fig. 1. In total, 224 cases were denoted as positive by all existing data interpretation methods. After validation, the PNBoBs™ assay theoretically detected 52 cases of an unbalanced chromosomal abnormality in the present sample sets. Supplementary Table SI provides further details on these positive cases.

A summary of the test results for all samples is provided in Fig. 2. The mean ± 3SD of the MNR value for the Y chromosome probe sets in male (n=1,220) and female samples (n=1,069) is presented in Fig. 2A. A statistical comparison of the autosomal controls and deviation probes in the selected true-positive samples (n=36) is provided in Fig. 2B, indicating the presence of a significant difference ($P<0.001$) among non-overlapping groups. The threshold values calculated with 99.7% confidence limits are listed in Table I.

Performance of data interpretation using existing methods. A detailed description of each existing data interpretation method is given in Table II. Of all 224 positive cases, 143 had three or more probes within a given target region exceeding the 2x trimmed SD threshold. According to the instructions of the PNBoBs™ assay kit, these samples were interpreted as positive. After validation, 94 of these (65.7%) were identified as false-positive results and yielded an FPR of 4.2% (Table III). In addition, two cases with partial copy number variations in the microdeletion syndrome regions were classified as 'missed detection' due overlooked by the instruction method due to the presence of < three deviation probes depite being positive (Fig. 1B and C). The statistical results indicated that the 'n-1 or greater probes' rule had the highest specificity (99.7%) in all existing methods and the second-highest FNR (11.5%). Comparison of the results suggested that the 'fixed threshold' rule had the lowest sensitivity (71.2%) and the second-highest specificity (99.3%). Compared to the other three approaches, the 'trimmed SD threshold' method had the highest sensitivity (98.1%), with only one overlooked case of low-level mosaicism. The same FPR (4.2%) was observed between this approach and the instruction method, although the composition of these two false-positive groups was markedly different. In the former, the false-positive results were mainly derived from the microdeletion syndrome regions (57.4%, 54/94), while in

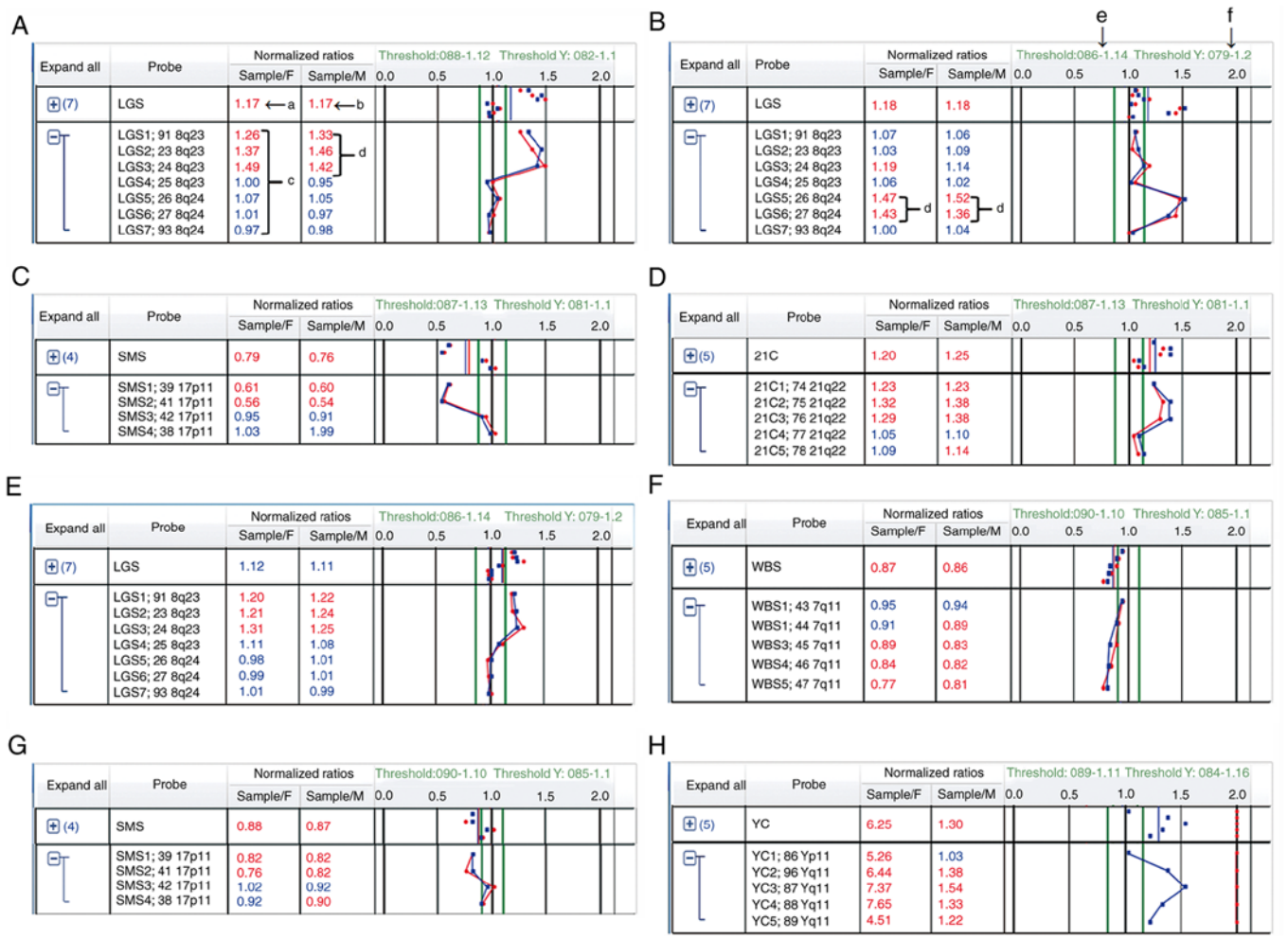

Figure 1. Representative prenatal BACs-on-Beads™ results for true-positive and false-positive cases. (A) The profiles indicated that both of the MNR of all the probes in the LGS region exceeded the threshold with three consecutive deviation probes and this was confirmed by CMA as a true-positive result. (B) The profiles suggested that both of the MNR of all the probes in the LGS region exceeded the threshold with two consecutive deviation probes and this was confirmed by CMA as a true-positive result. (C) The profiles indicated that both of the MNR of all the probes in the SMS region exceeded the threshold with two consecutive deviation probes and this was confirmed by CMA as a true-positive result. (D) The profiles suggested that both of the MNR of all the probes in the 21q22 region exceeded the threshold with three consecutive deviation probes and this was confirmed by karyotyping as a trisomy 21. (E) The profiles indicated that none of the MNR of all the probes in the LGS region exceeded the threshold despite having three consecutive deviation probes and this was verified by CMA as a false-positive result. (F) The profiles suggested that both of the MNR of all the probes in the WBS region exceeded the threshold despite having three and four consecutive deviation probes and this was verified by CMA as a false-positive result. (G) The profiles indicated that both of the MNR of all the probes in the SMS region exceeded the threshold with two consecutive deviation probes and this was verified by CMA as a false-positive result. (H) The profiles suggested that the MNR of the Y chromosome to male reference reached the fixed threshold of 1.3 with four consecutive deviation probes and was verified by karyotyping as a false-positive result. a, Representative mean normalized ratio of the sample-to-female reference; b, Representative mean normalized ratio of the sample-to-male reference; c, Representative normalized ratio of each probe in the target region of the sampel-to-reference; d, Representative normalized ratio of consecutive deviation probes in the target region of the sample-to-reference; e, Representative threshold values for the X chromosome and microdeletion syndrome region; and f, Representative threshold values for the Y chromosome. The numbers in red indicate that the value exceeds the threshold. The numbers in blue indicate that the value is within the range of threshold. The brackets on the right represent the range of the probes being analyzed. MNR, mean normalized ratio; CMA, chromosome microarray analysis; LGS, Langer-Giedion syndrome; SMS, Smith-Magenis syndrome; WBS, Williams-Beuren syndrome; 21C, Chromosome 21; YC, Y chromosome; M, male reference; F, female reference.

the latter, they were mostly derived from the Y chromosome (88.3%, 83/94).

Performance of the optimization method. As presented in Fig. 3, a pipeline was developed for the data interpretation of the PNBoBs™ assay results based on the comparison results described above. The first step of the analytic process consisted of the identification of the sex of the fetus by comparing the MNR of the Y chromosome in a sample to a fixed threshold of 0.24. According to this criterion, two out of the 1,069 female fetuses were classified as male fetuses due to the MNR of the Y chromosome slightly exceeding the cutoff (0.27 and 0.35, respectively). According to the analysis pipeline applied, the six cases with both MNR present outside of the 2X trimmed SD threshold in autosomal regions were classified as inconclusive rather than positive results, since the corresponding MNRs were at intermediate levels, namely between the upper limit of the threshold of one probe-set copy and the lower limit of two probe-set copies (data not shown). In addition, 82 out of the 83 false-positive results

Table I. Statistical results of mean normalized ratios of copy number variation cases.

Probe set	n	Mean	SD	Mean ± 3SD range
OCL/F[a]	10	0.67	0.05	0.52-0.82
OCL/M[b]	10	0.67	0.05	0.51-0.83
AUTO/F[a]	36	1.00	0.02	0.93-1.08
AUTO/M[b]	36	1.01	0.02	0.93-1.08
OCG/F[a]	26	1.29	0.06	1.12-1.46
OCG/M[b]	26	1.30	0.06	1.13-1.48
Chromosome Y/M[b] (female fetuses)	1,069	0.17	0.02	0.11-0.24
Chromosome Y/M[b] (male fetuses)	1,220	1.03	0.09	0.77-1.30

[a]Samples against the female reference. [b]Samples against the male reference. Although the female does not have the Y chromosome, in the PNBoBs™ test, in the Y chromosome probe region, female samples produced a ratio >0. However, it is significantly smaller compared with those produced by the male samples. Therefore, this value was used to determine the sex of the fetus in this proposed method. OCL, one copy loss; AUTO, autosomal control probes; OCG, one copy gain; SD, standard deviation; M, male reference; F, female reference.

Table II. Existing data interpretation methods presented for the prenatal BACs-on-Beads™ assay in the literature.

Study	Specimen type tested	Positive call criteria for non-sex chromosome syndromes	Positive call criteria for sex chromosome syndromes	(Refs.)
Miao *et al*	PC	[a]Three or more probes within a given target region exceeding the ±2x trimmed SD.	[a]Three or more probes within a given target region exceeding the ±2x trimmed SD for the X chromosome, and ±3x trimmed SD for the Y chromosome, respectively.	(20)
Fang *et al*	PC			(16)
Gross *et al*	CL	Ratios of n-1 or greater probes within a given target region exceeding the ±2x trimmed SD. to both references	Ratios of n-1 or greater probes within a given target region exceeding the ±2x trimmed SD cutoff for the X chromo some and ±3x trimmed SD for the Y chromosome, respectively.	(3)
Dang *et al*				(19)
Huang *et al*	PC	Both of the mean normalized ratios within a given target region exceeding the ±2x trimmed SD.	Both of the mean normalized ratios within a given target region exceeding the ±2x trimmed SD cutoff for the X chromosome and ±3x trimmed SD for the Y chromosome, respectively.	(17)
Choy *et al*	PC			(10,11)
Cheng *et al*	CL and PC			(9)
Vialard *et al*	PC			(5,8)
Li *et al*	PC	Either of the mean normalized ratio within a given target region equal to or exceeding 1.3 or 0.8 for duplication and deletion, respectively.	Not mentioned.	(18)
Garcia-Herrero *et al*	PC			(12)
Rosenfeld *et al*	POC	No details.	Not mentioned.	(14)
Shaffer *et al*	PC			(4)
Grati *et al*	PC	Not Mentioned.	Not mentioned.	(15)
Piotrowski *et al*	PC			(13)
Leung *et al*	PC			(6)

[a]Representative of the instruction method. PC, prenatal cases; CL, cell lines; POC, products of conception; SD, standard deviation.

with the Y chromosome, detected by using the 'trimmed SD threshold' method, were excluded in the combination method due to the presence of fewer than four deviation probes. Based on this data analysis pipeline, the overall sensitivity of the present optimization method was 98.1%, with an FPR of 0.4%.

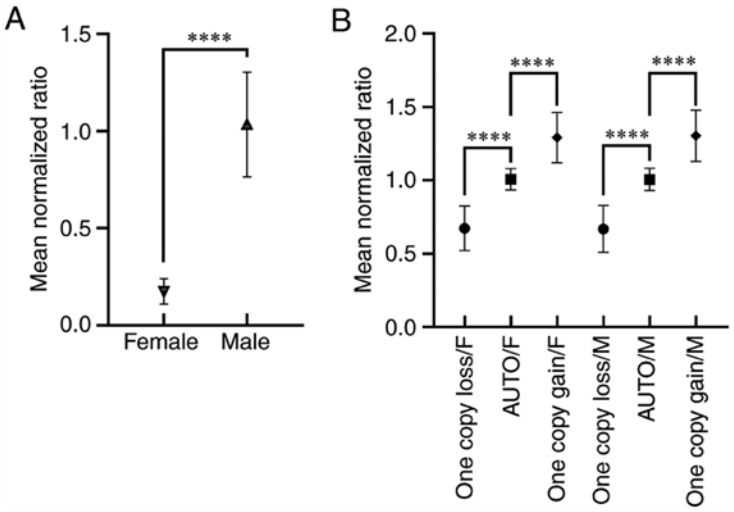

Figure 2. Statistical results of the mean normalized ratio from probes in the Y chromosome and deviation targets. (A) Profiles of the MNR ± 3SD of the Y chromosome to the male reference (female fetuses, n=1,069; male fetuses, n=1,220). The symbols represent the mean value, while error bars represent ±3SD for each column. (B) The MNR ±3SD profiles of deviation probes and autosomal control probes of abnormal cases to the female reference and the male reference. Symbols represent the mean value, while error bars represent ±3SD for each column. ****P≤0.0001. MNR, mean normalized ratio; SD, standard deviation; M, male reference; F, female reference; auto, autosomal control probes.

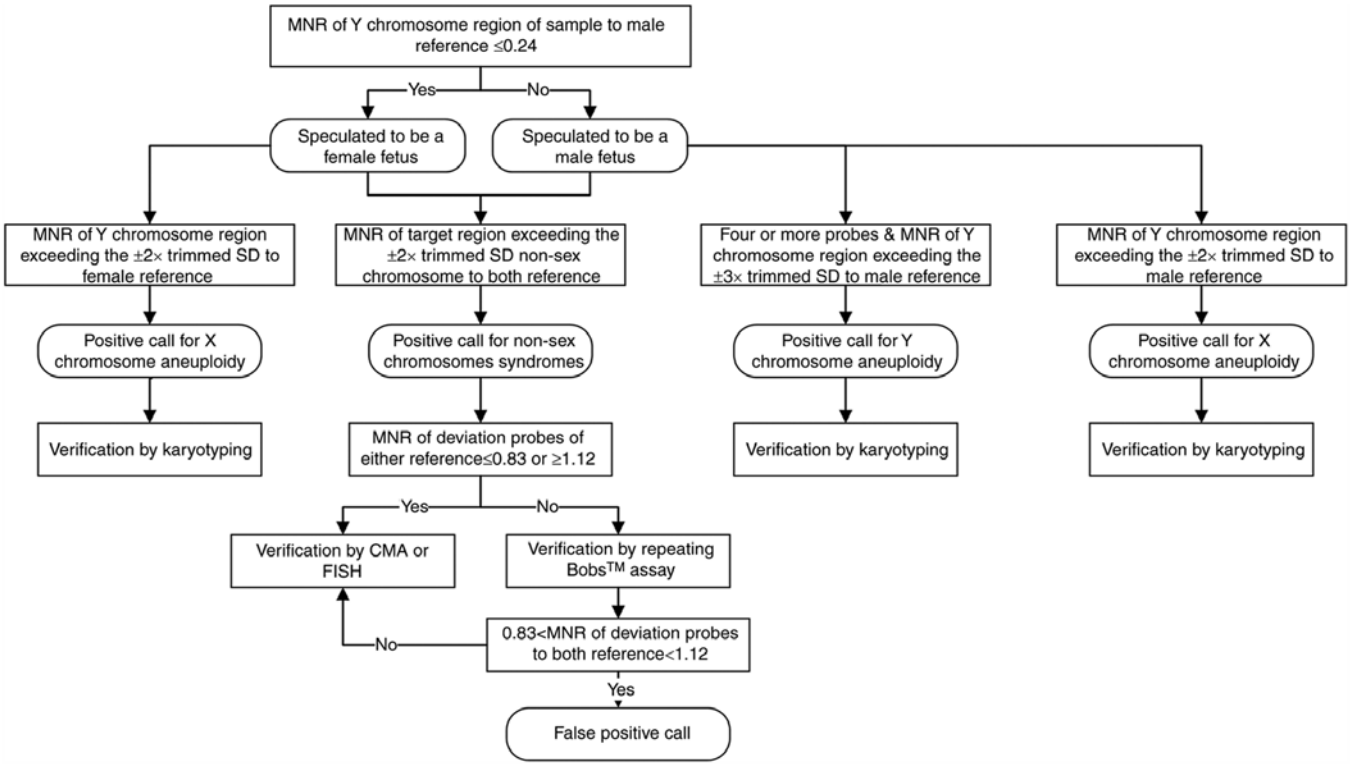

Figure 3. Flowchart of optimal data interpretation and verification procedure for the Prenatal BoBs assay. MNR, mean normalized ratio; SD, standard deviation; CMA, chromosome microarray analysis; FISH, fluorescence *in situ* hybridization; BoBs, BACs-on-Beads™.

Discussion

Following the QF-PCR and MLPA techniques, the PNBoBs™ assay has been widely utilized in recent years due to its superiority in extending the scope of rapid prenatal diagnosis of common diseases from fetal aneuploidy to microdeletion syndromes. In one study from the USA (14), which included ~2,900 prenatal samples, neither false negatives nor false positives were observed in the data obtained using the PNBoBs™ test. Miao *et al* (20) performed a prospective study on >4,800 prenatal cases in China and reported false-positive events; however, no data were provided. A multi-center retrospective study (15) involving a cohort of 9,500 pregnancies conducted in EU countries reported an FPR and FNR of 0.19 and 0.14%, respectively. However, the preliminary test of the present study indicated recurrent false-positive results when

Table III. Comparison of different methods on data interpretation for prenatal BACs-on-Beads™ assay.

	Instruction method			'N-1 or greater probes' method			'Fixed threshold' method			'Trimmed SD threshold' method			Optimization method		
Target region	TP	FP	FN	TP	FP	FN	TP	FP	FN	TP	FP	FN	TP	FP	FN
Chr21	15	10	0	14	0	1	8	0	7	15	2	0	15	0	0
Chr18	3	2	0	3	0	0	3	0	0	3	1	0	3	0	0
Chr13	3	0	0	3	0	0	1	0	2	3	0	0	3	0	0
Chr15(PWS)	2	24	0	2	0	0	2	0	0	2	0	0	2	0	0
Chr8(LGS)	1	7	1	0	0	2	2	0	0	2	0	0	2	0	0
Chr17(MDS)	1	12	0	1	1	0	1	0	0	1	0	0	1	0	0
Chr22(DGS)	4	0	0	4	0	0	3	0	1	4	0	0	4	0	0
Chr4(WHS)	2	4	0	2	1	0	2	0	0	2	0	0	2	0	0
Chr5(CDC)	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1
Chr7(WBS)	0	6	0	0	0	0	0	0	0	0	2	0	0	0	0
Chr17(SMS)	0	1	1	0	0	1	1	0	0	1	1	0	1	0	0
ChrX	12	18	0	11	2	1	8	0	4	12	5	0	12	5	0
ChrY	5	10	0	5	2	0	5	16	0	5	83	0	5	3	0
Total	49	94	3	46	6	6	37	16	15	51	94	1	51	8	1
Statistical indicators (%)															
Sensitivity		94.2			88.5			71.2			98.1			98.1	
Specificity		95.8			99.7			99.3			95.8			99.6	
FPR		4.2			0.3			0.7			4.2			0.4	
FNR		5.8			11.5			28.8			1.9			1.9	

PWS, Prader-Willi syndrome; LGS, Langer-Giedion syndrome; MDS, Miller-Dieker syndrome; DGS, DiGeorge syndrome; WHS, Wolf-Hirschhorn syndrome; CDC, Cri du Chat syndrome; WBS, Williams-Beuren syndrome; SMS, Smith-Magenis syndrome; Chr, chromosome; SD, standard deviation; TP, true positives; FNR, false negative rate; FPR, false positive rate.

using the included instruction method for data interpretation. Therefore, a retrospective comparison of the literature was performed, revealing one possible reason for these discrepancies: The criteria for data interpretation described in the studies were inconsistent.

Gross *et al* (3), the inventors of the BoBs™ technique, used the rule of 'n-1 or greater probes' beyond the threshold as a criterion for denoting a positive call. In practical terms, the researchers specifically indicated that this rule was based on results obtained from known syndromic cell lines rather than those from clinical samples. Compared with the instruction method, in which three deviated probes were sufficient for calling positive results, using the 'n-1 or greater probes' rule theoretically provided a higher specificity, as most of the desired syndrome regions (11/14) of the PNBoBs™ kit had at least five probes and at least four deviated probes are required to call for positive results under this rule. The results of the present analysis were consistent with this hypothesis that the 'n-1 or greater probes' rule had the highest specificity (99.7%) among the methods available. At the same time, six true-positive cases were overlooked under this rigid criterion, which resulted in a higher FNR (11.5%) compared to the rate (5.8%) obtained using the instruction method.

In the literature, there were no false results reported in studies that used the 'fixed threshold' rule as the interpretation method for data obtained using the PNBoBs™ assay (12,18). However, in the present analysis, 28.8% (15/52) of the positive samples were overlooked when using this approach. In addition, 16 false-positive cases with a Y chromosome aneuploidy were called using this method, as the Y chromosome ratio of the sample reached or exceeded the threshold of 1.3. Given the poor performance in terms of sensitivity observed in the present study, it is not recommended using the 'fixed threshold' rule alone to interpret data obtained from a PNBoBs™ assay.

Among the previous studies, the 'trimmed SD threshold' rule was the most popular approach for interpreting data obtained from a PNBoBs™ test (5,8-11,17). In this approach, a specific sample threshold is used to compare the MNR of the target region instead of a fixed threshold. Regarding the performance of this approach, Vialard *et al* (8) reported high sensitivity (97.3%) and specificity (100%) in retrospective samples and slightly lower sensitivity (95.6%) and specificity (99.7%) in prospective samples. In a retrospective study performed by Choy *et al* (10), the sensitivity of PNBoBs™ was 96.7%, with a specificity of 100%. In terms of sensitivity performance, the present result of 98.1% was somewhat higher than that obtained in earlier studies. The only false-negative result was from a low-level mosaicism case with an abnormal cell proportion of 6.57%, which is below the detection limit of the BoBs™ assay (9). Two cases with a partial deletion, located in the Langer-Giedion and the Smith-Magenis syndrome regions, respectively, were classified as positive results under this rule (i.e. using the trimmed SD threshold). Vialard *et al* (8) proposed that at least two consecutive probes should be observed to be evidently abnormal (ratio <0.8 or >1.3) when calling a partial copy number variation in microdeletion syndrome regions. These two realistic cases appeared to be consistent with this criterion. However, given the limited data, it is difficult to determine which approach is better for presenting a partial copy number variation at the present time. In terms of specificity, the results observed in this study were lower compared to those reported by other studies (5,10). A total of 94 false-positive results were observed using this rule, resulting in a high FPR compared with that of the existing methods (4.2%).

A comparison of the results described above revealed that none of the existing methods offered optimal levels of performance for the interpretation of data obtained from the PNBoBs™ assay, indicating a requirement to optimize these approaches in order to improve the accuracy of this technique when used in prenatal diagnosis. To ensure a high degree of sensitivity and a low rate of false-positives, an analysis workflow was developed by adopting the advantages and eliminating the disadvantages of the existing methods. In the analysis pipeline developed, the first step consists of identifying the sample sex in order to choose the correct reference sample. Generally, the MNR of the X chromosome in a normal male sample against the female reference sample is always less than the 2x trimmed SD threshold; thus, the sample makes sense only when compared to male reference DNA. Similarly, the MNR of the X chromosome in a female fetus should only be compared to the female reference in the present method. In addition, the upper limit of 99.7% was selected for the confidence interval of MNR of the Y chromosome rather than the maximum value that was observed in order to improve the performance in calling for sex-chromosome mosaicism. Due to its high sensitivity properties, the 'trimmed SD threshold' rule was used as the framework for the interpretation of data from autosomes. In a study by Slater *et al* (2), using an MLPA for rapid prenatal diagnosis, the threshold values from a dataset of normal and abnormal samples were used to assign the test results to different categories. In the present study, a similar approach was used to further differentiate the cases with MNR exceeding the 2x trimmed SD. Verification of results obtained in the present study demonstrated that in cases where the MNR of the deviation probes in the autosome region was present outside of the reference intervals, a repeated PNBoBs™ assay was more consistent with the original intention of using this technique than with a CMA verification. Compared to the existing methods, one of the major advantages of the present optimization approach was that it substantially reduces false positives from the Y chromosome by a comprehensive analysis of the number of deviation probes and MNR values, while maintaining a high degree of sensitivity. Re-analysis of the data using the method optimized herein indicated a high specificity (99.6%), with a sensitivity that was also at a high level (98.1%).

In conclusion, a reasonable balance between sensitivity and specificity was obtained for the interpretation of data of the PNBoBs™ assay, using all possible methods to reduce the FPR and providing a basis for reducing the turnaround time and cost associated with the use of this assay in clinical practice. However, there are two potential limitations of the present study. First, the reference intervals established may not be directly used in another laboratory. Researchers intending to use this method are encouraged to consider establishing in-house reference thresholds based on local samples. Furthermore, it should be noted that a sufficient number of confirmed positive and normal cases should be included in the reference sample set to meet the desired statistical requirements. In addition, the clinical implications of microduplications/microdeletions detected in the

present study were not discussed. Consequently, further studies are required in order to provide substantial evidence for future guidelines and recommendations for the PNBoBs™ assay.

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Availability of data and materials

Partial output data from the PNBoBs™ assay software (BoBsoft™) are included in the attached Table SI of this published article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YJ contributed to writing the manuscript. YJ and JZ performed the BoBs™ assay. LW helped with the karyotype analysis of chromosomes. WW and QG performed the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained from the Ethical Review Committee of the Women and Children's Hospital Affiliated to Xiamen University (Xiamen, China). Each participant provided written informed consent in compliance with the Declaration of Helsinki prior to being included in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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