PRENATAL DIAGNOSIS
Prenat Diagn 2005; **25**: 1032–1039.
Published online 17 October 2005 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.1247

Rapid detection of chromosomal aneuploidies in uncultured amniocytes by multiplex ligation-dependent probe amplification (MLPA)

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Objective To test whether multiplex ligation-dependent probe amplification (MLPA) can be used for the detection of aneuploidy of chromosomes 13, 18, 21, X, and Y in uncultured amniocytes.

Methods We performed a prospective study based on 527 amniotic fluid samples. Chromosome copy numbers were determined by analysing the relative amount of PCR product of chromosome-specific MLPA probes. Results were available within 48 h and were compared with those of karyotyping.

Results There were 517 conclusive MLPA tests. In 514 tests, results were concordant with those of karyotyping. There were two cases of 69,XXX triploidy that could not be detected by MLPA and there was one false-positive result. Here, MLPA indicated a 47,XXY fetus, whereas the karyotype was 46,XY. We correctly identified all 23 cases of autosomal trisomy and the single case of monosomy X in samples collected from 16 up to 36 weeks of gestation. In 10 cases (2%), the result was inconclusive owing to an insufficient amount of DNA.

Conclusion Sensitivity, specificity, and failure rate of MLPA were comparable to those of FISH and QF-PCR. Aneuploidy screening in uncultured amniocytes by MLPA is feasible in a clinical diagnostic setting, yielding an informative and rapid result in 98% of cases. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: aneuploidy; multiplex ligation-dependent probe amplification (MLPA); uncultured amniocytes; prenatal diagnosis

INTRODUCTION

Trisomy of chromosome 13, 18, and 21 and sex chromosome aneuploidy account for 60–80% of abnormal fetal karyotypes detected in cultured amniotic fluid cells (Hook, 1992). Rapid prenatal screening for these aneuploidies in uncultured amniocytes is performed worldwide either by multicolour interphase fluorescence *in situ* hybridization (FISH) (Kuo *et al*., 1991; Klinger *et al*., 1992; Ward *et al*., 1993; Philip *et al*., 1994; Tepperberg *et al*., 2001; Witters *et al*., 2002) or by multiplex quantitative fluorescence PCR (QF-PCR) based on polymorphic, short tandem repeat markers (Mansfield, 1993; Adinolfi *et al*., 1995; Mann *et al*., 2001, 2004; Cirigliano *et al*., 2004). These techniques have a high sensitivity and specificity, and can be useful when a rapid result (1–2 working days) is required in case of abnormal ultrasound findings or for obstetric management. Although informative only for the common aneuploidies, such a quick result is also valued as a relief of parental stress during the 7- to 14-day waiting period needed for cell culture and conventional cytogenetic analysis (Bui *et al*., 2002; Grimshaw *et al*., 2003).

Recently, multiplex ligation-dependent probe amplification (MLPA) has emerged as an alternative PCR-based technique for the relative quantification of genomic DNA sequences (Schouten *et al*., 2002). On the basis of a prospective, clinical study of 527 amniotic fluid samples, we investigated whether MLPA can be used for the detection of common chromosomal aneuploidies in uncultured amniotic fluid cells.

MATERIALS AND METHODS

Patient group and study design

Between February and May 2003, a total of 256 samples, and, between January and October 2004, a total of 271 samples were collected from the amniocenteses performed at the Department of Perinatology and Gynaecology of the University Medical Center Utrecht. Referral reasons were representative for the corresponding patient population, including both low-risk patients (maternal age $\geq$36 years) and patients with a high risk, based on ultrasound abnormalities or a high risk for Down syndrome based on serum screening and/or nuchal translucency measurement. The first 2 mL of amniotic fluid drawn was discarded. Samples were included only if the volume aspirated was at least 20 mL and if there was no visible blood contamination. Of all samples,

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Received: 11 January 2005
Revised: 10 June 2005
Accepted: 11 June 2005
Published online: 17 October 2005

88% was obtained between 15 and 17 weeks, and 18 samples were obtained during the third trimester (range 25–37 weeks). The study was designed to test MLPA prospectively. Results were available within 48 h after amniocentesis and they were interpreted without prior knowledge of FISH or karyotyping results.

Preparation of amniocyte lysate for MLPA

For MLPA, 1 mL from each sample was taken and transferred to a 1.5-mL Eppendorf vial. Amniocytes were collected by centrifugation for 5 min at 8000 rpm and washed twice with buffer (10 mM tris HCl, pH 8.3, 50 mM KCl). For cell lysis, 1.5 μL of proteinase K (10 mg/mL) was added and cells were incubated at 55 °C for 3 h. Proteinase K was inactivated by heating at 100 °C for 5 min. For each sample, 15 μL lysate was stored at 4 °C. The size of the genomic DNA fragments was variable, but less than 1000 nt in all cases (data not shown).

MLPA reaction

The P001 kit (MRC-Holland, Amsterdam, The Netherlands) used throughout this study contains MLPA probes for 40 genomic targets: eight probes each for chromosome 13, 18, and 21, and four each for the X and Y chromosomes. The other probes correspond to target sequences on chromosomes 1, 2, 3, 5, 8, and 15 (see Table 1, and the MRC-Holland website at www.MRC-Holland.com). MLPA reactions were performed in 96-well microtiter plates, containing a series of 15–45 consecutive samples per assay, all following instructions provided by the manufacturer. PCR products were separated by capillary electrophoresis using an ABI 3100 genetic analyser and ROX 500XL size standards (Perkin Elmer Applied Biosystems, Foster City, CA, USA). On the basis of titration experiments using genomic DNA obtained from blood, we found that a conclusive result by MLPA can be obtained if the minimum amount of input DNA is 10 ng (data not shown). This corresponds to the genomic DNA of about 1500 cells.

MLPA data analysis

Test results were defined as conclusive if, by visual inspection of peak profiles, the MLPA quality control fragments indicated that sufficient genomic DNA was present (see MRC-Holland website for details). For each MLPA probe, the size and peak area were analysed using Genescan 3.7 and Genotyper 3.6 software (Perkin Elmer Applied Biosystems, Foster City, CA, USA) and exported to a Microsoft Excel spreadsheet. Calculation of relative probe signals followed Schouten *et al.* (2002). In chromosomally balanced individuals, all relative signals for probes located on the autosomes are expected to be 1.0 because there are two copies of the target sequence in the genome. For calculating relative probe signals for probes on the sex chromosomes, we separately processed samples showing probe signals for Y chromosomal probes from those that did not. A monosomy is indicated by a relative probe signal ≤ 0.7, a trisomy by a relative probe signal ≥ 1.3. Inter-assay standard deviations were calculated on the basis of relative probe signals obtained in different assays. Intra-assay standard deviations were based on four replicates of the same sample in the same assay. Software details can be found at the MRC-Holland website.

RESULTS

We performed MLPA in 527 amniotic fluid samples (Table 2). For 10 samples (1.9%), an inconclusive test result was obtained, with the MLPA quality control fragments indicating an insufficient amount of genomic DNA. There was no correlation between MLPA failure and gestational age at sampling. There were 517 conclusive tests (98.1%). In 23 tests, a trisomy of either chromosome 13, 18, or 21 was found (Table 3), using the criterion that at least four of eight chromosome-specific probes have a relative probe signal ≥ 1.3 (Table 4). There was one case of monosomy X, in which all four probes specific for the X chromosome showed a relative probe signal ≤ 0.7 (data not shown). In all conclusive tests, the MLPA result for chromosomes 13, 18, and 21 was concordant with that of conventional cytogenetic analysis, with the notable exception of two cases of 69,XXX triploidy. The sex chromosome constitution, as indicated by MLPA, was consistent with that of karyotyping in all but one case, yielding a false-positive result. Here, MLPA indicated a 47,XXY karyotype based on the criterion that at least two of the four X chromosome–specific probes have a relative probe signal ≥ 1.3 (i.e. 1.45 for probe AR; 1.33 for probe PPEF1), whereas karyotyping indicated a 46,XY karyotype. On the basis of these results, the overall specificity of the MLPA test is 99.8%. There were 18 samples obtained during the third trimester (25–37 weeks of gestation). In all of these, a conclusive result was obtained, including one case of trisomy 13, one case of trisomy 18, and three cases of trisomy 21 (Table 3).

To evaluate the performance of the different probes, we determined the average relative probe signal and the inter- and intra-assay standard deviation for each of the 40 probes in normal samples (Table 1). All probes had an average relative probe signal of about 1.0, whereas inter-assay standard deviations varied more than threefold (range 0.051–0.168). The average inter-assay standard deviation was 0.091. There was no correlation between PCR-product size and inter- or intra-assay standard deviation (data not shown). In all cases, the intra-assay standard variation was lower than the inter-assay standard deviation (average 0.044). Overall, 98.3% of the relative probes signals in the normal samples were within the normal range, that is, between 0.7 and 1.3.

On the basis of 517 samples with a conclusive result, we determined the sensitivity and specificity for all autosomal trisomy probes (Table 5A). Whereas only a few probes have 100% sensitivity, the percentage of false positives for most probes is below 0.5%. Several autosomal probes (ING1, DCC, CDH2, TFF1, S100B) had

Table 1—Performance of MLPA probes for aneuploidy detection in uncultured amniocytes with normal karyotype

Probe	Chromosomal position	PCR product size (nt)	Relative probe signal (0.7< normal <1.3)		
			Average	Inter-assay standard deviation	Intra-assay standard deviation
BCAR3	1p22.1	418	0.987	0.088	0.055
F3	1p21.3	328	0.985	0.068	0.029
PRKCE	2p21	160	1.114	0.080	0.033
MME	3q25.2	283	1.021	0.072	0.016
XRCC4	5q14.3	373	1.012	0.156	0.093
IL4	5q23.3	130	1.005	0.073	0.034
MYC	8q24.21	238	0.989	0.090	0.059
B2M	15q21.1	194	0.997	0.068	0.031
ZNF198	13q12.11	265	1.001	0.095	0.072
BRCA2	13q13.1	355	1.005	0.078	0.017
CCNA1	13q13.3	178	1.017	0.065	0.045
RB1	13q14.2	220	0.997	0.071	0.047
DLEU1	13q14.2	400	0.997	0.082	0.032
ABCC4	13q32.1	148	1.012	0.060	0.042
ING1	13q34	445	1.005	0.087	0.035
P85SPR	13q34	310	0.976	0.086	0.023
TYMS	18p11.32	301	0.991	0.077	0.035
GATA6	18q11.2	391	1.011	0.094	0.037
CDH2	18q12.1	142	1.023	0.088	0.077
DCC	18q21.2	436	0.984	0.107	0.066
PMAIP1	18q21.32	172	0.981	0.129	0.043
BCL2	18q21.33	211	0.982	0.081	0.037
SERPINB2	18q21.33	346	1.016	0.089	0.051
NFATC1	18q23	256	0.987	0.065	0.033
STCH	21q11.2	247	1.007	0.137	0.055
NCAM2	21q21.1	166	1.013	0.077	0.026
APP	21q21.3	337	1.000	0.074	0.029
TIAM1	21q22.11	427	0.996	0.051	0.034
SIM2	21q22.13	136	0.995	0.081	0.042
TFF1	21q22.3	292	0.980	0.071	0.042
TMEM1	21q22.3	382	0.987	0.077	0.040
S100B	21q22.3	202	1.012	0.168	0.037
PPEF1	Xp22.13	229	0.977	0.094	0.050
AR	Xq12	154	1.041	0.141	0.036
PDCD8	Xq25	409	0.960	0.077	0.061
L1CAM	Xq28	319	0.984	0.111	0.049
SRY	Yp11.31	274	1.010	0.122	0.020
DBY	Yq11.21	364	1.008	0.092	0.082
UTY	Yq11.21	184	1.036	0.109	0.092
EIF1AY	Yq11.222	454	1.005	0.117	0.035

Table 2—Summary of prospective clinical study in 527 amniocenteses

	MLPA	Karyotyping
No relative copy number change 13, 18, 21, X,Y	493	501[a]
Trisomy 13	2	2
Trisomy 18	7	7
Trisomy 21	14[b]	15
Monosomy X	1	1
Inconclusive result	10	1
Total	527	527

[a] Including two cases of 69,XXX triploidy.
[b] One case of trisomy 21 gave an inconclusive result by MLPA.

a much higher percentage of false positives. Table 5B shows the percentage of false positives for probes on the sex chromosomes. In general, the percentage of false positive results for autosomal probes is lower compared to that of sex chromosomal probes.

Because the MLPA test was designed to detect copy number changes only of chromosomes 13, 18, 21, X, and Y, other chromosomal abnormalities with potential clinical significance for the fetus could only be detected by karyotyping of metaphases of cultured amniocytes. In our clinical series, there were seven abnormal karyotypes with consequences for the fetus that were not detected by MLPA, including the two cases of 69,XXX triploidy mentioned earlier. The other five cases concern structural

Table 3—MLPA detection of previously unknown aneuploidies

Result of MLPA	Result of karyotyping	Referral reason(*s*)[a]	Week of pregnancy at sampling[b]
Tri-13	47,XX,+13	US	26.3
Tri-13	47,XY,+13	FTS/NT	16.5
Tri-18	47,XX,t(3;5)(p10;q10)pat,+18	MA38/IUGR	18.2
Tri-18	47,XY,+18	MA42	17.6
Tri-18	47,XY,+18	MA41	17.3
Tri-18	47,XY,+18	US	35.6
Tri-18	47,XX,+18	MA36	16.3
Tri-18	47,XY,+18	IUD	19.0
Tri-18	47,XY,+18	MA42	18.6
Tri-21	47,XY,+21	MA36	16.3
Tri-21	47,XX,del(6)(q?)*de novo*,+21	US/IUGR	35.0
Tri-21	47,XY,+21	US	19.1
Tri-21	47,XX,+21	NT	16.0
Tri-21	46,XY,der(14;21)(q10;q10)mat,+21	Known familiar translocation	16.2
Tri-21	47,XY,+21	MA36	16.2
Tri-21	47,XY,+21	MA36	16.3
Tri-21	47,XY,+21	US	32.4
Tri-21	47,XY,+21	MA42	16.1
Tri-21	47,XX,+21	US/IUGR	31.6
Tri-21	47,XX,+21	FTS/MA42	17.1
Tri-21	47,XY,+21	NT/MA36	15.6
Tri-21	47,XY,+21	NT/MA36	16.0
Tri-21	47,XY,+21	FTS/MA42	16.0
Mono-X	45,X	MA37	16.2

[a] MA, advanced maternal age, followed by age in years; IUGR, intra-uterine growth retardation; US, abnormal ultrasound findings; IUD, intra-uterine death; NT, increased nuchal translucency; FTS, increased risk of chromosomal abnormalities as indicated by first-trimester serum screening test.
[b] Week and day.

chromosome aberrations or mosaicism of an additional chromosome of abnormal structure. All seven cases were high-risk cases with ultrasound abnormalities including abnormal nuchal translucency measurements, or serum screening indicating a high risk for Down syndrome.

DISCUSSION

We have developed a simple and rapid procedure for the determination of the relative copy numbers of chromosomes 13, 18, 21, X, and Y in uncultured amniotic fluid cells using MLPA. The procedure was validated on a clinical series of 527 amniocenteses. Conclusive results were obtained for samples obtained from week 16 up to late in third trimester, showing that the test is applicable until late during pregnancy. Lysis of amniocytes by proteinase K yielded DNA fragments with a size less than 1000 bp. This is not unexpected as more than 80% of the fetal cells in amniotic fluid are either dead or dying, releasing several enzymes including DNase (Gosden, 1992). Apparently, the DNA degradation did not interfere with MLPA, presumably because the genomic targets to which the dual MLPA probes hybridise are only 50–70 nt. In 10 cases (1.9%), the MLPA test failed to yield a conclusive result due to an insufficient amount of genomic DNA. Alternatively, the DNA may have been degraded to a larger extent compared to samples with a conclusive result. Because 8 of these 10 samples were from the first part of the clinical series, the inconclusive results also reflect our initial inexperience with the test. We conclude that 1 mL of amniotic fluid is sufficient to perform a reliable MLPA test in more than 98% of cases.

Amniotic fluid samples were processed in series of 15–45 consecutive samples. This ensures that aneuploidies, which more frequently occur in high-risk cases, can be identified against a background of low-risk maternal age cases, most of which show relative probe signals of ~1.0 for most probes. In 493 cases, where MLPA predicted a normal copy number for chromosomes 13, 18, and 21, the results by MLPA were concordant with those of karyotyping in all but two cases of 69,XXX triploidy. Here, there is no relative copy number change for any of the tested chromosomes. Therefore, MLPA results are indicative of a 46,XX karyotype in cases of 69,XXX triploidy. All cases involving an autosomal trisomy were correctly identified by MLPA, using the criterion that at least four of eight corresponding chromosome-specific probes were indicative of the trisomy. In addition, using the same criterion, there were no false-positive results for any of the autosomal trisomies. Also, the single case of monosomy X was correctly identified. Using the criterion that at least two of the four sex chromosome–specific probes indicated an abnormality, there was only one false-positive result, indicating a 47,XXY karyotype in a normal male fetus. In conclusion, the sensitivity of the MLPA test was 100% and the specificity was 99.8%.

Table 4—Relative probe signals[a] in cases of trisomy 13, 18, 21

Chromosome 13 probes	Relative probe signal		Chromosome 18 probes	Relative probe signal						
	Case 1	Case 2		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
ZNF198	1.19	1.37	TYMS	1.38	1.39	1.43	1.16	1.43	1.23	1.30
BRCA2	1.28	1.43	GATA6	0.97	1.17	1.17	1.24	1.65	1.47	1.36
CCNA1	1.22	1.40	CDH2	1.47	1.38	1.36	1.32	1.63	1.43	1.21
RB1	1.36	1.42	DCC	1.48	1.11	1.28	1.72	0.63	0.73	1.27
DLEU1	1.10	1.46	PMAIP1	1.42	1.34	1.00	1.10	1.60	1.06	1.31
ABCC4	1.33	1.22	BCL2	1.59	1.30	1.31	1.41	1.23	1.35	1.34
ING1	1.55	1.44	SERPINB2	1.15	1.39	1.14	1.29	1.45	1.20	1.26
P85SPR	1.35	1.25	NFATC	1.20	1.24	1.44	1.36	1.52	1.74	1.27

Chromosome 21 probes	Relative probe signal													
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14
STCH	1.35	1.53	1.36	1.38	1.29	1.57	1.26	1.16	1.31	1.18	1.32	1.28	1.14	1.27
NCAM2	1.20	1.37	1.12	1.42	1.26	1.45	1.35	1.26	1.13	1.30	1.23	1.36	1.20	1.21
APP	1.18	1.28	1.31	1.30	1.24	1.26	1.32	1.45	1.55	1.21	1.27	1.27	1.30	1.30
TIAM1	1.35	1.34	1.42	1.29	1.33	1.42	1.21	1.50	1.38	1.33	1.50	1.41	1.21	1.52
SIM2	1.38	1.32	1.13	1.15	1.35	1.18	1.30	1.35	1.12	1.52	1.31	1.35	1.45	1.28
TFF1	0.93	1.12	1.09	1.18	1.34	0.79	1.48	1.49	1.47	1.43	1.52	1.28	1.31	1.42
TMEM1	1.35	1.13	1.47	1.33	1.18	1.32	1.16	1.54	1.48	1.31	1.47	1.35	1.30	1.39
S100B	1.20	1.15	1.04	1.15	1.31	1.09	1.41	1.55	1.38	1.26	1.31	1.20	1.26	1.20

[a] Relative probe signal is normal if between 0.7 and 1.3; grey shading indicates a relative probe signal ≥1.3.

Table 5A—Sensitivity and specificity[a] of probes for detection of autosomal trisomies

Tri-13 probes	Sensitivity (%)	False positives (%)	Tri-18 probes	Sensitivity (%)	False positives (%)	Tri-21 probes	Sensitivity (%)	False positives (%)
RB1	100	0.2	BCL2	86	0.4	TIAM1	79	0.2
ING1	100	1.2	CDH2	86	1.2	TMEM	79	0.4
BRCA2	50	0.0	TYMS	71	0.2	SIM2	64	0.8
ABCC4	50	0.2	NFATC	57	0.2	TFF1	57	1.4
P85SPR	50	0.2	PMAIP1	57	0.8	STCH	50	0.2
ZNF198	50	0.4	GATA6	43	0.2	APP1	50	0.6
CCNA1	50	0.6	SERPINB2	29	0.6	NCAM2	43	0.0
DLEU1	50	0.8	DCC	29	1.9	S100B	36	2.3
Based on 517 samples/2 abnormal			Based on 517 samples/7 abnormal			Based on 517 samples/14 abnormal		

Table 5B—Sensitivity and specificity[a] of probes for detection of sex chromosomal aneuploidies

X chromosomal probes							Y chromosomal probes	
45,X	Sensitivity (%)	False positives (%)	47,XXX[b]	False positives (%)	47,XXY[b]	False positives (%)	47,XYY[b]	False positives (%)
PDCD8	100	0.8	PDCD8	0.0	PPEF1	0.8	DBY	0.0
PPEF1	100	0.8	L1CAM	0.8	PDCD8	1.5	EIF1AY	0.0
AR	100	0.8	PPEF1	1.2	AR	2.3	SRY	1.9
L1CAM	100	0.8	AR	3.2	L1CAM	4.2	UTY	2.3
Based on 251 samples/1 abnormal[c]			251 samples/0 abnormal[c]		266 samples/0 abnormal[d]		266 samples/0 abnormal[d]	

[a] Specificity is expressed as the percentage false-positive results.
[b] Sensitivity could not be determined because there were no results with this abnormal karyotype.
[c] Based on 251 samples with no PCR-products for Y chromosomal probes.
[d] Based on 266 samples with PCR-products for Y chromosomal probes.

During collection and analysis of the data, two tests of MLPA were published by others, based on 492 (Slater *et al*., 2003) and 809 amniotic fluid samples (Gerdes *et al*., 2005). The major outcome of our study is a critical evaluation of the performance of the different MLPA probes. We found that probes designed to detect trisomies of chromosomes 13, 18, and 21 have a widely diverging sensitivity (range 30 to 100%, see Table 5). Also, in the study by Slater *et al*. (2003), certain probes from the chromosome-specific sets failed to show the autosomal trisomy, although the authors did not document which probes. The finding that certain probes for trisomy detection show a higher percentage of false-negative results compared to others is unexpected, because all probes were designed to detect a single copy sequence per haploid genome. Perhaps, in relatively crude DNA lysates from amniocytes, relative probe signals ≤ 0.7 in normal samples may be due to incomplete denaturation of the target sequence area or to a decreased hybridisation efficiency of the probe to the target. Also, a point mutation at a critical nucleotide position in the target sequence may prevent probe hybridisation (Schouten *et al*., 2002). These factors may also explain the relative probe signals <1.3 in trisomic samples for the probes for the corresponding trisomy. Relative probe signals ≥ 1.3 in normal samples may be explained by probe hybridisation to a similar DNA sequence in the M13 vector used for preparation of the long oligonucleotide (J.P. Schouten, personal communication). For example, the X chromosomal probe AR indicates the presence of an additional X chromosome in normal 46,XX and 46,XY samples at a particularly high frequency (Table 5B), which, in our series, may have been responsible for the single false-positive result. Alternatively, these unexpected results could also be due to large-scale copy number variations of over 100 kb of genomic DNA that have recently been shown to occur in normal, unrelated individuals, and that can be found on virtually all chromosomes (Iafrate *et al*., 2004; Sebat *et al*., 2004). For example, we have found that probe NFATC1 (chromosomal position 18q23) is located within a region that is deleted and duplicated at a frequency of 1% each in unrelated dutch individuals (M. Poot, M. Eleveld and R. Hochstenbach, unpublished observations). Further research may discriminate among these possibilities. Improvement of the first-generation probe set should be undertaken by substituting probes with a low sensitivity and probes located in regions frequently showing copy number variation in the population. In addition, more sophisticated procedures for the calculation of relative probe signals are under development (Gerdes *et al*., 2005; www.MRC-Holland.com).

In our clinical series, there were seven abnormal karyotypes with potential consequences for the fetus that escaped detection by MLPA. From the evaluation of large clinical series of interphase FISH on uncultured amniocytes, it appeared that a molecular test cannot replace conventional cytogenetic analysis (Evans *et al*., 1999; Lewin *et al*., 2000; Thein *et al*. 2000). Therefore, we recommend MLPA to be used as a screening test only in conjunction with fetal karyotyping because structural

Table 6—Comparison of molecular methods for aneuploidy detection in uncultured amniocytes

Method	MLPA	QF-PCR	FISH
Number of markers each on chromosome 13, 18, 21	8	3–4	1
Number of markers each on chromosome X, Y	4	1	1
Size of genomic target DNA sequence	50–70 nt	2 × 25 nt	Several kb
Number of genomic targets per sample	40	13	∼5
Depends on informative markers	No	Yes	No
Amount of amniotic fluid needed	1–2 mL	1–2 mL	1–2 mL
Number of samples per assay	Up to 96	Up to 96	∼10
Automation/large throughput possible	Yes	Yes	No
Cost of consumables per sample (Euro)	12	14	50
Time needed to report result	<48 h	<24 h	<24 h
Sensitivity in clinical setting	100%	95.65%	99.7%
Specificity in clinical setting	99.8%	99.97%	97.6%
Test failure rate	1.9%	<1%	Up to 7%
Detection of mosaicism	ND	Yes	Yes
Detection of triploidy 69,XXX	No	Yes	Yes
69,XXY	ND	Yes	Yes
69,XYY	ND	Yes	Yes
Maternal contamination detectable	ND	Yes	Only if XY

ND: not determined
Bryndorf *et al*. (1997); Tepperberg *et al*. (2001); Grimshaw *et al*. (2003); Mann *et al*. (2004).

unbalanced aberrations, rarer aneuploidies, mosaicism, and triploidy 69,XXX cannot be identified by the current MPLA probe set. This follows the recommendations issued for the clinical use of interphase FISH by the American College of Medical Genetics (1993). Among the trisomies detected by MLPA in our series, one trisomy 18 was associated with a balanced familiar translocation that was unknown prior to karyotyping (Table 3). This illustrates that balanced parental rearrangements that predispose to abortions also go undetected if only MLPA is used.

Rapid aneuploidy screening is performed either by interphase FISH or multiplex QF-PCR in most clinical genetic laboratories (reviewed by Bui *et al*., 2002). Both FISH and QF-PCR are established, robust methods, with a high sensitivity and specificity (Mann *et al*., 2001, 2004; Tepperberg *et al*., 2001; Grimshaw *et al*., 2003; Cirigliano *et al*., 2004). A comparison of MLPA with these other techniques is shown in Table 6. MLPA and QF-PCR are less labour-intensive compared to FISH, more cost-effective, and more suitable for throughput of large numbers of samples. In contrast to QF-PCR and FISH, MLPA is unable to detect 69,XXX triploidy, and there is limited potential to discriminate maternal contamination from 69,XXY and 69,XYY triploidy. It also remains to be demonstrated whether chromosomal mosaicism can be detected by MLPA. However, there are two major advantages compared to QF-PCR. First, the number of genomic targets that can be addressed by single-tube multiplex QF-PCR is limited to about 13 (Mann *et al*., 2004), whereas by MLPA, up to 40 different genomic loci can be investigated. Second, QF-PCR is dependent on polymorphic markers that are informative in most, but not all cases. Non-polymorphic target genes located on the X chromosome (Cirigliano *et al*., 2002) and chromosomes 13, 18, and 21 (Rahil *et al*., 2002) have been developed, but these have not been adopted yet by the majority of laboratories. Because of these two major advantages, MLPA can be more easily exploited for other applications in prenatal diagnosis than QF-PCR. For example, it should be possible to use MLPA for the identification of other chromosomal abnormalities, such as (micro)deletions and unbalanced subtelomeric rearrangements.

In summary, this study documents that, in a clinical setting, MLPA can be used for the rapid determination of common chromosomal aneuploidies in uncultured amniocytes. The sensitivity, specificity, and test failure rate are comparable to those of FISH and QF-PCR. The technique is inexpensive and can be performed using equipment available at most clinical genetic laboratories. We see a relief of parental anxiety during the waiting period for the result of conventional cytogenetic analysis as the major benefit resulting from its clinical application.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Jan P. Schouten and Dr Martin Poot for discussions about the MLPA technique and data interpretation. The gift of MLPA kits for test implementation by MRC-Holland is gratefully acknowledged.

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