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Desirable quality-control materials for the establishment of qualified external quality assessment on prenatal diagnosis of chromosomal aneuploidies

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ABSTRACT

Objective: To prepare desirable quality-control materials for the establishement of qualified external quality assessment on fluorescence in situ hybridization (FISH)-detected prenatal diagnosis of chromosomal aneuploidies.

Methods: Four types of amniotic fluid cell suspensions (13-trisomy, 18-trisomy, 21-trisomy and 47,XXY) were mixed together by ratio to produce mosaicism with the percentages of each aneuploidy as 10%, 20%, 30% and 40%, respectively. After being stored in liquid nitrogen of −196 °C for six months, randomly selected samples were incubated in 37 °C water, followed by cultivation, hypo-osmosis and fixation. Finally, FISH detetion was applied on them before and after external laboratory mailing, in step with detection on conventional case samples.

Results: Before mailing, the positive rates of each aneuploidy described above were 12.8%, 23.6%, 33.8%, 44.0%, while 12.6%, 23.8%, 34.0%, 43.5% after mailing. *t*-test, criteria for stability assessment of quality-control materials in CANS-GL03:2006, showed no significant effect of external mailing on mosaicism since corresponding t values are lower than threshold with significance level α as 0.05 and degree of freedom as 10.

Conclusion: As FISH detection showed, the mosaic cell strains prepared in current study exhibited excellent stabilities after cryopreservation in −196 °C, subculture, hypo-osmosis, fixation and external laboratory mailing, demonstrating them as reliable and promising quality-control materials for the establishment of a qualified external quality assessment on prenatal diagnosis of chromosomal aneuploidies.

1. Introduction

Fluorescein-labeled nucleic acids are adopted as probes during the fluorescence in situ hybridization (FISH) detection to specifically bind a chromosome based on base complementary, and then emitted fluorescent signals was recorded for further luminescent qualitative analysis. Rapid as it is, FISH can greatly complement the deficiency of traditional cytogenetic diagnosis, such as failures in cell culture, time consuming and long reporting cycle. As a result, this method was widely applied in prenatal diagnosis of chromosome aneuploidies towards noncultured amniotic fluid cells in recent decades [1]. As an entry criteria for experimental diagnosis projects [2], the quality control of molecular genetic diagnosis, like FISH, has drawn great attention in the past few years [3,4]. As reported, Bastien P and co-workers [5] conducted a study on external quality assessment of prenatal molecular diagnosis in 23 laboratories from France and the conclusion was that external quality assessment played an important role in promoting technical communication between laboratories, problem discovery and diagnostic quality improvment. Additionally, Ramsden SC group [6] extracted 15 samples of DNA in non-cultured amniotic fluid or villous cells to perform a 3-year quality assessment on genotyping and clinical reports from 9 laboratories participated initially and 27 laboratories participated later. Obtained results highlighted the importance and necessity of external quality assessment on common molecular diagnosis of chromosomal aneuploidies. Since no mosaic amniotic fluid cell strains of chromosomal aneuploidies have been used as quality control materials for the external quality assessment on FISH-detected prenatal diagnosis of chromosomal aneuploidies, we attempt to generate such mosaic cell strains with desirable feathures as quality control materials to facilitate the establishment of corresponding external quality

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Table 1
FISH detections of chromosomal aneuploidies on quality control cell strains (from 6 identical tubes) before external mail and after external mail.

Tube number	Positive rates before external mail (%)				Positive rates after external mail (%)			
	13-trisomy	18-trisomy	21-trisomy	XXY	13-trisomy	18-trisomy	21-trisomy	XXY
1	12	23	35	43	13	22	32	42
2	8	24	33	44	12	25	35	43
3	15	26	36	47	9	27	36	46
4	14	20	32	43	13	21	33	44
5	13	25	34	42	14	24	33	41
6	15	24	33	45	12	24	35	45
Average (%)	12.8	23.6	33.8	43.8	12.1	23.8	34.0	43.5

assessment.

2. Materials and methods

2.1. Preparation for cell strains of chromosomal aneuploidy

Recombinant vector SV40Ltag-pcDNA3.1(−) was transfected into cell PT67 mediated by liposome to construct immortalized cell strains PT67 [7]. Then primary amniotic fluid cells seperately containing chromosome karyotype 13-trisomy, 18-trisomy, 21-trisomy and 47,XXY were transfected by SV40LT, which was generated from immortalized cell strains PT67. After positive clones screening via G418 and serial passage, cell strains derived from the 10th generation were harvested and cryo-preserved in liquid nitrogen of −196 °C according to the cell longevity, number of generations and the maximum cell amounts during passage.

2.2. Preparation of chromosomal aneuploid cell strains suspension as quality control materials for external quality assessment

Four types of chromosomal aneuploid cell strains (13-trisomy, 18-trisomy, 21-trisomy and 47,XXY) were prepared as cell suspension with concentration of 1×10^5 cells/ml, respectively. Subsequently they were mixed together in the proportion of 1:2:3:4 to afford mixture, of which the percentage for each type cell strains was 10%, 20%, 30% and 40%.

2.3. Cryopreservation and recovery of chromosomal aneuploid cell strains as quality control materials for external quality assessment

Cell mixture prepared above were suspended with freezing medium at a dose of 1×10^5 cells per tube and added into the frozen stock tubes. Then these tubes were stocked by liquid nitrogen in −196°Cthrough programmed cryopreservation. After random selection, 6 tubes were taking out for water-bath heating at 37 °C and washed twice with human amniotic cells medium. Then these cells were suspended with fixatives and stored in −20 °C after conventional low permeability and fixation.

2.4. Posting of chromosomal aneuploid cell strains as quality control materials for external quality assessment

Cryopreservation tubes containing target cell strains suspended in fixatives (methanol and acetic acid, 75/15, v/v) were enclosed into ordinary express envelopes and sent to the participating laboratories. Then these tubes were send back immediately, which took three to five days at temperature of 10 °C to 30 °C.

2.5. Detection of chromosomal aneuploidies on cell strains for external quality assessment

After receipt from participating laboratories mentioned above, received cell strains together with other cell strains prepared in current study but without suffering external mail were all frozen in −20 °C followed by detection of FISH. Meanwhile, conventional case samples were also subjected to FISH detection as control.

2.6. Reagents

FISH kit was purchased from Beijing JinPuJia medical technology limited company. 13/21 dual color probe (GLP): GLP 13 locates at 13q14, marked as green signal; GLP 21 locates at 21q22, marked as red signal. 18/X/Y tri-color probe (CSP) locates respectively at the centromere parts of chromosome 18, chromosome X and chromosome Y, with corresponding colors of signals as sky-blue, green and red, respectively. The fixative consist of 75% methanol and 15% acetic acid.

3. Results

3.1. Detection rates of chromosomal aneuploidies on quality-evaluation cell strains keep consistent before and after external laboratory mailing

The chromosomal aneuploidies of quality-evaluation cell strains stored respectively in six tubes were detected by FISH, and the results showed that the average detection rate of 13- trisomy, 18-trisomy, 21-trisomy and 47,XXY cell strains before and after external laboratory mail was 13%, 23.66%, 33.83%, 44% and 12.67%, 23.83%, 34%, 43.5%, respectively (Table 1). As the criteria for stability assessment of quality-control materials in CANS-GL03:2006 [8], *t*-test was performed based on the following Eq. (1). Looking-up table shows the threshold $\mathbf{t_{\alpha(n1+n2-2)}}$ to be 1.812 with significance level α as 0.05 and degree of freedom as 10. Since calculated t value was lower than this threshold, *t*-test result demonstrated that there was no significant difference in 13-trisomy, 18-trisomy, 21-trisomy, 47, XXY testing results before and after external laboratory posting (Table 2).

$$t = \frac{|\bar{x}_2 - x_1|}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2} \cdot \frac{n1+n2}{n1 \cdot n2}}} \quad (1)$$

As shown in Eq. (1), $\bar{x}_1$ and $\bar{x}_2$ represent the average values for the 1st and the 2nd detections, respectively. s_1 and s_2 represent the standard deviations for the 1st and the 2nd detections, respectively. n_1

Table 2
Average positive rates comparison between quality control cell strains (from 6 identical tubes) before external mail and after external mail.

Aneuploid karyotype	Average positive rates (%)		Standard deviation		*t*-test
	Before mail	After mail	Before mail	After mail	
13-trisomy	12.8	12.1	0.0264	0.0172	0.5116
18-trisomy	23.6	23.8	0.0207	0.0214	0.3200
21-trisomy	33.8	34.0	0.0147	0.0155	0.1954
XXY	43.8	43.5	0.0183	0.0187	0.3084