

# Fresh and aged human lymphocyte metaphase slides are equally usable for GTG banding

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**Abstract:** The identification of chromosomes for routine cytogenetic analysis is based on quality of metaphases and good banding pattern. Fresh slides of human lymphocytes have been shown to produce good bands for the identification of chromosomes morphology. G-bands by Trypsin using Giemsa (GTG) banding of aged slides is generally considered hard to get desired band pattern of chromosomes persistently. The current study is focused on GTG banding of aged slides. A total of 340 subjects including 290 primary infertile and 50 fertile were selected. The blood samples were drawn aseptically for cytogenetic analysis. Lymphocytes were cultured and GTG banding was done on 1440 glass slides. Giemsa trypsin banding of aged slides were done by adjusting average trypsin time for each month according to the slide age and metaphase concentration. Correlation analyses showed a significant and positive correlation between slide ageing and trypsin pre-treatment time. The results of this study suggest that, the fresh and aged human lymphocyte metaphases are equally usable for GTG banding.

**Keywords:** Cell culturing, chromosomes, cytogenetic, GTG banding, karyotyping.

## INTRODUCTION

Well spread metaphases are pre-requisite for cytogenetic analysis. Many culturing and spreading techniques have been developed from time to time using simple methods to complex models (Ronne, 1989; Spurbeck *et al.*, 1996; Hliscs *et al.*, 1997; Henegariu *et al.*, 2001; Deng *et al.*, 2003). Numeric anomalies can be determined by conventional Giemsa or Orcein staining methods but structural anomalies identification needs good banding techniques. Good banding meant for distinct alternate light and dark band pattern that allows identification of discrete region along each chromosome (Smith 2006). Only a few studies were done before the advent of chromosome banding (Philip *et al.*, 1970). In early 1970s, different banding protocols were established as Q banding, G banding and C banding for precise identification and pairing of each chromosome in the human karyotype (Caspersson *et al.*, 1970; Pardue and Gall, 1970; Seabright, 1971; Matsui and Sasaki, 1973). Technique of G-bands by trypsin using Giemsa (GTG) was introduced in 1971 which was further modified by many workers (Seabright, 1971; Sumner, 1971; Chiarelli *et al.*, 1972; Wang and Fedoroff, 1972; Korf *et al.*, 1976). GTG banding is most frequent, basic and stable method involving pre-treatment with trypsin and staining with Giemsa (Sumner *et al.*, 1971). Pre-treatment with trypsin hydrolyzes chromatin proteins. This technique produces a series of alternating dark and light bands along with each chromosome. Dark bands appear in AT rich region, while

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light bands in GC rich region (Holmquist, 1989; Holmquist, 1992; Moore and Best, 2001) as seen in fig. 1 a-d. The gross chromosomal anomalies are visualized by staining of the chromosomes, as gain and loss of whole or partial chromosome, presence of extra chromosomal material, ring chromosome, addition, deletion, translocation and inversion (Bayani and Squire, 2004; Yogesh *et al.*, 2011). GTG banding of aged slides is tricky. Each sample and slide needs specific optimization for human lymphocytes. Cytogeneticist prefers to make fresh slide for chromosomal evaluation to avoid laborious work. In countries like Pakistan, limited work has been done in the field of human cytogenetics (Moatter *et al.*, 2007; Aziz and Qureshi, 2008). This study was designed to perform GTG banding of aged slides which are hitherto generally considered useless.

## MATERIALS AND METHODS

A total of 340 subjects including 290 primary infertile and 50 fertile subjects were selected. The study was approved by ethical review committee of the institutes concerned. Informed consent was taken from subjects. The patients and sex matched controls were selected from Jinnah Postgraduate Medical Center (JPMC), Karachi and Infertility Clinic, Quetta Hospital, Quetta, Pakistan. Peripheral blood of subjects and controls were drawn in sodium heparin tube. Blood samples were cultured for 72 hours in RPMI-1640 (Cat No. 11875-119 Invitrogen) added with fetal bovine serum (cat no. 10100-139 Invitrogen), Streptomycin and penicillin (Cat. No.

SV30010 HyClone) and Phytohemagglutinin (Cat No. 10576-015 Invitrogen) as mitotic activating factor (Moorhead *et al.*, 1960). Freshly prepared Carnoy's solution was used for lymphocytes fixation. Milky white granules of lymphocytes from blood were obtained in the form of pellet. A couple of drops of lymphocytes suspension were dropped from a height of 20cm with pasteur pipette. Slides were left to dry on hotplate for few seconds or overnight, at room temperature. Lymphocyte suspension was kept in Carnoy's solution in -20°C for further use.

**G-bands by Trypsin using Giemsa**

All the slides (1490) were prepared on day one. Fifty (50) slides were stained on day 4 and the remaining slides were stained over a period of 12 months to see the quality of GTG banding in old slides. In each month 120 slides and in each week 30 slides were prepared to maintain accuracy and consistency. Slides were stored in a safe, dust and moisture-free environment at 20-30°C. The factor of humidity was excluded. Numeric aberrations were evaluated by conventional staining technique but structural aberrations needed fine banding. For GTG banding, 55mg trypsin (Cat No.27250-018 Invitrogen, USA) dissolved in 80ml phosphate buffer saline (PBS)

(Cat no. P4417, Sigma, USA) was used. After specific time for trypsin pre-treatment (table 1), slides were rinsed with PBS for few dips and then stained by Giemsa (Cat No.10092-013 Invitrogen, USA) for 5 minutes. After drying, slides were observed under bright field microscope (DM LS2 Leica, Germany). The trypsin time was noted in case of appearance of good bands; otherwise, trypsin time was adjusted depending upon metaphase morphology. All slides were treated for GTG banding in the same manner and were mounted by dibutyl phthalate in xylene (DPX) (Cat No.58402, Merck, Pakistan). Random slides were picked and visualized by Ikaros Metasystem version 5.84 attached with Nikon microscope (50i, eclipse, Japan). From each slide of patient and control, more than twenty metaphases were analyzed and GTG banding was observed.

**STATISTICAL ANALYSIS**

The Pearson's correlation coefficient was used to describe the relationship between the trypsin pre-treatment time and age of slide. Statistical analysis was done by using statistical package for social sciences (SPSS) version 18.0 for Windows (Chicago, IL. USA).

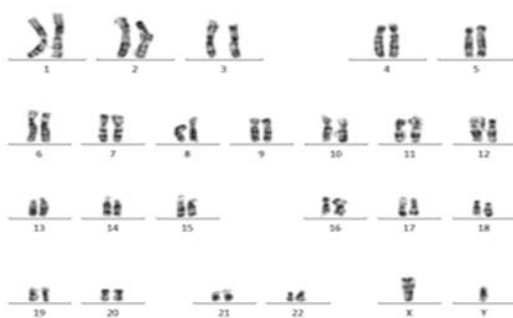


Fig. 1a

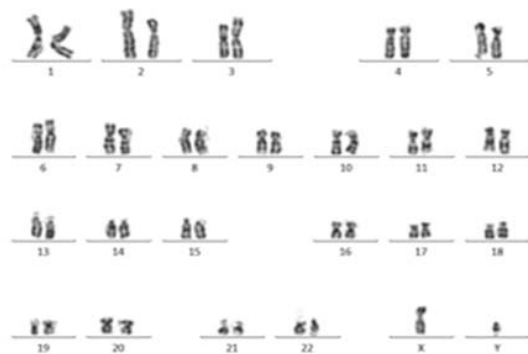


Fig. 1c

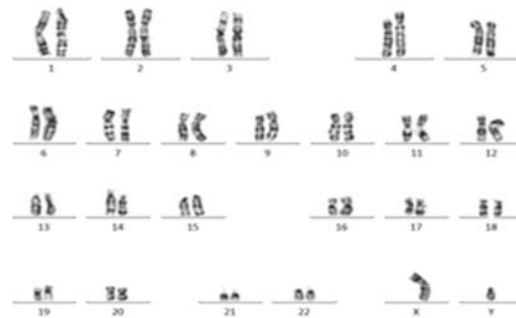


Fig. 1b

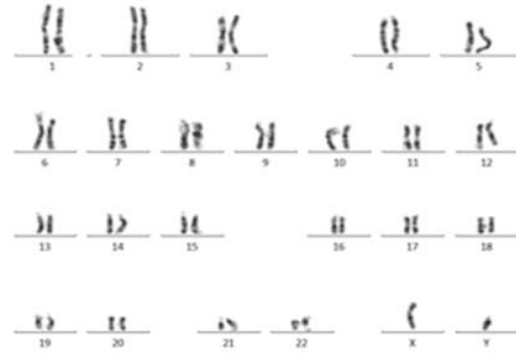


Fig. 1d

**Fig. 1:** Karyotype showing GTG banding; (a) four days old slide (b) one month old slide (c) six month old slide (d) twelve month old slide.

**Table 1:** Relationship among age of slide, trypsin pre-treatment time and number of metaphase

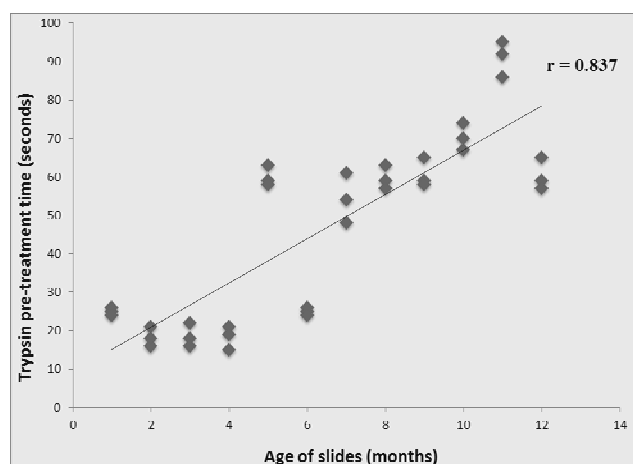
S. No.	Age of slides	No. of stained slides	Trypsin pre-treatment (Seconds)	Mean±S.D (seconds)	No. of metaphase	GTG Bands
1	4 days (Fresh)	19	40	34.86±4.71	High	Good Bands
		14	35		Medium	Poor Bands
		17	29		Low	Poor Bands
2	1 month	36	26	25.15±0.66	High	Poor Bands
		66	25		Medium	Good Bands
		18	24		Low	Poor Bands
3	2 months	26	21	18.22±1.67	High	Poor Bands
		26	16		Medium	Poor Bands
		68	18		Low	Good Bands
4	3 months	33	18	20±2.42	High	Poor Bands
		69	22		Medium	Good Bands
		18	16		Low	Poor Bands
5	4 months	36	21	18.13±2.52	High	Poor Bands
		40	19		Medium	Poor Bands
		44	15		Low	Good Bands
6	5 months	54	59	60.32±2.11	High	Good Bands
		45	63		Medium	Poor Bands
		21	58		Low	Poor Bands
7	6 months	40	26	25±0.82	High	Poor Bands
		40	25		Medium	Poor Bands
		40	24		Low	Good Bands
8	7 months	45	61	55.32±4.96	High	Poor Bands
		49	54		Medium	Good Bands
		26	48		Low	Poor Bands
9	8 months	34	63	59.62±2.29	High	Poor Bands
		55	59		Medium	Good Bands
		31	57		Low	Poor Bands
10	9 months	34	65	60.40±2.93	High	Poor Bands
		51	59		Medium	Good Bands
		35	58		Low	Poor Bands
11	10 months	34	74	70.08±2.78	High	Poor Bands
		44	70		Medium	Good Bands
		42	67		Low	Poor Bands
12	11 months	35	92	90.3±3.88	High	Poor Bands
		34	95		Medium	Poor Bands
		51	86		Low	Good Bands
13	12 months	34	65	59.88±3.34	High	Poor Bands
		37	59		Medium	Poor Bands
		49	57		Low	Good Bands

## RESULTS

All glass slides were GTG banded, keeping at laboratory temperature between 20-30°C. Chromosomal bands showed resolution in the range of 450 to 500 bands (ISCN 2009). No morphological differences of G bands were observed between slides of fertile and infertile having normal karyotype. For convenience, more than 12 metaphases at a view were assumed as high number metaphase slide, 6-11 metaphases as medium and less

than 6 metaphases as low number metaphase slide on 20X objective. The relationship among slide ageing, number of stained slides, trypsin pre-treatment time and number of metaphases (table 1) have been evaluated. A good GTG banding was observed in 4 days old slides, having good bands at high number of metaphase, mean trypsin pre-treatment time was 34.86 seconds and standard deviation was ±4.71 seconds (fig. 1a). In one month old slides, good GTG banding was observed with medium number of metaphases, mean trypsin pre-treatment time was 25.15

seconds with  $\pm 0.66$  seconds standard deviation (fig. 1b). Six months old slides produced good GTG banding upon pre-treatment of trypsin for 25 seconds, standard deviation was  $\pm 0.82$ , with low number of metaphases (fig. 1c). The oldest slides, about 12 months old, demonstrated good GTG banding with low number of metaphases when treated with trypsin for about 59.88 seconds having  $\pm 3.34$  seconds standard deviation (fig. 1d). The correlation ( $r = 0.837$ ,  $p < 0.001$ ) was significant between ageing and trypsin pre-treatment time (fig. 2).



**Fig. 2:** Correlation between age of slides and trypsin pre-treatment time.

## DISCUSSION

The most common stain used in cytogenetics is Giemsa (Maly *et al.*, 2012). Current G banded karyotyping is applicable for most chromosomal analyses. High quality chromosomes are equally important in diagnosing different type of chromosomal anomalies and provide further help in disease management (Kannan and Alwi 2009, Qu *et al.*, 2012). This GTG banding is also used, to study the homology between two comparable organisms (Borisov and Orlov 2012).

Storage of lymphocyte suspension at  $-20^{\circ}\text{C}$  after harvesting is a general practice in cytogenetic laboratory. Lymphocyte suspension and unstained slides remains stable and usable for GTG banding or FISH more than a year if kept at  $-20^{\circ}\text{C}$  in 100% ethanol jar (Henegariu *et al.*, 2001). The lymphocyte suspension at  $-20^{\circ}\text{C}$  and unstained slides of patients and healthy subjects were observed equally stable till month one to twelve even at  $20-30^{\circ}\text{C}$  without ethanol. Lymphocyte or unstained slides can be kept longer for re-assessment or for research purpose although initial results would have been communicated to the patients and, hence will not be used for clinical interpretation. When a chromosomal analysis is required, slides are prepared from the stored suspension and GTG banding is performed. Good morphology of bands is achieved from freshly prepared slides (fig. 1a).

Therefore, fresh slides are required for chromosomal analyses. However, slides can be prepared from the suspension after harvesting and can be stored unstained and when chromosomal analysis is required, these slides can be used for GTG banding periodically. The band morphology is similar in freshly prepared, as well as in aged slides (fig. 1 a-d). The trypsin pre-treatment time of slides (table 1) needs to be adjusted with ageing. The length of enzyme treatment is a crucial variable for quality bands (Dolan 2011). The correlation analysis was performed from month one to twelve. A significant correlation coefficient ( $r = 0.837$ ) at ( $p < 0.001$ ) was found between ageing and trypsin pre-treatment time.

One, 3, 7, 8, 9 and 10 month aged slides with medium number of metaphases showed good banding. The 2, 4, 6, 11 and 12 month old slides with low number of metaphases also produced good banding and 4<sup>th</sup> day and 5<sup>th</sup> month aged slides of high metaphase number produced good bands. For good banding low and medium number of metaphase slides are best but the high metaphase number slides can also produce good bands suggesting that any number of metaphase slides (high, medium, low) can produce good bands but the better results are obtained in slides with low and medium metaphase number. The range of standard deviation of trypsin pre-treatment time was from  $\pm 0.66$  to  $\pm 4.7$  seconds from month one to twelve (table 1). Deviation in trypsin pre-treatment time for each month stained slides was due to variable factors as different number of lymphocytes/metaphase in each sample, length of chromosomes, etc.

It is concluded that, similar to fresh human lymphocytes metaphase slides, aged slides can also be used to produce good bands for chromosomal analyses.

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