SELECTED BANDING TECHNIQUES IN THE IDENTIFICATION OF HUMAN CHROMOSOMES

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INTRODUCTION

Detailed analysis of the structure of normal and abnormal human chromosomes has advanced rapidly since the advent of the banding techniques, permitting successful identification of individual chromosomes (Paris Conference, 1971). Characteristic banding patterns have been shown along each metaphase chromosome though the biochemical basis for the staining reactions involved are still not clear.

Bands were first demonstrated in human chromosomes, using a fluorescent technique involving 'staining' with quinacrine mustard (Caspersson *et al.*, 1970). This was later replaced by a more easily available antimalarial drug, quinacrine dihydrochloride (atebrin). Such Q-bands are easily differentiated from G-bands produced by a non-fluorescent technique using Giemsa dye as a staining agent (Seabright, 1971). Another technique involving heat denaturation, results in a Giemsa staining banding pattern opposite to that obtained by the G-banding methods. This is referred to as the Reverse-staining Giemsa method, introduced by Dutrillaux and Lejeune (1971), resulting in R-bands. C-bands, unlike Q-, G- and R- bands, represent constitutive heterochromatin areas of human chromosomes located around the centromeres, secondary constrictions and the long arm of the Y chromosome. Routine C-banding procedures (Arrighi and Hsu, 1971; Sumner, 1972; Yunis *et al.*, 1971) have revealed considerable variability in the size of C-bands in the long arm of the Y chromosome and the centromeric regions of homologues of chromosome pairs 1, 9 and 16. For purposes of practical chromosomal identification, the C-banding technique is limited in demonstrating only certain segments of the genome. It is best used in combination with G-banding.

This article does not intend to review all the banding techniques introduced but to comment on the more useful ones relevant to chromosome identification in a routine diagnostic laboratory.

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RELEVANCE OF GOOD CHROMOSOME PREPARATIONS

A number of factors may influence the success of good banded preparations. What works well for one laboratory may not necessarily be applicable to another, unless the variable factors are standardised to a certain extent. The following salient points must be considered:-

Over-Contracted Chromosomes

To get a good number of metaphases, cell populations are usually treated with a mitosis arresting agent which inhibits spindle formation. The resulting metaphases, if too contracted, would band poorly. The right concentrations of colchicine or colcemide or velban in a short term 2-hour treatment prior to harvesting, should be sufficient.

Air-Dried Preparations

Though flame dried slides have been successfully banded in some laboratories; in the author's experience, air-dried specimens are preferred, invariably providing more superior banding preparations. Fixed cell suspensions should be dropped onto wet slides and air-dried.

Aging of slides

Freshly prepared slides vary in their response to the different banding treatments. It is also true that very old slides do not band well. The author finds 3 to 10 day old slides, most often band consistently well with the different techniques.

CHOICE OF BANDING TECHNIQUES

A cytogeneticist has at his/her disposal, a wide range of banding techniques for chromosome identification. Any attempt here, at a detailed review encompassing most techniques would be futile. The choice of technique must depend on the type of study and the chromosomes to be analysed. Some techniques provide a good picture of the entire karyotype (G-, Q- & R- banding) while others may demonstrate only certain segments of the genome (C-banding).

Chromosomal anomalies, non-numerical in nature, cannot be easily identified by the conventional orcein or giemsa staining of the human karyotype. With good banded preparations, one can recognise and pinpoint chromosomes or chromosome segments involved. At least one banding technique must be applied for each karyotype study, and because of its simplicity, either the G-or R- banding techniques would be suitable. A sus-

pected chromosomal anomaly once demonstrated, can be further substantiated by other banding techniques eg. a translocation by centric fusion or pericentric inversion calls for C-banding and polymorphic Y chromosomes require Q or RFA (Reverse-staining method using fluorescence and acridine orange stain) banding.

Listed below are selected banding procedures with modifications successfully applied by the author. It is hoped that interested research workers can get good preparations. The original methods may involve several steps and are time consuming. Here, some steps are modified to get good clear bands in the shortest time possible.

G-Banding

For a routine karyotype analysis, G-banding is simple and most often gives good clear bands.

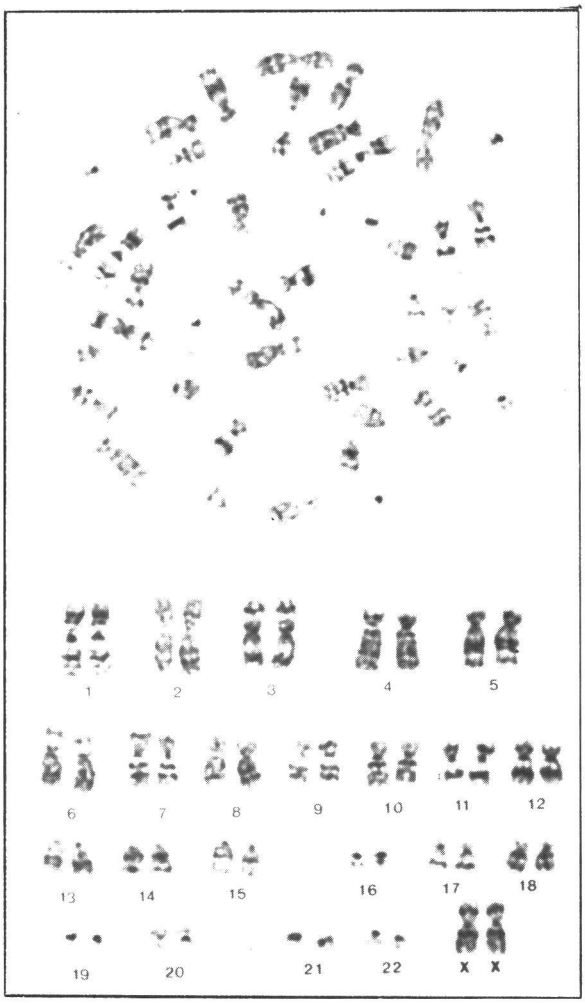

Fig. 1. Normal human female karyotype obtained from blood culture. Trypsin G-Banding.

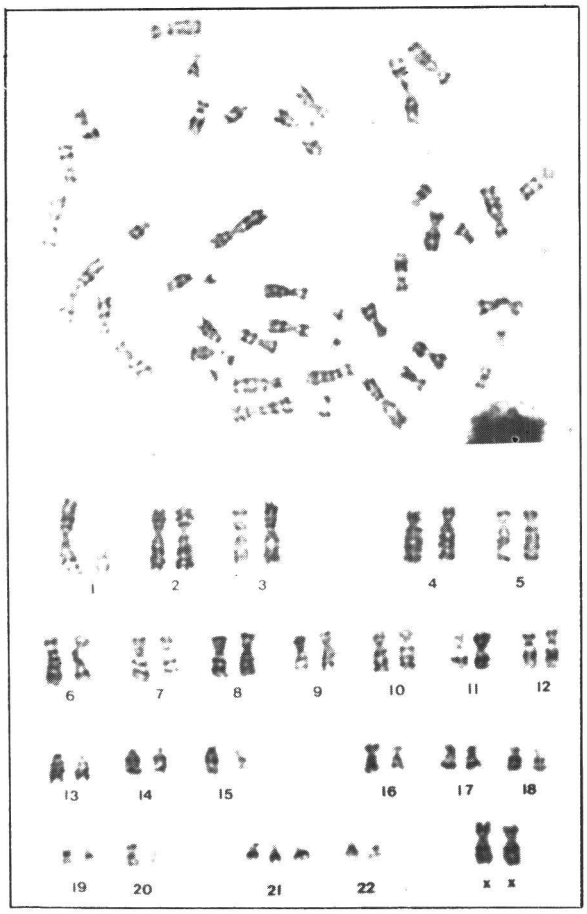

Fig. 2. Female trisomy 21 (Down's syndrome) karyotype with primay trisomy. Trypsin G-Banding.

Trypsin digestion is the preferred procedure. First, it is best to pretreat the slides with 2xSSC at 60°C for an hour. Some workers use hydrogen peroxide (H_2O_2) or no pretreatment at all. Slides are then to be flooded in 0.25% trypsin prepared in saline. Depending on laboratory temperatures, lower concentrations can be attempted. This trypsin step is important, each batch of slides can vary and be dependent on the different batches of trypsin used. Generally, a 30 second period of digestion with trypsin should be adequate. With practise, one can get good bands with the right timing. Slides should then washed at least twice before staining in 2% Giemsa for 10 minutes. Should the slides be underbanded, they can be flooded in trypsin for a variable time period, until bands produced are satisfatory (Fig. 1 & 2). All cell preparations were from cultures grown using RPMI 1640 and fetal calf serum. (Gibco)

In place of trypsin, a variety of chemicals can be used, even commercially available laboratory detergents eg. lipsol (Stephen, 1977).

C-Banding

Very often, it is advisable to counter check on the same specimen should the chromosomal anomalies involve the centromeric regions (e.g. centric translocations, pericentric inversions) or the length of the Y chromosome. A sequential analysis involving firstly G-banding, subsequently followed by C-banding would be useful in confirming the above. The modified BSG (Barium hydroxide/saline/giemsa) technique of Chandley and Fletcher (1973) can be performed on slides already G-banded. Slides first immersed in 0.2M HCl at room temperature for an hour, should be treated with 5% barium hydroxide at 60°C before being stained in 2% Giemsa for about 10 minutes. Fig. 3 shows chromosomes C-banded directly, the constitutive heterochromatin areas (i.e. C-bands) are highly polymorphic in chromosome pairs 1, 9 and 16 in this particular individual.

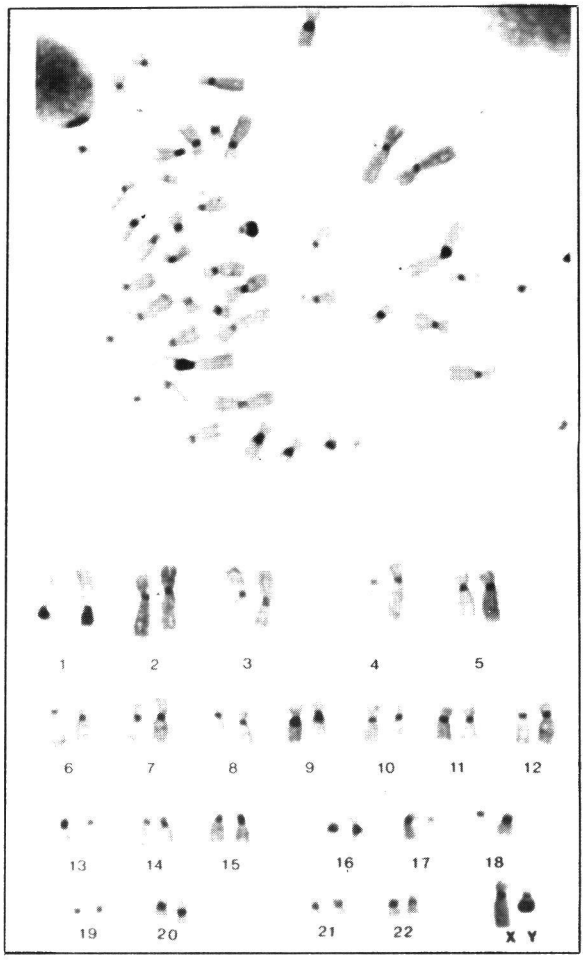

Fig. 3. Normal human male karyotype, C-banded with chromosome pairs 1, 9 and 16 highly polymorphic. Y chromosome distinctly C-banded.

R/RFA Banding

R-banding provides bands which are invariably opposite in staining intensity to G-bands though not as clear. Slides immersed in phosphate buffer, pH 6.5 at 85°C for 10 to 20 minutes could then be stained in 2% Giemsa. Heat denaturation in Earle's medium, pH 6.5 can be used instead of buffer (Dutrillaux, 1973).

Of late, a number of fluorescent techniques are available to produce R-banding patterns. Intensive yellow green fluorescence are observed at the sites of these R bands. Fluorescence procedures however, need microscopic accessories which may not be within the budget of some laboratories. In addition to technical difficulties, they are handicapped by rapid fading; hence preparations are not permanent, requiring photographic evidence to be taken immediately. Nevertheless, fluorescence techniques are superior in recognising chromosomes polymorphic for colour intensities and band sizes.

In differentiating such polymorphic chromosomes, the author finds the RFA technique of Verma and Lubs (1976) and Verma *et al.* (1977) superior to Q banding. This technique, using fluorescence is the same for ordinary R-banding, except that 0.01% acridine orange staining is used instead of giemsa. An 8 to 10 minutes staining should be sufficient. It is especially good for detecting fluorescence polymorphisms of human acrocentric chromosomes (chromosomes 13, 14 & 15 and chromosomes 21 & 22), clearly differentiated in terms of colour intensities and sizes of satellites stained.

RFA banding may be successfully applied to tracing the parental origin of the extra chromosome 21 in trisomy 21 (Down's syndrome) individuals (Yip, 1978). Fig 4 shows the karyotype of a trisomy 21 individual with the three chromosome 21s clearly polymorphic. Here, in this example, family studies were informative. The parental origin of the extra chromosome 21 could be determined since 2 of the 3 chromosome 21 variants were identically present in the mother, testifying to a maternal nondisjunctional error at meiosis. Also, the fact that these two chromosome 21s were dissimilar and not an identical duplicate of either one of the maternal chromosome 21s, pinpoints to a meiotic I error.

Q-Banding

This involves a simple fluorescence procedure, most useful for studying Y chromosome polymorphisms. Slides can be stained in 1% atebrin (quinacrine dihydrochloride) for 5 to 10 minutes, washed with distilled water, temporarily mounted in phosphate buffer for observations under the fluorescence microscope.

In conclusion, considering the range of banding

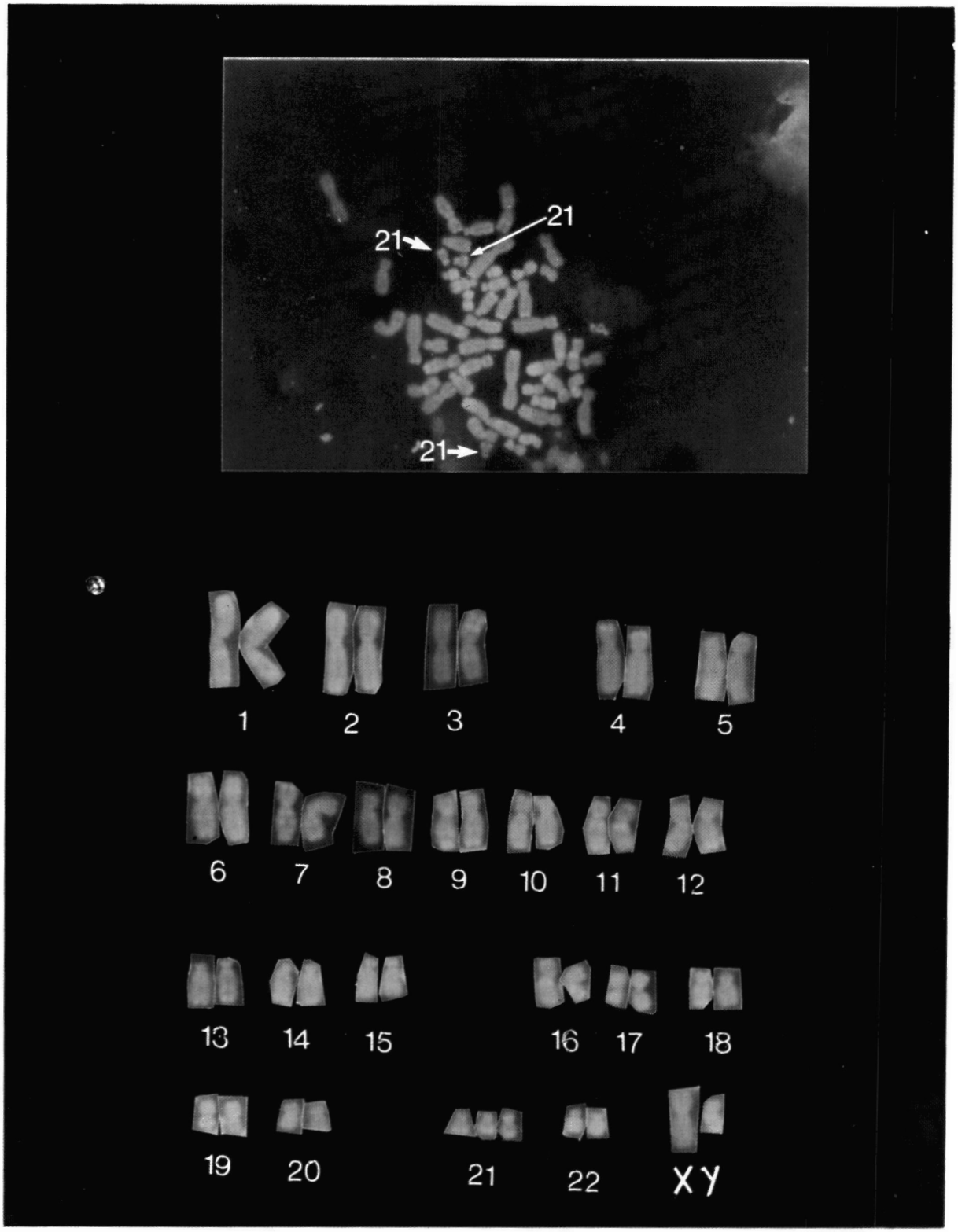

Fig. 4. Trisomic 21 cell and karyotype showing the three polymorphic chromosome 21s, varying in fluorescence intensities on both the short arms and the distal part of the long arm (RFA technique).

techniques available and its application to the understanding of chromosomal abnormalities prevalent in populations, the case for advocating the importance of chromosomal diagnostic laboratories need hardly be emphasized. The reliability of the new banding techniques has rendered simple morphological identification of human chromosomes inadequate. Laboratories have to decide as to which technique would be most suitable for their purpose. In that the Giemsa staining methods are highly reliable, requiring only bright-field microscopes and preparations permanent, research workers may have to use their discretion in combining a choice of other techniques when the need arises. Undoubtedly, the discovery of new banding techniques has provided useful tools for the recognition of breaks and rearrangements along chromosomes, confirmed many an established syndrome and is helping in the understanding of new ones.

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