

## METHODOLOGY

# Recuperation and improvement of chromosome preparations stained with silver nitrate

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## ABSTRACT

Both periodic acid and photographic reagents have been used to remove the silver nitrate residues from cytological preparations. We used potassium ferricyanide to remove AgNO<sub>3</sub> salts in cases of excessive chromosome impregnation. This method produced partial decolorization, with contrast enhancement. Counterstaining with Giemsa also promoted a better contrast between chromosome arms and NORs, which were preserved and retained a dark color. Application of this procedure to chromosome preparations treated sequentially for CBG banding/AgNO<sub>3</sub> staining promoted complete decolorization of C+ regions, leading to "reverse" C-banding, generally with high contrast.

## INTRODUCTION

Improvement of chromosome banding techniques has been important for advancement in cytogenetics. In spite of their generalized use, the action of the different chemical agents applied to chromosome preparations is still not clear.

The effects of both C and NOR banding on chromosome preparations seem to be irreversible. However, Dev (1979), using photographic chemical agents and Denton (1989), using periodic acid, have improved cytological preparations treated with argentic impregnation.

## MATERIAL AND METHODS

Cytological preparations of human and fish (*Lepidosiren paradoxa*) cells, obtained with leukocyte culture (Moorhead *et al.*, 1960) and with direct methods (Foresti *et al.*, 1993), were tested. These preparations were stained with silver nitrate, according to the technique described by Howell and Black (1980). For Ag-NOR banding they were then treated with an aqueous K<sub>3</sub>Fe(CN)<sub>6</sub> solution (1% ppv) during a few seconds (1-5 sec), and gently rinsed in water.

These procedures were also applied to previously C-banded chromosome preparations according to Sumner (1972) and sequentially treated with AgNO<sub>3</sub>. Finally, the material was stained with a Giemsa solution (5%, 2-3 min).

## RESULTS AND DISCUSSION

The potassium ferricyanide applied moderately to chromosome preparations, previously stained

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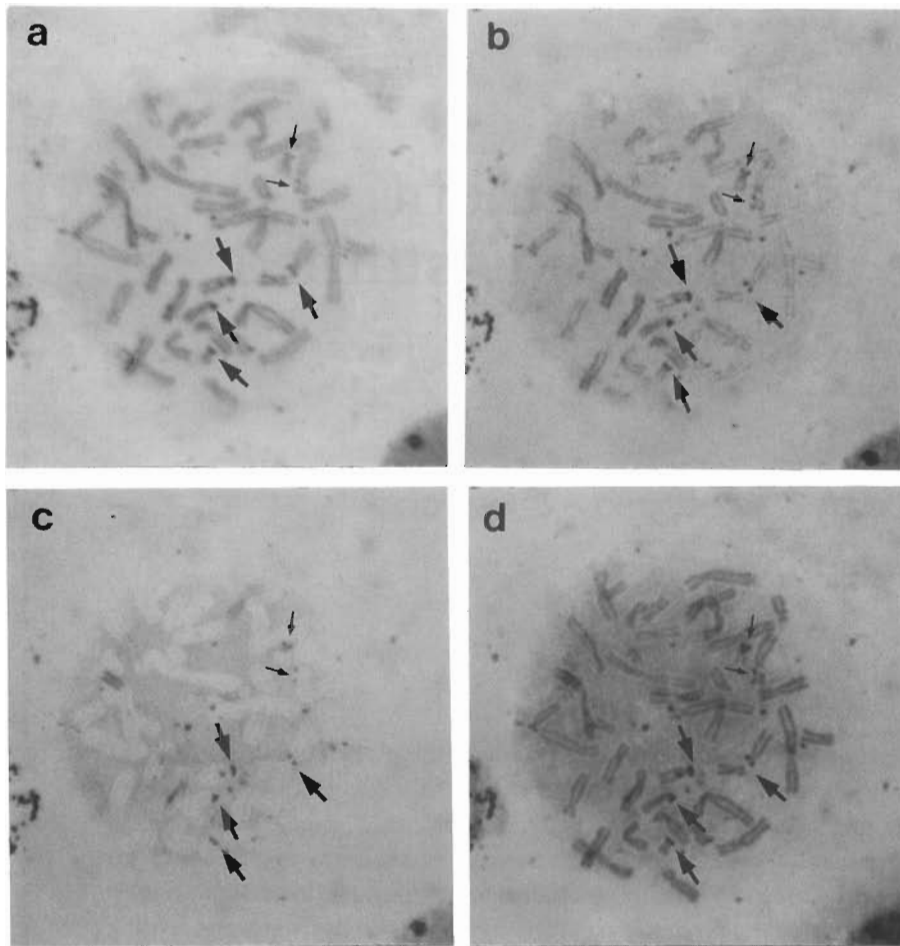


Figure 1 - Human metaphase plates. a, Ag-NOR stained. b, c, The same plate successively destained with  $K_3Fe(CN)_6$ . d, Giemsa counterstaining. Note the improvement of the NOR positive dots. The arrows indicate the NORs.

with  $AgNO_3$  caused selective removal of precipitated silver, apparently without any structural chromosome modifications. The chromosome arms progressively lost the brown color but the NORs retained their dark  $AgNO_3$  marks. Longer treatments with potassium ferricyanide promoted complete decolorization of the

chromosome preparations demonstrates the high affinity between silver salts and NOR. In these zones removal of precipitated silver salts was found to be very difficult. This fact could be related to the presence of acid proteins (C23) associated with chromatin (Ochs and Buchs, 1984).

cytological preparations, except for the NORs (Figure 1). These preparations can be stained again, either with  $AgNO_3$  or Giemsa, in order to improve the results.

Chromosomes stained successively for both C-banding (CBG) and  $AgNO_3$  were also treated with potassium ferricyanide. This procedure promoted differential removal of the characteristic brown gold color from chromosome arms, especially in C bands where all the constitutive heterochromatin "appears" to be removed. In these cases a "reverse" C-banding effect is observed (Figure 2). A peculiar staining pattern resulted with chromosomes in brown, NORs in black and C-positive bands in white, allowing a more accurate determination of C-band location and limits (Figure 2). Counterstaining with Giemsa showed that the heterochromatic regions in these preparations were not significantly affected as they again took on a bright blue color (Figure 3).

The lack of effect of  $K_3Fe(CN)_6$  on the  $AgNO_3$ -stained chromosome preparations demonstrates the high affinity between silver salts and NOR. In these zones removal of precipitated silver salts was found to be very difficult. This fact could be related to the presence of acid proteins (C23) associated with chromatin (Ochs and Buchs, 1984).

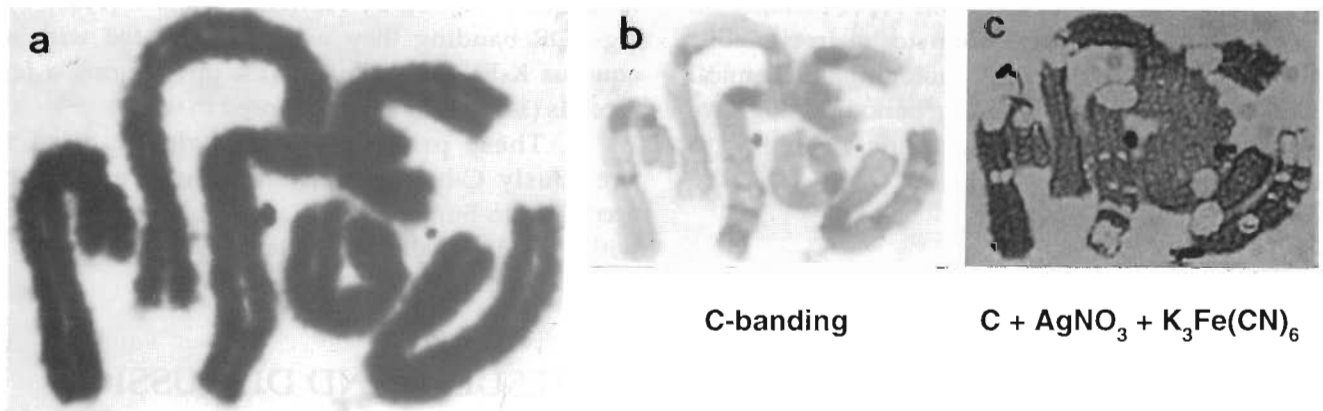
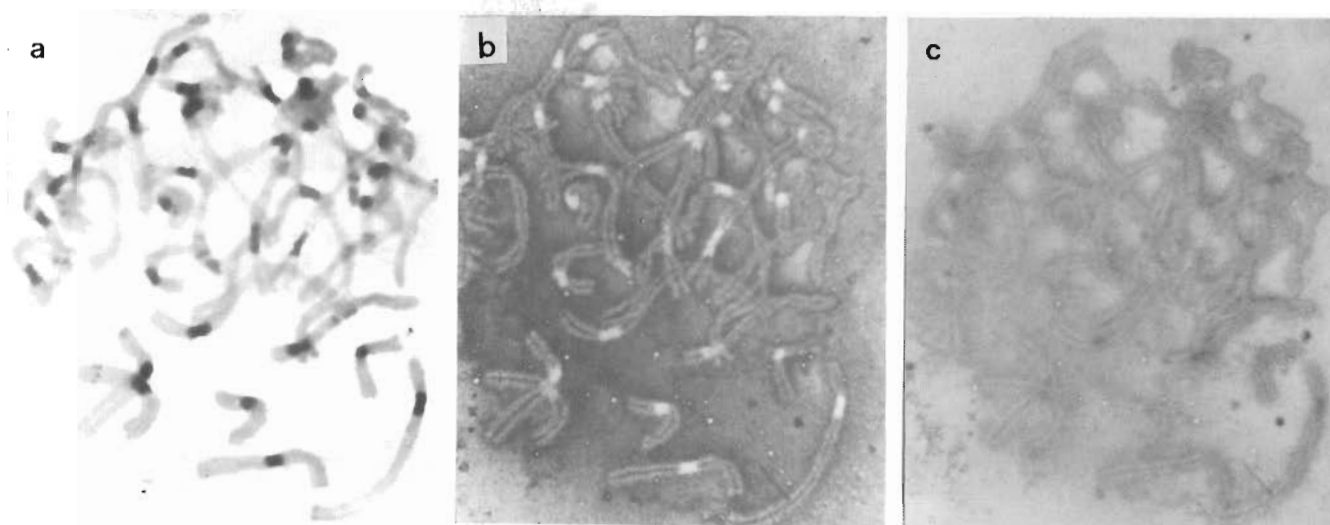


Figure 2 - *Lepidosiren paradoxa* partial metaphase plate. a, Conventional Giemsa staining. b, C-banding. c, "Reverse" C-banding obtained after successive treatments with  $AgNO_3$  and  $K_3Fe(CN)_6$ .



**Figure 3** - *Lepidosiren paradoxa* metaphase plate. a, C-banding. b, "Reverse" C-banding ( $\text{AgNO}_3/\text{K}_3\text{Fe}(\text{CN})_6$  successive treatment). c, Giemsa counterstaining. Note that the C+ bands reacquired coloration.

The present method produced fast and easy silver salts removal from  $\text{AgNO}_3$ - and C-banded-treated chromosomes.

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## RESUMO

O ácido periódico e reagentes fotográficos têm sido usados para remover resíduos de nitrato de prata de preparações citológicas. Nós usamos ferricianeto de potássio para remover sais de  $\text{AgNO}_3$  em casos de impregnação cromossômica excessiva. Este método produziu descoloração parcial, acentuando o contraste. Coloração subsequente com Giemsa também causou um contraste melhor entre os braços cromossômicos e NORs que foram preservados e retiveram uma cor escura. O uso deste procedimento para preparações cromossômicas tratadas sequencialmente por bandamento CBG e coloração por  $\text{AgNO}_3$  levou a descoloração global das regiões C+, causando um bandamento C "reverso", geralmente com alto contraste.

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