Uncultured Blood Samples can be Labeled by PRINS and Ready for Chromosome Enumeration Analysis 1 H After Collection


ABSTRACT

We describe a rapid technique to determine numerical abnormalities of chromosomes that can be applied to slides prepared from fresh, uncultured blood samples. Primed in situ synthesis is a method that has previously been utilized as a rapid alternative to conventional fluorescence in situ hybridization for localizing repeated DNA sequences on metaphase chromosomes and interphase nuclei prepared from cultured lymphocytes. By applying the technique to uncultured preparations from fresh blood, aneuploidy analysis can be completed in less than 3 h from the collection of the blood sample.

INTRODUCTION

The technique known as primed in situ synthesis (PRINS) was first described in 1989 (2), and since then it has developed into a very rapid and sensitive method for detecting numerical abnormalities of chromosomes. Whereas fluorescence in situ hybridization (FISH) is generally performed over two days (7), PRINS can be completed in less than 30 min. A PRINS reaction is performed by hybridization of an unlabeled oligonucleotide primer to the target chromosomal DNA. Hybridized primers then direct the enzymatic synthesis of labeled DNA at the target site, by extension from the 3′ end, incorporating labeled nucleotides, which are subsequently visualized by fluorescence microscopy. Developments such as multi-color PRINS (6) and cycling of the PRINS reaction (1) have increased the versatility and sensitivity of the PRINS technique. Detection of single-copy gene sequences has also been reported by using a polymerase chain reaction (PCR)-based derivative of the technique (5). This raises the possibility that newly discovered genes could be mapped onto chromosomes very rapidly by using synthetic oligonucleotides derived from partially sequenced DNA (e.g., sequence tagged sites and expressed sequence tags).

Previous reports (2–4) have demonstrated PRINS labeling of specific DNA sequences within metaphase chromosomes. Metaphase spreads are prepared by culturing peripheral blood lymphocytes stimulated with phytohemagglutinin (PHA) for 2–3 days, then arresting the cells at metaphase with colcemid. The resulting cells are swollen by using a hypotonic treatment and fixed with methanol:acetic acid. PRINS also labels centromeres within interphase nuclei present in these preparations, when the signals appear as discrete spots (3). In this report, we

Figure 1. PRINS labeling of chromosome 1. (A) Metaphase spread and interphase scanned at high power (×63). Two copies of chromosome 1 have each been labeled at the centromeres and in the nucleus. (B) Low power (×25) scan of direct blood preparation. Two spots corresponding to each copy of the chromosome are visible in most nuclei, but note the nucleus at the upper left shows only one signal. Focusing to a different plane displays the second signal in this nucleus and increases the strength of the second signal in its neighbor, but it loses some of the signals in the remaining nuclei, because of the limited focal depth of the scanning system. (C) High power (×63) scan of interphases.
demonstrate that PRINS can be used successfully to label specific chromosomes in uncultured cells from fresh blood samples. Preparation time is therefore reduced to a minimum, so that sample can be ready for complete aneuploidy analysis following chromosome labeling by PRINS in less than 1 h from collection from the patient.

MATERIALS AND METHODS

Metaphase spreads were prepared from peripheral blood lymphocytes provided by normal male volunteers, cultured by standard methods, fixed in methanol:acetic acid (3:1), dropped onto acid/alcohol cleaned glass slides and then air-dried. Direct blood slides were initially prepared (from the same samples) by putting a drop of lithium heparin-treated fresh blood on a clean glass slide, spreading a film with the edge of a second slide and, after air-drying, fixing directly in methanol:acetic acid (3:1) for 2–3 min. This method produced identifiable signals, but they were weaker than the comparable signals on cultured metaphase cells (data not shown). More recently, the signal quality of direct preparations has been improved by incorporating a hypotonic step: 0.5 mL of lithium heparin-treated fresh blood is placed in a conical-based, screw-cap polycarbonate 15-mL centrifuge tube, and 9.5 mL of 0.075 M KCl are added drop by drop while mixing on a vortex mixer (adding the hypotonic KCl too quickly can cause the nuclei to lyse). The sample is left at room temperature for 10–15 min and then centrifuged at 200×g for 5 min. The supernatant is removed by aspiration, and 5 mL of freshly made methanol:glacial acetic acid (3:1) fixing mixture are added drop by drop while mixing on a vortex mixer. Adding the mixture too rapidly can make the cells aggregate. The suspension is again left for 5–10 min at room temperature, centrifuged at 200×g for 5 min and the supernatant removed by aspiration. A second treatment with 5 mL of the mixture is carried out, but this time the mixture can be added all at

![Figure 2. PRINS labeling of chromosome 7.](image)

(A) Metaphase spread. Two copies of chromosome 7 have each been labeled at the centromere. (B) Direct blood preparation scanned at ×25. Two spots corresponding to each copy of chromosome 7 are visible in each nucleus. (C) Blood film scanned at ×63. Again, two spots are visible in each nucleus, but the intensity of the spots within a nucleus varies because of the differing focal planes of the signal.

![Figure 3. PRINS labeling of chromosome 9.](image)

(A) Metaphase spread and interphase scanned at ×63. Two copies of chromosome 9 have each been labeled at the centromere and in the nucleus. (B) Blood film scanned at ×25. Two spots corresponding to each copy of chromosome 9 are visible in all but one nucleus. (C) Blood film scanned at ×63. Two spots are visible in each nucleus, but there is some variation in signal intensity.
once and the suspension mixed afterwards, followed by immediate centrifugation. Changes of fixing are repeated until the nuclear pellet is white (rather than the dirty brown color of fixed hemoglobin), indicating that all the red cells have both been lysed and the debris removed. Thereafter, the preparation is treated as for cultured cells—placing 20 µL of the preparation on a slide and allowing it to air-dry. The fixed suspension can be stored at -20°C for several months, if necessary, for further investigations. Although this method takes a little longer (up to 40 min), it results in clearer signals and a greater density of larger nuclei (Figures 1–4).

PRINS reactions were performed by using Chromosome Prints kits from Advanced Biotechnologies Ltd. (Leatherhead, England, UK) according to the manufacturer’s instructions. The kits contain primers, enzyme, labeling mixture (buffered solution of dNTPs), stop solution and wash buffer for ten slides. The labeling mixture was completed by the addition of biotin-16-dUTP (Boehringer Mannheim UK, Lewes, England, UK). DNA denaturation, primer annealing and labeling extension were performed on the flat block of a Hybaid OmniGene® In Situ Thermal Cycler (Hybaid Ltd., Teddington, England, UK). After the labeling and stop reactions, avidin-fluorescein isothiocyanate (FITC) (Vector Labs, Burlington, CA, USA), diluted 1:500 in blocking buffer, was added, and the slides were incubated at 37°C for 30 min. Slides were then washed 3× 2 min in wash solution and then mounted in VectaShield® (Vector Labs) containing 4,6-diamidine-2-phenylindolide (DAPI) at 7.5 µg/mL and propidium iodide at 150 ng/mL.

Results were visualized on a Leitz Ortholux 2 microscope under UV illumination with Ploemopak® filters for epifluorescence (Wild Leitz GmbH, Wetzlar, Germany). Analysis was performed by eye on this microscope, but for record purposes, cells were scanned by using an MRC® 600 Confocal Laser Scanning System (Bio-Rad, Hertfordshire, England, UK) mounted on the same microscope. The resulting images were stored on the SyQuest Removable Hard Disk Drive (SyQuest Technology, Fremont, CA, USA) and photographed with a Freeze Frame Video System (Polaroid, Cambridge, MA, USA).

RESULTS

PRINS was performed by using the Chromosome Prints kits specific for chromosomes 1, 7, 9 and Y. On the metaphase chromosomes, the signals appeared as discrete fluorescent spots at or adjacent to the centromere for chromosomes 1 (Figure 1A), 7 (Figure 2A), 9 (Figure 3A) or at the distal region of the long arm for chromosome Y (Figure 4A). The specificity of the chromosome labeling was confirmed by examination of the chromosome morphology and bands. Interphase nuclei were also present within these chromosome preparations, and here, two spots could be clearly seen in 93% of the nuclei labeled for chromosomes 1 (Figure 1, A and B), 88% for chromosome Y in 97% of the nuclei (Figure 4A).

The results obtained when PRINS was performed on direct blood specimens were very similar. In samples, which were fixed directly, nuclear morphology was less well defined than that of the interphase nuclei present in metaphase preparations, which have been swollen with hypotonic solution before fixation. However, discrete fluorescent spots were clearly visible, and chromosome copies could easily be counted. The labeling efficiency of this method was marginally lower than that for cultured cells. More recent experiments, using the preparation method detailed above, resulted in better morphology, stronger signals and a higher rate of labeling efficiency, comparable to that obtained with cultured cells (Figure 1, B and C, Figure 2, B and C, Figure 3, B and C and Figure 4, B and C). Note that the images in Figures 1–4 were obtained with a confocal laser scanning microscope. This system has very limited focal depth, so that nuclei showing two signals when viewed directly, with the ability to change focus, may appear to have only a single signal in the figure. Both signals can be displayed by refocusing for a second scan.

DISCUSSION

PRINS is a rapid alternative to FISH for fluorescent labeling of chromosomes. Although in its present form, it is not generally applicable for analyzing structural abnormalities of chromosomes (e.g., translocations), it provides a very rapid and simple method for detecting numerical abnormalities. A complete PRINS reaction can be completed in less than 1 h, whereas a typical FISH procedure is performed over the course of two days.

Previous reports have demonstrated that PRINS can be applied to metaphase chromosomes prepared by cell culture from peripheral blood lymphocytes and to undividing nuclei in those preparations (3). In this study, we have demonstrated that PRINS can also be applied to slides prepared from a fresh, uncultured blood sample. Sample pre-

Figure 4. PRINS labeling of chromosome Y. (A) Metaphase spread and interphase scanned at ×63. The distal arm of chromosome Y has been labeled, and the interphase shows a single signal. (B) Blood film scanned at ×25. One spot is visible in each nucleus corresponding to chromosome Y. (C) Blood film scanned at ×63. Again there is a single signal in each nucleus, but the one at the lower right is weaker than the others, being in a different focal plane.
paration can be completed in 30–40 min, so that a complete PRINS reaction may be performed in less than 1 h after taking the blood sample, and analysis may be completed in only a few minutes more. The resulting time saving of several days over both FISH analysis and conventional PRINS on cultured metaphase preparations makes this technique an attractive method for rapid chromosome enumeration. The potential applications of this technique include aneuploidy analysis for prenatal diagnosis, evaluation of the aneuploidy status of metastatic tissues and identification of the human chromosome complement in somatic cell hybrids. There are obviously cases that need to be evaluated with some care, particularly where only a subset of the cells present in the sample demonstrate aneuploidy. In such cases, it may be necessary to combine the PRINS analysis with immunocytochemical analysis (ICC) to define specific cell populations. The combination of PRINS with ICC has been successfully performed and is described elsewhere (4).

REFERENCES


