

## REVIEW ARTICLE

### TECHNIQUES FOR BIOMONITORING OF HUMAN POPULATIONS FOR GENETIC EFFECTS\*

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#### ABSTRACT

At present there are several techniques available for monitoring human exposure to genotoxic agents. The most sensitive one is the determination of DNA adducts or protein (haemoglobin) adducts. Among the biological end points, chromosomal changes appear to be most sensitive, especially the analysis of cells containing a high frequency of sister chromatid exchanges (SCEs). The HPRT-mutation assay, though not very sensitive, can be very useful for detecting exposure to specific genotoxic agents, which can be revealed by the occurrence of hot spots.

Environmental, occupational or accidental exposure of human populations to genotoxic agents can lead to genetic effects. Lymphocytes and erythrocytes from human peripheral blood are target cells which can be easily obtained and studied for possible somatic genetic changes. The consequences of exposure of blood in circulation as well effects on the stem cells in the bone marrow can be estimated by taking blood samples shortly or several months after the exposure event. The end points that are currently

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studied are (1) chromosomal aberrations and micronuclei in lymphocytes, (2) aneuploidy in lymphocytes, (3) HPRT-mutations in lymphocytes, (4) glycoporphin mutations in erythrocytes and (5) hemoglobin mutations in erythrocytes. The techniques available to estimate these end points will be discussed briefly below.

The sensitivity of a given system to detect a genotoxic effect depends on the target size as well as the spontaneous frequency of events in question. For example, in measuring chromosomal aberrations or micronuclei, which are on the order of 0.5 to 2%, one is dealing with the whole genome. HPRT-mutations which include single base pair changes as well as gross rearrangements and deletions, measure events that occur in a single gene comprised of about 45 kilo bases, at a frequency of one to five per million lymphocytes. Glycophorin mutations, which like HPRT mutations, represent loss of function of the gene, also occur at a frequency of one to five per million erythrocytes. Hemoglobin mutations which represent single base pair changes in a single codon, occur at a frequency of one in two-three hundred million erythrocytes. Thus, the smaller the size of the target, the rarer the occurrence of changes. Therefore techniques to detect an increase in these rare events should aim at screening large number of cells with ease. Automation has played an important role in achieving this aim and some of the techniques developed in our and other laboratories in this direction are discussed in this paper.

### *Chromosomal aberrations*

In general, the types and frequencies of induced chromosomal aberrations depend on the type of genotoxic exposure and the cell cycle stage at the time of exposure. In peripheral blood lymphocytes, there are several classes with different half lives. Most of them are long lived. Ionizing radiation induce DNA strand breaks directly and some of them in turn are converted into chromosome-type aberrations, namely dicentrics, reciprocal translocations, rings and acentric fragments which show up in metaphases when the lymphocytes (predominantly T lymphocytes) are stimulated to divide *in vitro* in the presence of the mitogen phytohaemagglutinin. The frequency of radiation-induced chromosomal aberrations is dose dependent and therefore can be used to estimate absorbed radiation dose in case of accidents (IAEA Technical Report, 1986). This technique also allows one to evaluate cases such as partial body irradiation, mixture of high and low LET radiations, chronic and acute exposure etc. This method was effectively employed in a recent radiation accident in Goiania, Brazil (Ramalho *et al.*, 1989). The frequency of radiation-induced aberrations due to external exposure diminishes, with time whereas the frequency due to internal contamination increases initially and then declines (Ramalho *et al.*, 1990). Unstable chromosomal aberrations induced in lymphocytes of atom bomb survivors of Hiroshima and Nagasaki can be detected even after about 40 years (Awa, 1983), indicating the presence of long-lived lymphocytes in peripheral blood.

Most of the chemical mutagens induce lesions in the DNA (covalent adducts, cross-links etc.) of lymphocytes. These are removed by cellular repair processes and the non-repaired fraction of lesions give rise to chromatid type aberrations, when the lymphocytes are stimulated *in vitro* (Natarajan and Obe, 1980; Carrano and Natarajan, 1988). An increase in the frequency of chromosomal aberrations has been found following occupational exposure to carcinogens (such as ethylene oxide, vinyl chloride) as well as following treatment with cytostatics (Natarajan and Obe, 1980; Tates *et al.*, 1991). There are several confounding factors which can interfere with the effective usage of this technique and they have been discussed in detail elsewhere (Carrano and Natarajan, 1988).

Improved staining methods have become available which can increase the resolution power of conventional chromosomal aberration evaluation. Chromosome specific DNA libraries have been generated which can be used to specifically paint individual human chromosomes by non-radioactive *in situ* hybridization. When one chromosome is exclusively stained, it is possible to detect translocations involving this chromosome easily and accurately (Pinkel *et al.*, 1986). Translocations involving chromosome 2 in the lymphocytes of individuals involved in the radiation accident in Goiania, Brazil have been accurately detected using this "chromosome painting" technique (Natarajan *et al.*, 1991).

### *Micronuclei*

Micronuclei are formed from chromosomal fragments or chromosomes lagging in anaphase which are not included in the nuclei of the daughter cells. In contrast to chromosomal aberrations, micronuclei are easy to detect and therefore less time consuming. However, to quantify and make comparative evaluations, it is necessary to identify the lymphocytes which have undergone a division, since for the formation of micronuclei a cell division is necessary. This identification of divided cells has become possible by growing the lymphocytes in the presence of cytochalasin B, which arrests cytokinesis leading to binucleated cells (Fenech and Morley, 1985). The latter method is preferred in view of the ease with which the cells once divided can be recognized and because of its sensitivity (Ramalho *et al.*, 1988). Attempts have been made to automate scoring of micronuclei in binucleated lymphocytes. In our laboratory, using specific staining for the cellular protein (naphthol yellow) and DNA (Gallocynin) and an image analysis system (Leytas-MIAC), it has been possible to automate the identification of binucleated cells and micronuclei (Tates *et al.*, 1991). In view of the fact that more cells (in thousands) can be scored for the presence of micronuclei in comparison to the scoring of chromosomal aberrations (in hundreds), it is likely that in the future micronuclei techniques will replace scoring for chromosomal aberrations in population monitoring

studies. However, this relative sensitivity can vary depending on the population under study (Tates *et al.*, 1991).

### *Sister chromatid exchanges*

Sister chromatid exchanges (SCEs) are cytological manifestations of DNA double strand breakage (induced directly or indirectly) and rejoining at homologous sites between two chromatids of a chromosome. SCEs can be easily detected by differential staining of chromosomes in cells grown for two cycles in a medium containing 5-bromodeoxyuridine. This technique has been used extensively in human population monitoring. In view of several confounding factors, such as smoking, which can lead to an increase in the frequency of SCEs, it is sometimes difficult to interpret a small increase in the frequency of SCEs (for a discussion see Carrano and Natarajan, 1988). However, by only using cells with high frequencies of SCEs (HFC), it has been possible to discern differences in exposed populations (Carrano and Moore, 1982, Tates *et al.*, 1991).

### *HPRT-mutations in lymphocytes*

Mutations in the HPRT (hypoxanthine-guanine phosphoribosyl-transferase) locus, which is located on the X chromosome, can be easily detected and quantified in human lymphocytes using 6-thioguanine (6 TG) as the selective agent. Lymphocytes with a functional HPRT gene will phosphorylate this toxic purine analogue and incorporate it into their DNA and RNA, leading to their death. Mutants which have lost the function of the HPRT gene will grow in the medium containing thioguanine. Strauss and Albertini (1979) used tritiated thymidine in the culture medium to detect HPRT-mutants in human lymphocytes with autoradiographic techniques. It is also possible to use 5-bromodeoxyuridine and differential staining, or antibodies against 5-bromodeoxyuridine-DNA containing, for detecting HPRT-variants.

It is now feasible to clone T and B lymphocytes *in vitro*, by adding growth factors such as interleukin 2 to the medium. The mutant frequency is calculated from the number of colonies found growing in the presence 6 TG, in comparison to the cloning efficiency in the absence of TG. The reproducibility of this technique is very high, though it is time consuming and costly. The advantage of this technique, however is that DNA or RNA from the colonies can be isolated and HPRT mutants can be sequenced in order to determine the exact nature of the mutagenic event (Rossi *et al.*, 1990). Such a mutation spectrum can be very valuable to detect chemical-specific hot spots for mutations within this gene, as has been recently shown for ethylene oxide (Vrieling *et al.*, 1992).

## *Erythrocytes*

### *Mutations in the glycoporphin A locus*

This assay detects loss of gene expression at the Glycophorin A locus (GPA). This cell surface glycoprotein occurs in two allelic forms, i.e., M and N, and is codominantly expressed. Monoclonal antibodies for individual allelic forms are conjugated with a different fluorescent dye and used to label fixed erythrocytes from heterozygous MN donors. A fluorocytometric sorting system is used to estimate the frequency of cells that lack the expression of one of the GPA alleles (Langlois *et al.*, 1987). The shortcoming of this method is that only about 50% of the population (namely the heterozygotes) can be screened. In addition the nature of the mutation event cannot be determined at the DNA level.

### *Hemoglobin mutations*

Many hemoglobin mutations, such as sickle cell anaemia, occur in nature. These mutations involve single base pair changes of a codon in the hemoglobin molecule. Monospecific polyclonal antibodies have been generated against several of these mutants and these can be used to detect spontaneously occurring or mutagen-induced mutations in erythrocytes. Since these mutants represent single-base pair changes, their frequencies are very low, 1 in 10 million cells. Automated techniques have been developed to detect these rare events (Bernini *et al.*, 1990). Using this assay, increased mutation frequencies have been detected in the erythrocytes of the victims of the radiation accident in Goiania, Brazil (Natarajan *et al.*, 1991).

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## RESUMO

Atualmente existem diversas técnicas disponíveis para monitorar a exposição humana a agentes genotóxicos. O mais sensível deles é a determinação de DNA ou proteína (hemoglobulina) "adducts". Entre os pontos finais biológicos, mudanças cromossômicas parecem ser as mais sensíveis, especialmente a análise de células com alta frequência de permuta de cromátides irmãs. O exame de mutação HPRT embora não muito sensível, pode ser muito útil para detectar exposições a agentes genotóxicos específicos, os quais podem ser revelados pela ocorrência de pontos quentes (hot spots).

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