**HUMAN IMMUNODEFICIENCY VIRUS DETECTION AND DIFFERENTIAL LEUKOCYTE COUNTS ARE ACCURATE AND SAFER WITH FORMALDEHYDE-FIXED BLOOD**

*To the Editor:*

We have discovered a procedure for analyzing peripheral blood leukocytes (PBLs) that effectively addresses the urgent need to protect healthcare workers and scientists from infection with blood-born pathogens. The method involves hypotonic formaldehyde fixation of anticoagulated blood at the time of venipuncture. The red blood cells (RBCs) lyse in the presence of formaldehyde concentrations (2.2% to 3.2%) reported to inactivate important pathogens such as human immunodeficiency virus (HIV). Routine clinical centrifugation of the fixed blood leads to recovery of a stable suspension of morphologically normal PBLs, freed of RBCs and hemoglobin, and easily differentiated by standard blood smear staining techniques (Fig 1a). They specifically react in conventional immunostaining procedures with at least two commercially available monoclonal antibodies (MoAbs): HLe-1, which binds all fixed leukocytes; and LC, which binds fixed lymphocytes and monocytes (Fig 1b). A comparison of anticoagulated blood at the time of venipuncture. The red blood cells (RBCs) lyse in the presence of formaldehyde concentrations (2.2% to 3.2%) reported to inactivate important pathogens such as human immunodeficiency virus (HIV). Routine clinical centrifugation of the fixed blood leads to recovery of a stable suspension of morphologically normal PBLs, freed of RBCs and hemoglobin, and easily differentiated by standard blood smear staining techniques (Fig 1a). They specifically react in conventional immunostaining procedures with at least two commercially available monoclonal antibodies (MoAbs): HLe-1, which binds all fixed leukocytes; and LC, which binds fixed lymphocytes and monocytes (Fig 1b). A comparison of differential cell counts by morphology or immunostaining of fixed, normal donor PBLs yielded values typical of normal blood, suggesting that accurate differential blood cell counts, including lymphocyte subtypes such as CD4+, may be obtained with fixed cell preparations and appropriate antibodies.

In addition, HIV and human genomic DNA sequences are amplifiable by the polymerase chain reaction (PCR) performed on aliquots of the fixed PBLs. One thousand fixed cells are sufficient to generate PCR products in one round of amplification of single-copy genes (eg, β-globin) in quantities readily detectable by routine ethidium bromide staining of electrophoretically separated DNAs in polyacrylamide gels. As few as five copies of control HIV plasmid DNA added to 10^5 fixed PBLs were detected following three rounds of PCR amplification of HIV_gag gene sequences using a nested primer strategy. These results are consistent with detecting on the order of one HIV-infected cell in 10^6 without the need for hybridization of PCR products to labeled HIV DNA probes.

These approaches have allowed us to quantify the number of HIV-infected PBLs in seropositive hemophiliac blood collected and formaldehyde-fixed at sites remote from the laboratory. The analyses did not require infectious virus containment or radioisotope facilities and were without the laboratory risks associated with purifying HIV-infected cell DNAs. An example of such a quantitation on the remainder of a blood sample submitted for routine lymphocyte subtyping is shown in Fig 2. Aliquots of 1 × 10^4 and 3 × 10^4 cells (lanes 2 and 3), but not 1 × 10^5 cells (lane 4), were positive for HIV_gag DNA, suggesting 1 in 2 to 3 × 10^6 PBLs were HIV infected. Because
7% of these PBLs were reportedly positive for CD4 antigen, the PCR results indicate that one in 1 to 2 x 10^3 CD4+ PBLs were HIV infected in this individual.

Such analyses offer a safer, sensitive, quantitative approach to determining concentrations of HIV-infected cells in stable cell suspensions. This provides a potentially powerful tool with which to monitor clinical course and/or the efficacy of therapy, as well as infected cells in research settings.

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REFERENCES

Human immunodeficiency virus detection and differential leukocyte counts are accurate and safer with formaldehyde-fixed blood [letter]

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