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Rapid, Stable Ambient Storage of Leukocyte RNA from Whole Blood

To the Editor:

The purification of intact RNA is the first critical step for mRNA analysis. Because of the instability of RNA, the process requires labor-intensive multiple steps while guarding against RNases. Although many techniques and commercial kits are available, these are techniques for research laboratories and are not suitable when RNA is collected from patients in clinics, hospitals, operating rooms, or even the emergency room and stored until use.

Here we report a rapid method for the preparation of RNA from blood. Mononuclear leukocytes were first separated by conventional density gradient centrifugation or equivalent methods. The cell suspensions were filtered through glass fiber membranes, and the resultant membranes were immediately dried under vacuum. After dried membranes were stored at room temperature for 1 week, RNA was isolated by acid-guanidinium/phenol/chloroform (AGPC) extraction (1). As a control, cell suspensions were centrifuged, and pellets were homogenized by AGPC. The quality of RNA was tested by three criteria: (a) agarose gel electrophoresis to confirm 18S and 28S rRNA bands (2), (b) measurement of total mRNA by the method we developed (3), and (c) reverse transcription-PCR (RT-PCR) (4) of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA. As a result, rRNAs and G3PDH-specific mRNA in dried samples were stable at room temperature for 1 week (Fig. 1A), and the amount of total mRNA was similar to that of the control (mean ± SE, 5.3 ± 0.4 vs 4.6 ± 0.6 µg/L blood). More importantly, RNA was easily released from membrane-trapped cells by simply passing them through AGPC solution, whereas vigorous homogenization (pipetting/vortex-mixing) was required to recover RNA from cell pellets in control experiments.

In contrast to mononuclear leukocytes, whole blood is much more difficult to handle, probably because of the presence of RNase-rich granulocytes. The stability of RNA may be improved by selective lysis of erythrocytes before recovery of the nucleated cells by centrifugation (5). In the present study, erythrocytes were selectively lysed in hypotonic solution followed by filtration through glass fiber filter membranes. When cell-trapped membranes were dried and were stored at room temperature, we identified substantial amounts of degradation of RNA, although some rRNA bands remained (data not shown). We also tested various organic solvents to accelerate drying in conjunction with RNase inhibitors, but none of the procedures prevented RNA degradation (data not shown). We then applied a denaturing solution [4 mol/L guanidine thiocyanate, 25 mmol/L sodium citrate, 5 g/L N-lauroylsarcosine (Na salt, Sarksyl), and 0.1 mol/L 2-mercaptoethanol] to the membranes immediately after cells were trapped, and the RNA-containing pass-through fraction (guanidinium-stabilized RNA) was stored at room temperature for 2 weeks. RNA was then isolated by phenol/chloroform extraction.

As shown in Fig. 1B, we obtained high-quality RNA even after a 2-week storage period at room temperature in terms of rRNA bands in agarose gel electrophoresis (Fig. 1B, upper panel) and RT-PCR of G3PDH (Fig. 1B, lower panel). Furthermore, there were no differences in the amounts of total mRNA among 0, 1, 7, and 14 days after ambient storage (mean ± SE, 16.2 ± 3.9, 15.7 ± 1.5, 13.8 ± 3.2, and 14.5 ± 3.0 µg/L blood, respectively). In control experiments, leukocytes were pelleted by centrifugation, followed by conventional RNA extraction (1). However, we could recover neither mRNA (Fig. 1B, upper panel) nor mRNA from whole blood (<1 µg/L blood), and subsequent RT-PCR failed to amplify G3PDH mRNA (Fig. 1B, lower panel).

By capturing cells with glass fiber membranes, we could eliminate centrifugation, which is a major hurdle when assay automation is considered. Furthermore, this method overcomes the problems of irreproducibility of homogenization of cell pellets. In the case of mononuclear cells, the storage of RNA on dried membrane is convenient, and the membranes are easily transported to test/research facilities from the clinical sites. We hope this methodology
encourages physician scientists to bring more clinical specimens into research fields to bridge between basic science and clinical medicine.

References

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Comparison of the Abbott IMx Tacrolimus I and Tacrolimus II Assays

To the Editor:
Tacrolimus (FK-506, Prograf, Fujisawa Pharmaceutical Co.), first approved in the United States for liver transplants, is now widely used in other organ transplants (1). Its immunosuppressive potency is 10- to 100-fold greater than cyclosporine A. Because subtherapeutic concentrations are associated with organ rejection and high concentrations are associated with nephrotoxicity, neurotoxicity, and opportunistic infections, therapeutic drug monitoring of FK-506 is well-accepted and is a common laboratory practice (2).

Currently, there is no consensus on the therapeutic range for FK-506. Substantial amounts of the drug are present intracellularly, with a red blood cells-to-plasma ratio >4:1. In addition, clinical effects correlate better with whole blood concentrations than with serum or plasma concentrations (3). Therefore, routine laboratory assays determine concentrations on whole blood. The whole blood therapeutic range was originally thought to be 15–25 μg/L, but has subsequently been modified at most US centers to 5–20 μg/L (1). Several methods are available for monitoring the concentration of FK-506. These include bioassays, radio-receptor assays, HPLC, ELISA (Incstar Corp.), and microparticle enhancement immunoassay (MEIA; Abbott Diagnostics) (4). HPLC, the