Isolating RNA From Clinical Samples With Catrimox-14 and Lithium Chloride

Donald E. Macfarlane* and Christopher E. Dahle

*Department of Medicine, University of Iowa College of Medicine, Iowa City, and
Iowa Biotechnology Corp., Coralville, Iowa*

RNA is a highly informative molecule that has great potential as a target for diagnostic studies. This potential can be reached only when reliable methods for isolating RNA are available in the clinical environment. Cationic surfactants lyse cells and precipitate nucleic acids. We have described a novel cationic surfactant (tetradecyltrimethylammonium oxalate, Catrimox-14™), which is particularly effective in precipitating RNA from cells and which can be applied to clinical specimens. We examine the utility of a method of recovering RNA from the surfactant-nucleic acid precipitate, in which 2 M lithium chloride is used to extract the DNA and surfactant from the precipitate; RNA (being insoluble in lithium chloride solution) remains in the pellet.

The yield of RNA from peripheral blood mononuclear cells by the Catrimox-LiCl method we describe was the same yield by a conventional method using guanidine thio-

cyanate, phenol, and chloroform (GPC). The quality of the RNA, judged by agarose gel electrophoresis, A260/280 ratio and its ability to serve as a target for reverse transcription and PCR, was the same. RNA was isolated and amplified from blood stored for at least 2 weeks in Catrimox solution at room temperature. RNA was also easily isolated with the Catrimox-LiCl method in good yield from frozen sections of mouse liver, spleen, kidney and brain, and from core biopsies of liver and kidney. RNA isolated from needle aspirates of liver, spleen, kidney, pancreas, and brain was easily amplified by RT-PCR. The Catrimox-LiCl method is simple and does not call for the use of corrosive reagents. The Catrimox-LiCl method removes 98% of the DNA.

We conclude that the Catrimox-LiCl method is suitable for use in clinical applications of RNA-based diagnosis. *J. Clin. Lab. Anal.* 11:132–139, 1997. © 1997 Wiley-Liss, Inc.

Key words: RNA isolation; cationic surfactant; diagnosis by PCR

INTRODUCTION

Isolating intact RNA was one of the most difficult procedures carried out in molecular biology laboratories, requiring meticulous attention to detail to prevent degradation of the desired product by ubiquitous RNases. The use of chaotropic guanidine salts and the partition of proteins, lipids, and DNA into the organic phase of phenol-chloroform mixtures have greatly simplified the routine isolation of RNA from most sources (1–4). Despite these advances, the analysis of RNA is not often used in clinical diagnostic studies, and it is doubtful if the corrosive phenol-containing reagents employed in these methods will be acceptable in clinical areas.

We have reported a novel method for isolating RNA, taking advantage of the ability of certain cationic surfactants (including Catrimox-14™) to lyse cells and precipitate nucleic acids (5). We reported that RNA could be recovered from the precipitate by a variety of methods (6,7).

We report here studies exploring the use Catrimox-14 (a solution of tetradecyltrimethylammonium oxalate) followed by LiCl to isolate RNA from several tissues likely to be

submitted to clinical pathology departments. We compare our results with those obtained using a conventional guanidine thiocyanate-phenol-chloroform method (GPC) (4).

MATERIALS AND METHODS

Peripheral Blood Mononuclear Cells

Blood from human volunteers was anticoagulated with acid-citrate-dextrose. The blood (duplicate samples of 7.5 or 3.0 ml) was layered onto a density gradient (Histopaque 1077, Sigma Chemical Co., St. Louis, MO), and centrifuged. The peripheral blood mononuclear cells were collected from the interface and diluted with 10 ml phosphate-buffered saline (PBS). The cells were counted, centrifuged, and resuspended in 0.2 ml PBS.

*Correspondence to: Donald E. Macfarlane, Internal Medicine, University of Iowa, Iowa City, IA 52242.

Received 9 July 1996; Accepted 25 October 1996

Cryosections

Female Balb/c mice, at least 6 weeks old, were anesthetized with ether. Tissues were removed, mounted on corks with OCT mounting medium (Miles Laboratories, Elkhart, IN), and frozen immediately in liquid nitrogen. Frozen sections of tissue 5, 10, or 20 μm thick were prepared using a cryostat (2800 Frigocut N, Reichert-Jung). Cryosections were transferred into the cold box on a cotton swab from the microtome blade into a microfuge tube cooled to $\sim -10^\circ\text{C}$, containing either 1 ml Catrimox (Iowa Biotechnology Corporation, Coralville, IA) or 0.75 ml GPC reagent (TriReagent LS[®], Molecular Research Corp., Cincinnati, OH) and mixed immediately before warming to room temperature.

Core Biopsies

Needle-core biopsies of tissues were obtained under direct vision from anesthetized mice using 14 G Tru-Cut[®] needles. The tissue cores were mixed in a pre-weighed vial of 1 ml Catrimox or 0.75 ml GPC reagent, re-weighed, and homogenized with a hand-held polypropylene pestle.

Fine-needle aspirates were obtained from organs of anesthetized mice using 22 G needles attached to 1 ml syringes. The aspirates were mixed with 1 ml Catrimox, by flushing the needle and syringe with the Catrimox solution.

Isolation of RNA From Cells

Catrimox-LiCl method. Samples mixed with Catrimox were centrifuged for 5 min at 1,000 $\times g$. The supernatant was aspirated and discarded, and 0.5 ml 2M LiCl was added to pellet. The mixture was vortexed and centrifuged for 5 min at 16,000 $\times g$. The supernatant (containing DNA) was removed, and the pellet (containing RNA, which is insoluble in 2M LiCl) was washed with 70% EtOH and air dried.

GPC method. RNA was isolated from the samples using the GPC reagent (0.75; q.s. to 1.0 ml with H₂O) as described in the manufacturer's instructions.

Agarose Gel Electrophoresis

RNA was denatured in 10 μl of MOPS-formaldehyde-formamide (10 parts 10 $\times$ MOPS buffer, 15 parts formaldehyde, 50 parts formamide) by heating at 65°C prior to electrophoresis on a formaldehyde gel (10 by 6.5 cm, 1.2% agarose, 1 $\times$ MOPS buffer, 75 min, 75 volts). The resulting gel was stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$ in 50 mM NaOH, 10 min) and photographed in 302 nm light after destaining in 0.2 M Na acetate for 1 h (8).

Reverse Transcription PCR Amplification

Complementary DNA was synthesized by incubating the RNA with 1 μg random hexamers (Pharmacia, Piscataway, NJ) at 70°C for 10 min, then at 37°C for 1 h with 200 U

Moloney murine leukemia virus (MMLV) reverse transcriptase (BRL, Gaithersburg, MD), 0.5 U RNasin (Promega, Madison, WI), 8 mM dithiothreitol, 0.4 mM dNTP's (Pharmacia), and the buffer supplied by the enzyme manufacturer in a volume of 50 μl . The reaction was stopped by heating for 5 min at 95°C . The cDNA was precipitated in ethanol and redissolved in 50 μl sterile H₂O.

Polymerase Chain Reaction

PCR was performed for 35 or 45 cycles with 2.5 U AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT), 0.2 mM dNTP's, 1.5 mM MgCl₂, in buffer supplied by the enzyme manufacturer, and 5 μl of the cDNA (or serial dilutions as indicated), in a final volume of 50 μl , using a PTC-100 thermal cycler (MJ Research, Watertown, MA). The primers (2.5 $\mu\text{g}/\text{ml}$) amplified sequences encoding either protein kinase C beta (expressed in low abundance in blood cells) (9) using a temperature cycle of 94°C , 30 s; 55°C , 30 s; 72°C , 45 s; or the *bcr/abl* hybrid protein found in cultured K562 cells (6), using a temperature cycle of 94°C , 30 s; 63°C , 30 s; 72°C , 45 s. A portion (24 μl) of the amplified product was electrophoresed on a 1% agarose gel in TBE buffer containing ethidium bromide for 1.5–2 h at 50 volts. The gel was photographed in 302 nm light.

DNA Content of RNA Preparations

The DNA content of isolated RNA was estimated by dissolving the RNA in 50 μl DEPC H₂O and incubating it with 1 μg Hoechst 33258 DNA specific fluorescent dye in 2 ml 2 M NaCl, 50 mM NaPO₄, pH 7 for 15 min in the dark. Fluorescence (360 nm excitation, 460 nm emission) and absorbance (A₂₆₀ and 280 nm) were measured and compared to standards consisting of dilutions of calf thymus DNA and yeast tRNA (Boehringer Mannheim, Indianapolis, IN). The DNA content of a sample of the supernatant of the 2 M LiCl extraction of the Catrimox-precipitated nucleic acid pellet was also estimated by fluorescence.

Other Materials. [³H]-Thymidine was from DuPont-NEN (Boston, MA). Yeast tDNA was from Boehringer Mannheim. Agarose electrophoresis gels were prepared using SeaKem ME agarose (FMC, Rockland, ME). DEPC and calf thymus DNA was from Sigma. LiCl (Fisher, Pittsburgh, PA) was dissolved at 2 M in DEPC-treated water.

RESULTS

Peripheral Blood Mononuclear Cells

We compared the yield and purity of RNA isolated from paired samples of peripheral blood mononuclear cells from normal donors prepared by density-gradient centrifugation. Comparison of the Catrimox-LiCl and GPC methods shows that they yield much the same amount of RNA from these cells (Fig. 1a) and that the resulting RNA has the same A₂₆₀/

280 ratio (Fig. 1b) and essentially the same appearance on agarose gels (Fig. 2).

To evaluate the suitability of the RNA for PCR amplification, we reverse-transcribed the RNA prepared by the Catrimox-LiCl method and the GPC method using random primers and prepared serial dilutions of the resulting cDNA. The appropriate product was amplified without difficulty by PCR between primers specific for the sequence encoding a protein expressed in relatively low abundance in blood cells (one of the isozymes of protein kinase C). There was no difference in the limit of detection of the cDNA between RNA prepared using the Catrimox-LiCl method and the GPC method (Fig. 3).

Whole Blood

Whole blood contains a low concentration of RNA, which can be isolated effectively using the Catrimox-LiCl method. To determine the stability of RNA in blood-Catrimox mixtures, we added cultured K562 cells to blood and mixed it with Catrimox. This mixture was allowed to stand on the benchtop at room temperature for up to 2 weeks. Storage for 2 weeks resulted in no reduction in the sensitivity of amplification of PKC beta RNA and a reduction of about one dilution (fivefold) in the sensitivity of the amplification of the *bcr/abl* mRNA expressed in the K562 cells during the 2 weeks of storage (Fig. 4). In similar experiments, we successfully amplified RNA from blood samples stored in Catrimox for 6 months at 4°C and -20°C (data not shown).

Cryosections

Useful amounts of RNA were obtained without difficulty from cryosections. The yields varied with the type of tissue. In general, yields were higher with the Catrimox method than with the GPC method (Fig. 5), and they compared favorably with published RNA content of tissues, e.g., rat liver $3.68 \pm 0.08 \mu\text{g}/\text{mg}$ (10), or 3.69 ± 0.49 (11), and in rat spleen 3.89 ± 0.23 or kidney 2.23 ± 0.11 , or brain 1.43 ± 0.09 (10). Good quality RNA was isolated from sections of liver, kidney, and brain (Fig. 6). Sections of spleen or pancreas gave RNA of variable quality when using either isolation reagents (data not shown).

The RNA isolated from cryosections was successfully amplified by RT-PCR (Fig. 7). RNA isolated with Catrimox could be amplified from liver, kidney, and brain. RNA isolated with GPC could be amplified from brain sections.

After mixing with Catrimox or the GPC reagent, sectioned tissues were left on the benchtop for 2 weeks before extracting the RNA by further processing. Amplification of this RNA by RT-PCR was successful in spleen and brain tissue when isolated with Catrimox. No amplification was evident in samples stored in the GPC reagent for 2 weeks before processing (data not shown).

Solid Tissue

We added small fragments of mouse tissues (~50 mg) to Catrimox or GPC reagent and ground the tissue coarsely with

Fig. 2. Electrophoresis of RNA from peripheral blood mononuclear cells. RNA isolated from the blood cells of three donors was prepared by the Catrimox and the GPC method in duplicate as described in the legend of Fig. 1. Samples (0.8–1.9 μ g) were separated by denaturing electrophoresis. The RNA standard (Stds) range in size from 0.24 to 9.5 kb.

Fig. 3. RT-PCR amplification of RNA. RNA was isolated from paired samples of peripheral blood mononuclear cells from three donors using the Catrimox and GPC methods. The RNA was used as a template for reverse transcriptase. The resulting cDNA was serially diluted tenfold in amplification buffer to the equivalent the number of cells indicated, and amplified by PCR for 35 cycles between primers for protein kinase C beta. The figure shows agarose gel electrophoresis of the amplified product (expected to be 312 bp). The standards (Stds; multiples of 123 bp) are shown in the left lane. The right lane is the result of omitting cDNA. No amplification product was observed when reverse transcriptase was omitted (not shown).

Fig. 4. RT-PCR amplification of RNA isolated from blood-Catrimox mixtures stored at room temperature. Blood samples (6 samples, 0.1 ml) each containing 10^5 cultured K562 cells was added to 1 ml Catrimox, and the mixtures were stored at room temperature for 0, 3, 7 and 14 days. RNA was then isolated from blood-Catrimox mixtures, and was reverse transcribed. The cDNA was serially diluted as indicated, and subjected to PCR using primers for protein kinase C beta (left, 312 bp) or *bcr/abl* (right, 219 bp). One of three experiments giving similar results is shown.

a pestle. Although quite respectable yields of RNA were obtained (Fig. 5) and the RNA could be amplified by amplified by RT-PCR, examination of the RNA by agarose gel electrophoresis showed it to be partially degraded. Unless sophisticated preparation of the tissue can be performed under clinical conditions to prevent degradation before mixing with the reagents, we do not recommend this approach.

Core Biopsies

Core biopsies of liver and kidney are often obtained in clinical practice. The yields of RNA from mouse liver and kidney core biopsies shown in Figure 5 and the quality of this RNA are shown in Fig. 8. RT-PCR amplification of RNA derived

Fig. 5. RNA yields from mouse cryosections, tissues and needle biopsies. RNA was isolated using either Catrimox-14 or GPC reagent. The yields were calculated from measurement of the absorbance at 260 nm. For cryosections, the weight of the tissue was estimated by measuring the area of the tissue section and calculating its volume, then assuming that 1 mm³ of tissue is equivalent to 1 mg. The weight of other samples was estimated by weighing. The figure shows means and standard errors of six RNA isolations for each tissue.

by the Catrimox method from needle core biopsies of mouse tissues was successful with liver and kidney (Fig. 9).

Fine-Needle Aspirates

Figure 10 shows RT-PCR amplification of RNA isolated with Catrimox from fine-needle aspirates of liver, spleen, kidney, pancreas, and brain. Fine-needle aspirates contain in variable numbers of cells, and very few cells are obtained from aspirating normal muscle.

DNA Contamination

Cationic surfactants precipitate both RNA and DNA. In preliminary studies, we found that Catrimox precipitates ~85% of the radioactivity of [³H]thymidine prelabelled cells and that most of this radioactivity is extracted from the surfactant-nucleic acid pellet by 2 M LiCl (data not shown). To determine the extent to which DNA contaminates RNA isolated from cells with the Catrimox-LiCl method, we measured the fluorescence in the recovered RNA using the DNA-specific fluorescent dye, Hoechst 33528. These studies show that the Catrimox-LiCl method removes >98% of the DNA from the RNA. Of the recovered nucleic acid, <1.5% is found to be DNA (Table I).

DISCUSSION

RNA is a highly informative molecule that has great potential as a target for diagnostic use. This potential can be realized only when RNA can be isolated reliably from clini-

Fig. 6. RNA isolated with Catrimox or GPC reagent from cryosections of different tissues. RNA was isolated from two 20 µm cryosections of mouse liver, kidney and brain. About 2 µg RNA from each section was applied to an agarose gel for electrophoresis and staining with ethidium bromide.

Fig. 7. RT-PCR from cryosections of mouse tissues. The protein kinase C beta sequence was amplified by RT-PCR from RNA isolated (in duplicate) with Catrimox or GPC reagent from cryosections of mouse liver, spleen, kidney and brain. The lane marked Neg was amplified in the PCR reaction without a template.

Fig. 8. RNA from needle core biopsies of mouse liver and kidney. RNA was isolated with Catrimox or GPC reagent from about 10 mg of tissue obtained with a Tru-Cut biopsy needle. The RNA was electrophoresed in 1.2% agarose.

cal specimens. RNA is unstable, and it is prone to hydrolysis by ubiquitous RNase. It is wise, therefore, to protect RNA from degradation as soon as the specimen is collected. Adding the specimen to a mixture of phenol and guanidinium achieves this objective, but the reagents used are corrosive and may be unacceptable in patient care areas. Several authors have successfully detected RNA by in situ RT-PCR in paraffin embedded sections (12–15), but this technique is unlikely to find widespread acceptance in clinical practice.

Fig. 9. RT-PCR of RNA from needle core biopsies. The RNA isolated from mouse liver and kidney tissue with a Tru-Cut biopsy needle shown in Figure 8 was reverse transcribed and amplified by PCR between primers for protein kinase C beta. The products were electrophoresed in 1% agarose.

Cationic surfactants are widely used in cosmetics, shampoos, and other health care products, attesting to their acceptability in the clinical area. The use of Catrimox-14 as a precipitant of RNA from biological specimens is becoming accepted (e.g., 16).

We have described methods for recovering the RNA from the surfactant-nucleic acid pellet using either high ionic strength or an organic solvent to disrupt the forces binding the surfactant to nucleic acid. In this report, we explore the use of lithium chloride to release the surfactant from the nucleic acid. The ionic effect of the salt solubilizes the surfactant (as the chloride salt), and DNA (as the lithium salt). The RNA in the pellet is converted to the lithium salt, but since RNA is insoluble in concentrated LiCl solution (17), it remains in the pellet. After decanting the LiCl, the RNA is easily dissolved in water or enzyme buffers. This method of isolating RNA is simple and (as this report shows) it is effective.

The yield of RNA by the Catrimox-LiCl method from cultured cells is the same as achieved by the conventional GPC method, and the purity seems to be equivalent. The RNA isolated by either technique could be amplified without difficulty by reverse transcription and PCR.

Frozen sections are frequently obtained to enable rapid histological diagnosis during surgery, and for certain immu-

TABLE I. DNA Content of RNA Isolated Using Catrimox

Cell number	RNA yield $\mu\text{g}/10^6$ cells, mean $\pm$ s.d.	DNA yield $\mu\text{g}/10^6$ cells mean $\pm$ s.d.	DNA in RNA fraction, $\mu\text{g}/10^6$ cells, mean $\pm$ s.d.	DNA eliminated percent, mean $\pm$ s.d.
0.25×10^6	12.4 ± 4.8	13.9 ± 1.4	0.12 ± 0.06	99.0 ± 0.4
0.50×10^6	14.1 ± 2.0	13.0 ± 1.0	0.15 ± 0.05	98.9 ± 0.4
1.0×10^6	14.0 ± 1.8	11.3 ± 0.8	0.12 ± 0.03	99.1 ± 0.2
2.0×10^6	13.6 ± 1.7	10.4 ± 1.1	0.15 ± 0.02	98.9 ± 0.1

RNA was isolated from the indicated number of K562 cells using the Catrimox-LiCl method ($n=9$). The RNA was dissolved and assayed for RNA by absorbance, and for DNA using the Hoechst 33258 dye method. The content of DNA in the lithium supernatant was also assayed using the fluorescence method.

Fig. 10. RT-PCR from RNA isolated by needle aspirates from mouse tissues. The RNA isolated with Catrimox-14 from needle aspirates of mouse liver, spleen, kidney, pancreas, muscle and brain was amplified by RT-PCR using protein kinase C beta primers. The cDNA was used at the normal dilution (first lane of each pair) and at a 1:10 dilution (second lane of each pair).

nohistochemical staining. We found reports of in situ identification of mRNA in frozen sections (18,19), but we have not found reports of their use as a source of clinical material for RNA isolation. We found that RNA can be isolated from frozen sections with Catrimox or the GPC reagent. Good quality RNA was obtained from sections of 5–20 μ m thick, and the thicker sections were easier to handle. RNA from frozen sections was isolated in yields similar to published values for the tissues examined, and the RNA isolated from most tissues with the Catrimox method was easily amplified by RT-PCR.

Core biopsies have been used to detect hepatitis C virus in liver tissue by RT-PCR (20,21). Our experiments with needle core biopsies show that there is good amplifiability of RNA isolated with Catrimox. Fine-needle aspirates are increasingly used for histological diagnosis of tumors, etc. The yield of cells is greatly affected by the nature of the tissue and the technique used. We obtained amplifiable RNA from cellular aspirates using the Catrimox-LiCl method without difficulty.

The processing of clinical specimens is rarely ideal. It may be convenient to add the specimen directly to Catrimox solution in the clinical area and ship it to a remote laboratory for processing. Considerable expense is spared if the specimen can be shipped without refrigeration. We investigated whether delay in shipping would result in destruction of the RNA in a blood sample if it is left in contact with Catrimox at room temperature. Blood is a hostile environment for RNA. It contains sufficient RNase to destroy naked RNA in a few seconds, and it contains a high concentration of iron, which catalyses oxidant events. Despite this, we demonstrate that there is little reduction of the amounts of PCR-amplifiable cDNA that can be prepared from RNA harvested after 2 weeks storage at room temperature. It would, therefore, appear quite feasible to ship specimens mixed with Catrimox to a remote processing site by mail.

Minor DNA contamination of RNA preparations is generally of no consequence, because experiments can be designed to distinguish between (processed) RNA and (genomic) DNA. The Catrimox-LaCl method removes most of the DNA from the sample. Further processing (such as the use of a DNase) can be used to remove the residual DNA from the RNA when necessary. Parenthetically we note that the “house-

keeping” genes GAPDH and actin (which are commonly used as internal standards to control RNA recovery) have fully processed pseudogenes in the human genome (22). They should not be used to prove the integrity of RNA in a specimen intended for RT-PCR, because the pseudogenes in contaminating DNA may act as a template just as effectively as RNA. It is for this reason that we selected other genes for this work.

The Catrimox-LiCl method is suitable for isolating RNA in amounts up to 30 μ g from small samples. Extracting RNA from larger specimens results in the formation of a bulky surfactant-nucleic acid pellet (which should be centrifuged at low speeds to avoid over compaction) and the release of so much DNA into the lithium chloride solution that it becomes markedly viscous, requiring the DNA to be sheared through a 25-gauge needle. The method we described using a guanidinium salt to solubilize the RNA may be more suitable to isolate higher amounts of RNA (6,7).

We look forward to an expansion in the use of RNA as a diagnostic target, enabling the detection of a wide range of pathogens, tumors, and pathological states characterized by qualitative or quantitative abnormalities of protein production. In an era of cost containment, we are confident that mixing clinical specimens directly to Catrimox in the clinic, followed by shipment to a reference laboratory opens a range of diagnostic procedures that have the potential for accurate, rapid, cost-effective diagnosis.

REFERENCES

- MacDonald RJ, Swift GH, Przybyla AE, Chirgwin JM: Isolation of RNA using Guanidinium Salts. *Meth Enzymol* 152:219–227, 1987.
- Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987.
- Chomczynski PA, Sacchi N: Single-step isolation from cultures cells and tissues. In *Current Protocols in Molecular Biology*. FM Ausubel, RE Kingston, DD Moore, JA Smith, JG Seidman, K Struhl, eds. New York: John Wiley & Sons, 1991.
- Chomczynski P: A reagent for the single step simultaneous isolation of RNA, DNA, and proteins from cells and tissue samples. *BioTechniques* 15:523–537, 1993.
- Macfarlane DE, Dahle CE: Isolating RNA from whole blood: The dawn of RNA-based diagnosis? *Nature* 362:186–188, 1993.
- Macfarlane DE, Dahle CE: Use of a novel cationic surfactant in the isolation of RNA. *Biomed Products* 18:60–62, 1993.
- Dahle, CE, Macfarlane DE: Isolation of RNA from cells in culture using Catrimox-14™ cationic surfactant. *Biotechniques* 15:1102–1105, 1993.
- Selden RF: Analysis of RNA by Northern hybridization. In *Current Protocols in Molecular Biology*, Vol. Unit 4.9 (Supplement 13). FMAusubel, RE Kingston, DD Moore, JA Smith, JG Seidman, K Struhl, eds. New York: John Wiley & Sons, 1987.
- Macfarlane DE, Manzel L: Activation of beta-isozyme of protein kinase (PKC β) is necessary and sufficient for phorbol ester-induced differentiation of HL-60 promyelocytes: Studies with PKC β defective PET-mutant. *J Biol Chem* 269:4327–4331, 1994.
- Too HP, Maggio JE: Simultaneous extraction of total RNA and peptides from tissues: applications to tachykinins. *Peptides* 16:45–53, 1995.
- Fang ZA, Castro CE: Simple modification of the single-step method of RNA isolation from rat liver. *BioTechniques* 10:734–736, 1991.

12. de Andrés B, del Pozo V, Gallardo S, de Arruda-Chaves E, Cárbeda B, Martín-Orozco E, Posada M, Palomino P, Lahoz C: Improved method for mRNA extraction from paraffin-embedded tissues. *BioTechniques* 18:42–44, 1995.
13. Diamond DA, Davis GL, Qian KP, Lau JY: Detection of hepatitis C viral sequences in formalin-fixed, paraffin-embedded liver tissue: Effect of interferon alpha therapy. *Med Virol* 42:294–298, 1994.
14. Finke J, Fritzen R, Ternes P, Lange W, Dölken G: An improved strategy and a useful housekeeping gene for RNA analysis from formalin-fixed paraffin-embedded tissues by PCR. *BioTechniques* 14:448–453, 1993.
15. Stanta G, Schneider C: RNA extracted from paraffin-embedded human tissues is amenable to analysis by PCR amplification. *BioTechniques* 11:304–308, 1991.
16. Liedtke W, Battistini L, Brosnan CF, Raine CS: A comparison of methods for RNA extraction from lymphocytes for RT-PCR. *PCR Methods Applic* 4:185–187, 1994.
17. Wallace DM: Precipitation of nucleic acids. *Meth Enzymol* 152:41–48, 1987.
18. Bucana CD, Radinsky R, Dong X, Sanchez R, Brigati DJ, Fidler IJ: A rapid colorimetric in situ mRNA hybridization technique using hyperbiotinylated oligonucleotide probes for analysis of mdrl in mouse colon carcinoma cells. *J Histochem Cytochem* 41:499–506, 1993.
19. Hodges E, Howell WM, Tyacke SR, Wong R, Cawley MI, Smith JL: Detection of T-cell receptor beta chain mRNA in frozen and paraffin embedded biopsy tissue using digoxigenin-labelled oligonucleotide probes in situ. *J Pathol* 174:151–158, 1994.
20. el-Batanouny MH, Savage K, Jacobs R, el-Refai AO, Squadrito GG, Brown D, Saleh SM, Raouf AA, Amer KM, Dusheiko GM et al: Hepatitis C virus-polymerase chain reaction of routinely processed liver biopsies. *J Med Virol* 43:380–385, 1994.
21. Marin MG, Cariani E, Salmi A, Rangoni G, Chiodera PL, Pizziccolo G, Albertini A: HCV-RNA detection in ultrasound-guided fine needle biopsies of liver nodules and surrounding tissue. *J Virol Meth* 48:125–132, 1994.
22. Garbay B, Bove-Grabot E, Garret M: Processed pseudogenes interfere with reverse transcriptase-polymerase chain reaction controls. *Anal Biochem* 237:157–159, 1996.