The Notes section welcomes the following types of contributions: (1) practical innovations or solutions to everyday practice problems, (2) substantial updates or elaborations on work previously published by the same authors, (3) important confirmations of research findings previously published by others, and (4) short research reports, including practice surveys, of modest scope or interest.

Notes should be submitted with AJHP’s manuscript checklist. The text should be concise, and the number of references, tables, and figures should be limited.

Bioactivity of cryopreserved alteplase solutions

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Alteplase, a recombinant tissue-type plasminogen activator, is a serine protease with potent fibrinolytic properties. It is approved in the United States for use in the management of acute myocardial infarction, acute ischemic stroke, and pulmonary embolism. Various thrombolytic agents, including alteplase, have also been used to restore the patency of occluded central venous catheters. Urokinase, the drug most commonly used for this indication, is produced from cultures of renal cells harvested from deceased neonates. Recently, the U.S. Food and Drug Administration (FDA) announced that it has detected serious deviations from current good manu-
turing practice regulations in the manufacture of the only urokinase product marketed in this country. These deviations in manufacture, according to FDA, may result in the transmission of infectious agents capable of posing a potentially serious health hazard. While adverse effects linked to the manufacture of urokinase have not been documented to date, limitations in the product’s distribution have resulted in a national shortage.

Two articles suggest that a 2-mg dose of alteplase 1 mg/mL may be a useful alternative to urokinase for the management of occluded central venous catheters. Alteplase (Activase, Genentech) is supplied as a sterile, lyophilized powder in 50-mg (29 million-IU) and 100-mg (58 million-IU) vials. Once reconstituted to a concentration of 1 mg/mL according to the manufacturer’s instructions, this preservative-free solution must be used within eight hours. Since relatively small doses are needed for catheter clearance, preparation and freezing of small portions of alteplase would be economical. However, the current product labeling does not include information about the activity of alteplase after it has been frozen and thawed.

Therefore, we evaluated the bioactivity in vitro of cryopreserved alteplase.

Methods. The bioactivity of alteplase was determined by using an assay that measures the fibrindependent conversion of plasminogen to plasmin. This assay was adapted from a method developed to measure alteplase concentrations in plasma samples. The assay is widely available in its commercial form. However, the commercial assay was not optimal for our purposes, since it is designed for much lower concentrations of alteplase than we planned to measure.

Preparation of solutions. Lyophilized alteplase was reconstituted aseptically with Sterile Water for Injection, USP, to a final concentration of 1 mg/mL. A 1-mL portion of this solution was stored in a polypropylene tube at −20 °C for six months. On a separate occasion, alteplase was reconstituted with Sterile Water for Injection, USP, to a final concentration of 1 mg/mL in a biological safety cabinet in a cleanroom environment. Three 2-mL portions of this solution were stored in 5-mL clear-glass molded type 1 borosilicate vials at −70 °C for two weeks. These vials were thawed at 37 °C for five minutes in a water bath incubator, kept at room temperature (22–24 °C) for 24 hours, and then refrozen at −70 °C. All the samples
were kept frozen until the day their activity was measured. On the day of the assay, all the samples were thawed at 37 °C and then kept at 22–24 °C.

**Assay of bioactivity.** The activity of all alteplase test samples was compared with that of an alteplase* 1-mg/mL solution that had been freshly prepared on the day of the assay and never frozen. This control solution was diluted to 100 IU/mL (2.4 nM), 50 IU/mL, and 20 IU/mL with Tris-buffered sodium chloride solution (0.1 M sodium chloride, 0.04 M Tris hydrochloride, and 0.01 M Tris base, pH 7.35) containing 1 mg of bovine serum albumin per milliliter. Each alteplase sample was diluted in exactly the same way to a nominal concentration of 50 IU/mL (1.2 nM). In the wells of a microtiter plate, 20-μL portions of each diluted sample were mixed with 10 μL of 18 μM des-Arg-fibrinogen.1 To these mixtures were added 200 μL of a mixture of 0.93 μM human plasminogen and 0.5 M H-o-norleucyl-hexhahydroxytrypsin-lysine-p-nitroanilide diacetate (0.5 mM),5 a chromogenic substrate for plasmin. At this point, the microtiter plate was immediately placed in an automated microplate reader at 37 °C and agitated for 15 seconds. After 20 minutes there was a linear relationship between the alteplase concentration and the optical density (OD) at 405 nm. Therefore, at this time the reaction was stopped by adding 25 μL of a solution containing 0.8 M potassium acetate and 3.2 M guanidine hydrochloride (pH 3.7), and the OD at 405 nm was read immediately. From seven replicate samples at each of three alteplase concentrations (20, 50, and 100 IU/mL), the assay's coefficient of variation was determined to be 6%.

**Results.** The bioactivity of all the treated samples was indistinguishable from that of alteplase in the freshly prepared control solution (Table 1). As expected, when des-Arg-fibrinogen (a fibrin analogue) was omitted from the assay, no color developed.

**Discussion.** The assay we used measures the bioactivity of alteplase by testing two of its critical molecular interactions: its binding to fibrin (i.e., fibrin specificity), shown by the lack of color development in the absence of a fibrin analogue; and its conversion of plasminogen to the fibrinolytic enzyme plasmin, shown by the reactivity with a plasmin-specific chromogenic substrate. The treated samples were not tested for the possible formation of soluble or insoluble aggregates. Since this product is free of preservatives, however, care must be taken to ensure the safe preservation, storage, and handling of alteplase solutions.

**Conclusion.** Alteplase 1 mg/mL was bioactive in vitro after storage for six months in a polypolyethylene container at −20 °C and for two weeks in glass vials at −70 °C.

**Table 1.** Activity of Alteplase 1 mg/mL at −20 and −70 °C

<table>
<thead>
<tr>
<th>Test Condition</th>
<th>Mean ± S.D. % Alteplase Bioactivity Remaining Compared with Control</th>
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<tr>
<td>In polypolyethylene tube for 6 mo at −20 °C</td>
<td>98.7 ± 6.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>In glass vials for 2 wk at −70 °C, then thawed once and maintained at 22–24 °C for 24 hr (and then stored at −70 °C for 19 days until assay)</td>
<td>102.2 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup>The mean actual initial bioactivity of the control solution was 10.3 millioplates per International Unit of Alteplase.

<sup>b</sup>Determined from four samples from a single tube.

<sup>ab</sup>Determined from 11 samples from three vials.

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Information on the freezing of alteplase solutions is now available from Genentech upon request.

Activase 50-mg vial, Genentech, Inc., South San Francisco, CA, lot E0930A.

Abbott Laboratories, Chicago, IL, lot 15-1855-DK.

Activase 50-mg vial, Genentech, lot E0930A.

Abbott Laboratories, lot 47-496-DK.

Desitin-S 5 mg/mL, American Diagnostica, Inc., Greenwich, CT.

Spectrozyme PL, American Diagnostica.

VEI400 Bio Kinetic Reader, Bio-Tek Instruments, Inc., Winooski, VT.

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**References**


