Simplified method of collecting and processing whole blood for quantitation of plasma catecholamines.

**Title**: Laboratory Medicine 1990 January Vol. 21 No. 1 pp. 26-29

**Abstract**

DTIC Document Classification: Unclassified, Same as Report

**Distribution**: Approved for public release; distribution is unlimited.

**Monitor Organization**: Naval Medical Command

**Report Number**: AD-A219 365

**Author(s)**: D'Alesandro MM, Reed HL, Robertson R, Lewis SB

**Type of Report**: Journal Article

**Date of Report**: 1990

**Page Count**: 4
Best Available Copy
Simplified Methods of Collecting and Processing Whole Blood for Quantitation of Plasma Catecholamines

Michele M. D'Alessandro, PhD; H. Lester Reed, MD; Robert Robertson; and Stephen B. Lewis, MD

Plasma catecholamine measurements are important in the diagnosis and management of nervous system related disorders,12 hypertension,14 pheochromocytoma,5 and to assess patient resuscitation following shock and or trauma.7,8 Due to the chemical structure of the catechol ring and its susceptibility to spontaneous oxidation in buffered solutions, catecholamine stability has been of particular concern when determining quantitative levels.7 Quantitative analysis has been achieved by fluorometric techniques,9 radioenzymatic assays,10,11 and high-pressure liquid chromatography (HPLC).12,13 HPLC with electrochemical detection has shown the least interlaboratory variability when compared to other analytical techniques.14 Different methods of blood collection including the choice of anticoagulant, the addition of antioxidants, and various sample processing techniques have also been reported.10,13,16

The need to rapidly process plasma catecholamines to prevent their degradation has limited their use in the clinic, especially when attempting a comprehensive analysis of critical care medicine.17 This work was undertaken to characterize further the effects of two common anticoagulants (heparin and EDTA) and the antioxidant glutathione (GSH/EGTA) as a preservative on plasma catecholamine stability. We analyzed NE and EPI stability at room temperature (24°C) and −70°C. In addition, we measured the changes in NE and EPI levels after delaying plasma separation from whole blood, which may occur with field studies. HPLC with electrochemical detection, which affords increased sensitivity compared with other detection methods, was used to quantitate catecholamine levels. We designed these studies to establish reasonable procedures for the management of routine clinical and research laboratory samples, including samples collected in field studies conducted at some distance from the laboratory. Realistic time limitations and choice of anticoagulant for quantitating NE and EPI from whole blood and plasma are presented.

Materials and Methods
Sample Collection
Blood samples were collected from nonfasted healthy women and men, aged 25 to 55 years. Venous blood (80 mL) was drawn into tubes containing either sodium EDTA (4 mmol/L, final concentration) or heparin (14.3 USP units/mL) as anticoagulant. All blood samples except those to be used for whole-blood degradation studies were centrifuged and aliquoted within 1 hour of collection.

Blood was centrifuged at 3,000 rpm (1,000 Xg) for 10 minutes at 4°C in a Sorvall RC-3 centrifuge to pellet cells and platelets. Plasma was pooled, placed on
ice, and aliquoted 1 mL into 1.5-mL microcentrifu
tubes with or without the addition of 1.6, 1.8, 3
mmol/L final concentration and glutathione reduced,
6.3 mmol/L final concentration, as an
antioxidant preservative. Plasma samples
were either frozen immediately at
-70°C as controls or 30 minutes for
analyis after long-term storage, or incubated
between 5 minutes and 24 hours at
24°C to measure decay rates. Plasma for
the whole blood studies was serially anal-
yzed following continuous, gentle rota-
tion at 24°C for 1, 6, 24, and 48 hours
prior to removal of plasma and was stored at
-70°C.

A single prospective analysis was con-
ducted with the single pooled samples of
plasma. Plasma for room temperature and
long-term -70°C degradation analysis
was pooled from a minimum of six in-
dividuals. The subject pools were differ-
cent for the various experiments. This ac-
counts for the differences in reported
control values.

Extraction and Quantitation of
Catecholamines
Plasma samples were extracted as origin-
ally described by Anton and Saxer. In
brief, alumina adsorption of catechola-
mine from plasma was complete by 30
minutes at room temperature. Following
thorough washing of the adsorbed alumina,
desorption was complete by addition of
100 mL of 1 N acetic acid containing
0.1% EDTA and 0.1% sodium dode-
Ctane (DDHBA) was used as an internal standard to quantitate the
catecholamines. The extraction effici-
cy of NE, EPI, and DDHBA was rou-
tinely 66% to 70% based on the extract-
ion of known standards. After extrac-
tion, samples were immediately assayed
by HPLC using a Waters 460 electro-
chemical detector interfaced with a Dig-
tal 380 computer utilizing Waters Expert
Chromatography software (Version 5.2).

The extraction and HPLC methods allow
for reliable detection of NE and EPI if
values are greater than 20 pg/mL of plas-
ma. The intra-assay coefficient of varia-
tion was 5% for NE and 8% for EPI. As-
says were run in duplicate and data were
expressed as the mean pg/mL ± SE. The
conversion factor for expressing NE as
mmol/L is 0.00591, and 5.458 for express-
ing EPI in pmol/L. Statisticall differ-
ces were determined by ANOVA with Dun-
can's text between means for repeated
measures.

Results
Degradation of NE and EPI in Plasma
(Heparinized Blood)
Plasma NE and EPI levels from hepar-
inized blood were measured after 24
hours at 24°C (Table I). Without preser-
vative, NE and EPI decreased by 0.6 ± 5
and 0.3 ± 1.9 pg in 24 hours, respec-
tively. The addition of preservative did not
significantly alter the degradation of NE
and EPI which were 14 ± 4 and 1.7 ± 1.8
pg, respectively, in 24 hours. These
decay rates are not concentration depen-
dent, since there was no change in the degra-
dation rate at 24 hours at 24°C when plas-
ma NE and EPI was increased to 1,000
and 600 pg/mL, respectively, by addition of
exogenous catecholamine (data not
shown).

Degradation of NE and EPI in Plasma
(EDTA Blood)
Plasma NE in whole blood collected with
EDTA was stable for up to 6 hours at
24°C with or without the addition of pre-
servative (Table I). However, both NE
and EPI rapidly decayed following incuba-
tion for greater than 6 hours at 24°C.
NE decreased 50% and EPI in the ab-
ence of preservative, respectively, after 24
hours at 24°C (Table II). When compared with heparinized plasma, the NE
decay rate in EDTA plasma was 30-fold greater in the absence of preser-
vative and 17-fold greater in the pres-
ence of preservative (Table II). EPI de-
graded to 45% and 48% of the control
value in the absence and presence of pre-
servative, respectively, when compared to

| Table I: Changes in the Concentration of Plasma Norepinephrine (NE) and Epinephrine (EPI) (± SE) Following Incubation at Room Temperature (24°C) |
|-----------------|-----------------|-----------------|
| Plasma          | GSH/EGTA*       | Concentration, pg/mL | Decay Rate (pg/24h, %) |
|                 |                 | 0 h               | 6 h               | 24 h               |
| Heparin         |                 |                   |                   |                   |
| NE             | +               | 335 ± 8           | 324 ± 2           | 326 ± 7           | 6 ± 5 (2.7)   |
| EPI            | +               | 299 ± 2           | 285 ± 0           | 282 ± 9           | 14 ± 4 (5.7) |
| EDTA           |                 |                   |                   |                   |
| NE             | +               | 43 ± 0.8          | 43 ± 0.4          | 42 ± 2            | 0.3 ± 1.9 (0.7) |
| EPI            | +               | 37 ± 0.0          | 33 ± 2.2          | 36 ± 0.4          | 1.7 ± 1.8 (4.6) |

*Minus signs indicate plasma without preservative; plus signs, plasma with reduced glutathione (GSH) (6.5 mmol/L) and EDTA (8 mmol/L) as a preservative.

Table II: Changes in Norepinephrine (NE) in Whole Blood Incubated at Room Temperature (24°C)

<table>
<thead>
<tr>
<th>Time, h</th>
<th>NE</th>
<th>Heparin, pg/mL</th>
<th>EDTA, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>435</td>
<td>394</td>
<td>394</td>
</tr>
<tr>
<td>1</td>
<td>404</td>
<td>392</td>
<td>392</td>
</tr>
<tr>
<td>6</td>
<td>435</td>
<td>409</td>
<td>409</td>
</tr>
<tr>
<td>24</td>
<td>159</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>48</td>
<td>106</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

*Decay rates for the 48-hour period were determined by linear-regression analysis. No significant change was observed in the NE concentration of EDTA or heparinized plasma for 0 to 6 hours.

Laboratory Medicine January 1990 27
a negligible loss in heparinized plasma (Table I). Since control values of EPI measured in the EDTA plasma were below the level of detection (<20 pg/mL), exogenous EPI (440 pg/mL) was added to the pooled samples prior to incubation.

Degradation of NE in Whole Blood
NE values in whole blood anticoagulated with heparin or EDTA are stable up to 6 hours at 24°C (Table II). After delaying plasma separation between 6 and 48 hours, NE values are lower than those of controls. Decay rates of 7.34 ± 1.58 and 6.03 ± 0.59 pg/h in heparin or EDTA whole blood, respectively, were similar. However, increasing the time to 72 and 96 hours before heparinized plasma is separated resulted in a further decline in NE to less than 15% of control. EPI was below the level of detection possible with HPLC.

Plasma NE and EPI Stability During Long-term Storage
NE and EPI plasma values were stable when stored at −70°C for up to 228 days with heparin (Fig 1) and 205 days with EDTA (Fig 2) as the anticoagulant. No advantages were noted with the addition of GSH/EGTA as a preservative. There was no statistical difference in the decay rates (pg/d) of NE and EPI between heparin and EDTA plasma (Table III).

Comment
Our results support the previous findings that catecholamine analysis does not require elaborate collection, processing, and storage procedures. We have, however, extended these findings with a comprehensive comparison of the stabilizing effect of preservatives added to both EDTA and heparin plasma and contrasted these with the effects of cellular elements of whole blood on plasma catecholamine values. Pettersson et al previously reported catecholamines were stable in plasma from heparinized blood for 22 hours at room temperature in the absence of thiols or antioxidants. De Vera et al also found no degradation of NE in heparinized plasma without addition of antioxidants for 10 hours at 37°C. Weir et al showed no effect on plasma catecholamines from EDTA anticoagulated blood after 3 hours at room temperature. We find no significant change of NE or EPI values in heparinized plasma samples for up to 24 hours after separation. In

Fig 1. Plasma norepinephrine (NE) and epinephrine (EPI) levels from heparinized blood following long-term storage at −70°C with and without reduced glutathione and EGTA (GSH/EGTA) as preservative. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); and EPI with GSH/EGTA (closed triangles).

Fig 2. Plasma norepinephrine (NE) and epinephrine (EPI) levels from EDTA-treated blood with and without the addition of reduced glutathione and EGTA (GSH/EGTA) as a preservative following long-term storage at −70°C. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); and EPI with GSH/EGTA (closed triangles).
contrast, NE and EPI concentrations in EDTA-anticoagulated blood were stable for 6 hours at 24°C but decreased significantly by 24 hours. EDTA plasma kept at 24°C for longer than 6 hours, a likely consequence of freezer malfunction or long-distance transport, will show markedly reduced plasma NE and EPI values when assayed with our HPLC system.

NE and EPI are rapidly degraded when stored in Tris-HCl (pH 7.4) buffer in the absence of exogenously added stabilizing agents. However, since plasma catecholamines are relatively stable, the presence of endogenous plasma antioxidants may be inferred. Therefore, adding preservatives prior to storage of plasma is not required if EDTA plasma samples are stored frozen at −70°C within 6 hours or if heparinized plasma is frozen within 24 hours. Furthermore, we show that NE and EPI values from plasma anticoagulated with either heparin or EDTA and stored at −70°C are stable for more than 200 days without the addition of a preservative.

Previous reports of delayed plasma separation from whole blood for up to 3 hours showed no change in catecholamine levels. Our data extend this 3-hour limit to 6 hours and expand the finding to include either heparin or EDTA as the anticoagulant. We suggest collecting clinical and field blood samples for plasma NE and EPI analysis with heparin as the anticoagulant. This technique allows for long-distance transport of whole blood (6 hours) or lengthy delays after plasma separation (24 hours) without significant in vitro degradation.

**Acknowledgment**

We wish to thank Dr. L. Homer for reviewing the manuscript and the excellent technical assistance of W. Smart and D. Smith. This study was supported by NAVYDRSRCH HDFXGOM #623333NMM316C30-A1-0102.

**References**


**Suppliers**


b. Waters Millipore Corporation, Bedford, MA 01730.

c. Digital Equipment Corporation, Milford, MA 01757.

d. STAFAK, Northwest Analytic, Inc., Portland, OR 97209.

---

Table II: Decay Rates of Norepinephrine (NE) and Epinephrine (EPI) from Heparinized and EDTA Blood Following Long-Term Storage at −70°C

<table>
<thead>
<tr>
<th>Plasma</th>
<th>GSH/EGTA*</th>
<th>Decay Rate, pg/g +/−SE†</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>NE</td>
<td>−</td>
<td>0.17 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>EPI</td>
<td>+</td>
<td>0.03 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>−</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>NE</td>
<td>+</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>EPI</td>
<td>+</td>
<td>0.14 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>EPI</td>
<td>+</td>
<td>0.05 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>EPI</td>
<td>+</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>EPI</td>
<td>+</td>
<td>0.02 ± 0.04</td>
</tr>
</tbody>
</table>

*Minus signs indicate plasma without 6.5 mmol/L GSH and 8 mmol/L EGTA; plus signs, plasma with 6.5 mmol/L GSH and 8 mmol/L EGTA.

†Decay rates were determined by linear-regression analysis.