

# Assay of Free Anticonvulsant Drugs

■ With separation by Centrifree® Micropartition Device

The binding of anticonvulsant drugs (phenytoin, valproic acid and carbamazepine) to a patient's blood proteins can have a significant effect on the pharmacokinetic and pharmacodynamic characteristics of the drug. This, of course, can considerably affect how the patient responds to the drug as well as the physician's approach and adjustment of drug therapy. For drugs largely bound to proteins in the blood plasma (mostly albumin), it is the unbound (free) concentration of the drugs which is biologically active and is, therefore, significant for the patient's pharmacologic response and for the physician's determination of drug administration.

Normal changes in physiology and diseases as well as the drug therapy itself can alter the degree of protein binding, thereby changing the important concentration of free drug in the blood stream. When patient response to anticonvulsant drug therapy does not correspond to the measured total drug concentration, knowledge of the unbound drug concentration enables the clinician to make appropriate adjustments of the patient's therapeutic regimen.

## Indications For Free Drug Measurement

*A good rule of thumb: Whenever the patient's clinical status does not correlate with what is indicated by given total drug levels, altered protein binding and altered free drug concentration should be suspected.*

The following patient conditions suggest the need for determination of free (as well as total) anticonvulsant drug levels:

- Hypoalbuminemia: Low albumin concentrations lead to increased free drug levels which may produce undesirable effects.
- Hyperalbuminemia: High albumin concentrations lead to decreased free drug levels which may cause therapeutic failure.
- Renal failure: Albumin/protein binding sites are altered. Increased free drug levels can produce toxicity even though total concentrations are within the therapeutic range.
- Drug/drug interactions: Drugs with greater affinity for protein binding sites displace an anticonvulsant drug from its protein binding sites and increase free drug concentrations. For example, valproate displaces other anticonvulsant drugs from their protein binding sites.
- Saturation kinetics: Valproic acid and disopyramide exhibit nonlinear (zero order) binding kinetics. The free level can vary remarkably between patients, at the same total drug concentration.
- Change in normal physiological status: Any age-related change in physiologic factors (pregnancy, disease, aging) can alter free drugs. These changes can also alter how a receptor responds to a given drug concentration



Centrifree Disposable Micropartition Device

## Glossary

The terminology of drug protein binding can be confusing. Note the distinction between free drug *concentration* and *fraction*:

Free drug *concentration* is the actual concentration of drug not bound to protein, in plasma.

Free drug *fraction* is the portion of the drug concentration in plasma which is not bound to protein. It is

determined by dividing free drug concentration by total drug concentration. Multiplying the free drug fraction by 100 gives the % of free drug present in the specimen. Percent of free drug (percent free) is often used interchangeably with "free drug fraction" in the older scientific literature.

## Free/Bound Separation Now Easy

In the past, free drug levels in serum or plasma samples were not widely measured, partly for want of a convenient means of separating free from protein-bound drugs. The technique of choice was generally equilibrium dialysis, a time-consuming procedure, subject to effects of dilution and buffers. It does not directly indicate the free drug concentration in the sample. Such other techniques as ultracentrifugation and gel filtration are no less time-consuming and there is inadequate standardization of results.

Today's better alternative for free/bound separation is ultrafiltration (UF) with Millipore's Centrifree micropartition device. Centrifree employs a membrane filter with controlled porosity which retains more than 99.9% of serum protein and lets free drugs readily pass the membrane for collection and analysis. This is done rapidly in a standard laboratory centrifuge (preferably angle-head), with samples in the range of 0.15 to 1 mL. The sample is not diluted in the process. Multiple samples are conveniently handled, typically in 10 minute runs per set. With the availability of Centrifree micropartition, combined with assay calibration and control kits, there is no longer any practical impediment to the ready availability of *unbound* as well as total anticonvulsant drug concentration measurement. As stated by Dr. C. E. Pippenger, "Any clinical laboratory can perform free level analyses for antiepileptic drugs, using a simple, one-step filtration process: Centrifree micropartition."

## Free Drug Assay Kits Readily Available

Determinations of free phenytoin, valproic acid and carbamazepine levels are just as easy to run via TDx as total level assays. The reagent system is actually identical. Only calibrator and control kits are different. TDx assays for the quantitative measurement of the unbound fractions of anticonvulsant drugs in serum or plasma samples are available from Abbott Laboratories. They utilize fluorescence polarization immunoassay technology, as discussed in the TDx operation manual. For detailed kit options, see page 3. Other standard analytical methods may also be used, with low-level standards.

## Use of Centrifree Is Simple, Efficient

The Centrifree micropartition device is a disposable unit. It contains Millipore's Amicon YMT hydrophilic ultrafiltration membrane which has virtually no non-specific adsorptivity for anticonvulsant drugs. The pressure generated by centrifugation forces plasma water and free drug molecules (typically < 1,000 MW) convectively through the ultrafilter, then into a filtrate collection cup. The larger protein molecules remain in the sample reservoir above the membrane. Free drug concentration in the collected ultrafiltrate (plasma water) is quantified by routine analytical techniques. Operation at an angle causes the protein layer that normally forms on the membrane to slide radially during centrifugation, maintaining high filtration efficiency (see Fig. 3).

Figures 1 and 2 illustrate the relationship between free and bound drug before and during UF. During ultrafiltration, convective transport of only free drug assures that equilibrium between free and protein-bound drug is maintained. This equilibrium is largely a function of the free drug concentration which does not change during filtration and to a lesser degree of the bound fraction which

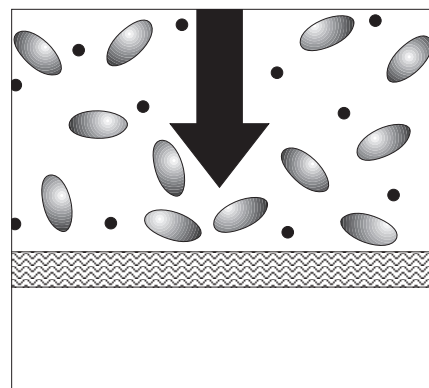


Figure 1. Before ultrafiltration, the drug (small dots) is partly free in the serum, partly bound to serum protein (large bodies).

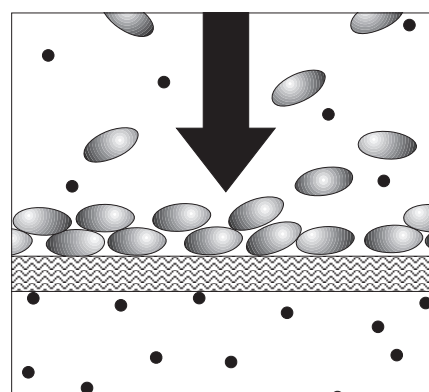


Figure 2. During ultrafiltration in Centrifree, free drug permeates the membrane. Its concentration in the filtrate is the same as in the sample above the membrane. The drug/protein molar binding ratio is unchanged. During centrifugation in a fixed-angle rotor, the proteins on the membrane surface are forced to slide laterally, maintaining relatively free access to the membrane.

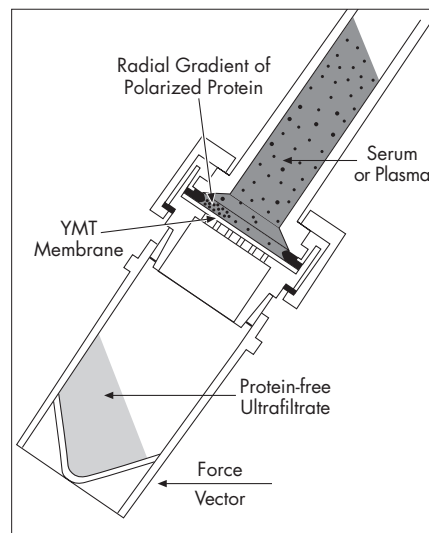


Figure 3. Polarization control, using a 35° fixed-angle rotor.

will not play a role unless large filtrate volumes are removed. Although total drug concentration in the sample reservoir increases during UF, the drug/protein molar binding ratio remains constant, as does the drug concentration in the filtrate. Therefore, drug concentration in the filtrate is the same as free drug concentration at equilibrium in the plasma sample before UF. Because the drug/protein molar binding ratio remains constant, drug concentrations in the ultrafiltrate are also independent of the volume collected.

### Technical Considerations

When determining free drug concentrations by UF, one should remember factors that can alter drug/protein binding *in-vitro*. They include:

*Temperature:* Drug binding decreases with rising temperature. The centrifuge should produce minimal temperature rise in its chamber. Generally, run times are so short that this is not a problem.

*pH:* Protein binding of some drugs is pH-dependent. Exposure of plasma to air can raise its pH, increasing free drug concentration. pH changes can be prevented by closing the sample reservoir with the provided cap before the run.

*Fatty acids:* Free fatty acids are released by lipolysis of triglycerides during prolonged plasma storage. They can displace drugs from their binding sites, increasing free drug concentration. Sample UF prevents those effects.

## TDx Anticonvulsant Drug Assays

All Abbott calibrations, controls and assay systems are run on the TDx system.

Item	Quantity	Abbott Catalog No.
<b>Free phenytoin</b>		
Free phenytoin calibrators	50 runs, 6 calibrators per kit	9530-01
Free phenytoin assay system	100 test kit	9507-69
Free phenytoin control	50 runs, 3 controls per kit	9530-10
<b>Free valproic acid</b>		
Free valproic acid calibrators	50 runs, 6 calibrators per kit	9537-01
Free valproic acid assay system	100 test kit	9514-69
Free valproic acid control	50 runs, 3 controls per kit	9537-10
<b>Free carbamazepine</b>		
Free carbamazepine calibrators	50 runs, 6 calibrators per kit	9538-01
Free carbamazepine assay system	100 test kit	9515-69
Free carbamazepine control	50 runs, 3 controls per kit	9538-10

For more information regarding the above contact Abbott laboratories, North Chicago, IL 60064 (phone: 1-800-527-1869)

### Low Cost

The only difference in laboratory cost between performing free and total drug assays is the cost of the disposable Centrifree device and the time required to ultrafilter the specimen by centrifugation, typically 10 minutes. The analytical system for measuring both total and free drug level is the same, except that free level controls are run to verify system performance.

### How To Order

*For the Physician:*

To order tests, please contact your clinical laboratory. If the lab is not familiar with the contents of this publication, please ask them to order it.

*For the Clinical Laboratory:*

Description	Catalogue No.
Centrifree Microconcentrators, Filtrate cups and caps. Box of 50 units.	4104

## To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:

In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**

In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**

On the Internet

<http://www.millipore.com>

E-mail: [tech\\_service@millipore.com](mailto:tech_service@millipore.com)

## Millipore Worldwide

### AUSTRALIA

Tel. 1 800 222 111  
or (02) 9888 8999  
Fax (02) 9878 0788

### AUSTRIA, CENTRAL EUROPE, C.I.S., AFRICA, MIDDLE EAST AND GULF

Tel. (43) 1 877-8926  
Fax (43) 1 877-1654

### BALTIC COUNTRIES

Tel. +358 9 804 5110  
Fax +358 9 256 5660

### BELGIUM AND LUXEMBOURG

Tel. (02) 726 88 40  
Fax (02) 726 98 84

### BRAZIL

Tel. (011) 548-7011  
Fax (011) 548-7923

### CANADA

Tel. 1-800-645-5476  
Fax 1-800-645-5439

### CHINA, PEOPLE'S REPUBLIC OF

Beijing:  
Tel. (8610) 6500-8063  
Fax (8610) 6500-7372  
Guangzhou:  
Tel. (8620) 8755-4021  
Fax (8620) 8755-4350  
Hong Kong:  
Tel. (852) 2803-9111  
Fax (852) 2513-0313  
Shanghai:  
Tel. (8621) 5306-9100  
Fax (8621) 5306-0838

### CZECH REPUBLIC

Tel. 02-205 138 41  
02-205 138 42  
Fax 02-205 14294

### DENMARK

Tel. 70 10 00 23  
Fax 70 10 13 14

### FINLAND

Tel. (09) 804 5110  
Fax (09) 256 5660

### FRANCE

Tel. (01) 30.12.70.00  
Fax (01) 30.12.71.80

### GERMANY

Tel. (06196) 494-0  
Fax (06196) 43901

### HUNGARY

Tel. 1-205 9784  
Fax 1-205 9792

### INDIA

Tel. (91) 80-839 46 57  
Fax (91) 80-839 63 45

### ITALY

Vimadrone (Milano):  
Tel. (02) 25.07.81  
Fax (02) 26.50.324  
Roma:  
Tel. (06) 52.03.600  
Fax (06) 52.95.735

### JAPAN

Tel. (03) 5442-9711  
Fax (03) 5442-  
9736 Analytical  
9737 BioProcess  
9734 Lab Water

### KOREA

Tel. (822) 551-0990  
Fax (822) 551-0228

### MALAYSIA

Tel. 603-757 1322  
Fax 603-757 1711

### MEXICO

Tel. (525) 576-9688  
Fax (525) 576-8706

### THE NETHERLANDS

Tel. 076-5022000  
Fax 076-5022436

### NORWAY

Tel. 22 67 82 53  
Fax 22 66 04 60

### POLAND

Tel. 22-669 12 25  
22-663 70 31  
Fax 22-663 70 33

### PUERTO RICO

Tel. (787) 273-8495  
Fax (787) 747-6553

### SINGAPORE

Tel. (65) 842 1822  
Fax (65) 842 4988

### SPAIN AND PORTUGAL

Madrid:  
Tel. 917 283 960  
Fax 917 292 909  
Barcelona:  
Tel. 934 525 530  
Fax 934 516 048

### SWEDEN

Tel. 08-628 6960  
Fax 08-628 6457

### SWITZERLAND

Volketswil:  
Tel. (01) 908-30-60  
Fax (01) 908-30-80  
Lausanne:  
Tel. (021) 648 51 18  
Fax (021) 648 51 37

### TAIWAN

Taipei:  
Tel. (886-2) 700-1742  
Fax (886-2) 755-3267  
Hsin Chu City:  
Tel. (886-3) 571-0178  
Fax (886-3) 572-9520

### U.K. AND IRELAND

Tel. (01923) 816375  
Fax (01923) 818297

### U.S.A.

Tel. (781) 533-6000  
Fax (781) 533-3110

### IN ALL OTHER COUNTRIES

Millipore Intertech (U.S.A.)  
Tel. +1 (781) 533-8622  
Fax +1 (781) 533-8630

# MILLIPORE

Millipore and Centrifree are trademarks of Millipore Corporation or an affiliated company

Lit. No. AN1002EN00 Printed in U.S.A. 12/99 99-297

Copyright 1999 Millipore Corporation or an affiliated company, Bedford, MA. All rights reserved.