Standards of laboratory practice: antiepileptic drug monitoring

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Discussion and development of standards for appropriate monitoring led to the following key recommendations for ordering, sampling, and analyzing antiepileptic drugs: Monitoring should usually be done on trough specimens after steady-state has been reached and always with an appropriate medical indication; non-steady-state concentrations may be indicated in selected situations. Monitoring of free phenytoin and free valproic acid is indicated in specific situations and should be done in serum. The metabolite of primidone, phenobarbital, should be measured concurrently with parent drug, but the active metabolite of carbamazepine does not need to be monitored unless the patient is exhibiting an unusual toxic response that cannot be otherwise explained. Assays used for antiepileptic drug monitoring should display a long-term CV of <10% and preferably <5%. Subtherapeutic and supratherapeutic drug concentrations should be investigated on a regular basis as part of a quality assurance process.

Epilepsy is one of most common dysfunctions of the nervous system, with a prevalence of 2 million affected individuals in the US (1). Patients with epilepsy are often noncompliant or incompletely compliant with their medication regimens for a variety of reasons. The effects of antiepileptic therapy can be assessed only through evaluation of the patient’s seizure frequency, a sometimes time-consuming process, especially if seizures are infrequent. Drugs for which relationships between blood concentration and therapeutic effect have been established can be evaluated through use of therapeutic drug monitoring, which can quickly determine whether the patient has achieved the desired drug concentration. This helps expedite the process of establishing a drug regimen for an individual patient. Monitoring is also helpful in this class of drugs because it is sometimes difficult to differentiate between drug toxicity and uncontrolled disease.

When monitoring is performed, the therapeutic ranges that have been established for the drugs in this class should be used only as guides. Several articles indicate that a strict use of the therapeutic range cutoffs to classify patients as subtherapeutic, therapeutic, or toxic will result in considerable numbers of misclassifications (2, 3). A therapeutic concentration is one that stops seizures or decreases seizure frequency with acceptable side effects in an individual patient.

Recently, carbamazepine and valproic acid have been reported to be effective in the treatment of bipolar disorder. Therefore, in addition to being classified as antiepileptic drugs, they are now also classified (along with lithium) as mood stabilizers or thymoleptics. Therapeutic ranges for the mood-stabilizing action of these drugs have not as yet been established, but most practitioners are using the therapeutic ranges established for the antiepileptic activities of these agents (4, 5).

In this paper we will focus on the most widely used antiepileptic drugs for which the value of monitoring blood concentrations is well established, namely, carbamazepine, phenytoin, phenobarbital, primidone, and valproic acid. Some information on lamotrigine, gabapentin, and topiramate is included, although the value of monitoring for these drugs has not yet been established.

**General Information**

Tables 1 and 2 provide a detailed summary of general information and pharmacokinetic data for the antiepileptic drugs.

**CARBAMAZEPINE**

Carbamazepine is effective in treating both seizures and bipolar disorder. It is thought to act through inhibiting nerve impulse transmission through the thalamus, blunting high-frequency, repetitive neuronal firing. This inhibition is likely mediated through a delay in the recovery rate of voltage-dependent neuronal sodium channels (6).
Carbamazepine exhibits several “idiosyncratic” adverse effects that may require cessation of the drug. If a rash develops and the only signs are a mild, nonpainful pruritic exanthem, carbamazepine can be continued cautiously or discontinued. If the drug is continued and any additional signs develop, carbamazepine should be immediately discontinued and, if the condition does not quickly resolve, consultation should be obtained, given the possibility of Stevens–Johnson syndrome (7). Carbamazepine also causes a relatively common modest suppression of leukocyte count (range: 3000–4000 cell/mm³) in the first 2 months of treatment that does not require action. Many clinicians feel that a complete blood count every 2 weeks during this period is sufficient to document

### Table 1. Antiepileptic drugs: general information.

<table>
<thead>
<tr>
<th>Generic/brand name</th>
<th>Conditions treated</th>
<th>Most common adverse effects</th>
<th>Major toxic effects</th>
<th>Other monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine/ Tegretol®</td>
<td>Complex partial, generalized tonic-clonic seizures; in combination for multiple seizure types. Also tic douloireux and other neuralgias (42). Recently applied to bipolar affective disorders.</td>
<td>Nausea, vomiting, drowsiness, dizziness, ataxia, vertigo, diplopia, rash (25). GI transit time can affect concentrations when sustained-release preparation is used.</td>
<td>Chronic toxicity leads to seizures (21). Hematologic dyscrasias, aplastic anemia possible (1/200 000–1/600 000) (22). Hepatic failure, Stevens–Johnson, leukopenia.</td>
<td>Hepatic and renal function (21) and baseline CBC (42).</td>
</tr>
<tr>
<td>Lamotrigine/ Lamictal®</td>
<td>Adjunctive treatment for partial seizures in adults.</td>
<td>CNS depression, rash, abnormal thinking, diplopia, dizziness, ataxia, nausea, nervousness, somnolence, vomiting.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin/Dilantin®</td>
<td>Primary drug for all types of seizures except absence seizures. Not effective for toxic seizures caused by theophylline.</td>
<td>Nystagmus, ataxia, impaired concentration; also drowsiness, seizure, rash. Chronic use leads to gingival hyperplasia, acne, or hirsutism in −50% of patients (21).</td>
<td>Stevens–Johnson, seizure lymphadenopathy, hemato logic dyscrasias, aplastic anemia.</td>
<td></td>
</tr>
<tr>
<td>Valproic acid/ Depakene®</td>
<td>Simple and complex absence seizures, complex partial seizures, tonic-clonic seizures, and multiple seizures in combination (21). Also manic episodes associated with bipolar disorder and migraine prophylaxis (12).</td>
<td>Nausea, vomiting, abdominal cramps, somnolence, dizziness most common; also tremor, ataxia, malaise, weakness, lethargy, facial edema, anorexia, weight gain (12, 25). Inhibits platelet aggregation, which may increase bleeding time (21). Side effects profile may change for Depakene versus Depakote.</td>
<td>Hepatic dysfunction, pancreatitis, metabolic disturbances (e.g., hyperammonemia) nystagmus, headache, ataxia, tremor, hallucinations, or changes in vision (21, 25).</td>
<td>Hepatic function should be checked at frequent intervals first 6 months of therapy.</td>
</tr>
</tbody>
</table>

* All anticonvulsant drugs are Category C: use in pregnancy only when benefit outweighs the risk. Teratogenesis occurs with all standard anticonvulsants drugs and is estimated to have an incidence of 2–4%. Neural tube defects are highest with valproate (~1%) and carbamazepine (~0.5%); it is suggested that this incidence can be lessened through the administration of folic acid before and during pregnancy. Teratogenic effects of new anticonvulsant medications are expected to be similar.

*GI*, gastrointestinal; CBC, complete blood count; and CNS, central nervous system.
that the leukocytes have reached a stable value. However, if a decline continues to <2500 leukocytes/mm³ or <1000 granulocytes/mm³, carbamazepine should be discontinued; it can produce aplastic anemia or agranulocytosis on rare occasions. Patients taking carbamazepine who develop sudden febrile illnesses, ecchymoses, mucosal bleeding, or any other systemic symptoms that are not easily explained require a stat blood count and differential (7).

Carbamazepine may also cause mild increases in liver function test results that tend to resolve over time, although many clinicians consider that increases greater than three times the upper limits of normal are an indication for discontinuation of the drug.

Table 2. Antiepileptic drugs: pharmacokinetic information.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Half-life</th>
<th>Time to ss* (1–5 t₁/₂)</th>
<th>Vₚ L/kg</th>
<th>Protein binding, %</th>
<th>Therapeutic range, µmol/L (μg/mL)</th>
<th>Toxic conc., µmol/L (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>24–48 h (44), decreases to 10–30 h due to autoinduction (21)</td>
<td>Variable, due to autoinduction that occurs in 4–6 wks</td>
<td>1.2 (45)</td>
<td>72 (44)</td>
<td>17–51 (4–12) (28)</td>
<td>Initial: &gt;63 (15) (21); serious: 200 (50)</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>5–7 h</td>
<td>1–2 days, depending on renal function</td>
<td>58±6</td>
<td>&lt;3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>7.5–23.1 with inducer; 11.6–61.6 monotherapy; 30.5–88.8 with valproate</td>
<td>30 h–2 wks (widely variable)</td>
<td>0.9–1.3</td>
<td>55</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1–5 days (44)</td>
<td>4–20 days</td>
<td>Adult: 0.55–0.7 (28, 44); neonate: 1.0 (47)</td>
<td>51 (44)</td>
<td>Adult: 64–172 (15–40); infant: 90–270 (20–60)</td>
<td>Initial: &gt;225 (50) (21); serious: 450 (100) (46)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Not 1st-order, varies with drug conc.; 6–60 h adult (44); 7–29 h child (21)</td>
<td>1–3 wks depending on conc. (48)</td>
<td>Adult: 0.6–0.8 (44); neonate: 0.8–1.2 (49)</td>
<td>90 (44); may be lower in neonates (49)</td>
<td>Adult: 40–80 (10–20); infant: 24–44 (6–11); free drug (at 25 °C): 4–8 (1–2)</td>
<td>Total: &gt;119 (30)²; free: &gt;12 (3)²; serious: 160 (40) (46)</td>
</tr>
<tr>
<td>Fosphenytoin</td>
<td>Prodrug of phenytoin, used for intravenous administration: t₁/₂ = 15 min, then phenytoin kinetics take over</td>
<td>See phenytoin</td>
<td>See phenytoin</td>
<td>See phenytoin</td>
<td>See phenytoin</td>
<td>See phenytoin</td>
</tr>
<tr>
<td>Primidone</td>
<td>3–12 h (44)</td>
<td>1–2 days (44)</td>
<td>0.8 (44)</td>
<td>19 (44)</td>
<td>23–55 (5–12)</td>
<td>Initial: &gt;84 (15) (21); serious: 225 (40) (46)</td>
</tr>
<tr>
<td>Topiramate</td>
<td>21 h</td>
<td>4–5 days</td>
<td>Not given</td>
<td>−15</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>9–18 h adult (44); 17–40 h infants; 7–13 h children (50)</td>
<td>2–4 days (21)</td>
<td>0.14 (44)</td>
<td>90, decreasing at higher total drug conc. (44)</td>
<td>347–833 (50–120)²</td>
<td>Serious: &gt;1428 (200) (46)</td>
</tr>
</tbody>
</table>

* SS, steady-state; conc., concentration; and ND, not determined.

² Values for water-soluble drugs will be greater in infants and young children than in adults because of greater proportion of body water in infants and children.

² Because carbamazepine induces its own metabolism ~2-fold in the 1st 1–2 wks of therapy (28), the t₁/₂ decreases after the patient has been taking the drug for a period of time. t₁/₂ at start will be ~36 h, will decrease to 10–20 h with autoinduction, and will decrease further to 8–12 h if phenobarbital or phenytoin is added (29).

Data for these drugs were obtained from package inserts and Graves (53).

Because the elimination of phenytoin is not first-order, t₁/₂ not a very useful concept: It changes as concentration changes and is increased in the elderly (28).

Critical values that trigger a call to both a clinician and a clinical pharmacist in institution of A.W.

Fosphenytoin, a prodrug for phenytoin, is converted to phenytoin with a 15-min half-life. Drug monitoring of phenytoin concentrations after use of the prodrug requires that 2 h elapse from the end of an intravenous infusion and 4 h from an intramuscular injection before samples are obtained. This time interval ensures that the elimination of phenytoin is not first-order, t₁/₂ not a very useful concept: It changes as concentration changes and is increased in the elderly (28).

Primidone is metabolized to phenobarbital. Both drugs should be monitored and concentrations evaluated in light of the patient’s response.

There is significant variation in the half-life of this drug. Generally, in children and patients taking enzyme-inducing drugs, valproic acid has a short half-life and more frequent dosing is required (21).

A minimal concentration has been defined: 347 µmol/L (50 μg/mL). The therapeutic range was not evaluated in a large sample of patients. Further, the therapeutic concentrations depend on whether mono- or polytherapy is being used. The maximum concentration is being evaluated, with some clinicians advocating concentrations of 1041–1214 µmol/L (150–175 μg/mL) (11).
PHENYTOIN
Phenytoin is used to treat all types of seizure disorders except absence seizures; it is also used as prophylaxis after brain injury, although it has not been shown to be effective for other than short-term prophylaxis. Its unique feature is its nonlinear kinetics of action. Other features are numerous drug interactions and the potential for causing a wide variety of adverse effects. Although the mechanism of action is not established, phenytoin is theorized to act by blocking sodium channels in neuronal tissue, causing in prolongation of their rate of recovery and reduction in the frequency of sustained repetitive firing of action potentials (6).

When attempting to increase plasma concentrations of phenytoin, the dose should be increased by <100 mg/day if the concentration is ≥28 μmol/L (7 μg/mL) (8). Phenytoin dosage in obese patients should be based on the adjusted body weight (ABW), calculated as follows:

\[
ABW = \text{IBW} + 1.33 \times (\text{actual wt.} \; - \; \text{IBW})
\]

where IBW is the ideal body weight (9).

Because children are faster metabolizers than adults, the dose (mg/kg) that was effective in a child will need to be decreased after puberty.

Long-term complications of phenytoin therapy include hirsutism, acne, coarsening of facial features, folate deficiency, vitamin D deficiency, and gingival hyperplasia, which can occur even if phenytoin is kept at “therapeutic” concentrations. Signs of toxicity include lethargy, drowsiness, nystagmus, diplopia, ataxia, vertigo, neuropsychological impairment, and nausea.

PRIMIDONE AND PHENOBARBITAL
Primidone is effective for the treatment of tonic-clonic simple, partial, and complex partial seizures and seizures in neonates. It is also used for treatment of essential tremor, particularly in the elderly. Primidone has an elimination half-life of 2–12 h, being rapidly transformed into its active metabolite, phenobarbital. Because of its longer half-life, phenobarbital will accumulate during primidone therapy. When a patient is receiving primidone therapy, therefore, both primidone and phenobarbital should be measured. The primidone concentration will achieve steady-state in ~2 days, whereas phenobarbital will not reach steady-state until after 20 days.

Phenobarbital is an antiepileptic drug in its own right and is used to treat tonic clonic and partial seizures. As an effective stimulator of P450 enzymes, its use leads to increased metabolism of several drugs, including other antiepileptics, carbamazepine, and valproic acid. The long elimination half-life of phenobarbital means that the drug must be administered in a loading dose to rapidly achieve a therapeutic blood concentration.

Because phenobarbital distributes into lipid tissue, obese patients may require a loading dose based upon their actual weight (10). The drug is addictive, and evidence of a withdrawal “syndrome” may present if the drug is discontinued suddenly. The most troublesome adverse effects of phenobarbital are sedation and negative effects on cognition, particularly in children. Because phenobarbital may impair learning in some children when used for a long time, it is important to keep concentrations as low as possible. The half-life of phenobarbital in neonates is longer than in children or adults—probably because of the larger volume of distribution and decreased metabolic capacity of infants—and needs to be considered in calculating appropriate dosing regimens. The half-life of phenobarbital is also reported to increase in patients over 70 years of age, indicating that doses should be gradually decreased as a patient ages (11).

VALPROIC ACID
Valproic acid, like carbamazepine, is effective in treating both seizures and bipolar disorder; it is also used for migraine prophylaxis. It is theorized to act by increasing the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) within the central nervous system through either inhibition of GABA degradation or enhancement of GABA synthesis and release. Other postulated mechanisms are inhibition of excitatory neurotransmitters or action at sodium and calcium channels to reduce sustained neuronal firing (6).

An unusual feature of this drug is that once the plasma concentration reaches ~536 μmol/L (75 μg/mL), the free fraction of valproate increases rapidly if the dose is increased. Because it is the unbound drug that is available for metabolism, any amount of increasing free valproate is rapidly metabolized, so that the total plasma concentration increases very little with increasing dose. At lower doses, there is a linear increase of blood concentration in response to a dose increase. To illustrate: If a dose of 700 mg produces a concentration of 179 μmol/L (25 μg/mL), a doubling of the dose to 1400 mg would be expected to produce a blood concentration of 358 μmol/L (50 μg/mL). To increase the concentration to 715 μmol/L (100 μg/mL), however, more than a doubling of the dose would be required (12).

Careful clinical monitoring should be performed during the first 6 months of therapy with valproic acid. Some relatively frequent but usually clinically insignificant transient and chronic effects not clearly related to dose are seen, including hair loss, increased results for liver function tests, and a reduced platelet count accompanied by a mildly prolonged coagulation time. This latter effect needs to be considered if the patient is receiving other drugs that influence coagulation or is to undergo surgery. As with carbamazepine, most clinicians feel that an increase of liver function test results greater than three times the upper limit of normal may require discontinuation of the drug (7).

Children younger than 2 years who are receiving anticonvulsant polytherapy or who have a known metabolic problem are at increased risk for developing fulmi-
nant hepatitis, possibly resulting from a toxic metabolite (12). Valproate should be used with extreme care in patients with known hepatic disease or significant hepatic dysfunction. The drug is a probable teratogen, leading to an increase of neural tube defects during pregnancy, and should be avoided in such a case.

NEW ANTIEPILEPTIC DRUGS
Information on several of the new antiepileptic agents is summarized in the tables. The value of concentration monitoring has not yet been established for these agents. A recent review provides more detailed information on gabapentin and lamotrigine (13).

Practice Issues
Control of epilepsy with a single drug—monotherapy—is the goal. If a patient fails to meet the therapeutic goal (no seizures with acceptable adverse effects) with a single agent, a second drug should be added and the first drug discontinued. Polytherapy is usually indicated only if the patient has failed two or more drugs as single agents (11). In many patients, adverse effects and seizure control must be balanced.

Compliance is a major problem with patients who require long-term therapy for a seizure disorder. A recent survey of patients with epilepsy revealed that 49% of patients were dissatisfied with their current regimen because of adverse drug effects. The rate of noncompliance to phenytoin therapy is 15–60%, with adolescents more likely to be noncompliant than adults (14). Most patients cite the unwanted cosmetic side effects associated with phenytoin as the primary reason for discontinuing their medication intake.

In an attempt to become more cost-effective, managed care organizations are developing drug formularies that list the drugs for which the most favorable prices have been negotiated each contract period and which, therefore, are required to be used in their patients. This practice can provide economies in the prescription budget, but for patients and physicians it can lead to increased expenditures if the managed care organization requires the switching of patients controlled on a given formulation of antiepileptic drug to another formulation. Some patients are very sensitive to small changes in bioavailability that may occur with such drug switching. Differences in the formulations of generic carbamazepine and phenytoin vs the brand names are known to result in variations in bioavailability. The practical result of this is that a patient who has been titrated and stabilized on a specific dose of Dilantin® or Tegretol® may, upon switching to a generic formulation, develop toxicity or become subtherapeutic. In general, then, it may be more cost-effective overall to maintain patients with epilepsy on the drug formulation on which they were initially titrated. Indiscriminate switching between brands of antiepileptic drugs should be discouraged (11).

Several clinical trials indicate that phenytoin, carbamazepine, and sodium valproate have relatively equivalent efficacy in seizure prevention; however, they vary in their adverse effects and patient acceptance (15–17). In one trial involving all four drugs, the incidence of adverse effects with phenobarbital was so high that it was dropped from the study (17). Another study reported that patients randomized to phenytoin were more likely to withdraw (9%) than were patients receiving either carbamazepine (4%) or valproate (4%) (18).

A major concern, particularly when these drugs are used to treat children, is their effect on cognition. The anticonvulsant with the greatest adverse effect on cognition is phenobarbital, but phenytoin, carbamazepine, and valproate may also impair cognition in individual patients (19).

THERAPY INITIATION
A seizure is a transient change in neurological function caused by paroxysmal firing of groups of neurons. Epilepsy is a condition characterized by recurrent seizures; a single seizure does not represent epilepsy. Whether to treat a first seizure is a controversial topic. A recent randomized, prospective study (20) demonstrated a reduction in the risk of recurrent seizures after a first seizure was treated with an antiepileptic drug. However, antiepileptic drug treatment of a first seizure should take into account the risk of seizure recurrence and the risk of acute and chronic adverse effects of the medication. Chronic treatment is usually unnecessary if a reversible cause of the seizure is found.

DISCONTINUATION OF THERAPY
Patients often do not need to remain on antiepileptics for life and a long-term goal is to be able to withdraw drug therapy. After 3–5 seizure-free years, discontinuation of therapy can be considered (11).

ANTIEPILEPTIC DRUG INTERACTIONS
Because antiepileptic drugs are frequently used together in polytherapy, knowledge of the major interactions between these drugs is of interest.

- Carbamazepine causes decreased concentrations of phenytoin and valproic acid.
- Phenobarbital stimulates P450 enzymes, leading to enhanced metabolism and thus lower concentrations of primidone, phenytoin, carbamazepine, and valproic acid.
- Valproic acid leads to increased phenobarbital concentrations. The acidification of urine by valproate enables reabsorption of phenobarbital, which is also acidic. The resulting increase in the t1/2 of phenobarbital leads to a 10–20% (up to 40%) increase in its concentration after 24–26 days (21).
- Phenytoin enhances the conversion of primidone to phenobarbital (22).
OTHER DRUG INTERACTIONS
Many drug interactions have been identified between various antiepileptic drugs and drugs from other classes. Only a few are presented here. More detailed information on drug interactions can be obtained from such sources as Young et al. (23) and the Physicians Desk Reference (Medical Economics Co.). In addition, most dispensing pharmacies in both hospitals and the retail environment use computer programs to identify potential interactions before a drug is dispensed.
- Salicylate, phenylbutazone, and sulfonyleureas can increase the free fraction of phenytoin.
- Salicylate can increase the free fraction of valproic acid.
- Erythromycin interacts with valproic acid, leading to increased blood concentrations of valproic acid.
- Chloramphenicol, dicoumarol, disulfiram, isoniazid, cimetidine, and some sulfonamides cause increased phenytoin concentrations through enzyme inhibition (21).

Indications for Monitoring
Several studies have documented that the monitoring of antiepileptics is frequently done inappropriately. D’Angio et al. (24) found that 78% of specimens submitted for concentration monitoring in their institution were obtained either before steady-state or without noting the draw time, and 31–83% of specimens were obtained without a clear medical indication. A study at another institution determined that 73% of requests for antiepileptic drugs were made without an appropriate medical indication (25). These studies indicate that there is a great deal of waste in the current approach to therapeutic drug monitoring. Laboratory tests ordered without a rational indication are unlikely to improve patient care, and they consume scarce resources. Therefore, developing and then applying appropriate guidelines for ordering determinations of antiepileptic drug concentrations is an important step toward improved patient care and better use of testing resources.

MONITORING FREE (UNBOUND) DRUG
Routine drug monitoring involves measuring the concentration of total drug. In some cases, however, the total drug concentration may be misleading and a free drug concentration is needed. Measurement of free drug concentrations of antiepileptics is not needed routinely. Free drug measurement may be important in selected cases for the following reasons:
- Drug that is bound to serum proteins is pharmacologically inactive, whereas free drug is active.
- An equilibrium exists between bound and free drug that is based on drug and protein concentration.
- A change in binding, due (e.g.) to decreased protein concentration, can alter the fraction of free drug (a) and thus lead to changes in the pharmacological response at a given total concentration of drug.

There are two main criteria for determining when measurement of the free drug concentration may be clinically useful:
- The drug is bound to plasma proteins by >85–90%.
- For drugs that are so highly bound, a relatively small decrease in the amount of protein available for drug binding may have a correspondingly large impact on the amount of free drug present.
- The extent of binding of the drug is known to vary as a result of changes in protein or drug concentration, availability of or competition for binding sites, and binding affinity.

The two antiepileptic drugs that fulfill these criteria are phenytoin and valproic acid, with free phenytoin being the measure more frequently used because of clinicians’ greater familiarity with it. Measurement of free carbamazepine, of which 70–80% is bound to albumin and \( \alpha_1 \)-acid glycoprotein, is not clinically indicated.

Both phenytoin and valproic acid bind to serum albumin. Phenytoin is 90% bound, but its binding can be affected by the presence of other acidic drugs that also bind to albumin such as valproic acid, salicylate, phenylbutazone, and sulfonyleureas. Valproic acid is also 90% bound at low therapeutic concentrations, but as the total valproic acid concentration increases, the binding becomes saturated and increasing amounts of free drug are present. Salicylate is the only acidic drug known to effectively compete for albumin binding sites with valproic acid (26). Uremia can cause a two- to threefold increase in free drug concentrations over what would be seen in a nonuremic patient with the same albumin concentration (26).

In the pediatric population, a decrease in drug protein binding may occur in patients with malnutrition or chronic renal failure or in neonates, especially those with hyperbilirubinemia (27). In these situations a given concentration of total drug may produce a greater effect than expected.

RECOMMENDATIONS
General indications for monitoring antiepileptics. Measuring a serum concentration of an antiepileptic drug is most appropriate when the blood sample is drawn after steady-state conditions have been reached, i.e., after 4–5 half-lives on an unchanged dose regimen. The following are appropriate clinical reasons for obtaining a drug concentration measurement:
- As a baseline measurement after starting antiepileptic drug therapy (The exact concentration at which phenytoin reaches zero-order kinetics in a given patient cannot be stated with certainty but in many patients it does occur within the range of concentrations designated as the therapeutic range. Because of this, a good strategy is to check concentrations several days after starting the drug and again after 2–3 weeks to verify that the concentration is not continuing to increase slowly, leading to delayed toxicity (28).)

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As a control measurement after a change in the dose regimen
- After adding a second drug with a potential for interaction with the antiepileptic drug
- After a change in the patient’s liver, cardiac, or gastrointestinal tract function

Non-steady-state concentrations may be indicated in emergency treatment of serial seizures or status epilepticus. Measuring a serum concentration is usually appropriate:
- Within 6 h after a seizure recurrence in a controlled patient
- After an unexplained change in seizure frequency
- In suspected dose-related drug toxicity
- In suspected patient noncompliance

Although monitoring antiepileptic drugs with a same-day turnaround time is generally sufficient, stat testing is indicated in the following situations:
- The patient is currently experiencing seizures.
- The patient has recently suffered multiple seizures.
- Substantial toxicity is suspected but not yet demonstrated.

Indications for monitoring free drug concentrations. Monitoring of free phenytoin or free valproate may be indicated in the following situations:
- A patient with a drug concentration within the therapeutic range is exhibiting unexpected toxicity.
- The patient is elderly, pregnant, or a neonate.
- The patient is uremic.
- The patient has an albumin concentration <377 μmol/L (<2.5 g/dL).
- The patient is taking concomitant medications that are known to compete for protein binding sites, e.g., valproic acid, salicylate, phenytoin combinations.

### Analytical Issues

#### TYPE AND TIMING OF SAMPLES
Table 3 provides a summary of sampling information for the antiepileptic drugs. Serum is always an appropriate sample for monitoring antiepileptic drugs and must be used when the free drug is being measured. Generally, use of plasma for total drug measurement depends on the analytical method chosen; however, citrate and oxalate anticoagulants decrease the total concentration of phenytoin and valproic acid and should be avoided (29, 30). In general, before using plasma, it is necessary to verify that a particular anticoagulant does not interfere with the analysis. Because some anticoagulants interfere with drug–protein binding, plasma is not an appropriate specimen for the measurement of free drugs. Whole blood is an acceptable specimen only if the analytical method has

### Table 3. Samples for therapeutic monitoring of antiepileptic drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample timing</th>
<th>Sample type*</th>
<th>Sample stability*</th>
<th>Metabolite monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Predose, consistent time of day</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>Active epoxide metabolite not</td>
</tr>
<tr>
<td></td>
<td>after steady-state</td>
<td></td>
<td>stable at least</td>
<td>measured routinely</td>
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<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Gabapentin</td>
<td>Value of monitoring not yet</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>No metabolites formed</td>
</tr>
<tr>
<td></td>
<td>established</td>
<td></td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Value of monitoring not yet</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>Inactive glucuronide only metabolite</td>
</tr>
<tr>
<td></td>
<td>established</td>
<td></td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Any time during dosage interval</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>No active metabolites</td>
</tr>
<tr>
<td></td>
<td>after steady-state</td>
<td></td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Predose (oral dosing); 1–4 h post-</td>
<td>Serum, do not</td>
<td>Separated serum</td>
<td>No active metabolites</td>
</tr>
<tr>
<td></td>
<td>IV loading dose. At least 2 h</td>
<td>use SST</td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-IV dose or 4 h post-IM dose of</td>
<td>tubes</td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fosphenytoin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primidone</td>
<td>Predose after steady-state</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>Phenobarbital: active metabolite,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>stable at least</td>
<td>measured</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td>Only 30% of dose is metabolized</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Value of monitoring not yet</td>
<td>Serum, plasma</td>
<td>Separated serum</td>
<td>None monitored</td>
</tr>
<tr>
<td></td>
<td>established</td>
<td></td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Predose, consistent time of day</td>
<td>Do not use</td>
<td>Separated serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>after steady-state</td>
<td>citrate or</td>
<td>stable at least</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>oxalate</td>
<td>30 min at 60 °C</td>
<td></td>
</tr>
</tbody>
</table>

* Serum is always an acceptable specimen for antiepileptic drug monitoring. Citrate and oxalate should be avoided because of their effects on total concentrations of valproic acid and phenytoin (29, 30). Whether other types of plasma can be used depends on the effect of the anticoagulant on the assay system chosen; whole blood would be acceptable only if the instrumentation and method used have been adapted to whole blood; the therapeutic range might need to be adjusted for such samples. Antiepileptic drugs have been measured in saliva, which may have some clinical applicability, but this has not moved into routine use.

The stability reported here is from two studies in which samples were tested for stability to heating sufficient to destroy HIV.

Data for these drugs was obtained from package inserts and Graves (53).

IV, intravenous; IM, intramuscular.

Because of the short $t_{1/2}$, serum concentrations of valproate can fluctuate widely. Therefore, collection of blood samples must be timed carefully in relation to dosing.
been developed for analyzing whole-blood samples. If whole blood is used, the therapeutic range may need to be adjusted if the blood cells have a dilutional effect.

The issue of whether tubes containing serum separator gels are appropriate for the collection of drug specimens remains largely unresolved because of the lack of a large-scale definitive study. In our opinion, as a rule of thumb, decreases in concentration that exceed 10% for antiepileptic drugs should be considered clinically significant. Several studies in the literature indicate that gel-containing tubes are generally acceptable for drug monitoring specimens (31–34); however, concentrations of phenytoin were clinically significantly decreased in two of the studies when SST® (Becton Dickinson) tubes were used for specimen collection (31, 34). If serum separator tubes are used, any absorbance of drug in vitro can be minimized by filling the tubes completely and processing and removing the serum promptly.

Predose or trough samples are the most appropriate samples to use when monitoring orally dosed antiepileptic drugs. Proper timing of samples is required for the therapeutic range to be valid and is most critical for drugs that have a short half-life. Recording the correct times (dosing and sampling) can alleviate some of the problems caused by mistimed samples. Because valproic acid and carbamazepine concentrations are affected by circadian rhythm, both are best monitored in samples drawn at a consistent time of day.

Postdose or peak sampling can be done after intravenous administration of a loading dose. Recently, a prodrug of phenytoin for parenteral use has been introduced, fosphenytoin. This phosphorylated form of formylphenytoin must undergo hydrolysis to phenytoin before its activity will be evident. The half-life of fosphenytoin is ~15 min. The recommended sampling time to check a loading dose is 2 h postintravenous dosing or 4 h postintramuscular dosing.

ANALYTICAL PRECISION

Table 4 summarizes representative precision data indicating what is currently achievable with immunoassay reagents on automated analyzers. The assay precision required for antiepileptic drug concentrations to be used to calculate appropriate dosage regimens depends on several factors, most importantly the toxicity of the drug and the dosing precision that can be achieved. Although these drugs can be toxic, the margin of safety between the upper end of the therapeutic range and onset of toxicity in most patients is broader than for some other drugs (e.g., lithium). In addition, because these drugs are usually given in solid dosage forms that are available in only a few sizes, the ability to calculate an appropriate dose is almost always going to be more precise than is the actual ability to administer a specific dose. Taking into account the relative toxicity and the dosing imprecision inherent in using solid dosage forms, concentration results within ±10% of the actual result are probably acceptable for all of the anticonvulsant drugs when total drug is being measured. This means that assays must be able to demonstrate routinely an interrun precision with a CV of 5–10% or better.

A similar standard can also be applied to the measurement of free phenytoin and free valproic acid. Because free drug concentrations are so much lower than total drug concentrations, such measurements are potentially prone to greater variability—although this is more of a problem with free phenytoin than with free valproate. Data from the College of American Pathologists Surveys indicate that the precisions for free and total valproate measurements are comparable, whereas that for free phenytoin is inherently higher than that observed for the total drug. Some of the imprecision encountered in free phenytoin analysis may result from poor control of temperature during the ultrafiltration step (see Free drugs below).

METABOLITES

Metabolites are candidates for monitoring if they are active. In actual practice, however, the metabolites that are routinely measured are those for which a convenient method is available. In the case of phenobarbital, a metabolite of primidone, concurrent monitoring with parent drug is routinely performed. However, the active epoxide metabolite of carbamazepine is not routinely monitored, in part because measurement of the metabolite requires an HPLC method. Although the relationship of the epoxide to adverse effects has not been clearly defined and the role of the epoxide in possible idiosyncratic toxicity also remains largely unexplored (35), the metabolite is known to be active and is a drug in its own right for the treatment of tic doloreaux (36). The ratio of epoxide metabolite to parent drug is variable, although under steady-state conditions, plasma epoxide concentrations tend to be ~20–25% of the concomitant concentration of parent carbamazepine (37). Despite its activity and potential for toxicity, routine monitoring of carbamazepine epoxide does not appear to be indicated at this time.

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**Table 4. Representative precision of immunoassays for antiepileptic drugs.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>CV, % (at concentration, mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Interlaboratory</strong></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>3.7 (4–8)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>3.2 (20–65)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>3.8 (8–14)</td>
</tr>
<tr>
<td>Free phenytoin</td>
<td>11.9 (1)</td>
</tr>
<tr>
<td>Primidone</td>
<td>3.9 (7–11)</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>3.1–4.4 (49–115)</td>
</tr>
<tr>
<td>Free valproic acid</td>
<td>5.4 (78)</td>
</tr>
</tbody>
</table>

* Represents the best CV, independent of method, reported on the CAP ZA 1996 survey for >100 laboratories.

† Representative data obtained with AxSym or TDx/FLx instrumentation in the laboratory of A.W.
for most patients. Monitoring in occasional patients may be useful in sorting out the etiology of a toxic response.

FREE DRUGS
The measurement of free drug concentrations is most easily performed by use of an ultrafiltration device. The most appropriate sample for free drug analysis is serum, given the effects of some anticoagulants on either free fraction or total drug concentration (38).
- Citrate and oxalate anticoagulants substantially decrease the total concentration of phenytoin and valproic acid and should be avoided when either total or free drug measurements are desired (29, 30).
- The effect of EDTA is not established; results of studies are discrepant (29, 30, 39).
- In vivo heparin use produces spuriously high free fraction results for most drugs because heparin activates lipoprotein lipases, which causes an increase in circulating free fatty acids that displace drugs from albumin (39).
- Effective use of ultrafiltration devices requires the use of a fixed-angle centrifuge and temperature-controlled centrifugation. Phenytoin–protein binding is particularly sensitive to changes in temperature.

QUALITY ASSURANCE
A result less than the detectable limit for a therapeutic drug measurement may indicate one or more of the following problems: patient noncompliance; incorrect drug assay ordered; specimen mix-up; presence of an interfering substance in the specimen; or instrument malfunction. Laboratories should develop protocols for determining whether one of these types of errors has occurred if a therapeutic drug is not detected in a specimen.
- A result that indicates that the patient may be toxic needs to be verified through repeat analysis and then communicated to the physician as soon as possible. In hospitalized patients, such results are communicated as soon as they are obtained, whatever the time of day or day of the week, generally by paging the attending physician. Laboratories doing outpatient testing face a dilemma when the value obtained has been designated as critical. Calling a physician in the middle of the night to report a critical drug concentration on a sample obtained sometime during the previous afternoon from a patient the physician may be unable to contact until morning can lead to unhappy interactions between laboratories and physicians. Given that the outpatient was probably seen by the physician at the time the drug sample was obtained, life-threatening toxicity should have already been recognized. If there is any doubt, however, it is better to contact the attending physician. This is an extremely difficult issue for many laboratories and physicians, given the possibility of harm to the patient as well as the potential for legal action. Approaches that may help are: In consultation with physicians and clinical pharmacists, the laboratory should develop appropriate critical value concentrations for outpatients; the laboratory can also establish protocols, approved by referring physicians, that provide for early-morning reporting of any results obtained during the night.

RECOMMENDATIONS
Specimens and timing
- Choose serum, plasma, or whole blood as the sample for analysis of total drug, according to the requirements of the analytical method chosen. Avoid citrate and oxalate in samples obtained for phenytoin and valproic acid analysis.
- Measure free drug in serum because several anticoagulants interfere with drug–protein binding.
- Draw samples after oral dosing of anticonvulsants immediately before the next dose for drugs with short half-lives; for drugs with long half-lives (≥24 h), the draw time during the dosing interval is less critical. Always ascertain and report the time of sample draw.
- Obtain samples for valproic acid and carbamazepine concentration measurement at a consistent time of day because of the presence of a circadian rhythm effect.
- Obtain samples after intravenous dosing of phenytoin at 1–4 h postdose or at 2 h postintravenous or 4 h postintramuscular dosing with fosphenytoin.
- Avoid tubes containing the SST brand gel separator for specimens containing phenytoin.

Analytical precision
- Use assays for therapeutic drug monitoring precise enough to produce a long-term CV of no more than 10% and preferably <5%.

Metabolites
- The metabolite of primidone, phenobarbital, must be measured concurrently with the parent drug.
- The active metabolite of carbamazepine is not measured unless the patient exhibits an unusual toxic response that cannot be otherwise explained.
- Manufacturers of immunoassays are encouraged to develop assays that have no cross-reactivity with either inactive or active metabolites or that measure drug and active metabolites with a cross-reactivity proportional to the total pharmacological activity.

Measurement of free drugs by ultrafiltration. When ultrafiltration is used to separate free drug, the following guidelines should be followed:
- Centrifuge at a fixed angle to maximize the efficiency of ultrafiltration device.
- Maintain constant temperature during centrifugation, especially when measuring free phenytoin.
- If storing samples, ultrafilter before freezing, because freezing can affect protein binding (39).
- Adjust the detection limits of the assays to measure the lower concentrations of the free drugs.
- Use the appropriate therapeutic range for free phenytoin, according to the temperature of the ultrafiltration
procedure (40): 4–8 μmol/L (1–2 μg/mL) at 25 °C, 6–12 μmol/L (1.5–3 μg/mL) at 37 °C.

**Quality assurance**
- Investigate subtherapeutic drug concentrations on a regular basis as part of a quality assurance process because such values frequently represent some type of error.
- Define critical values for each drug for a given institution whenever possible through joint discussions involving laboratory scientists, physicians, and clinical pharmacists.
- Communicate critical values for potential drug interactions to the patient’s caregiver as soon as they are verified.
- Develop policies for reporting critical values in the outpatient setting in conjunction with the referring physicians and laboratory legal counsel.

**Reporting Issues**
Appropriate therapeutic ranges need to be provided to the clinician—along with comments if there is an indication that such ranges may not be applicable for a specific sample. Although therapeutic ranges should not serve as absolutes, they are an important guide. The following are representative therapeutic ranges in wide use: carbamazepine, 34–51 μmol/L (8–12 μg/mL); phenytoin, 40–80 μmol/L (10–20 μg/mL); free phenytoin, 4–8 μmol/L (1–2 μg/mL; 25 °C); phenobarbital, 64–172 μmol/L (15–40 μg/mL); primidone, 23–55 μg/mL (5–12 μg/mL); and valproic acid, 347–833 μmol/L (50–120 μg/mL).

If sufficient data are available to the laboratory to indicate that the sample timing is not correct, a comment to that effect should be appended to the report. In most cases, however, laboratories do not have all of the information available to support such an assessment. For phenytoin reports, a potentially useful comment to append would be: Because of the variable half-life and time to steady-state, plasma phenytoin concentrations should always be interpreted with a knowledge of how long the patient has been taking the current dose.

Other comments that may be useful would be ones referring to potential drug interactions, if the laboratory has this information on the patient. Drug interactions are of interest to the clinical laboratory if they have the potential to cause either of the following effects: a falsely increased or decreased drug concentration measurement (assay interference), or an actual change in concentration of drug without a dose change. For most laboratories, trying to append comments to reports regarding potential drug interactions is not a practical strategy. However, having information available on such interactions is useful when dealing with physicians’ questions about why the concentrations being reported do not fit with the patient’s picture.

Our recommendations in reporting test results are as follows:
- Include therapeutic ranges for the antiepileptic drugs on the report containing the value obtained in the patient.
- Append comments to the report to indicate situations known to the laboratory that may affect the interpretation of the drug concentration.

We acknowledge all of the participants of the National Academy of Clinical Biochemistry meeting for their comments and discussion, which have added immeasurably to the development of this Standard of Laboratory Practice. In addition, we thank everyone who reviewed the earlier drafts of this material and commented before the meeting.

**References**

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4 The range for free phenytoin has not been established as such; rather, it results from multiplying the accepted therapeutic range for the total drug by the free fraction (α = 0.1).