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Standards of laboratory practice: cardiac drug monitoring

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In this Standard of Laboratory Practice we recommend guidelines for therapeutic monitoring of cardiac drugs. Cardiac drugs are primarily used for treatment of angina, arrhythmias, and congestive heart failure. Digoxin, used in congestive heart failure, is widely prescribed and therapeutically monitored. Monitoring and use of antiarrhythmics such as disopyramide and lidocaine have been steadily declining. Immunoassay techniques are currently the most popular methods for measuring cardiac drugs. Several reasons make measurement of cardiac drugs in serum important: their narrow therapeutic index, similarity in clinical complications and presentation of under- and overmedicated patients, need for dosage adjustments, and confirmation of patient compliance. Monitoring may also be necessary in other circumstances, such as assessment of acetylator phenotypes. We present recommendations for measuring digoxin, quinidine, procainamide (and *N*-acetylprocainamide), lidocaine, and flecainide. We discuss guidelines for measuring unbound digoxin in the presence of an antidote (Fab fragments), for characterizing the impact of digoxin-like immunoreactive factor (DLIF) and other cross-reactants on immunoassays, and for monitoring the unbound (free fraction) of drugs that bind to α_1-acid glycoprotein. We also discuss logistic, clinical, hospital, and laboratory practice guidelines needed for implementation of a successful therapeutic drug monitoring service for cardiac drugs.

Three major groups of drugs are used for treatment of cardiac complications: drugs for treatment of angina *(1)*, drugs for treatment of arrhythmias *(2)*, and drugs for treatment of congestive heart failure *(3)*. For treatment of angina, organic nitrates such as nitroglycerin (Nitrostat); Ca^{2+}-channel blockers such as diltiazem (Cardizem CD); or β-adrenergic antagonists such as metaprolol (Lopressor) are generally used. Drugs for treatment of arrhythmias, antiarrhythmics, as proposed by Vaughan Williams in 1970 *(4)*, are divided into four major classes, based on their effect on the cardiac action potential. A fifth class is added by some authors to include those drugs that alter membrane responsiveness *(5)*.

The Vaughan Williams classification of antiarrhythmics and examples of representative agents are as follows (although, as discussed elsewhere *(5)*, this classification has certain limitations): class I, Na^+-channel blockade (Harrison's modification *(6)* further divides this class of antiarrhythmics into three subclasses: IA (quinidine and procainamide), IB (lidocaine and phenytoin), and IC (flecainide)); class II, β-adrenergic blockade, propranolol; class III, prolonged repolarization, amiodarone (Cordarone) or bretylium (Bretylol); class IV, Ca^{2+}-channel blockade, verapamil or diltiazem; class V, altering cell membrane responsiveness, digoxin and atropine.

Drugs used for treatment of congestive heart failure include cardiac glycosides such as digoxin and digitoxin, diuretics (e.g., thiazides), and angiotensin-converting enzyme inhibitors (e.g., captopril) *(1)*.

General Considerations

In this report, our focus is on the cardiac glycosides and those antiarrhythmics that are frequently monitored in the current therapeutic drug monitoring (TDM)[3] practice. Cardiac drugs for which commercial automated immunoassays are available on various platforms for use in clinical laboratories (ranging from small physicians' offices to large referral laboratories) include digoxin, procainamide and *N*-acetylprocainamide (NAPA), quinidine, lidocaine, disopyramide, flecainide, and digitoxin. In addition, various chromatographic methods (e.g., HPLC) are also reported in the literature for quantification of these drugs.

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[3] Nonstandard abbreviations: TDM, therapeutic drug monitoring; NAPA, *N*-acetylprocainamide; MEGX, monoethylglycylxylidine; and GX, glycinexylidine.

Table 1 includes information on the general use and side effects of cardiac drugs. Selected pharmacokinetic information is included in Table 2, and requirements for samples as well as monitoring of metabolites are presented in Table 3. Analytical issues such as precision requirements and interference issues are listed in Table 4. Finally, interactions of drugs and possible mechanisms for these interactions (if known) are summarized in Table 5.

Although several other cardiac drugs in use are not described in this report, a few general attributes and pharmacokinetic information regarding propafenone, amiodarone, and mexiletine are included in Tables 1 and 2.

Propafenone is a racemic drug with active sodium-channel blocking, β-adrenergic antagonistic, and calcium-channel antagonistic properties *(7)*. After oral administration, propafenone is subject to first-pass metabolism. The hydroxy metabolite of propafenone is pharmacologically inactive and is formed in part by the activity of cytochrome P4502D6, which exhibits polymorphism *(8)*. Coadministration of low doses of quinidine has been shown to inhibit cytochrome P4502D6 and lead to increased propafenone plasma concentrations in individuals who are extensive metabolizers *(8)*. Plasma concentrations of propafenone and the hydroxy metabolite can be monitored simultaneously by HPLC *(9)*, which has reportedly been useful in assessing patient compliance, identifying poor metabolizers, and guiding antiarrhythmic therapy *(10)*. Both propafenone enantiomers have the same sodium-channel blocking activity, and the R-enantiomer impairs the disposition of the S-enantiomer *(11)*.

Table 1. General information.[a]

Generic/trade name	Conditions treated	Most common side effects	Major toxic effects	Other monitoring required
Amiodarone/Cordarone®	Life-threatening recurrent ventricular arrhythmias without response to adequate doses of other antiarrhythmics	Photosensitivity, corneal microdeposits, thyroid abnormalities	Hypersensitivity, alveolar or interstitial pneumonitis, liver injury, worsened arrhythmias	Liver and thyroid functions, ECG,[b] PFTs, eye exam
Digitoxin/Crystodigin®	Heart failure, atrial flutter/fibrillation, and supraventricular tachycardia	Anorexia, nausea, vomiting	Mental status changes, vomiting, bradycardia, heart block	Serum K^+; in acute overdose, serum [K^+] decreases but increases in chronic overdose
Digoxin/Lanoxin®, etc.	Heart failure, atrial flutter/fibrillation, and paroxysmal atrial tachycardia	Nausea, vomiting, visual disturbances, weakness	Atrioventricular (AV) block, premature cardiac contraction, arrhythmia, vomiting	Monitor [K^+] and ECG
Disopyramide/Norpace®	Documented life-threatening ventricular arrhythmias	Heart failure, hypotension	Apnea, arrhythmias, loss of consciousness	Serum glucose, renal function, ECG
Flecainide/Tamboco™	Paroxysmal ventricular tachycardia, atrial fibrillation/flutter, supraventricular tachycardia	Proarrhythmic effects	Nausea, vomiting, hypotension, syncope bradycardia, heart failure	Renal function, ECG
Lidocaine/Xylocaine®	Arrhythmias	Excitatory or depressive CNS effects, allergic reaction, bradycardia	Cardiovascular depression, convulsions, hypoxia	ECG
Mexiletine/Mexitil®	Documented life-threatening ventricular arrhythmias (e.g., sustained ventricular tachycardias)	Nausea, vomiting, heartburn, lightheadedness, tremors, coordination difficulties, changes in sleep habits	Nausea, hypotension, sinus bradycardia, paresthesia, seizures, left branch bundle block, asystole	ECG, liver function, CBC
Procainamide/Procanbid®	Documented ventricular arrhythmias	Hypotension, vomiting, lupus erythematosus-like syndrome, neutropenia	Ventricular extrasystoles and tachycardia	Monitor serum [NAPA], ECG, renal function
Propafenone/Rythmol®	Life-threatening ventricular arrhythmias	Unusual taste, dizziness, constipation, dyspnea, nausea/vomiting, anxiety	Supraventricular tachycardia, atrial flutter, tinnitus, apnea	ECG, pacemaker function, renal and hepatic functions
Quinidine/Quinidex®, etc.	Conversion of (and decreased relapse into) atrial fibrillation and flutter; suppression of ventricular arrhythmias	Diarrhea, headache, palpitations, rash, tremors	Ventricular arrhythmias, hypotension, vomiting, diarrhea, tinnitus	ECG

[a] From *Physician's Desk Reference*, 1997 edition.
[b] ECG, electrocardiogram; CBC, complete blood count; PFT, pulmonary function test; CNS, central nervous system; and CBC, complete blood count.

Table 2. Pharmacokinetic information.

Drug	Half-life, h[a]	Time to steady-state[b]	V_d, L/kg[a]	% protein binding[c]	Therapeutic range, mg/L[d]	Toxic concentration, mg/L
Amiodarone	13–37 days	130–535 days[c]	66 ± 44	96–97	0.5–2.0	>2.5
Digitoxin	100–200	1 month	0.54 ± 0.14	90	0.01–0.03	>0.045
Digoxin	26–52	5–7 days	7.30[c]	23	0.0005–0.002	>0.003
Disopyramide	5.0–7.0	1–2 days	0.59 ± 0.15	28–68	2.0–5.0	>7.0
Flecainide	8.0–14	3–5 days[e]	4.9 ± 0.4	32–58	0.2–1.0	>1.0
Lidocaine	1.4–2.2	0.5–1.5 h (with loading dose); 5–10 h (without loading dose)	1.1 ± 0.4	43–60	1.5–5.0	>6.0
Mexiletine	7.1–11.3		4.9 ± 0.5	60–66	0.5–2.0	>2.0
Procainamide	2.4–3.6	15–25 h	1.9 ± 0.3	15	4.0–8.0	>10
NAPA	5.5–6.2		1.38[c]	10	10–20	>40
Propafenone	3.4–7.6 (normal metabolizers); 9–25 (slow metabolizers)	4–5 days[e]	3.6 ± 2.1	85–95[a]	<1.0	>4.8[f]
Quinidine	4.4–9.0	2 days (oral dosing)	2.7 ± 1.2	70–80	2.0–5.0	>6.0

[a] From Benet LZ, Oie S, Schwartz JB. Design and optimization of dosage regimens; pharmacokinetics data. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, eds. *The Goodman and Gilman's Pharmacological Basis of Therapeutics*, 9th ed. Appendix II, New York: McGraw Hill, 1996:1707–92.

[b] Evans WE, Oellerich M, eds. *Therapeutic Drug Monitoring Guide*. Chicago: Abbott Laboratories, 1984.

[c] Tietz NW. Therapeutic drugs. In: Tietz NW, Pruden EL, McPherson RA, Fuhrman SA, eds. *Clinical Guide to Laboratory Tests*, 3rd ed. Section 3, Philadelphia: WB Saunders Co., 1995:787–897.

[d] Roden *(2)*.

[e] *Physicians Desk Reference*, 1997 ed.

[f] Five h postingestion. Baselt RC, Cravey RH. Propafenone. In: Baselt RC, Cravey RH, eds. *Disposition of Toxic Drugs and Chemicals in Man*, 4th ed. Foster City, CA: Chemical Toxicology Institute, 1995:653–5.

Amiodarone, a class III antiarrhythmic, has a very slow onset of action, a large volume of distribution, and a half-life measured in weeks *(12)*. A highly lipophilic drug, the lipid:plasma concentration ratio of amiodarone is >300:1 *(2)*. Because amiodarone is structurally similar to thyroid hormone, its toxicity may involve interaction with nuclear thyroid receptors *(2)*. Its major metabolite is desethylamiodarone, which is pharmacologically active and has a half-life of ~129 days *(13)*. Serum monitoring of amiodarone and its active metabolite can be performed by HPLC *(14)* and has been suggested to be useful in follow-up of arrhythmic patients chronically treated with the drug *(15)*. For a given patient, the distribution of amiodarone (and desethylamiodarone) in plasma and erythrocytes is highly variable. Therefore, measured plasma concentrations of this drug should be interpreted with such limitations in mind *(16)*.

The antiarrhythmic activity of mexiletine is similar to that of lidocaine and decreases the maximal velocity of phase 0 by blocking the fast sodium channels *(17)*. Mexiletine is metabolized in the liver to *p*-hydroxymexiletine, hydroxymexiletine, and corresponding alcohols, which are not pharmacologically active *(18)*. The elimination half-life of mexiletine is reduced by hepatic inducers such as rifampicin and phenytoin *(18)*. Coadministration of mexiletine and theophylline causes a considerable increase in plasma theophylline concentrations because of the competitive inhibition of demethylation of theophylline by mexiletine *(19)*. Monitoring serum concentrations of mexiletine has been shown to be clinically helpful *(20)*, and several HPLC *(21, 22)*, and gas chromatography–mass spectrometry *(23)* methods for therapeutic monitoring of this drug have been reported.

CARDIAC GLYCOSIDES

Cardiac glycosides (digoxin and digitoxin) are used for their positive inotropic effects and for treatment of heart failure, atrial fibrillation, atrial flutter, and paroxysmal atrial tachycardia *(24)*. Although the main drug in this class is digoxin (Lanoxin, etc.), in European countries digitoxin (Crystodigin) is also used for similar indications. Cardiac glycosides elicit their pharmacological activities by inhibition of the ion-transport activity of the membrane-associated sodium pump (Na^+,K^+-ATPase) *(25)*. Digoxin is administered intravenously or can be taken orally. Incomplete or variable bioavailability of digoxin in conventional tablet or elixir formulations has been reported. Soft gelatin capsules (Lanoxicaps) have yielded more consistent serum concentrations *(26)*; the plateau in serum concentration of digoxin is reached 8–12 h after intravenous, intramuscular, or oral administration *(27)*. Because of its high affinity for binding to its receptor (the sodium pump), digoxin distributes extensively into tissues, as evidenced by its large volume of distribution in humans (5–9 L/kg) *(24)*. About 20% of digoxin is bound to proteins in serum. Metabolism of digoxin involves deglycosylation, reduction of the lactone ring, oxidation, epimerization, and conjugation to several more polar

Table 3. Recommended collection logistics and samples for therapeutic monitoring.

Drug	Sample timing	Sample type	Sample stability	Metabolite monitoring
Digitoxin	>6 h after the last dose	Serum (no SST tubes); plasma (heparin, fluoride, oxalate); affected by EDTA or citrate plasma	24 h at 2–8 °C, 1–2 weeks at −20 °C	Immunoassays with active metabolites cross-reacting in proportion to their biological activities are desired
Digoxin	8–12 h (or more) after the last dose	Serum or plasma (heparin, EDTA); SST tubes should not be used	24 h at 2–8 °C, 1–2 weeks at −20 °C	Immunoassays with active metabolites cross-reacting in proportion to their biological activities are desired
Disopyramide	Sample to be collected 1 h before next dose (trough)	Serum or plasma (heparin, EDTA); SST tubes should not be used		Mono-*N*-dealkyldisopyramide is active and can be detected by HPLC
Flecainide	Sample to be collected 1 h before next dose (trough)	Serum or plasma (heparin, EDTA); SST tubes should not be used		Assay should not detect the metabolite, which is inactive
Lidocaine	>30 min after intravenous dosing for up to 5–10 h after start of drug administration	Serum or plasma (heparin, EDTA); SST tubes should not be used	24 h at 2–8 °C, 1–2 weeks at −20 °C	MEGX and GX are active metabolites; they may not cross-react in immunoassays but can be detected by HPLC
Procainamide	Sample to be collected 1 h before next dose (trough)	Serum or plasma (heparin, EDTA, oxalate)	24 h at 2–8 °C, 1–2 weeks at −20 °C	NAPA should also be monitored on the same sample; NAPA and procainamide concentrations should not be summed
NAPA	Sample to be collected 1 h before next dose (trough).	Serum or plasma (heparin, EDTA, oxalate)	24 h at 2–8 °C, 1–2 weeks at −20 °C	Procainamide should also be monitored on the same sample
Quinidine	Sample to be collected 1 h before next dose (trough)	Serum or plasma (EDTA); SST tubes should not be used	1–2 weeks at −20 °C	Dihydroquinidine (impurity), quinine, and hydroxyquinidine may interfere in immunoassays

Source: Tietz NW. Therapeutic drugs. In: Tietz NW, Pruden EL, McPherson RA, Fuhrman SA, eds. *Clinical Guide to Laboratory Tests*, 3rd ed. Section 3, Philadelphia: WB Saunders, 1995:787–897.

metabolites *(28)*. The biotransformation of digoxin seems to take place mainly in the stomach and the intestines. In the stomach, digitoxose sugars of digoxin are removed by gastric acid to form deglycosylated congeners *(29)*. Intestinal flora have been indicated in the metabolism of digoxin to its reduced form, dihydrodigoxin *(30)*. Importantly, although the biological activity of some digoxin metabolites (e.g., digoxigenin) is low, the immunoreactivity of the metabolites in some digoxin immunoassays may show greater cross-reactivity than digoxin itself *(31)*.

Potassium ions compete for binding of cardiac glycosides to the sodium pump. Thus, the biological activities of cardiac glycosides are linked to serum K^+ concentrations. Digoxin overdose may cause hyperkalemia as a result of decreased sodium pump activity. Therefore, in cases of suspected digoxin overdose, it is important to measure potassium in whole blood or serum.

Reported mechanisms for interaction of other drugs with digoxin include increased absorption of digoxin *(32)*, inhibition of biliary clearance of unchanged digoxin *(33)*, and decreased renal tubular secretion of digoxin by inhibition of P-glycoprotein *(34)*. Quinidine has been indicated to alter the pharmacokinetics of digoxin by all three of these mechanisms, whereas verapamil inhibits the biliary and renal elimination of digoxin. Coadministration of quinidine and digoxin is reported to greatly increase observed digoxin toxicity, even when the serum digoxin concentrations are well within the therapeutic range *(35)*. Therefore, before initiation of quinidine, the digoxin dose must be reduced; serum digoxin concentrations should be monitored as well as clinical signs of digitalis poisoning, to allow for dosage adjustments *(36)*. Interactions of other drugs (e.g., amiodarone and clarithromycin) with digoxin are included in Table 5.

Although several methods, including radioreceptor assays and inhibition of Na^+,K^+-ATPase or Rb^+ uptake, have been used for measuring digoxin in biological fluids, immunoassay is the prominent method currently used. However, because of measurement interferences caused by endogenous and exogenous substances, the lack of a well-defined therapeutic reference range, overlap between toxic and nontoxic concentrations, a narrow therapeutic index, and undefined subtherapeutic concentrations, the therapeutic monitoring of digoxin continues to have substantial difficulties. Details of these problems have been summarized in a recent review *(37)*.

Table 4. Analytical issues in monitoring cardiac drugs.

Drug	Analytical precision required (CV, %)[a]	Analytical interferences	Comments
Digitoxin	7	Digoxin is a minor metabolite of digitoxin that may also interfere in immunoassays. Cardiac glycoside-like poisons (e.g., oleander) may also interfere.	In case of overdose, antidote (Fab fragments) may be administered. Such patients should be monitored only if the immunoassay used is not affected by the presence of the antidote.
Digoxin	7	Deglycosylated metabolites of digoxin (with less bioactivity) may have cross-reactivity greater than digoxin in some immunoassays. Cardiac glycoside-like poisons (e.g., oleander) may also interfere. Digoxin-like immunoreactive factors may interfere and should be eliminated.	Interference from poisonous cardiac glycosides (e.g., oleandrin) may in some clinical settings be desired because the antidote may also be effective in treating such poisonings.
Disopyramide	5	*N*-Desmethyldisopyramide may cross-react in immunoassays.	Monitor unbound drug concentrations in renal failure patients.
Flecainide	7	Metabolite should not cross-react in the immunoassay.	Urinary acidification may increase and alkalinization may decrease elimination.
Lidocaine	5	MEGX and GX (active metabolites) should also be measured.	Because lidocaine is primarily bound to α_1-acid glycoprotein, variations in concentration of this protein alter unbound fraction of the drug.
Procainamide	8	Hemolysis, lipemia, and icterus may affect the immunoassays. HPLC and GC are not affected by such interferences.	(NAPA) equal to or greater than (procainamide) on the sample collected 3 h after the last dose in a patient with normal renal function indicates fast acetylator status.
NAPA	8	Hemolysis, lipemia, and icterus may affect the immunoassays.	NAPA accumulates in renal failure and may compete with procainamide for renal excretion.
Quinidine	8	Dihydroquinidine, hydroxyquinidine, quinine, and similar compounds may interfere in immunoassays.	Metabolism is affected by hepatic function.

[a] The analytical precision required was calculated as one-third of the testing limit set by the College of American Pathologists surveys for therapeutic drug monitoring (1996). For analytes that are not graded, a testing limit of 20% was used for calculations. For disopyramide, the calculated precision required is 3.3%; however, an imprecision limit of 5% is recommended.

PROCAINAMIDE AND NAPA

Procainamide (e.g., Procanbid) is utilized to treat ventricular and supraventricular arrhythmias. It reduces the velocity of impulse conduction in atria, His–Purkinje fibers, and ventricles by increasing their refractory periods. Its vasodilatory properties offset its depressive actions on the heart. This drug is available for oral, intramuscular, and intravenous administration. Extended-release tablets of procainamide provide a sustained release at a constant rate from the small intestine *(38)*. The plasma protein binding of procainamide is ~20%, and ~70% of the administered dose of procainamide is eliminated in the urine unchanged. Increased urine pH can decrease renal elimination of procainamide *(2)*.

Hepatic conjugation of procainamide (catalyzed by *N*-acetyltransferase), which results in the formation of its major active metabolite (NAPA), exhibits genetic polymorphism. The fast acetylator phenotype occurs in 10–20% of Asians; 50% of Americans (blacks and whites); and 60–70% of Northern Europeans *(39, 40)*. During therapeutic monitoring of procainamide, determination of the serum concentration of NAPA is essential because NAPA alters both the elimination and the electrophysiological actions of procainamide. Both procainamide and NAPA are actively secreted by the proximal tubules of the kidney; competition between NAPA and procainamide for renal secretion results in decreased elimination of the parent drug *(41)*. The N-acetylation of procainamide is inhibited by various substances, including *p*-aminobenzoic acid *(42)*. On the other hand, cimetidine and ranitidine have been reported to reduce renal elimination of procainamide and NAPA by competing for proximal tubule cationic transport *(43, 44)*. The pharmacodynamics of procainamide and NAPA also differ, in that the parent drug prolongs both the QRS and QTc intervals, whereas NAPA may prolong only the QTc interval *(41)*. This may be clinically significant because using total concentrations of procainamide and NAPA to assess pharmacological activity or toxicity may be misleading.

In fast acetylators and in patients with impaired renal function, NAPA accumulates, and its serum concentration may exceed that of the parent drug *(45)*. A patient (without renal impairment) is considered a fast acetylator if a specimen collected 3 h after dosing has a NAPA concentration equal to or greater than procainamide *(46)*. Lima and Jusko *(47)* propose an alternative method for determination of acetylator status, for use in renal failure patients; they calculate the apparent acetylation clearance by using serum concentrations of procainamide at steady-state and urinary excretion rates of NAPA.

When procainamide is administered as a slow-release formulation, some individuals may exhibit delayed absorption of the drug. This possibility should be considered in determining the acetylator status of patients.

Table 5. Drug interactions observed for cardiac drugs.

Drug	Interacting drug	Mechanism of interaction	Laboratory action required
Digitoxin	Phenobarbital, phenylbutazone, and other hepatic inducers	Enhance hepatic metabolism of digitoxin	When phenobarbital is discontinued, serum digitoxin may rise and lead to toxicity. If such coadministration is known, notify the physician.
Digoxin	Quinidine, amiodarone, verapamil	Increased absorption, inhibit biliary and decreased renal tubular excretions (P-glycoprotein mediated excretion suspected)	Notify the physician if the information on coadministration of these drugs with digoxin is available.
	Flecainide	Increases serum digoxin concentration, mechanism not known	
	Amiodarone	Decreases renal excretion of digoxin	
	Clarithromycin	Decreases the conversion of digoxin to dihydrodigoxin by the gut flora	
Disopyramide	Erythromycin	Inhibits hepatic metabolism	Notify the physician if the information on coadministration of these drugs with disopyramide is available.
	Hepatic inducers (e.g., rifampin)	Increase metabolism and elimination	
Flecainide	Digoxin	Flecainide can cause an increase in serum concentrations of digoxin (13–19%); mechanism of this interaction is not known	If such coadministration is known, notify the physician.
Lidocaine	Hepatic enzyme inducers (rifampin)	Increased metabolism, causing decreased half-life	If such coadministration is known, notify the physician.
Procainamide	Cimetidine, ranitidine, NAPA	Competition for proximal tubule excretion	This may lead to increased drug half-life and induce toxicity. If such coadministration is known, notify the physician.
NAPA	Cimetidine, ranitidine	Competition for proximal tubule excretion	If such coadministration is known, notify the physician.
Quinidine	Rifampin and other hepatic inducers	Increased hepatic elimination	If such coadministration is known, notify the physician.

In addition to automated immunochemical methods (e.g., fluorescence polarization immunoassay and Emit), various HPLC methods capable of determination of procainamide and NAPA for routine clinical use in plasma *(48)* or whole blood *(49)* have been reported.

QUINIDINE

Quinidine (Quinaglute, Quindex, Cardioquinis) is the oldest primary antiarrhythmic agent currently used in clinical practice. In addition to blocking Na^+ and K^+ channels, it is weakly vagolytic and has α-adrenergic blocking properties. However, its ability to block Na^+ channels is the primary mechanism by which it delays reactivation of Na^+ channels, thus prolonging the effective refractory period of the action potential *(50)*. Quinidine is indicated for treatment of (and prevention of relapse to) atrial fibrillation and atrial flutter. It is also used to suppress ventricular arrhythmias *(51)*.

Quinidine, the D-isomer of quinine, also is a schizonticidal and gametocidal agent with antimalarial properties *(51)*. This drug is frequently administered orally; intravenous or intramuscular administration is not recommended. It is available in three different salt forms: sulfate, gluconate, and polygalacturonate, which contain 83%, 62%, and 60%, respectively, of the anhydrous quinidine. The gluconate form is available as a sustained-release preparation. After oral administration, quinidine is absorbed in the small intestine; gastric acidity has little effect on its bioavailability *(52)*. About 80% of the quinidine in plasma is bound to protein. Quinidine is extensively metabolized, and only 20% of the parent drug is excreted in the urine unchanged *(2)*. The urinary metabolites of quinidine include 2′-quinidinone, 3-hydroxyquinidine, quinidine-*N*-oxide, and quinidine-10,11-dihydrodiol *(53)*. The activity of dihydroquinidine is equal to that of quinidine, and this metabolite is present as an impurity (up to 15%) in commercial preparations of quinidine *(53)*. The 3-hydroxyquinidine metabolite also has activity comparable with that the parent drug, whereas the other metabolites have less activity *(2, 53)*.

Quinidine is most frequently measured by immunoassays but can also be analyzed by HPLC. The variable cross-reactivities of dihydroquinidine and metabolites of quinidine mean that values obtained by some immunoassay methods may be overestimated *(54)*. For measurements of unbound and total quinidine, such interferences can be eliminated by using HPLC methods *(53, 55)*. The presence of endogenous quinidine-like immunoreactive substances in rats with conditions such as hyperthyroidism has been reported *(56)*. The presence of such substances in humans has not yet been investigated.

LIDOCAINE

Lidocaine (Xylocaine), a Na^+-channel blocker, has been shown to lower the maximum rate of rise of phase 0 depolarization, decrease the duration of cardiac action potential, and depress membrane responsiveness *(52)*. Because lidocaine inhibits neuronal membrane ion flux, it is also used as a local anesthetic. Oral administration can cause abdominal discomfort and vomiting. Lidocaine is also available as an ointment for topical applications as well as in injection forms for regional anesthesia. After oral administration of lidocaine, its extensive first-pass metabolism means that about one-third of the dose reaches the general circulation. For antiarrhythmic use, therefore, lidocaine is administered intravenously *(57–59)*. Of the lidocaine in plasma, ~70% is bound to α_1-acid glycoprotein *(2)*. Renal excretion of unchanged lidocaine is minimal (<10% of the administered dose), and decreased renal function has little effect on its elimination. Because lidocaine is primarily metabolized by the liver, decreased hepatic blood flow may lower its elimination. Hepatic metabolism of lidocaine involves deethylation, leading to the formation of monoethylglycylxylidine (MEGX) and glycinexylidine (GX). MEGX and GX have ~83% and 10%, respectively, of the antiarrhythmic activity of the parent compound *(60)*.

Various HPLC and gas–liquid chromatographic methods capable of measuring lidocaine, MEGX, and GX in serum have been reported *(61, 62)*. Determination of lidocaine and MEGX simultaneously may be useful in evaluation of liver impairment.

DISOPYRAMIDE

Disopyramide (Norpace) increases the duration and the refractory periods of the cardiac action potential and lowers the amplitude as well as the maximum rate of increase of phase 0 depolarization *(52)*. Disopyramide is a racemic mixture and is indicated for treatment of established ventricular arrhythmias. Because of its proarrhythmic properties, however, particularly negative ionotropic effects, its use is limited to selected cases *(63)*. Disopyramide is available for oral administration in both immediate release and controlled-release formulations. It mainly binds to α_1-acid glycoprotein in a saturable manner *(62, 64)*. This saturable protein binding may account for significant variations in unbound serum drug concentrations that cannot be detected by total drug measurement *(64)*. Meffin et al. *(65)*, studying 12 patients on disopyramide therapy, reported that at any given total disopyramide concentration, there was an ~2-fold range in the concentration of the unbound fraction. Furthermore, Kishino et al. *(66)* reported that the number of disopyramide-binding sites per α_1-acid glycoprotein molecule decreased in patients with renal insufficiency. Whenever possible, therefore, free (unbound) disopyramide should be monitored, especially in patients with renal failure.

Hepatic mono-N-dealkylation is the major route of metabolism of disopyramide and leads to the formation of mono-*N*-dealkyldisopyramide. This metabolite has ~50% of the antiarrhythmic activity of the parent drug *(67)*, has 24-fold more anticholinergic activity than disopyramide, and accumulates in renal failure *(68)*.

Similar to disopyramide, mono-*N*-dealkyldisopyramide binds to protein binding sites in a concentration-dependent manner, and both forms compete for such binding. Therefore, changes in concentration of the metabolite can alter the unbound concentration of disopyramide in serum *(69)*. The unchanged drug (57%) and nordisopyramide (27%) are mainly excreted by the kidneys *(70)*.

Erythromycin has been shown to inhibit the hepatic metabolism of disopyramide to nordisopyramide, leading to increased serum disopyramide concentrations *(71)*. On the other hand, coadministration of rifampin with disopyramide can lead to a reduction in serum disopyramide concentrations *(72)*.

Various HPLC methods for determination of serum concentrations of disopyramide and its metabolite have been reported *(73, 74)*. The sensitivity of the HPLC methods is generally 0.5 mg/L. The fluorescence polarization immunoassay for disopyramide monitoring gives results that correlate acceptably with HPLC results *(75)*.

FLECAINIDE

Flecainide (Tambocor) blocks the Na^+ channels and in a rate- and time-dependent manner decreases the V_{max} of ventricular and Purkinje action potentials *(52)*. It prolongs the duration of ventricular action potential and slows the cellular conduction throughout the heart *(76)*. Flecainide is indicated for treatment of paroxysmal supraventricular tachycardias, paroxysmal atrial fibrillation or flutter (or both), and documented ventricular arrhythmias *(76)*. Flecainide may also be proarrhythmic and decrease cardiac contractility; its use should thus be reserved for selected patients. A history of acute myocardial infarction or atrioventricular block must be ruled out before flecainide is given. The combination of flecainide and amiodarone is effective for controlling refractory tachyarrhythmias in infants *(75)*. Flecainide is administered orally and, because of its long half-life (12–27 h), dosing does not exceed two times per day *(76)*. The fraction of flecainide bound to serum proteins is reportedly between 48% and 68%. Such protein binding correlates well with the serum albumin concentration, not with the α_1-acid glycoprotein values *(77)*.

About one-half of the administered dose of flecainide is excreted unchanged in urine. Hepatic metabolism of flecainide exhibits polymorphism by cytochrome P450 2D6 *(78)*. Flecainide is dealkylated to *m-O*-dealkylflecainide, which is then either conjugated and excreted, or oxidized to a lactam-ring-containing metabolite, which is also conjugated and excreted. Flecainide metabolites lack pharmacological activity *(79)*.

Various methods such as fluorescence polarization

immunoassay (Abbott Laboratories) and HPLC have been reported for measurement of serum flecainide concentration *(80, 81)*. For HPLC analysis, a solid-phase extraction procedure with Empore solid-phase extraction membranes has been reported *(81)*. The immunoassay results for serum flecainide concentrations <0.5 mg/L exhibit more imprecision and less accuracy than the HPLC results *(80)*.

Indications for Monitoring

CARDIAC GLYCOSIDES

The clinical side effects associated with high concentrations of cardiac glycosides (e.g., digoxin) in serum include paroxysmal atrial tachycardia with block, atrioventricular block, ventricular ectopy (e.g., bigeminy), and rarely, atrial arrhythmia. Because these effects resemble the clinical condition for which the drug is administered *(82)*, it is recommended that the serum concentration of digoxin or digitoxin be monitored to determine if the drug dose needs to be increased (if the patient is subtherapeutic) or if a possible overdose needs to be treated (if the serum concentration of the drug is above the therapeutic range). Other clinical and emergency situations in which serum digoxin monitoring is recommended include: (*a*) in cases of digitalis intoxication, to determine the amount of antidote (Fab fragment) needed; (*b*) in suspected poisoning by ingestion of plants such as oleandrin, to confirm the presence of digitalis-like poisons; (*c*) in patients with decreased renal function, to adjust digoxin dosage; and (*d*) in cases when other drugs known to interact with digoxin pharmacokinetics (e.g., quinidine, amiodorone, verapamil) are coadministered.

PROCAINAMIDE AND NAPA

In routine therapeutic monitoring, a sample collected 1 h before the next dose (trough) is recommended for determination of both procainamide and NAPA. To establish the acetylation status of a patient, procainamide and NAPA should be measured on the same sample (drawn 3 h after administration of the last dose).

Because NAPA competes with renal elimination of procainamide and is pharmacologically active, both the parent drug and its metabolite should be monitored frequently in cases of decreased kidney function. Also, in cases of overdose or when dosage is to be adjusted, monitoring both procainamide and NAPA is recommended.

QUINIDINE

Determination of serum quinidine concentrations is useful to confirm suspected toxicity, adjust the current dose, and establish patient compliance.

LIDOCAINE

Capacity of the liver to excrete lidocaine is reduced when the drug is administered by infusions for 24 h or longer *(83)*. Accumulation of MEGX can impair metabolism of lidocaine *(84)*. In addition, clearance of lidocaine is decreased in liver disease *(85)*, reduced hepatic blood flow, or coadministered propranolol *(86)*. The mean systemic clearance of lidocaine in patients with congestive heart failure is reportedly only 35% of usual clearance values in healthy subjects *(86)*. In these situations, the elimination half-life is prolonged, and the risk of toxicity becomes greater. Toxicity of lidocaine with regard to the central nervous system (e.g., seizures) could be evident at serum values >8 mg/L *(86)*. Therefore, when the patient is suspected of having altered hepatic blood flow or reduced ability to metabolize lidocaine, the serum concentration of the drug should be monitored.

DISOPYRAMIDE

It has been suggested that disopyramide is proarrhythmic *(52)*. In cases of suspected toxicity and to establish compliance, therapeutic monitoring of disopyramide is recommended. Because various drugs, such as erythromycin and rifampin, can alter hepatic metabolism of this drug, determination of its serum concentrations may be beneficial in obtaining effective concentrations in plasma. In renal failure, mono-*N*-dealkyldisopyramide accumulates, which is thought to contribute to the hypoglycemia induced by disopyramide in this group of patients *(68)*. The half-life of disopyramide may be prolonged to as much as 12 h in patients with recent myocardial infarction, possibly because of a reduced renal blood flow and decreased elimination *(87)*. Therefore, in such patients, therapeutic monitoring is recommended.

FLECAINIDE

Administration of flecainide has been shown to increase mortality in patients recovering from myocardial infarction who have left ventricular dysfunction *(88)*; thus, its long-term benefit in such patients is questionable. In patients with decreased renal function, elimination of flecainide is decreased. Drugs or agents affecting the activity of cytochrome P450 2D6 enzymes can potentially alter the metabolism of flecainide *(1)*. Therefore, therapeutic monitoring of this drug is recommended for patients who are coadministered drugs known to inhibit hepatic metabolism of flecainide, patients with myocardial infarction, and patients with decreased renal function.

Analytical Issues

DIGOXIN

Specimens for true and useful therapeutic monitoring of digoxin should be collected in the postdistribution period (at least 8–12 h after the last dose) because only then will the concentration have a linear relationship to the pharmacological activity. In a recent study *(89)*, in patients who routinely took digoxin at the same time every day, inappropriately drawn specimens constituted 55% of the tests performed—a remarkable waste of resources! On the other hand, in none of the patients who took their daily digoxin dose after 1700 and had blood drawn the next morning were the samples for digoxin inappropriately

drawn. Therefore, it is recommend that laboratory analysts and clinical staff establish procedures to assure the specimens for therapeutic monitoring of digoxin after oral or intravenous administration be collected at least 8 h and preferably 12 h after the last dose.

Biological activities of some metabolites of digoxin (e.g., digoxigenin) are low relative to the parent compound *(90)*; however, their immunoreactivities in some digoxin immunoassays may be greater than that of digoxin *(31)*. Knowledge of the cross-reactivities of digoxin metabolites, especially in renal failure patients (owing to accumulation as a result of decreased elimination), is prudent in interpretation of digoxin results. These findings raise the question of whether the bioactive metabolites of digoxin should be measured with potencies that stoichiometrically reflect their relative fractional bioactivities *(90)*. Although investigators have not yet determined whether immunoassays having proportional cross-reactivities to digoxin metabolites are better in predicting the true biological activity (or toxicity) of this drug, it is intuitive that cross-reactivities of digoxin metabolites in immunoassays should parallel their relative biological activities—because it is the cumulative effect of the drug and its metabolites at the sodium-pump receptor that is clinically relevant. This point remains controversial because only a few manufacturers of immunoassays have antibodies that react proportionally to biological activity *(90)*.

False-positive digoxin results in subjects who had not taken any digitalis compounds or drugs known to interfere with digoxin assays were reported as early as 1965 for receptor-based assays *(91)*. Analysts eventually discovered that false positives could be produced by immunoassays *(92)*. Thus, evaluation of digoxin immunoassay methods for cross-reactivity of endogenous substances (digoxin-like immunoreactive factors) that can cause such false positives is important *(37, 92)*. Furthermore, given the structural similarity between digoxin and certain cardenolides [e.g., Chinese medicine containing Ch'an Su, dried venom of the Chinese toad *(93)* or in plants such as *Nerium oleander (94, 95)*] can cause clinically life-threatening toxic episodes as well as interfere with the accurate quantitative measurement of digoxin in serum. Such cross-reactivity of oleander glycosides in digoxin immunoassays may be desired because the same antidote used for treatment of digoxin intoxication (i.e., Digibind®) can also be used for poisonings from ingestion of the oleander plant *(96–99)*. Substantial recent evidence indicates cross-reactivity can cause both positive as well as negative interferences in different immunoassays *(100)*. Therefore, we recommend that cross-reactivities of the suspected interferants be determined in the presence of digoxin (see below). Knowledge of the extent of these cross-reactivities and their potential interference is important in interpretation of serum digoxin results.

Specimens from patients treated with digoxin antidote (e.g., Digibind) give misleading values for digoxin concentrations by most immunoassays tested to date *(101)*. Determination of the unbound digoxin concentration in serum during treatment with the antidote may be efficacious *(102)*. However, in most digoxin immunoassays, Fab fragments interfere with various steps of the assay (e.g., binding to the tracer, and others) and thus give sporadically unpredictable and erroneous results. Interference from the antidote in serum digoxin measurements of a subject with renal impairment was noted to last >10 days after administration of the antidote *(103)*.

One method for measuring the unbound digoxin is to use ultrafiltration before the immunoassay. Although this procedure is cumbersome, it is has been used successfully *(104)*. For ultrafiltration, serum samples are preferred because serum has better ultrafiltration efficiency than plasma. Some immunoassays are affected by differences in matrix and exhibit bias in digoxin results for samples prepared by adding known concentrations of digoxin to serum or its ultrafiltrate (Fig. 1). Therefore, differences in matrix (ultrafiltrate vs serum) should be evaluated before determining unbound digoxin concentrations by ultrafiltration followed by immunoassay of the ultrafiltrate. Furthermore, no bias between serum and its ultrafiltrate for analysis of unbound digoxin should be set as the goal in development of new digoxin immunoassays.

Another approach is to develop immunoassays that will directly measure only the unbound fraction of digoxin in presence of Fab in serum. Although not established unequivocally, some digoxin immunoassays seem to have come close to this goal *(105, 106)*. We recommend that all manufacturers of digoxin immunoassays investigate and determine the effects of antidote on the measurement of unbound digoxin in their assays. We have observed that in vitro experiments (with digoxin and the antidote added to serum) do not represent a valid test of effects of antidote on unbound digoxin measurements (data not shown). Therefore, we also recommend that, in evaluating immunoassays for their ability to accurately measure unbound digoxin in the presence of antidote, samples should consist of serum collected from patients on digoxin who have also been treated with antidote. We propose the following protocol for evaluation of unbound digoxin analysis in the presence of antidote:

1. Determine if the immunoassay is affected by differences in matrix (i.e., ultrafiltrate of serum vs the serum itself). If the assay is not affected, one can analyze ultrafiltrate by the assay.

2. Using a Centricon concentrator (30-kDa cutoff) or equivalent, ultrafilter 1 mL of serum (at 25 °C and 1500*g* for 20 min) collected from a patient taking digoxin who has been treated with the antidote. Measure the ultrafiltrate for digoxin.

3. Analyze the serum directly by the immunoassay without ultrafiltering it. If results of several serum samples and their ultrafiltrates are not statistically different, the digoxin immunoassay may be used for determination of unbound digoxin in the presence of Fab fragments.

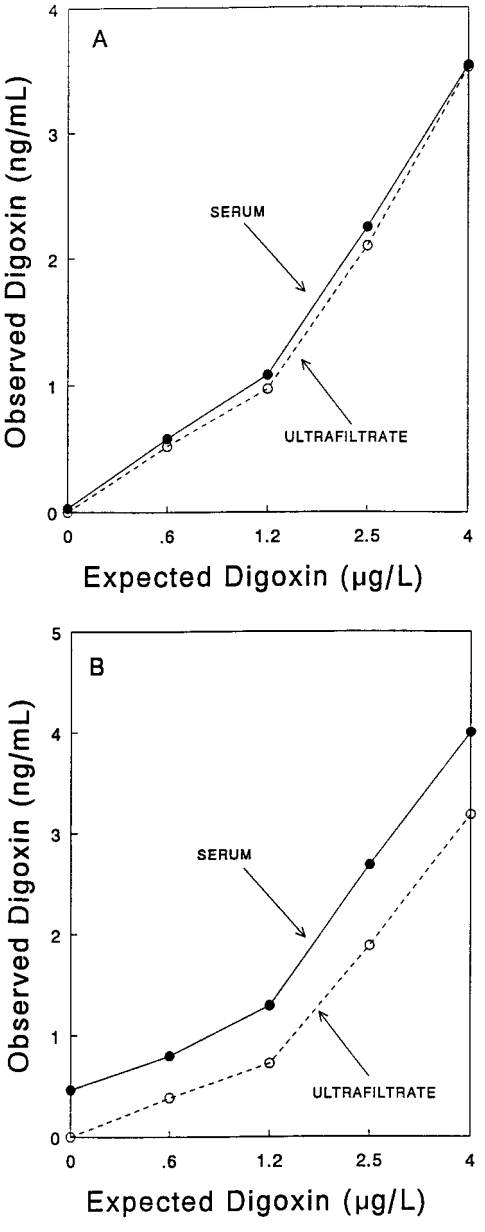

Fig. 1. Analysis of digoxin in the serum and its ultrafiltrate by an immunoassay with no significant matrix bias (*A*) and one with bias (*B*).

A pool of digoxin-free serum (~20 mL) was prepared, one-half of which was centrifuged at 1500*g* (25 °C) for 45 min in a fixed-angle centrifuge and the other half used to prepare serum controls containing various concentrations of digoxin. Identical amounts of digoxin were added to the ultrafiltrate to make similar concentrations of digoxin in the ultrafiltrate. Solutions were analyzed by the nonpretreatment AxSYM digoxin assay (Abbott Diagnostics; *A*) and by the Stratus II digoxin assay (Dade Behring; *B*). Each point is an average of two measurements. Note that the observed values of digoxin in the ultrafiltrate (○) are consistently lower than those in serum (●) in the Stratus digoxin assay but are not affected in the AxSYM assay.

Samples with various Fab and digoxin concentrations should be used in this evaluation.

4. Serum samples collected from patients treated with Fab fragments (with known unbound digoxin concentration as verified by ultrafiltration) can be used as controls for both ultrafiltration and direct measurements of unbound digoxin.

PROCAINAMIDE AND NAPA

Both procainamide and NAPA should be measured on the same sample. Concentrations of procainamide and NAPA can be determined either individually by automated immunoassays or in the same chromatographic run by HPLC *(48, 49)*.

Procainamide can be administered intravenously dissolved in dextrose solutions. However, Henry et al. reported that the procainamide–dextrose complex does not revert to the free procainamide hydrochloride (in vitro) during the first 8 h of incubation at physiological temperature *(107)*. Cross-reactivities of such complexes in procainamide immunoassays should therefore be evaluated.

CROSS-REACTIVITY STUDIES

Establishing the cross-reactivity of compounds in immunoassays is necessary in characterizing the specificity of these techniques. In this regard, because of the low-molecular-mass compounds in question, several issues are particularly important in TDM. In characterizing the cross-reactive patterns of drugs, several items need to be noted: Molar (and not mass) concentrations must be used in calculations *(108)*; cross-reactants should be evaluated in both the presence and absence of the principal ligand being measured *(37)*; and the evaluation should be done over a wide range of concentrations *(109)*. Despite the common belief that cross-reactants always introduce a positive bias in immunoassays, it has recently been reported that cross-reactants are capable of suppressing recovery of the expected immunoassay results *(100)*. This phenomenon has been shown to be related to the design of the assay and thus may have general implications. Therefore, we recommend that suspected cross-reactants (e.g., drug metabolites) be added and tested in the presence of the principal ligand and also be analyzed for their impact on assessing analytical recovery.

MONITORING UNBOUND DRUG

Several cardiac drugs have been shown to bind to α_1-acid glycoprotein in serum. In pathological conditions such as myocardial infarction, trauma, surgery, rheumatoid arthritis, cancer, and morbid obesity, α_1-acid glycoprotein concentrations increase *(55)*. Oral estrogen therapy and inflammation have opposite effects on the hepatic glycosylation of α_1-acid glycoprotein *(110)*. Furthermore, in nephrotic syndrome, serum concentrations of α_1-acid glycoprotein have been shown to decrease from 0.95 ± 0.28 (mean ± SD) to 0.34 ± 0.12 g/L during exacerbation vs the remission phase *(111)*. Because of significant binding to α_1-acid glycoprotein, the unbound amounts of several antiarrhythmic drugs, including disopyramide *(66)*, lidocaine *(66, 112, 113)*, and quinidine *(114)* vary as the concentrations of this protein change in serum. Because the unbound fractions of these drugs are responsible for their

pharmacological activities, monitoring the total drug concentrations may be misleading. Therefore, we recommend that, whenever possible, the unbound concentrations of these drugs be monitored. We also recommend that reference ranges for the unbound concentrations be established. Several ultrafiltration or equilibrium dialysis methods for separation of bound and unbound antiarrhythmics have been reported in the literature; however, they are time-consuming, and lack of matrix bias (between serum and its ultrafiltrate) has not been demonstrated (see Fig. 1) in many cases. Therefore, we recommend that manufacturers develop automated immunoassays to perform such tasks without the requirement that the operator separate the fractions. Immunoassays capable of measuring unbound drugs in serum can also have an impact on TDM in areas beyond monitoring cardiac drugs.

SERUM GEL SEPARATOR TUBES

Serum gel separator tubes should not be used to collect samples for any of the cardiac drugs unless any potential interference has first been evaluated. Recoveries of lidocaine, quinidine, phenobarbital, and phenytoin collected in Vacutainer Tube plastic or glass separator tubes are decreased, whereas recoveries of procainamide and NAPA are not affected *(115)*.

Practice Issues

Practice issues center around understanding the limitations of the assays, how reported results may be interpreted by clinicians, and how logistics of sample collection and transport affect reported results. Several examples pertaining to cardiac drugs are indicated and should be considered by laboratories establishing a cardiac drug TDM unit. One issue is that of reporting very low values, well below the therapeutic range, particularly values near or at the detection limits of the assays. Results below the lower analytical range of the assay should be considered suspicious and be further investigated. In the vast majority of cases, ordering an analysis implies the drug has been administered. Thus, a report of "not detectable" leads to suspicion of mixed samples or inappropriate collection of some kind, and so forth. We recommend that laboratories establish a mechanism for contacting the clinician to investigate the suspected result before making the final report.

Given the many difficulties and challenges in measuring digoxin *(37)*, we recommend that laboratorians and clinicians become aware of these difficulties so the serum digoxin results will be interpreted with these in mind. It is often difficult, if not impractical, for the laboratory to establish a priori if a sample was drawn appropriately (>8–12 h after the last dose). Digoxin results obtained on samples drawn before the completion of the distribution phase lack clinical value; therefore, the laboratory should establish a mechanism for contacting the ordering clinician, whenever possible, before reporting high digoxin values (e.g., >3.5 μg/L). If it is determined the sample was drawn inappropriately, then another specimen should be drawn at the correct time. If recollection is not possible, the current sample can be analyzed and reported with an annotation attached indicating the sample collection was inappropriately timed. Another important practice issue is that clinical users of this analysis must be aware that digoxin should not be measured on samples obtained from patients having recently (<2 weeks) been placed on antidotal therapy (Fab fragments). Otherwise, the laboratory must demonstrate that the method used is not adversely affected by presence of the antidote.

Serum concentrations of procainamide should not be assessed alone. NAPA should also be analyzed (on the same sample), and each analyte should be quantified with reference to its own reference range.

Coadministration of quinidine can increase serum digoxin, even at therapeutic concentrations *(54)*, by the previously discussed mechanisms. Amiodarone *(116)* and itraconazole *(117)* also reportedly increase serum digoxin concentrations. The mechanisms of amiodarone and itraconazole interactions with digoxin have not yet been characterized, but they can increase the serum digoxin concentrations to toxic values. Therefore, we recommend that serum digoxin be measured in patients currently taking digoxin before treatment with quinidine, amiodarone, or itraconazole is initiated. Subsequent reductions in the dosage of digoxin may also become necessary. Many other interactions with cardiac drugs are known. A compilation of such interactions is available *(118)*, and laboratory analysts should be aware of them.

Reporting Issues

Critical values (low or high) should be analytically verified by whatever mechanism the laboratory has established and the physician be notified immediately. This can help in quality assurance and in assuring intervention in the case of potential drug overdose.

Reporting results for digoxin samples collected <8 h after the last dose should be done only if redraw is not possible and the physician insists that the value be reported. If the result for such samples is to be reported, appropriate annotation should be included with the result.

Digoxin results on a serum from a patient treated with the antidote should be reported only if the method has been proved to be unaffected by the presence of Fab fragments.

Procainamide and NAPA have different pharmacokinetic as well as pharmacodynamic properties *(41)*. Although the parent drug is considered to be a class I antiarrhythmic, pharmacological actions of the metabolite (NAPA) more resemble the class III antiarrhythmic actions (e.g., K^+-channel blockade) *(41)*. The common practice of summing their concentrations as one value should be avoided. Although reference ranges for the total concentration are available, we recommended that the individual reference ranges for pro-

cainamide and NAPA be used for assessing their therapeutic efficacies and toxicities.

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